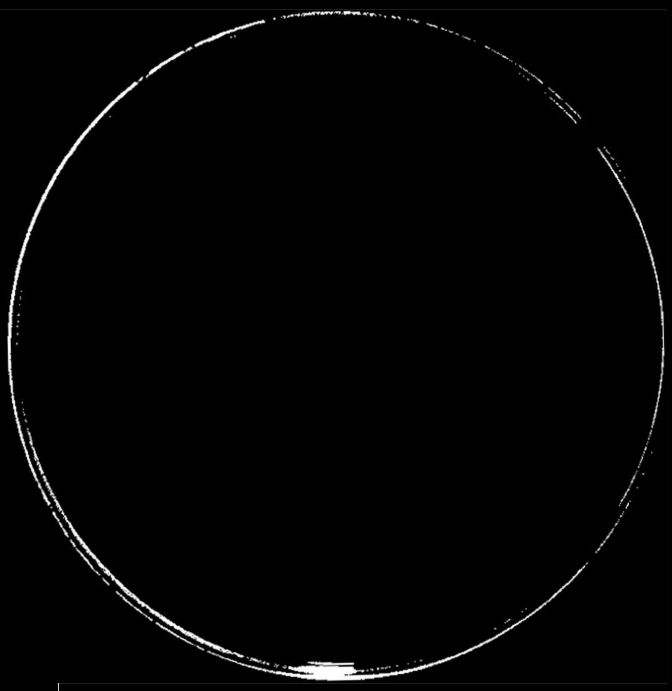
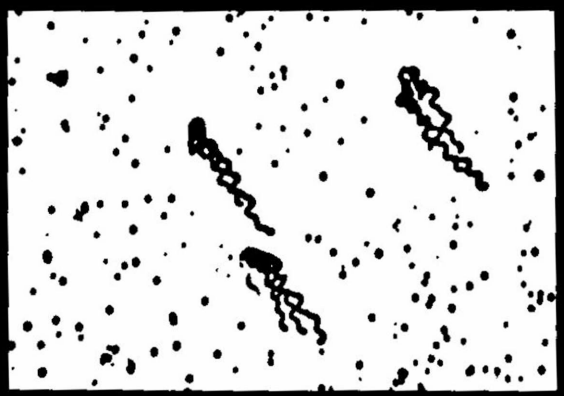
CDC

IDENTIFICATION OF
Unusual Pathogenic Gram-Negative Aerobic and Facultatively Anaerobic Bacteria







1984

PARTMENT OF HEALTH AND HUMAN SERVICES•Public Health Service•Centers for Disease Control

NOMENCLATURE CHANGES

Names Used in Book	New or Proposed Names
Achromobacter xylosoxidans	*Alcaligenes denitrificans* subsp. *xylosoxydans*
Agrobacterium radiobacter	*Agrobacterium tumefaciens*
Alcaligenes denitrificans	*Alcaligenes denitrificans* subsp. *denitrificans*
Alcaligenes faecalis	Classification unknown
Alcaligenes odorans	*Alcaligenes faecalis*
Flavobacterium sp. (□b)	*Flavobacterium indologenes*, *F. gleum*
Flavobacterium multivorum	*Sphingobacterium multivorum*
Flavobacterium spiritivorum	*Sphingobacterium spiritivorum*
Haemophilus equigenitalis	*Taylorella equigenitalis*
Pasteurella anatipestifer	*Moraxella anatipestifer* (species *incertae sedis*)
Pseudomonas maltophilia	*Xanthomonas maltophilia*
Pseudomonas marginata	*Pseudomonas gladioli*
Pseudomonas mesophilica	*Protomonas extorquens*
Pseudomonas putrefaciens	*Alteromonas putrefaciens*
Pseudomonas thomasii	*Pseudomonas pickettii*
Vibrio fluvialis (strains with gas from glucose)	*Vibrio furnissii*
∇a-1	*Pseudomonas pickettii*

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Identification of Unusual Pathogenic Gram-Negative Aerobic and Facultatively Anaerobic Bacteria

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with Photographs by James D. Howard and William A. Clark

OCTOBER 1984

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Elizabeth O. King (1912-1966)

PREFACE

DEDICATION– ELIZABETH O. KING

The research of bacteriologist Elizabeth O. King (1912-1966) forms the nucleus of this manual, which we dedicate to her.

Miss King was born in Atlanta in 1912. She received a Bachelor of Science in Zoology degree from the University of Georgia and a Master of Science in Medical Technology degree from Emory University. During World War II she served as a commissioned officer (bacteriology) in the Army at Fort Detrick, Frederick, Maryland. In 1946-1948 she worked in the Emory University Hospital. In 1948 Miss King joined the staff of the Communicable Disease Center, now the Centers for Disease Control (CDC), in Atlanta, where she worked until her untimely death from cancer in April 1966.

Miss King's first assignment at CDC was in the Diphtheria Laboratory, where she worked for 3 years with Dr. Elizabeth Parsons. In 1951 she transferred to the newly established General Bacteriology Laboratory, where she worked under the Director, Dr. Martha K. Ward. There she became intensely interested in the gram-negative rods which are associated with disease, but which do not belong to the family Enterobacteriaceae. Many of them were poorly classified. Having worked in two different hospital laboratories, Miss King was keenly aware of the diagnostic dilemma these bacteria presented. With characteristic concentration and determination, she set herself to the task of bringing some order into the classification of these organisms. This she did by means of a systematic approach, a keen power of observation, and a zeal for her task.

Miss King was internationally known as an authority on a variety of unusual bacteria. Her rational and systematic approach led to the characterization and establishment of *Flavobacterium meningosepticum* as a new species of bacteria and the proof that this organism was an etiologic agent of nosocomial epidemics of meningitis in infants.

The same approach was used to simplify the identification of such organisms as *Acinetobacter calcoaceticus* (then *Herellea vaginicola* and *Mimi polymorpha*), *Pasteurella* species, and *Campylobacter* (then *Vibrio*) *fetus*. Miss King was also among the first workers in the United States to perceive the importance of *Listeria* in human infections and to alert the medical profession to the prevalence of listeriosis.

Her observations were generously transmitted to others who sought her advice through correspondence, bench training, and formal courses at CDC.

Miss King's contributions and devotion to her profession were an inspiration to those who had the good fortune of knowing her. This manual brings together in a single volume the legacy of information from Miss King's own work and from that of her colleagues.

C88331

ACKNOWLEDGEMENTS

We acknowledge the support Dr. Albert Balows, Assistant Director for Science, Center for Infectious Diseases, Centers for Disease Control, gave us in writing this manual. In the spring of 1976, we approached him with the idea of bringing together in book form the information contained in the various files of the Special Bacteriology Laboratory at CDC and in the minds of its staff members. He replied that he had hoped this could be done.

Mr. Earl Long, Director (1961-1982) of the Laboratory Section, Georgia Department of Human Resources (GDHR), generously contributed facilities and resources for part of the work.

Mr. Harvey Tatum, through his close association as a laboratory investigator with Miss King and his meticulous work with the organisms dealt with in the manual, contributed greatly to the reliability of the data. He also provided valuable information for writing the historical background alluded to in the Preface and first chapter (''Introduction'') of the manual. The chapter on ''Miscellaneous Gram-negative Bacteria,'' written by Mr. Tatum (with W. H. Ewing and R. E. Weaver) in the second edition of the *Manual of Clinical Microbiology* (American Society for Microbiology, Washington, D.C., 1974) was a valuable source of information.

We also thank present and former members of the Special Bacteriology Laboratory staff for helping to develop these data, the secretaries at CDC and the Georgia Department of Human Resources for preparing the manuscript, and the editors, Ms. Edwina Davis and Ms. Charlotte Turner, for their editorial assistance, also Mr. Ralph Ramsey (GDHR) for his help with the photography.

We are also grateful for the patience, support, and understanding of our families during the preparation and writing of this manual.

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INTRODUCTION

The Special Bacteriology Laboratory, Division of Bacterial Diseases, Center for Infectious Diseases, Centers for Disease Control (CDC), Atlanta, Georgia, has on file the recorded characteristics of more than 60,000 unusual pathogenic bacteria which have been submitted for identification over the past 30 years. Until now, only a small portion of these data have been available in published or summary form. Bacteriologists in hospitals and clinical laboratories need ready access to these data to help them identify these strange and unusual organisms. To meet this need, this manual presents descriptions of species and as yet unnamed "groups", the compiled detailed characteristics of strains examined, laboratory media and methods used in identification, photographs of colonies and photomicrographs of stained preparations of selected strains of the various species and groups, sources of strains submitted for identification, and pertinent references.

In 1948, Miss Elizabeth O. King came to CDC when the organization, established to study malaria and the exotic diseases brought back by servicemen in World War II, was in its infancy. CDC needed a laboratory to study organisms which the State health departments were having difficulty identifying. So CDC established the General Bacteriology Laboratory under Dr. Martha K. Ward, and in 1951 Miss King was appointed to the laboratory staff because of her broad background in bacteriology.

Miss King spent much time in research, trying to match up these unidentified organisms with known cultures obtained from culture collections and from specialists throughout the world.

Each culture that was submitted for identification by the State laboratories was propagated, frozen, and stored at below $-42^{\circ}C$, thus preserving it for future study and comparison. The large collection thus amassed is still extant.

Each culture (as submitted and as a subculture) was stained for microscopic examination and inoculated into a battery of test media. The results were recorded over a 7-day period (some were observed for 2 to 3 weeks for certain characteristics helpful in identification). Known cultures for many named species were obtained and studied with the same battery of tests, so that valid comparisons could be made between known and unknown cultures. (Miss King would never send out a report identifying an organism with a named species until she had studied a known culture of that species. If she could not be quite sure, she would merely report "resembles" a certain organism.) Results were recorded on data cards illustrated in Fig. 1.

The culture data being collected were compiled first on McBee punch cards and later on cards designed by Dr. Ward and Miss King (Fig. 2). Data presented in the charts in this manual were compiled from the second set of cards.

In 1964 at a round table meeting entitled "Current Trends in Diagnostic Microbiology," Miss King first presented her "Identification of Unusual Gram-negative Pathogenic Bacteria." There, in Washington, D.C., at the annual meeting of the American Society for Microbiology, Miss King gave the results of research she had done since 1951 in the form of the "Round Table." From that time until the present the General Bacteriology Laboratory (now the Special Bacteriology Laboratory) has collected more information and data; its staff, under Dr. Robert E. Weaver, has revised and enlarged Miss King's original "Round Table" several times (1967, 1972, and 1974).

Miss King used the "Round Table" in teaching the Laboratory Methods in Special Medical Bacteriology Course at CDC, and the Bacteriology Training Branch, with the help of Dr. Weaver's staff, has continued this practice. Thus, since 1964, CDC has published and distributed this document to hundreds of clinical bacteriologists. Now, using the "Round Table" as a basis, we present this manual, an expanded and up-to-date volume, to clinical bacteriologists, as a further aid in identifying "strange and unusual bacteria" of clinical significance.

The user of this manual should keep in mind that many bacteriologists, working over a period of 25 years, recorded the culture results from which these data have been compiled. During that time there were changes in the formulations of some of the media and in the procedures for some of the tests. These circumstances have undoubtedly produced an unknown degree of error in the data.

CULTURE NO. | SENDER'S NO. | DATE REC'D. | IDENTIFIED AS:

SENDER: STATE H.D.

SOURCE: PATIENT:

MORPHOLOGY OF ORIGINAL CULT.: GRAM

PURE CULT.: H.I. SLANT (24 HRS.): GRAM

H.I. BROTH (24 HRS.): FLAGELLA:

TYPE OF GROWTH: BLOOD AGAR (H/R) 24 HR. CO_2):

A.F.: SPORE:

H.I. SLANT: H.I. BROTH:

GROWTH TEMP.: 5° 25° 35° 42° 52° O_2 REQUIREMENTS ODOR

BASE	24	48		1 wk		BASE	24	48		1 wk	BASE	24	48		1 wk	24
GLUCOSE						CATALASE					TSI SI / BUTT					LITMUS 48
D-XYLOSE						OXIDASE K/ROUT.					H_2S PAPER BUTT					MILK
MANNITOL						MAC CONKEYS					MR /VP ROUT.					1 wk
LACTOSE						S.S.					MR /VP SPEC.					PIGMENT:
SUCROSE						CITRATE					MOTILITY WP DIFCO					GROWTH
MALTOSE						CETRIMIDE					GELATIN					WATER SOLUBLE:
FRUCTOSE						UREA SERUM PLAIN					ESCULIN HYD.					FLO.
CONTROL						NITRATE INF. ROUT.					% 0					TECH.
10% SLANT LACTOSE						INDOLE INF. ROUT.					N_aCl 6					HIT.

CDC 56.16 (f. 3.96A)
12/81

LABORATORY DATA CARD

BASE	24	48		1 wk		BASE	24	48		1 wk		PAI:
GLUCOSE						GLYCOGEN						SEROLOGY, NOTES
D-XYLOSE						ERYTHRITOL						
MANNITOL						MELIBIOSE						
LACTOSE						MELEZITOSE						
SUCROSE						STARCH						
MALTOSE						BLANK						
GLYCEROL						FAT HYD.						
SALICIN						NUT. AGAR 25 35						
L-ARABINOSE						TOMATO JUICE						
ADONITOL						KETOGLUCONATE						
DULCITOL						ACETAMIDE						
D-GALACTOSE						SERINE						
FRUCTOSE						TARTRATE						
MANNOSE												
RHAMNOSE												
TREHALOSE												
RAFFINOSE						PPA.						
SORBITOL						LYSINE						
INOSITOL						ARGININE						
CELLOBIOSE						ORNITHINE						
INULIN						BLANK						
DEXTRIN						KCN						

Figure 1. Laboratory data card. Front (above), reverse (below).

GENUS _____ SPECIES _____ CARD NO. _____

Cult No.	Glu	Xyl	Man	Lact	Suc	Malt	Bk	Aes	Pen	Pig	O/F	10%/L	TSI S/BH$_2$S	Hem	Cat	O	A/S/T	MeC/SS	Cit/Cet	Urea	Nit	Ind	MR/VP	Gel	Milk	NaCl/Tol	PPA	S/A	Temp	Mol	Notes & Source	Sender

Nomenclature of Taxa Included in the Manual

In January 1980, the International Committee on Systematic Bacteriology (ICSB) published the "Approved Lists of Bacterial Names"—names which the Committee considered to have been validly published as of January 1, 1980.—in the *International Journal of Systematic Bacteriology*, volume 30, pages 225-240. Many of the names appearing in this manual are not in the "Approved Lists of Bacterial Names", and therefore the Committee holds that they have no standing in bacterial nomenclature. All names cited in the manual, however, are those which Miss King and her successors in the Special Bacteriology Laboratory, CDC, and clinical bacteriologists in general have used through the years. Hence, we have used them in the manual, even though some of them have not been validly published. (A number of these taxa have been validly named since the appearance of the "Approved Lists".)

For clarity, those names appearing in this manual which are in the "Approved Lists of Bacterial Names" are identified in the Index to "Round-Table" Charts, p. 43 by a double asterisk.

Evaluation of Clinical Bacterial Specimens Submitted for Characterization and Identification

In this chapter, we describe how clinical bacterial specimens have been evaluated through the years in the Special Bacteriology Laboratory, Division of Bacterial Diseases, Center for Infectious Diseases, Centers for Disease Control.

Bacterial growth from the submitted specimen is Gram-stained and streaked (quadrant method) onto a 5% defibrinated rabbit blood agar plate (when sheep blood is employed, the hemolytic reactions may vary from those obtained on rabbit blood). The agar is stabbed several times with the inoculating loop in the primary quadrant streak to create an area of reduced oxygen tension for enhancement of hemolysis. Then the plate is inverted and incubated overnight in a candle jar at 35°C. Reactions on blood agar are described below.

After overnight incubation (20-24 h), the blood plate is observed for the amount of growth, colonial morphology, and hemolytic reaction. An isolated colony of the organism is picked to a heart infusion broth (HIB), a heart infusion agar (HIA) slant, and a triple sugar iron (TSI) agar slant (streaked and stabbed). When colonies are very small, several are picked. All of the cultures are incubated for 18-24 h at 35°C in room atmosphere. If the organism appears to grow poorly on the blood agar plate, a blood agar slant is also inoculated; the four tubes of media are incubated in a candle jar at 35°C. After the colony is picked, the oxidase test is performed. After incubation, Gram stains are made from the HIA and HIB cultures, and the cellular morphology is noted. The TSI slant is examined to determine presumptively the culture's mode of carbohydrate utilization (see below, "Oxidation, fermentation, or nonutilization of carbohydrates").

The bacterial isolate, now of known cellular morphology and with a presumed mode of carbohydrate metabolism, is characterized biochemically by using a basic set of differential media: carbohydrates glucose, xylose, mannitol, lactose, sucrose, and maltose in appropriate base (enteric fermentation base is used for fermenters, and the OF base semisolid agar is used for non-fermenters of glucose); MacConkey agar slant; *SS* agar slant; Simmons' citrate agar; motility medium; urea agar (Christensen's); nitrate broths (routine and special); tryptone broth for indole; litmus milk; nutrient broth; nutrient broth with 6% sodium chloride; gelatin; HIA slant; esculin agar slant; three tryptose glucose yeast (TGY) agar slants for temperature range testing (25°, 35°, and 42°C); and TSI agar (a strip of lead acetate paper is suspended above the slant).

Additional media for **fermenters** are MR-VP broth for the methyl red test, an additional HIA slant to grow a culture for inoculating an MR-VP broth for the Voges-Proskauer test (Coblentz method), and a 10% lactose agar slant.

Additional media for the **oxidizers** and **nonutilizers** of carbohydrates are an OF-base control containing no carbohydrates, a cetrimide agar slant, "Flo" and "Tech" agar slants, and a heart infusion tyrosine agar slant.

Special media for *Neisseria* species include the carbohydrates glucose, lactose, sucrose, maltose, and fructose in CTA base.

The decarboxylase tests for lysine and ornithine, the dihydrolase test for arginine, and other tests are useful in characterizing certain microorganisms, as indicated in the tables of biochemical data.

Inoculation of differential media

With a straight needle and inoculum from the HIA slant, the Simmons' citrate is inoculated lightly on the slant, the motility medium by stabbing once about 5 mm under the surface, and the six OF base carbohydrates and the control tube and/or CTA carbohydrates by stabbing four times approximately 2.5 cm under the surface (for the *Neisseria* cultures a heavy inoculum is introduced with a loop and mixed throughout the entire top 5-10 mm of the CTA medium).

With a capillary pipette and the HIB culture, the litmus milk and the gelatin are inoculated with four drops of inoculum, and the remaining media with one drop of inoculum each. When the agar slants are inoculated they are rotated to insure that the inoculum is evenly distributed over the surface of the agar.

When growth of fastidious microorganisms needs to be enhanced, 1-2 drops of sterile rabbit serum are added to each of the media.

After the media are inoculated, they are incubated at 35°C in room atmosphere and maintained under those conditions, unless the growth temperature tests indicate that either 25° or 42°C is closer to optimal temperature.

Fastidious organisms are incubated in the candle jar or CO_2 incubator, unless they are shown not to require such an atmosphere.

All differential media are examined after 1, 2, and 7 days of incubation, with the following exceptions. (1) The TGY slants are examined after 18-24 h of incubation for comparative growth. The tube with the best growth is used for the catalase test. Negative catalase reactions are confirmed by testing all of the TGY slants having growth. If no growth appears at any temperature, the slants are reincubated, or HIA slants are inoculated and incubated at the prescribed temperatures. (2) The nitrate reduction and indole production tests are performed on 48-h cultures. (3) The methyl red test is performed on 48-h

cultures (poorly growing cultures are assayed after 4-5 days of incubation). (4) Gelatin liquefaction is tested at 1, 2, 5, 7, and 14 days before a negative reaction is reported.

Reactions on rabbit blood agar

Beta hemolysis is the same as that described for the streptococci, except that the total destruction of all the red blood cells within the hemolytic area is not as important as it is for the streptococci. Some microorganisms show little hemolysis surrounding individual colonies, but show marked hemolysis in the stabs. For example, *Listeria monocytogenes* shows very weak hemolytic action under the streaks and usually none at all under isolated colonies. In the stabs this organism shows a very distinct, narrow, clear zone of hemolysis at 24 h. Other microorganisms show a well-defined zone of hemolysis extending out from the heavy portion of the streak. Beta hemolysis usually has a very sharp and well-defined edge. The zones in this case seem to be devoid of hemoglobin and appear colorless.

Incomplete beta hemolysis is exemplified by *Gardnerella vaginalis*, which, after 3-4 days of incubation in a candle jar, shows wide but incomplete zones of hemolysis. Even upon prolonged incubation the hemolytic action never becomes completely clear, nor will the edge of the zone become well defined.

Lysis is the term used when the red blood cells are lysed but the hemoglobin is not decolorized (destroyed) as it is in beta hemolysis. In this case, the edge of the zone is not well defined. Some organisms lyse the whole area of the plate; some produce lysis under the heavy portion of the streak; well-isolated colonies never show any action on the blood.

Greening is the same general action as lysis, except that the lysed area has a greenish tinge.

Alpha hemolysis is pronounced greening under heavy growth and around well-isolated colonies, usually without a lysed area. Some microorganisms show this only on prolonged incubation. These usually show alpha hemolysis around stabs and a faint reaction under the heavy growth within 24 h. Generally, these microorganisms are poor growers. There is no lysis of red blood cells.

Browning is a reaction whereby the blood turns dark brown and completely opaque under the area of heaviest bacterial growth. Well-isolated colonies sometimes show alpha hemolysis.

Lavender-green is a reaction in which lavender and green appear under the areas of heavy growth. Very proteolytic organisms, such as *Pseudomonas aeruginosa*, *P. maltophilia*, *Aeromonas hydrophila*, and *Flavobacterium meningosepticum*, produce this reaction.

Soluble brown pigments are produced by some bacteria, such as melanin-producing strains of *P. aeruginosa*. These pigments diffuse from the heavy portions of growth.

Lysis-inhibition zones occur with some organisms; the blood immediately surrounding an isolated colony remains unchanged, but an area of lysis occurs outside the zone of intact cells.

Compacted red blood cells are noted with some organisms. A well-isolated colony has a very narrow zone of lysis immediately surrounding the colony, and beyond this a "compacted" and narrow, dense band of red blood cells occurs.

Oxidation, fermentation, or non-utilization of carbohydrates

Triple sugar iron (TSI) agar is generally used to differentiate fermentative from oxidative and noncarbohydrate-utilizing microorganisms. An organism which produces in 24 h an acid butt with or without an acid slant is considered fermentative. An oxidizer or non-utilizer of sugars produces no change in the butt and slant, or produces alkalinization of the slant. Occasionally, an oxidizer produces slight acidity on the slant. However, a poorly growing organism which produces a degree of acidity of the slant and no change in the butt is tentatively designated a fermentative organism. In addition, some fermentative organisms (usually fastidious) do not acidify either the butt or the slant of TSI agar.

In cases when there is no change in the butt of TSI agar or the interpretation of the reaction is doubtful, an oxidation-fermentation (OF) test is used to establish whether an organism ferments, oxidizes, or does not produce acid from glucose.

Enteric fermentation base medium with carbohydrates is used to detect acid production by fermentative microorganisms. Oxidative organisms usually do not produce a detectable amount of acid in the enteric base carbohydrate medium for one of two reasons: (1) the accumulation of acid products produced by oxidation generally is less than that produced by fermentation; or (2) sufficient alkaline products frequently are produced from the peptone in the medium to neutralize the acid produced by oxidative metabolism.

An OF-base medium is used to determine the pattern of carbohydrate metabolism of nonfermentative bacteria (the Special OF Medium [King] is the medium upon which carbohydrate reactions given in this manual are based). OF-base medium contains 0.3% agar and less peptone than does the fermentation base medium. The agar inhibits diffusion of the acid produced and concentrates it in the area of bacterial growth.

After the biochemical characteristics of an unknown organism are determined as described above, and with media and methods as detailed in the following chapter, the reactions are carefully recorded (see fig. 1). The keys then are used to select a group of species, the characteristics of which can be compared to those of the unknown to attempt to arrive at the correct identification.

Media* and Methods

List of Titles

- Acetate agar
- Acid dichromate cleaning solution
- Amylosucrase test with 5% sucrose agar
- Benedict's reagent (double strength)
- Blood agar
- Bordet-Gengou agar without peptone (Difco)
- *Brucella* broth for sodium chloride and glycine tolerance and H_2S production by *Campylobacter*
- 10% Carbohydrate agar slants
- Catalase reaction
- Cetrimide agar
- Charcoal yeast extract (CYE) agar
- Chocolate agar
- Citrate agar (Simmons') (Difco)
- Coccobacilli and cocci, differentiation of
- Cysteine agar (carbohydrate base for *Francisella*)
- CTA medium (BBL)
- "Decarboxylase tests" (Moeller's Method) (decarboxylases for lysine and ornithine, dihydrolase for arginine)
- Dye tolerance test for *Brucella*
- Enteric fermentation base (carbohydrate fermentation) medium (Difco)
- Esculin medium
- Feeley-Gorman (F-G) agar
- Flagella broth
- Flagella stains
- Fluid thioglycollate medium (BBL)
- Gas production from fluid thioglycollate medium (BBL)
- Gel formation (*Brucella canis*)
- Gelatin liquefaction
- Glucose cysteine peptone agar
- Gram stain
- Growth factor test for *Haemophilus*
- Heart infusion agar (Difco)
- Heart infusion broth (Difco)
- Heart infusion tyrosine (HIT) agar
- Indole spot test
- Indole test
- 2-Ketogluconate test
- 3-Ketolactonate medium for *Agrobacterium*
- Litmus milk
- MacConkey agar (Difco)
- Maintenance of bacteria in motility medium
- Methyl red (MR) test
- Motility test
- Nitrate broth, semiaerobic (Stanier)
- Nitrate reduction test
- Nitrite reduction test
- Nutrient agar (Difco) growth test for pathogenic *Neisseria*
- OF (Oxidation/Fermentation) medium
- ONPG (O-Nitrophenyl-Beta-D-Galactopyranoside) test
- Oxidase test
- Peptic digest of blood
- Phenylalanine agar
- Pigment production by pseudomonads
- Porphyrin test for *Haemophilus*
- Potassium hydroxide test for verifying the gram reaction of bacteria
- Preservation of bacteria by freezing
- Rapid sugar test for *Neisseria*
- Saline, buffered (pH 7.0)
- S S agar (Difco)
- Salt tolerance test
- Selective medium for pathogenic *Neisseria* (Thayer-Martin Medium-modified)
- "String" test for vibrios
- Thiosulfate citrate bile salts sucrose (TCBS) agar (BBL)
- Transformation assay for *Acinetobacter* and *Moraxella osloensis*
- Triple sugar iron agar (TSI agar)
- Tryptone glucose yeast extract (TGY) agar
- Urea agar (Christensen's)
- Voges-Proskauer (VP) test (Coblentz Method)
- Wayson's stain

*The composition of each commercially-prepared medium cited in this manual is stated with quantities of the ingredients present in one liter of the finished medium.

• ACETATE AGAR

This medium is used to determine whether the tested organism can use acetate as a sole source of carbon; breakdown of sodium acetate causes the pH of the medium to shift toward the alkaline range, turning the indicator blue.

The medium consists of sodium chloride, 5 g; sodium acetate, 2 g; magnesium sulfate, 0.2 g; monoammonium phosphate, 1 g; dipotassium phosphate (anhydrous), 1 g; bromthymol blue, 0.08 g; agar, 20 g; and distilled water, 1 liter. The pH is 6.7 ± 0.2.

The medium is tubed in 3-ml portions into 13- X 100-mm screwcapped tubes, autoclaved at 121°C for 15 min, and slanted for cooling. Each slant is inoculated with one drop of an 18- to 24-h HIB culture. These are incubated at 35°C and observed for up to 7 days. Positive reactions are indicated by the medium becoming alkalinized and blue.

Reference: Trabulsi LR, Ewing WH: Sodium acetate medium for differentiation of *Shigella* and *Escherichia* cultures. Pub Hlth Lab* 20:137-140, 1962.

• ACID DICHROMATE CLEANING SOLUTION

Sixty grams of potassium dichromate is dissolved in 300 ml of distilled water; 252 ml of concentrated sulfuric acid is added **cautiously**, with mixing. The Pyrex glass mixing container is placed in cold water to dissipate the heat. After repeated use (100-200 slides per 100 ml of solution), the cleaning fluid turns dark. At this point, it is discarded and replaced.

Before being discarded, this strong acid is either neutralized with NaOH or diluted by being poured into copious amounts of water.

• AMYLOSUCRASE TEST WITH 5% SUCROSE AGAR

The test is used to detect an amylosucrase enzyme produced by some *Neisseria* strains. The enzyme synthesizes an iodine-reacting polysaccharide from sucrose. *N. meningitidis*, *N. gonorrhoeae*, and *N. lactamica* do not produce the enzyme, but many other neisseriae do.

The medium consists of heart infusion agar (Difco) 20 g (for composition, see p. 15); sucrose, 25 g; and distilled water, 500 ml. Six-milliliter amounts are dispensed into 16- X 125-mm screwcap tubes and autoclaved at 121°C for 15 min. The medium is melted as needed, cooled for 50°C, and poured into 60- X 15-mm Petri dishes.

The culture to be tested is inoculated onto the agar plate and incubated at 35°C for 48 h. To the growth is applied 1-2 drops of a solution containing 0.2% iodine and 0.4% potassium iodide (Burke's modification of Gram's iodine solution) freshly diluted 1:5 with distilled water.

A positive test is indicated by a rapid darkening of the colonies (a dark reddish brown or blue-black develops, then fades; the color can be restored by reapplying the iodine solution). Corresponding colonies on sucrose-free medium do not react in this manner when tested with iodine.

Reference: Lennette EH, Spaulding EH, Truant JP (eds): *Manual of Clinical Microbiology*, 2nd ed. Washington, D.C. American Society for Microbiology, 1974, pp 119-120.

• BENEDICT'S REAGENT (DOUBLE STRENGTH)

The double-strength reagent is used for the 2-ketogluconate test; it may be diluted 1:1 with distilled water for use in the 3-ketolactonate test.

The ingredients are copper sulfate ($CuSO_4 \cdot 5H_2O$, 17.3 g; sodium citrate, 173 g; sodium carbonate (anhydrous), 100 g; and distilled water, to a final volume of 500 ml.

The citrate and carbonate are dissolved by heating in about 300 ml of water; the solution is then filtered; the copper sulfate is dissolved by heating in 50 ml of water; it is then poured slowly into the first solution, with constant stirring; the solution is cooled, made up to a final volume of 500 ml, and dispensed into a 500-ml ground-glass stoppered bottle. (This reagent keeps indefinitely.)

• BLOOD AGAR

The medium consists of heart infusion agar (Difco), 40 g (for composition, see p. 15); and distilled water, 1 liter; 250-ml amounts of the melted medium are added to each of four 500-ml Erlenmeyer flasks and autoclaved at 121°C for 15 min. After the mixture has cooled to 50°C, 12.5-ml amounts of sterile defibrinated rabbit blood are added aseptically to each flask. This is mixed by swirling with care, to keep bubbles from forming. Then, 20-ml portions are poured into Petri dishes.

Reference: King EO: The Identification of Unusual Pathogenic Gram-negative Bacteria. Atlanta, Communicable Disease Center, 1964.

• BORDET-GENGOU AGAR WITHOUT PEPTONE (DIFCO)

This medium is especially useful for growing *Bordetella pertussis*. *Bordetella pertussis* cultures inoculated onto the medium produce colonies surrounded by a beta-hemolytic zone. The hemolytic zone is more prominent around stabs made in the agar.

The medium is prepared according to the manufacturer's instructions (the dehydrated medium is composed of potato

*Abbreviations of journal titles are according to the *Bibliographic Guide for Editors and Authors* (American Chemical Society, Washington, D.C., 1974).

infusion, 125 g; sodium chloride, 5.5 g; and Bacto-agar, 20 g); in addition, 5 g of Bacto-agar per liter is added. The medium is tubed in 5-ml amounts into 16- X 125-mm screwcap tubes for slants, and in 15-ml amounts into 18- X 150-mm screwcap tubes for plates. The medium is autoclaved at 121°C for 15 min are cooled to approximately 50 C. Seventeen percent blood (that is, 1 ml if blood to 5 ml of medium) is added to the tubes, the contents of which are then mixed well, and either slanted or poured. Alternatively, the tubed medium can be stored in the refrigerator, melted and blood added as needed.

The slants and plates are inoculated and then incubated at 35°C in a candle jar for 24-48 h.

• *BRUCELLA* BROTH FOR SODIUM CHLORIDE AND GLYCINE TOLERANCE AND H_2S PRODUCTION BY *CAMPYLOBACTER*.

***Brucella* Broth**. The basal medium is *Brucella* broth (Difco), prepared according to the manufacturer's directions with 1.6 g agar added per liter of water. The *Brucella* broth medium consists of Bacto-tryptone, 10 g; peptamin, 10 g; dextrose, 1 g; yeast extract, 2 g; sodium chloride, 5 g; and sodium bisulfite, 0.1 g, in 1 liter of distilled water. The final pH is 7.0. The medium is dispensed in 10-ml amounts into 16- X 150-mm screwcap tubes and autoclaved at 121°C for 15 min.

Hydrogen sulfide production. *Brucella* broth is supplemented with 0.02% L-cysteine-hydrochloride or 0.05% L-cystine. The medium is inoculated with one drop of a 36- to 48-h broth culture, and a strip of lead acetate paper is suspended above the medium. The culture is incubated at 35°C for 6 days. Blackening of the paper indicates production of hydrogen sulfide.

Tolerance tests

a) Glycine tolerance. *Brucella* broth is supplemented with 1% glycine.

b) Sodium chloride tolerance. *Brucella* broth is supplemented with 3.5% sodium chloride.

Each tolerance test medium is inoculated with two drops of a 36- to 48-h broth culture or a broth suspension prepared from a 1-3 day blood agar culture (either equivalent to a McFarland standard #1). For consistent results the tolerance tests are incubated at 35°C for 3-6 days in an atmosphere of 5%-6% O_2. Cultural growth indicates a positive reaction.

References: Véron M, Chatelain R: Taxonomic study of the genus *Campylobacter* Sebald and Véron and designation of the neotype strain for the type species, *Campylobacter fetus* (Smith and Taylor) Sebald and Véron. Int J Syst Bacteriol 23:122-134, 1973.

Holdeman LV, Cato EP, Moore WEC (eds): Anaerobe Laboratory Manual. 4th ed. Blacksburg, Va., Southern Printing Co., 1977.

• 10% CARBOHYDRATE AGAR SLANTS

The medium consists of phenol red broth base (BBL), 15 g; Bacto-agar, 15 g; carbohydrate, 100 g; and distilled water, 1 liter (for the glucose slant, the pH is adjusted to 7.7 before being autoclaved). The media are tubed in 5-ml amounts into 15- X 125-mm tubes and autoclaved at 115°C for 12 min. Tubes are slanted for cooling.

Each slant is inoculated with one drop of an 18- to 24-h HIB culture and incubated at 35°C for up to 7 days. The tubes are examined daily for acid production (indicated by a yellow color).

Reference: Chilton ML, Fulton, M: Presumptive media for differentiating *Paracolon* and *Shigella* cultures. J Lab Clin Med 31:824-827, 1946.

• CATALASE REACTION

Purpose of the test: to detect the presence of the enzyme catalase, which promotes breakdown of hydrogen peroxide with the evolution of oxygen gas.

The reaction is determined on an 18- to 24-h culture grown on a tryptone glucose yeast extract (TGY) agar slant. Several drops of a 3% hydrogen peroxide solution are deposited on the agar slant growth. A positive test is indicated by the active liberation of gas bubbles from the bacterial growth exposed to the hydrogen peroxide. The reagent lasts indefinitely when refrigerated. Care should be taken not to use 30% hydrogen peroxide because the reaction can be more pronounced and might produce a contamination hazard. Growth media containing blood are avoided, because the catalase in blood sometimes gives false-positive results.

Reference: Levin M, Anderson DQ: Two new species of bacteria causing mustiness in eggs. J Bacteriol 23:337-347, 1932.

• CETRIMIDE AGAR

This medium contains cetrimide, a toxic substance which inhibits the growth of many bacteria but permits *Pseudomonas aeruginosa* and certain other bacteria to grow.

The medium consists of heart infusion agar (Difco), 40 g (for composition, see p. 15); cetrimide (hexadecyltrimethylammonium bromide) (Eastman Organic Chemicals, Rochester, New York), 4 ml of a 22.5% solution in distilled water; and distilled water, 1 liter. The medium is tubed in 5-ml amounts into 15- X 125-mm tubes, autoclaved at 121°C for 15 min, and slanted for cooling.

The slants are inoculated with one drop of an 18- to 24-h HIB culture and incubated at 35°C for up to 7 days. The slants are examined for bacterial growth (a positive reaction).

Reference: Lowburg EJL: The use of a new cetrimide product in a selective medium for *Pseudomonas pyocyanea*. J Clin Pathol 8:47-48, 1955.

• CHARCOAL YEAST EXTRACT (CYE) AGAR

This medium is used for the cultivation of *Legionella* species.

The medium consists of yeast extract (Difco), 10 g; activated charcoal (Norit SG-Sigma Chemical Co., St. Louis, MO, Catalog C5510), 2 g; L-cysteine HCl·H_2O, 0.4g; soluble ferric pyrophosphate, 0.25g; agar (Difco), 17 g; and distilled water, 1 liter. All ingredients except L-cysteine HCl and soluble ferric pyrophosphate are added to 980 ml of distilled water, dissolved by boiling, autoclaved at 121°C for 15 min, and cooled to 50°C in a water bath.

Fresh solutions of L-cysteine HCl (0.4g in 10 ml distilled water) and soluble ferric pyrophosphate (0.25 g in 10 ml distilled water placed in a 50°C water bath to dissolve) are prepared, sterilized by membrane-filtration, and added to the cooled (50°C) basal medium. **Note that the L-cysteine HCl must be added before the soluble ferric pyrophosphate.**

The complete medium at 50°C is adjusted to pH 6.9 by adding 4 to 4.5 ml of 1 N KOH.

(Recently a buffered charcoal yeast extract medium has been described which is useful for culturing *Legionella* (J Clin Microbiol 10:437-441, 1979.)

References: Feeley JC, Gibson RJ, Gorman GW, Langford NC, Rasheed JK, Mackel DC, Baine WB: CYE agar: a primary isolation medium for the Legionnaires' disease bacterium. J Clin Microbiol 10:437-441, 1979.

Feeley JC, Gorman GW, Gibson RJ: Primary Isolation Media and Methods. In Jones GL, Hébert GA (eds): *"Legionnaires"*, the Disease, the Bacterium, and Methodology. Atlanta, Center for Disease Control, October 1978, pp 107-117.

• CHOCOLATE AGAR

This medium, used to grow fastidious organisms like *Neisseria gonorrhoeae*, is prepared with heart infusion agar (Difco), 40g (for composition see p. 15); Bacto-supplement B (a sterile yeast concentrate), 10 ml; rabbit blood, 40 ml; and distilled water, one liter.

The heart infusion agar and water are mixed together and sterilized at 121°C for 20 min. This is cooled to 70°-75°C, and the rabbit blood is added. The mixture is kept at that temperature until the blood turns chocolate-brown. Then supplement B is added, and the mixture is cooled to approximately 55°C, then is poured into Petri dishes.

Reference: McLeod JW, Coates JC, Happold FC, Priestley DP, Wheatley B: Cultivation of the gonococcus as a method in the diagnosis of gonorrhoea with special reference to the oxydase reaction and to the value of air reinforced in its carbon dioxide content. J Path Bact 39:221-231, 1934.

• CITRATE AGAR (SIMMONS) (DIFCO)

This medium is used to determine whether an organism uses citrate when it is incorporated as the sole source of carbon.

The medium (composed of magnesium sulfate, 0.2 g; monoammonium phosphate, 1 g; dipotassium phosphate, 1 g; sodium citrate, 2 g; sodium chloride, 5 g; Bacto-agar, 15 g; and Bacto-brom thymol blue, 0.08 g) is prepared according to the manufacturer's instructions and tubed in 5-ml amounts into 12- X 125-mm tubes, autoclaved at 121°C for 15 min, and slanted for cooling.

The agar slant is inoculated lightly with a needle, by using growth from an 18- to 24-h HIA slant, and incubated at 35°C for up to 7 days. A positive reaction, denoting alkalinization, is indicated by the development of a dark blue color (growth without color change in the medium is not considered a positive test).

Reference: Simmons JS: A culture medium for differentiating organisms of typhoid-colon-aerogenes group and for isolation of certain fungi. J Infect Dis 39:201-214, 1926.

• COCCOBACILLI AND COCCI, DIFFERENTIATION OF

This procedure involves the microscopic (wet mount) examination of cells taken from rabbit blood agar or susceptibility-testing plates which have grown peripherally to discs of the antibiotics penicillin G, ampicillin, or sulfadiazine. True *Neisseria* species retain the coccoid form, but *Acinetobacter* and *Moraxella* cultures produce long rods and filaments. Low concentrations of the antibiotics block septum formation more strongly than synthesis of lateral cell-wall components. Therefore, bacteria which normally divide and produce short rods do not divide but continue to grow and produce filaments.

The cells to be examined are removed from the outer margins of the zones of growth inhibition surrounding the antibiotic discs on plates incubated 18-24 h. The cells are suspended in a small loopful of sterile broth and used to make a wet mount preparation (alternately, the smear is dried and stained for 30 sec with crystal violet). The preparation is examined by using the oil immersion objective and (for the wet mount) reduced illumination. The slide is scanned for filaments.

Reference: Catlin BW: Cellular elongation under the influence of antibacterial agents: a way to differentiate coccobacilli from cocci. J Clin Microbiol 1:102-105, 1975.

• CYSTEINE AGAR (CARBOHYDRATE BASE FOR *FRANCISELLA*)

A special base with cysteine is required to demonstrate acid production from carbohydrates by *Francisella*.

The medium consists of two solutions: solution A, consisting of Bacto-peptone, 10 g; beef extract, 1.5 g; NaCl, 5 g; and cysteine-HCl, 1 g; dissolved in 500 ml distilled water, pH 7.2; and solution B, consisting of agar, 15 g, melted in 500 ml distilled water. Solutions A and B are mixed, and 2 ml of a 1.5% aqueous solution of phenol red (dissolve 1.5 g phenol red in 5 ml 0.1N NaOH and add 95 ml of water) is added. The medium is sterilized at 121°C for 20 min, and filter-sterilized carbohydrate solution is added to a final concentration of 1%. The medium is tubed, 5 ml per 16- X 125-mm tube, and slanted.

Each slant is inoculated heavily with a loop of growth from a young glucose cysteine peptone agar culture of *Francisella tularensis*; this is incubated with caps loose in a 35°C incubator without CO_2.

• CTA MEDIUM (BBL)

This medium is used to determine carbohydrate utilization by *Neisseria* cultures.

The medium consists of cystine, 0.5 g; trypticase peptone, 20 g; sodium chloride, 5 g; sodium sulfite, 0.5 g; phenol red, 0.017 g; agar, 2.5 g; and distilled water, 1 liter (pH 7.3). The medium, in 5-ml amounts, is dispensed into 16- X 125-mm screwcap tubes and autoclaved at 121°C for 15 min. The medium is cooled to approximately 50°C, and filter-sterilized carbohydrates are added aseptically to a final concentration of 1%.

A heavy inoculum is introduced into the agar deeps with a loop and mixed throughout the entire top 5-10 mm of the medium. The inoculum is obtained from bacterial growth on an 18- to 24-h HIA slant. The tubes, with caps tightened, are incubated at 35°C for up to 7 days. Acid production is indicated by a yellow color.

Reference: Vera HD: A simple medium for identification and maintenance of the gonococcus and other bacteria. J Bacteriol 55:531-536, 1948.

• "DECARBOXYLASE TESTS" (MOELLER'S METHOD) (DECARBOXYLASES FOR LYSINE AND ORNITHINE, DIHYDROLASE FOR ARGININE)

The purpose of the test is to measure the enzymatic ability of an organism to decarboxylate (or hydrolyze) an amino acid, forming an amine, with resulting alkalinity.

The basal medium for this test is prepared with peptone (Orthana) (marketed as "Thiotone"), 5 g; beef extract, 5 g; bromcresol purple, 0.01 g; cresol red, 0.005 g; glucose, 0.5 g; pyridoxal, 0.005 g; and distilled water, 1 liter; the pH is 6.0.

The basal medium is divided into four equal parts: one part is tubed as a control; to each of the other three parts are added, respectively, 1% L-arginine monohydrochloride, 1% lysine dihydrochloride, and 1% L-ornithine dihydrochloride (if DL-amino acids are used, the concentration is doubled). The pH of the ornithine portion is readjusted before the medium is sterilized. The media are tubed in 13- X 100-mm screwcap tubes, 1.5 ml per tube, and autoclaved at 121°C for 10 min.

For nonfermenting organisms, very heavy suspensions (≥McFarland #8 turbidity standard) prepared with young bacteria, 18-24 h old, from blood agar cultures are made in each of the three decarboxylase media and the control medium; a 4-mm layer of melted petrolatum is added to each tube. The cultures are incubated at 35°-37°C for 5-6 days. Alkaline reactions are recorded as positive.

With fermentative organisms, the tubes are inoculated with a drop of an 18- to 24-h HIB culture.

Reference: Moeller V: Simplified tests for some amino acid decarboxylases and for the arginine dihydrolase system. Acta pathol Microbiol Scand 36:158-172, 1955.

• DYE TOLERANCE TEST FOR *BRUCELLA*

The purpose of the test is to determine whether cultures of *Brucella* species are inhibited by basic fuchsin and/or thionin dyes at concentrations of 1:50,000 and 1:100,000.

The basal medium, heart infusion agar (Difco) (for composition, see p. 15), is prepared according to the manufacturer's directions. Twenty-ml amounts of the medium are dispensed into 18- X 150-mm screwcap tubes and autoclaved at 121°C for 15 min; they are cooled to 50°C, and the prescribed concentration of dye is added. The warm agar is mixed and poured into Petri dishes. The solidified agar in plates is held in the incubator for 1-2 h before being used.

Dyes. Stock solutions, 0.1% in distilled water of both dyes, basic fuchsin and thionin, are prepared. Each dye solution is dispensed into an 8-oz screwcap bottle and autoclaved at 121°C for 15 min (the dye solutions are routinely discarded after 30 days). The final dye concentrations routinely employed in the media are 1:50,000 (0.4 ml of 0.1% dye solution) and 1:100,000 (0.2 ml of 0.1% dye solution).

The inoculum used is prepared by suspending a loopful of bacterial growth from a 24- to 48-h agar slant into 1 ml of sterile physiological saline. A sterile cotton swab is immersed into the bacterial suspension and used to inoculate the dye plates and one control plate containing no dye. A single streak is made across each of the dye plates and, last, the control plate, without reloading the swab. The plates are inverted and incubated at 35°C for up to 4 days (if the cultures require an increased carbon dioxide tension, the plates are incubated in a candle jar). The plates are then examined for bacterial growth.

Reference: Alton GG, Jones LM, Pietz DE: Laboratory Techniques in Brucellosis. 2nd ed., Geneva, World Health Organization, 1975, pp 42-44.

• ENTERIC FERMENTATION BASE (CARBOHYDRATE FERMENTATION) MEDIUM (DIFCO)*

This medium is used to determine the ability of an organism to ferment a specific carbohydrate which is incorporated in a basal medium, thereby producing acid or acid with visible gas.

The medium consists of peptone, 10 g; meat extract, 3 g; sodium chloride, 5 g; Andrade's indicator, 10 ml; and distilled water, 1 liter. The pH is adjusted to 7.2. The medium is tubed in 3-ml amounts into 15- X 125-mm tubes containing inverted Durham vials and autoclaved at 121°C for 15 min. After cooling, 0.3 ml of a 10% filter-sterilized carbohydrate solution is added aseptically to each tube (to give a final concentration of 1% carbohydrate).

Each tube is inoculated with one drop of an 18- to 24-h HIB culture and incubated at 35°C for up to 7 days. The tubes are examined for acid (indicated by a red color) and gas production.

Andrade's indicator consists of acid fuchsin, 2 g; sodium hydroxide (1N), 160 ml; and distilled water, 1 liter, autoclaved at 121°C for 20 min.

The fuchsin is dissolved in the distilled water, and the sodium hydroxide is added. If, after overnight standing, the fuchsin is not sufficiently decolorized (not darker than faint straw-colored when brought up into a 10-ml pipette), an additional 1 or 2 ml of alkali is added. Andrade's indicator improves somewhat on aging and should be prepared in a sufficiently large amount to last for several years. It should be prepared and aged for approximately 6 months before being used.

References: Ewing WH, Davis BR: Media and Tests for Differentiation of *Enterobacteriaceae*. Atlanta, Center for Disease Control, 1970.

Andrade E: Influence of glycerine in differentiating certain bacteria. J Med Res 14:551-556, 1905-06.

• ESCULIN MEDIUM

This medium is used to determine whether an organism will hydrolyze the glycoside esculin.

The medium consists of esculin, 1 g; ferric citrate, 0.5 g; heart infusion agar (Difco), 40 g (for composition see p. 15), and distilled water, 1 liter. The ingredients are heated to dissolve, then cooled to 55°C. The pH is adjusted to 7.0. The medium is tubed in 5-ml amounts into 15- X 125-mm tubes, autoclaved at 121°C for 15 min, and slanted for cooling.

The medium is inoculated with one drop of a 24-h HIB culture and incubated at 35°C for up to 7 days. The slants are examined for esculin hydrolysis (esculin fluoresces under the ultraviolet rays of a Wood's lamp; when esculin is hydrolyzed, the medium turns reddish black and the esculin no longer fluoresces). An inoculated control tube of the medium without esculin may be useful in interpreting results when there are problems.

Reference: Sneath PHA: Cultural and biochemical characteristics of the genus *Chromobacterium*. J Gen Microbiol 15:70-98, 1956.

• FEELEY-GORMAN (F-G) AGAR

This medium is used to cultivate *Legionella pneumophila* and to demonstrate this organism's production of soluble and fluorescent pigments.

The medium consists of casein (acid hydrolysis), 17.5 g; beef extractives, 3 g; L-cysteine HCl·H_2O, 0.4 g; soluble ferric pyrophosphate, 0.25 g; starch, 1.5 g; agar, 17 g; and distilled water, 1 liter. All ingredients except L-cysteine HCl and soluble ferric pyrophosphate are added to 980 ml of distilled water, dissolved by boiling, autoclaved at 121°C for 15 min, and cooled to 50°C in a water bath.

Fresh solutions of L-cysteine HCl (0.4 g in 10 ml distilled water) and soluble ferric pyrophosphate (0.25 g in 10 ml distilled water placed in a 50°C water bath to dissolve) are prepared, sterilized by membrane filtration, and added to the cooled (50°C) basal medium. **Note that the L-cysteine HCl must be added before the soluble ferric pyrophosphate.**

The complete medium at 50°C is adjusted to pH 6.9 and dispensed into Petri dishes, or tubes which are slanted. F-G agar also is prepared by supplementing Mueller-Hinton (M-H) agar (BBL, lot #105621) with L-cysteine HCl and soluble ferric pyrophosphate (other lots of M-H agar may not be satisfactory). A 38-g amount of M-H agar is added to 980 ml of distilled water, dissolved by boiling, autoclaved at 121°C for 15 min, and cooled to 50°C in a water bath. Solutions of L-cysteine HCl and soluble ferric pyrophosphate are prepared and added as described above.

Precaution. Soluble ferric pyrophosphate must be kept dry and stored in the dark; it is no longer usable if its color changes from green to yellow or brown. A freshly mixed solution of the compound must be prepared each time the medium is made. The compound is readily dissolved in water in a 50°C water bath. Do not heat above 60°C.

Reference: Feeley JC, Gorman GW, Weaver RE, Mackel DC, Smith HW: Primary isolation media for the Legionnaires' disease bacterium. J Clin Microbiol 8:320-325, 1978.

• FLAGELLA BROTH

This liquid medium is high in phosphate, which, according to Leifson, enhances flagella production by certain bacteria.

The medium consists of Bacto-tryptose (Difco), 10 g; dipotassium phosphate (anhydrous), 1 g; sodium chloride, 2.5 g; and distilled water, 1 liter. The pH is 7.0. The medium is tubed in 5-ml amounts into 15- X 125-mm tubes and autoclaved at 121°C for 15 min.

*This medium is commercially available (Difco C-836-01), but it is not included in the Difco catalog.

The medium is inoculated with a drop of an actively motile 18- to 24-h broth culture. Or, to enhance motility, bacteria are picked into flagella broth from the leading edge of young culture growth in a semisolid motility medium (Gard plate). The broth subculture is incubated at 25°C for 18-24 h. The organisms are stained with flagella stain to determine flagellar arrangement and morphology.

Reference: Leifson E: Staining, shape, and arrangement of bacterial flagella. J Bacteriol 62:377-389, 1951.

• FLAGELLA STAINS

Flagella stains are used to demonstrate the presence, the site of attachment, and the morphology of flagella. Both the Leifson stain and the silver stain have been used successfully in the Special Bacteriology Laboratory. The Leifson stain was used for all flagella stains photographed for this manual.

Leifson stain. The flagella stain is prepared as follows. Solution A consists of basic fuchsin (certified for flagella staining), 0.6 g, and 95% ethanol, 50 ml. Alternatively, solution A can be prepared by adding 0.45 g pararosanilin acetate and 0.15 g pararosanilin hydrochloride to 50 ml of 95% ethyl alcohol. The mixture is shaken, and the dye is allowed to dissolve overnight.

Solution B consists of sodium chloride, 0.75 g; tannic acid (chemically pure grade), 1.5 g; and distilled water, 100 ml. Solutions A and B are combined and mixed thoroughly. The pH is checked and, if necessary, adjusted to 4.8-5.0 with 1 N sodium hydroxide. The stain is stored at 4°C for 2-3 days to improve its effectiveness. Thereafter, 50-ml portions of the stain are chilled to −20°C or below in screwcap tubes for future use (the ultra-cold stain is stable indefinitely).

For use, the stain is warmed to room temperature (20-25°C) and remixed. Immediately after use, it is stored at 4°C (stain is stable for about 1 month at 4°C).

The culture. With most motile cultures, smears for flagella staining are made directly from young (usually 18-24 h) cultures on blood agar or a 25°C tryptone glucose yeast extract agar slant. With a needle, and avoiding the agar, a small amount of growth, equivalent to a 1-mm colony, is transferred to a test tube containing 3 ml of distilled water. The growth is emulsified into a droplet of water against the side of the tube, then mixed with the water in the tube to produce a faint opalescence. From this, a smear is prepared.

If the culture is not actively motile, or if it is poorly flagellated, the center of a tube or plate of motility medium (Gard plate) is inoculated with a small amount of the culture on a needle and incubated at the optimum temperature for motility. If the culture is motile, it begins to spread well away from the point of inoculum; a bit of growth from the actively growing edge of the culture is picked and transferred to flagella broth. The broth culture is incubated at the optimum temperature for motility for 18-24 h or until growth appears. Twenty-five hundredths milliliter of 37% formaldehyde is added to the broth culture (5 ml), which is allowed to stand for 15 min and centrifuged about 10 min in a table-top centrifuge. The supernatant is decanted, the packed cells are mixed in the drop or two of liquid left in the bottom of the tube, and distilled water is added to three-fourths the volume of the tube. After the mixture is centrifuged again, the water is decanted, and the cells are resuspended in sufficient water to attain a faint opalescence.

Slides. The flagella stain will not work unless the slides are scrupulously clean. New "precleaned" slides should be soaked for 4 days at ambient temperature (20°-25°C) in acid dichromate solution. For soaking, 10 slides are placed in a glass rack, then immersed in dichromate in a covered staining jar. Slides are rinsed in 10 changes of tap water, then 2 changes of distilled water, and then air-dried. Cleaned slides are handled with forceps and stored in a covered container.

Slides also can be cleaned satisfactorily by substituting 3% concentrated HCl in 95% ethyl alcohol for acid dichromate solution in the above procedure.

The smear. Before the smear is prepared, the acid-cleaned slide is passed slowly several times through the tip of a blue Bunsen flame to burn off any insoluble residue (a yellow flame will deposit carbon on the slide, rendering it unusable). The slide is cooled, flamed side up, on a paper towel. With a wax pencil, a thick line is drawn across the width of the slide to contain the stain in an area approximately 2.5 X 4.5 cm.

A large loopful of culture suspension is deposited onto the center of the slide adjacent to the wax line and allowed to run down the length of the tilted slide (slides which do not permit the droplet to glide down easily are discarded as unclean). The slides are air-dried on a level surface.

Staining. Without being heat-fixed, the slide is placed on a level staining rack. One milliliter of stain at ambient temperature is added, and a timer is set for the required time. (The optimum staining time is determined for each new batch of stain by staining three or more slides of a known flagellated organism for varying times [5-15 min].) The slide is washed with tap water, air-dried in an upright position, and examined with the oil immersion lens.

References: Leifson E: Staining, shape, and arrangement of bacterial flagella. J Bacteriol 62:377-389, 1951.

Clark WA: A simplified Leifson flagella stain. J Clin Microbiol 3:632-634, 1976.

Silver stain. The flagella stain is prepared as follows. The mordant consists of 25 ml saturated aluminum potassium sulfate (kept as a stock), 50 ml of a fresh 10% tannic acid solution, and 5 ml of 5% ferric chloride solution (kept as a stock); these ingredients are mixed together and stored in a dark bottle at 20°-26°C. This mordant is stable several weeks.

[A third flagella stain, the Ryu stain (J Clin Microbiol 16:948-952, 1982), has recently been found to be satisfactory. To keep background staining to a minimum, we recommend the staining time to be the shortest time that produces satisfactory staining of the flagella.

The silver stain consists of a 5% silver nitrate solution (90 ml) to which is added, drop by drop, 2-5 ml of concentrated ammonium hydroxide until the precipitate which forms dissolves. Then additional silver nitrate solution is added until the solution becomes faintly cloudy. This solution, when stored in a brown bottle at 20°-26 C, is stable for several months.

The culture, slides, and smears are prepared as described above for the Leifson stain. However, the requirement for scrupulously clean slides does not seem to be as stringent as it is in the Leifson procedure.

The slides are placed on a staining rack and flooded with the mordant, which is allowed to act for 4 min. The slide is rinsed with distilled water, then flooded with the silver stain. The stain is heated by passing a flame under the slide until steam begins to rise. Then the flame is removed and the stain is allowed to act for 2-4 min (background staining is lighter with the shorter time). The slide is rinsed with distilled water and air-dried.

Reference: West M, Burdash NM, Friemath F: Simplified silver-plating stain for flagella. J Clin Microbiol 6:414-419, 1977.

• FLUID THIOGLYCOLLATE MEDIUM (BBL)

This medium is used for a variety of reasons: for demonstrating growth characteristics, cellular morphology, and cellular arrangement, especially with streptococci, lactobacilli, *Gardnerella vaginalis*, and *Streptobacillus;* for demonstrating gas production (see "Gas Production from Fluid Thioglycollate Medium"); and for promoting growth of fastidious organisms (serum is added when required).

The medium consists of pancreatic digest of casein (trypticase - BBL), 15 g; L-cystine, 0.5 g; dextrose (anhydrous), 5 g; yeast extract, 5 g; sodium chloride, 2.5 g; sodium thioglycollate, 0.5 g; resazurin, 0.001 g; agar, 0.75 g; and distilled water, 1 liter; final pH was 7.1 ± 0.1.

The medium is prepared according to the manufacturer's instructions, tubed while hot, 9 ml per tube in 16- X 125-mm screwcap tubes, and autoclaved at 121°C for 15 min. It is stored in the dark at room temperature with caps tightly closed. It is discarded after a third of the medium in the tube has become oxidized (indicated by the pink color of the indicator).

• GAS PRODUCTION FROM FLUID THIOGLYCOLLATE MEDIUM (BBL)

This is a sensitive method for demonstrating gas production from a fermentable carbohydrate (glucose) when gas production is not obvious by other tests. In particular, the test is useful for detecting gas production by cultures of *Actinobacillus actinomycetemcomitans* and *Haemophilus aphrophilus*.

A 48- to 72-h fluid thioglycollate medium culture of the organism is used. A red-hot inoculating needle is plunged into the broth near the side of the glass tube. In a positive test gas bubbles appear along the line of insertion. (This test is performed in a biological safety cabinet.)

Reference: King EO, Tatum HW: *Actinobacillus actinomycetemcomitans* and *Haemophilus aphrophilus*. J Infect Dis 111:85-94, 1962.

• GEL FORMATION (*BRUCELLA CANIS*)

Brucella canis usually forms a stringy mass or "gel" of increased viscosity when suspended in phenolized saline.

A heavy suspension is prepared with 24- to 48-h bacterial growth in phenolized saline (0.5% phenol in buffered saline) in a 13- X 100-mm screwcap test tube and is allowed to stand for 15-30 min. (If the test is negative after this period, and *B. canis* is still suspected, the tube is incubated overnight.)

In testing for gel formation, a bacteriological loop is inserted into the suspension. If a gel has been formed, a short string is formed as the loop is withdrawn. The gel also can be detected by tilting the tube and comparing the viscosity of the suspension in the tube with that of buffered saline in a control tube.

Reference: Weaver RE, Tatum HW, Hollis DG: The Identification of Unusual Pathogenic Gram Negative Bacteria (EO King). Preliminary Revision. Atlanta, Center for Disease Control, 1972.

• GELATIN LIQUEFACTION

The purpose of the test is to determine the ability of an organism to produce proteolytic enzymes (gelatinases) which liquefy gelatin.

The test medium consists of 12% Bacto-gelatin in heart infusion broth (Difco) (for composition see p. 15), pH 7.4. The medium is tubed in 5-ml amounts into 16- X 125-mm screwcap tubes and autoclaved at 121°C for 15 min.

The medium is inoculated with 4-5 drops of a 24-h HIB culture and incubated at 35°C for up to 14 days. The medium is incubated at 25°C if the organism grows better at 25°C than at 35°C.

The culture is checked daily for liquefaction by placing the tube into an ice water bath or into the refrigerator, along with an uninoculated control. Liquefaction is assayed only after the control has hardened (gelled). If liquefaction occurs within 14 days, the culture is considered to be positive.

Reference: Ewing WH: *Enterobacteriaceae*, Biochemical Methods for Group Differentiation. U.S. Public Health Service, Atlanta, Publication 734 (revised), 1962.

• GLUCOSE CYSTEINE PEPTONE AGAR

This medium is used for the cultivation of *Francisella tularensis*.

The medium consists of (A) Bacto-peptone, 20 g; meat extract, 3 g; sodium chloride, 10 g; glucose, 50 g; cysteine-HCl, 2 g; and distilled water, 1 liter (the pH is adjusted to 7.2); (B) agar, 30 g; distilled water, 1 liter. (A) and (B) are combined, mixed thoroughly, dispensed in 500-ml amounts into 1,000-ml Erlenmeyer flasks, and autoclaved at 121°C for 20 min. The medium is cooled to 50°C, and 25 ml of defibrinated rabbit blood is added to each flask. Five milliliter quantities are dispensed into 15- X 125-mm screwcap tubes and 20-ml quantities into Petri dishes. The tubes are slanted.

• GRAM STAIN

The Gram stain enables us to differentiate bacteria into two major groups, depending upon the chemical nature of the cell wall: Gram-positive organisms, which retain the crystal violet-iodine complex (blue) in the cell wall during decolorization; and Gram-negative organisms, which do not; the latter take up safranin (red).

The solutions are prepared as follows:

Crystal violet consists of solutions A and B. Solution A is crystal violet powder (90% dye content), 13.87 g (the amount of dye used is adjusted in proportion to the dye content of the powder, stated on the label) and 200 ml 95% ethanol. Solution B is ammonium oxalate, 8 g in 800 ml distilled water. Solutions A and B are mixed and allowed to stand overnight or until the dye dissolves. The solution is filtered through coarse filter paper.

Gram's iodine consists of iodine crystals, 1 g; potassium iodide, 2 g; and distilled water, 300 ml.

The **decolorizer** is 95% ethanol.

The **counterstain** is safranin-O, 3.41 g; 95% ethanol, 100 ml; and distilled water, 900 ml.

The smears are prepared by depositing a thin suspension of bacteria on a clean glass slide and allowing it to air dry; smears are heat-fixed gently.

The staining procedure. The smear is covered with crystal violet for 30 seconds, then rinsed with tap water; the smear is covered with Gram's iodine for 30 seconds, then rinsed with tap water; the smear is decolorized with ethanol for about 30 seconds, or until the wash is clear, then is counterstained about 30 seconds with safranin.

Reference: Bartholomew JW: Variables influencing results, and the precise definition of steps in Gram staining as a means of standardizing the results obtained. Stain Technol 37:139-155, 1962.

• GROWTH FACTOR TEST FOR *HAEMOPHILUS*

The purpose of the test is to determine the requirement for different growth factors (X = heme, V = nicotinamide adenine dinucleotide) by various species of *Haemophilus*.

The medium used in the test is heart infusion agar (Difco) (for composition see p. 15); it was prepared according to the manufacturer's directions. The melted sterile medium is dispensed in 20-ml amounts into Petri dishes.

The inoculum is prepared by making a heavy suspension in sterile distilled water with the growth from an overnight (18- to 20-h) chocolate agar culture. The test plate is inoculated with three widely distributed 25-mm-diameter smears, with a loopful of suspension for each smear. A growth-factor strip (one of each, X, V, and XV) is laid in the center of each smear. (Strips are cut into quarters; one quarter is used for each assay.) The plates are incubated at 35°C for 18-24 h. If no growth occurs around any of the factors, then a newly inoculated factor plate is incubated in a candle jar for 18-24 h at 35°C.

Factor strips. The strips, X, V, and XV (Taxo, BBL), are obtained from Bioquest, Cockeysville, Md.

• HEART INFUSION AGAR (DIFCO)

The medium consists of heart infusion agar (Difco), 40 g (composed of beef heart infusion, 500 g; Bacto-tryptose, 10 g; sodium chloride, 5 g; and Bacto-agar, 15 g), and distilled water, 1 liter. The mixture is heated to dissolve the agar, then dispensed in 5-ml portions into 15- X 125-mm tubes. These are autoclaved at 121°C for 15 min, then cooled in a slanted position to obtain a 2.5-cm butt and a 4.0-cm slant.

• HEART INFUSION BROTH (DIFCO)

The medium is prepared as follows: heart infusion broth (Difco), 25 g (composed of beef heart infusion, 500 g; Bacto-tryptose, 10 g; and sodium chloride, 5 g) and distilled water, 1 liter. Four-milliliter amounts are dispensed into 15- X 125-mm test tubes and autoclaved at 121°C for 15 min.

• HEART INFUSION TYROSINE (HIT) AGAR

This medium is used to enhance the production of brown soluble pigments by certain bacteria.

The medium consists of heart infusion agar (Difco), 40 g (for composition see p. 15); L-tyrosine, 1 g; distilled water, 1 liter.

The medium is tubed in 7-ml amounts in 15- X 125-mm tubes, sterilized at 121°C for 15 min, and slanted.

Reference: King EO: The Identification of Unusual Pathogenic Gram Negative Bacteria. Atlanta, Communicable Disease Center, 1964.

• INDOLE SPOT TEST

This is a rapid test in which a minimal amount of reagent is used for determining the ability of a microorganism to produce indole on media containing tryptophane. In this laboratory the test is used specifically on suspected *Haemophilus* strains; generally it has not been used for other genera.

The reagent is 1% paradimethylaminocinnamaldehyde in 10% HCL (90 ml of distilled water with 10 ml of concentrated HCL added).

The culture is grown on a chocolate agar plate at 35°C for 18-24 h (we use a candle-jar atmosphere). No. 1 Whatman filter paper is placed in a Petri dish and moistened with a drop of the test reagent. The colonies to be tested are taken with a loop and spread over a small area of the moist filter paper. The development of a blue to blue-green color indicates the presence of indole. Development of any other color indicates a negative test.

References: Vracko MD, Sherris JC: Indole-spot test in bacteriology. Am J Clin Pathol 39:429-432, 1963.

Sutter VL, Carter WT: Evaluation of media and reagents for indole-spot tests in anaerobic bacteriology. Am J Clin Pathol 58:335-338, 1972.

• INDOLE TEST

The purpose of the test is to determine the ability of an organism to split the tryptophane molecule to form the compound indole.

The medium for culture growth is 2% Bacto-tryptone broth (rich in tryptophane) (pH 7.2 to 7.4). The broth is tubed in 4-ml amounts into 15- X 125-mm tubes and autoclaved at 121°C for 15 min. Heart infusion broth is used to test organisms which do not grow well in Bacto-tryptone broth.

The broth is inoculated with one drop of a 24-h HIB culture and incubated at 35 C for 48 h. Thereafter, 1 ml of xylene is added to the broth culture. The mixture is shaken vigorously to extract the indole and allowed to stand until the xylene forms a layer on top of the aqueous phase. Then 0.5 ml of Ehrlich's reagent (see below) is added down the side of the tube. The presence of indole is indicated by the development of a pink ring below the xylene layer.

Ehrlich's reagent consists of 95% ethanol, 95 ml; paradimethylaminobenzaldehyde, 1 g; and hydrochloric acid (concentrated), 20 ml. The aldehyde is dissolved in the alcohol, and then the acid is slowly added. The reagent is prepared in small quantities and stored at 4°C.

Reference: Böhme A: Die anwendung der Ehrlichsen Indolreaktion für bakteriologische Zwecke. Centr Bakt I Abt Orig 40:129-133, 1905.

• 2-KETOGLUCONATE TEST

The purpose of the test is to determine the ability of an organism to oxidize gluconic acid as a sole carbon source to the reducing compound 2-ketogluconate.

The medium consists of monopotassium phosphate, 5.4 g; potassium nitrate, 2 g; potassium gluconate, 20 g; and distilled water, 1 liter. The pH is 6.5. The mixture is filter-sterilized and dispensed aseptically in 1-ml amounts into sterile 13- X 100-mm screwcap tubes.

The medium is inoculated with one loopful of a 24-h HIB culture and incubated at 35°C for 3 days.

2-Ketogluconate reaction. Three-tenths of a milliliter of double-strength Benedict's reagent is added to the culture broth. The tube is placed in a boiling water bath for 10 min. In place of the Benedict's reagent, a Clinitest (Ames) tablet can be added to the culture broth (heating is not required). The presence of 2-ketogluconate is indicated by the appearance of a copper-colored precipitate.

Reference: Moore HB, Pickett MJ: The *Pseudomonas-Achromobacter* group. Can J Microbiol 6:35-42, 1960.

• 3-KETOLACTONATE MEDIUM FOR *AGROBACTERIUM*

This medium is used to determine whether an organism has the property, probably limited to members of the genus *Agrobacterium*, of producing 3-ketoglycosides from the corresponding disaccharides and bionic acids.

The medium consists of lactose, 10 g; yeast extract (Difco), 1 g; agar, 20 g; and distilled water, 1 liter. The medium is tubed in 20-ml amounts into 20- X 150-mm screwcap tubes and autoclaved at 121°C for 15 min. The contents of one tube are poured into a Petri dish.

A TGY agar slant is inoculated with the test culture and incubated at 25°C for 2-3 days. Then two 3-ketolactonate medium plates are inoculated by smearing some of the TGY slant growth onto the agar plate. The area of inoculation is never larger than a circle with a diameter of 1 cm. A positive control (that is, a strain of *Agrobacterium radiobacter*) is also inoculated onto the test plates. The plates are incubated at 25°C for 2-7 days. After 2 days of incubation, one of the plates is flooded with single-strength Benedict's solution. A positive reaction is indicated by a large yellow zone occurring, within 30 min, around the bacterial growth. If no zone appears, the other plate, after 7 days' incubation, is tested in the same manner.

Reference: Bernaerts MJ, DeLey J: A biochemical test for crown gall bacteria. Nature 197:406-407, 1963.

• LITMUS MILK

This medium is used to detect acid production from lactose fermentation, production of caseolytic enzymes, casein coagulation, and reduction of litmus indicator by bacteria.

Litmus milk (Difco), 105 g (composed of Bacto-Skim Milk, 100 g and Bacto-Litmus, 5 g), is mixed into 1 liter of distilled water to obtain a homogeneous suspension. The suspension is dispensed in 6-ml amounts into 15- X 125-mm tubes and autoclaved at 121°C for 10 min.

The medium is inoculated with 4-5 drops of a 24-h HIB culture and incubated at 35°C for up to 7 days. The culture is observed daily for changes in composition: a blue color indicates a pH shift to the alkaline range; pink indicates acid production; and loss of color, a reduction of the indicator. Also acid clot, rennet clot, or peptonization are noted.

Reference: King EO: The Identification of Unusual Pathogenic Gram Negative Bacteria. Atlanta, Communicable Disease Center, 1964.

• MACCONKEY AGAR (DIFCO)

This medium is used to detect an organism's ability to grow in a moderate concentration of bile salts, as in enteric environments.

The medium (composed of Bacto-peptone, 17 g; Proteose peptone (Difco)), 3 g; Bacto-lactose, 10 g; Bacto-bile salts #3, 1.5 g; sodium chloride, 5 g; Bacto-agar, 13.5 g; Bacto-neutral red, 0.03 g; and Bacto-crystal violet, 0.001 g) is prepared according to the manufacturer's directions, tubed in 5-ml amounts into 15- X 125-mm tubes, autoclaved at 121°C for 15 min, and slanted.

Slants are inoculated with a drop of a 24-h HIB culture and incubated at 35°C for up to 7 days. The slants are observed for growth, indicating a positive reaction.

• MAINTENANCE OF BACTERIA IN MOTILITY MEDIUM

Many strains of bacteria encountered in the Special Bacteriology Laboratory are maintained satisfactorily for extended periods (at least 1 year) at "room" temperature (around 25°C) in motility medium (see below). The medium in screwcap tubes is inoculated with a needle to 2 mm below the agar surface and incubated at optimal temperature until growth appears. Then the tube is closed to prevent evaporation and stored at room temperature.

• METHYL RED (MR) TEST

The purpose of the test is to determine an organism's ability to produce and maintain stable acid end products from glucose fermentation.

The medium for growing the organism is MR-VP medium (Difco) (composed of buffered peptone, 7 g; Bacto-dextrose, 5 g; and dipotassium phosphate, 5g). It is prepared according to the manufacturer's instructions, tubed in 4-ml amounts into 15- X 125-mm tubes, and autoclaved at 121°C for 10 min.

The broth medium is inoculated with 1-2 drops of a 24-h HIB culture and incubated at 35°C for 48 h (4 to 5 days for poorly-growing cultures).

Five to six drops of methyl red reagent (see below) are added to the broth culture. A positive test is a bright red (acid); a weakly positive test is a red-orange; and a negative reaction, yellow or orange.

The methyl red reagent is prepared by dissolving 0.1 g of methyl red in 300 ml of 95% ethanol. Distilled water is added to make a final volume of 500 ml.

Reference: Clark WM, Lubs HA: The differentiation of bacteria of the colon-aerogenes family by the use of indicators. J Infect Dis 17:161-173, 1915.

• MOTILITY TEST

Motility is a useful characteristic for classifying bacteria. The simplest test for motility is to observe the bacteria in a culture droplet under the microscope (see (1) below). If the organism appears nonmotile, it should be inoculated into motility medium to determine whether it spreads throughout the soft agar (motile) or remains at the site of inoculum (nonmotile) (see (2) below).

(1) Culture droplet. A line is drawn on a clean slide with a glass-marking pencil, and a loopful of culture suspension is placed on the slide adjacent to the line. The microscope with the 10X objective in place is focused on the line. The light is decreased by closing down the substage diaphragm, and the slide is moved to focus on the edge of the droplet.

The 44X objective is swung into place, and the organisms are observed. Motile organisms change position with respect to one another; in Brownian movement, the organisms, although quite active because of molecular bombardment, remain in the same relative position with respect to other organisms in the field.

(2) Motility medium. The medium consists of motility test medium (Difco), 16 g (composed of Bacto-tryptose, 10 g; sodium chloride, 5 g; and Bacto-agar, 5 g); nutrient broth (Difco), 4 g (composed of Bacto-beef extract, 3 g and Bacto-peptone, 5 g); sodium chloride, 1 g; and distilled water, 1 liter. (This medium contains a 0.4% final concentration of agar.) The medium is heated to melt the agar, 8-ml amounts are dispensed into 15- X 125-mm tubes, and the tubes are autoclaved at 121°C for 15 min. The inoculum, obtained from an 18- to 24-h HIA slant culture, is stabbed into the center of the tube to a depth of 5 mm. The tubes are incubated at 35°C (or 25°C if the organism did not exhibit motility at 35°C) and examined for up to 7 days. Motile organisms grow away from the inoculation site into the surrounding medium. Nonmotile organisms remain localized at the inoculation site.

If an organism does not appear motile in motility medium, one can check OF medium for motility, if previously inoculated. (Motility medium has 0.4% agar, and OF media have 0.2-0.3% agar; this lesser amount of agar further enhances expression of motility.)

The Gard plate also is useful for determining motility. A Petri dish containing motility medium is inoculated at the center of the plate with the test organism and incubated at 25°C for up to 7 days. If motile, the organisms spread away from the point of inoculation; a subculture is picked from the edge of the spreading growth (center of increased concentration of motile cells) into flagella broth and incubated at 25°C until growth appears. A flagella stain is then prepared.

Reference: Gard S: Das Schwarmphänomen in der Salmonella-Gruppe und seine praktische Ausnützung. Zeitschr Hyg Infektionskr 120:615-619, 1938.

• NITRATE BROTH, SEMIAEROBIC (STANIER)

This medium is used for testing *Pseudomonas* strains for denitrification. It is used selectively for organisms one would expect to produce gas from nitrate but which do not do so in ordinary nitrate broths.

The broth medium consists of disodium phosphate and monopotassium phosphate, 1 M, pH 6.8, 40 ml; vitamin-free mineral solution (Hutner) (see below), 20 ml; ammonium sulfate, 1 g; glycerol, 10 g; potassium nitrate, 10 g; yeast extract, 5 g; distilled water, to an 800-ml volume.

The ingredients are combined and heated slightly in a double water bath to effect solution; they are then filter-sterilized.

One gram of Ionagar #2 (Oxoid) or its equivalent is dissolved in 200 ml of glass-distilled water and autoclaved at 121°C for 15 min.

All ingredients are combined aseptically and dispensed in 5-ml volumes in sterile 16- X 125-mm screwcap tubes.

Hutner's vitamin-free mineral solution contains nitrilotriacetic acid, 10.0 g; magnesium sulfate, 14.45 g; calcium chloride ($2H_2O$), 3.335 g; ferrous sulfate ($7H_2O$), 0.099 g; ammonium molybdate ($4H_2O$), 0.0093 mg; and concentrated metals solution (see below), 50.0 ml.

The nitrilotriacetic acid is dissolved and neutralized with potassium hydroxide, about 7.3 g. The salts and 50 ml of concentrated metals solution are added, the pH is adjusted to 6.8, and glass-distilled water is added to make a final volume of 1 liter.

The concentrated metals solution consists of ethylenediaminetetraacetic acid, 2.50 g; zinc sulfate ($7H_2O$), 10.95 g; ferrous sulfate ($7H_2O$), 5.00 g; manganese sulfate (H_2O), 1.54 g; copper sulfate ($5H_2O$), 0.392 g; cobalt nitrate ($6H_2O$), 0.248 g; sodium borate ($10H_2O$), 0.177 g; sulfuric acid, a few drops; and distilled water to make 1 liter.

A tube of nitrate broth is inoculated with 5-7 drops of a 24-h HIB culture using a Pasteur pipette. The inoculation is initiated in the bottom of the tube and brought upward carefully to the medium surface, in order not to introduce air bubbles into the medium with the pipette. After 24 h incubation, the organism is subcultured in the same way into the same medium, melted and cooled to around 40°C. A 1-cm cap of sterile petroleum jelly is applied over the surface of the inoculated medium. The tubes are incubated at 35°C for up to 7 days. The tubes are examined daily for (1) gas production, resulting in the formation of a space between the petroleum jelly cap and the medium, and (2) cellular growth out from the area of the line of inoculation.

References: Stanier RY, Palleroni NJ, Doudoroff M: The aerobic pseudomonads: a taxonomic study. J Gen Microbiol 43:159-271, 1966.

Cohen-Bazire G, Sistrom WR, Stanier RY: Kinetic studies of pigment synthesis by non-sulfur purple bacteria. J Cell Comp Physiol 49:25-68, 1957.

• NITRATE REDUCTION TEST

The purpose of the test is to determine an organism's ability to reduce nitrate to nitrite, to free nitrogen gas, or to some other reduced nitrogen compounds.

The broth media for assaying nitrate reduction are (1) routine nitrate broth: Bacto-peptone, 20 g; potassium nitrate, 2 g; and distilled water, 1 liter; and (2) special nitrate broth (for the more fastidious organisms): heart infusion broth (Difco), 25 g; potassium nitrate, 2 g; and distilled water, 1 liter. In each case the pH is adjusted to 7.0.

Each of the broth media is dispensed in 4-ml amounts into 15- X 125-mm tubes containing inverted Durham vials and autoclaved at 121°C for 15 min.

The reagents for detecting the presence of nitrite are solution #1: sulfanilic acid, 2.8 g; distilled water, 250 ml; and glacial acetic acid, 100 ml; and solution #2: dimethyl-alpha-napthylamine, 2.1 ml; distilled water, 250 ml; and glacial acetic acid, 100 ml.

An alternative solution #2 is made up of distilled water, 120 ml; glacial acetic acid, 30 ml; and 1,6-Cleve's acid (5-amino-2-naphthalenesulfonic acid), 0.2 g. Water is added to the Cleve's acid, and the mixture is warmed and shaken frequently until most of the compound is dissolved; do not boil, because boiling will cause almost all of the compound to dissolve. The solution is filtered and cooled, and acetic acid is added to the filtrate. The reagent is stored in the refrigerator.

The broth tubes are inoculated with 1-2 drops of a 24-h HIB culture and incubated at 35°C for 48 h. After incubation, the Durham tube is observed for gas formation and the broth is tested for the presence of nitrite.

Test for Presence of Nitrite

To the nitrate broth culture are added 0.25 ml of nitrite test solution #1, then 0.25 ml of nitrite test solution #2. The presence of nitrite is evidenced by the development of a red color within 1-2 min. (Color produced with alternative solution #2 fades rapidly but can be restored by adding more of the reagent.)

If there is gas in the Durham vial and the mixture turns red after solutions #1 and #2 are added, nitrate has been reduced to nitrite, and part of the nitrite has been reduced to a gaseous substance.

If red is not observed, either (1) the nitrate has not been reduced, or (2) the nitrate has been reduced beyond the oxidation level of nitrite (if gas is produced, it is detected in the Durham vial). To determine which has taken place, a little powdered zinc is added to the tube. If nitrates are still present, they will be reduced by the zinc and a red color will develop in 5-10 min.

• NITRITE REDUCTION TEST

The purpose of the test is to determine whether an organism can reduce nitrites to gaseous nitrogen or to other compounds containing nitrogen.

The broth medium for assaying nitrite reduction consists of heart infusion broth (Difco), 25 g; potassium nitrite, 1 g; and distilled water, 1 liter. The pH is adjusted to 7.0.

The test for the presence of nitrite (see Nitrate Reduction Test) is performed on a 2- to 5-day-old nitrite broth culture. If the broth does not become red within 1 min, the nitrite has been reduced. Gas production from nitrite reduction is observed in the Durham vial. If the broth does not become red and no gas production is observed, zinc dust is added to insure that the nitrite has not been oxidized to nitrate. If it has, the mixture becomes red.

• NUTRIENT AGAR (DIFCO) GROWTH TEST FOR PATHOGENIC NEISSERIAE

Cultures of *Neisseria gonorrhoeae* and *N. meningitidis* will not grow on nutrient agar at temperatures of 25° or 35°C (a few strains of *N. meningitidis*, usually isolated from human carriers, will grow at 35°C). Most nonpathogenic *Neisseria* cultures grow at 35°C, but variable results have been noted at 25°C.

The medium, which does not contain NaCl, is prepared according to the manufacturer's directions, dispensed in 5-ml amounts into 15- X 125-mm tubes, and slanted for cooling.

One drop of an 18- to 24-h HIB culture or a light suspension of bacteria is used to inoculate each slant. The cultures are incubated in a candle jar at 35°C for up to 7 days. A blood agar slant, inoculated at the same time, serves as a positive control for culture viability.

Reference: Hollis DG, Wiggins GL, Weaver RE: *Neisseria lactamicus* sp. n., a lactose-fermenting species resembling *Neisseria meningitidis*. Appl Microbiol 17:71-77, 1969.

• OF (OXIDATION/FERMENTATION) MEDIUM

This medium is used to determine whether an organism produces acid from carbohydrates. The basal media for detecting carbohydrate utilization and mode of utilization are:

(1) Special OF Medium (King). (This is the medium upon which carbohydrate utilization is based in this manual): Bacto-casitone, 2 g; phenol red, 2 ml of a 1.5% aqueous solution (dissolve 1.5 g phenol red in 5 ml 0.1N NaOH and add 95 ml water); agar, 3 g; and distilled water, 1 liter (the pH is 7.3). (**Reference:** Riley PS, Tatum HW, Weaver RE: Appl Microbiol 24:798-800, 1972.)

(2) OF Basal Medium (Difco). (This medium also has been used successfully to determine carbohydrate utilization): 9.4 grams of the Difco product (Bacto-tryptone, 2 g; sodium chloride, 5 g; dipotassium phosphate (anhydrous), 0.3 g; bromthymol blue, 0.08 g; and agar, 2 g) and 1 liter of distilled water, made according to the manufacturer's instructions (the pH is 6.8-6.9; the color of the medium is green).

Each basal medium is tubed in 6-ml amounts into 15- X 125-mm test tubes and autoclaved at 121°C for 15 min. The basal media are cooled to approximately 50°C, and the filter-sterilized carbohydrates are added aseptically to a final concentration of 1%.

Production of acid from carbohydrates

For determining whether acid is produced from carbohydrates, the agar deeps are inoculated with bacterial growth from a 24-h HIA slant by stabbing a needle 4-5 times into the medium to a depth of 2.5 cm. The tubes are incubated at 35°C for up to 7 days. Acid production is indicated by the color indicator changing to yellow.

Nonfermentative bacteria are routinely tested for their ability to produce acid from six carbohydrates (glucose, xylose, mannitol, lactose, sucrose, and maltose) in Special OF Medium (King). In addition to the six tubes containing carbohydrates, a control tube containing the OF base without carbohydrate also is inoculated.

Weak acid formation can be detected by comparing the tube containing the medium with carbohydrate with the inoculated tube containing medium with no carbohydrate. Most bacteria that can grow in the OF base produce an alkaline reaction in the control tube. If the color of the medium in a tube containing carbohydrate remains about the same color as it was before the medium was inoculated and if the inoculated medium in the control tube becomes a deeper red (that is, becomes alkaline), the culture being tested is considered weakly positive—that is assuming the amount of growth is about the same in both tubes.

If screwcap tubes are used to contain OF media, the caps are loosened during incubation to allow for air exchange. Otherwise, the control tube and tubes containing carbohydrates which are not oxidized might not become alkaline.

Mode of acid production from glucose

For determining whether the culture oxidized or fermented glucose, duplicate tubes of 1% glucose OF medium are inoculated.* The medium in one of the tubes is then overlaid with 1-2 cm of sterile petroleum jelly; both tubes are incubated at 35°C for up to 7 days. Acid formation in both tubes indicates a carbohydrate fermenter. Acid only in the tube which is not overlaid with petroleum jelly indicates an oxidizer. The absence of acid in either tube indicates that either the organism did not use glucose, or that during glucose metabolism, the organism did not produce detectable amounts of organic acids.

Reference: Hugh R, Leifson E: The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram negative bacteria. J Bacteriol 66:24-26, 1953.

For a discussion of the relative merits of the use of bromthymol blue and phenol red indicators in OF media, see Webster JA and Hugh R: Int J Syst Bacteriol 29:336-337, 1979.

• ONPG (O-NITROPHENYL-BETA-D-GALACTOPYRANOSIDE) TEST

The purpose of the test is to detect "late" lactose fermenters which, though possessing the enzyme beta-galactosidase for cleaving the lactose molecule, do not have the enzyme

*When the Special OF Medium (King) is used for determining whether an organism oxidizes or ferments glucose, the amount of casitone in the medium is increased to 5 g per liter. If less casitone is used, some organisms which oxidize glucose may produce an acid reaction under the petroleum jelly seal. **Reference:** King EO: The Identification of Unusual Pathogenic Gram Negative Bacteria. Atlanta, Center for Disease Control, 1964.

galactosidase-permease for transporting lactose inside the cell, where beta-galactosidase can split the lactose. ONPG is hydrolyzed, if beta-galactosidase is present, to produce 0-nitrophenol, a yellow compound.

(1) Medium assay. The medium consists of Bacto-tryptose (Difco), 4 g; Bacto-yeast extract (Difco), 4 g; sodium chloride, 5 g; agar, 3 g; and distilled water, 800 ml. The medium is autoclaved at 121°C for 15 min. Then it is cooled to approximately 50°C, and to it is added aseptically 200 ml of a 1% ONPG solution (filter-sterilized). The medium is dispensed in 3-ml amounts into 12- X 100-mm screwcap tubes.

The agar deeps are inoculated with a 24-h HIA slant culture by stabbing a needle to a depth of about 1.5 cm directly into the center of the tube. The tubes are incubated at 35°C and observed at 24 and 48 h to determine if the area around the bacterial growth has become yellow.

(2) Tablet assay. One ONPG tablet (Key Scientific Products, Los Angeles) is added to 1 ml of distilled water. The suspension is inoculated with a heavy suspension of bacterial growth from a 24-h HIA slant and incubated at 35°C for 6 h. A positive reaction for beta-galactosidase activity is indicated by a yellow color.

Reference (medium assay): Negut M, Hermann G: A comparison of two methods for detecting beta-D-galactosidase. Pub Health Lab 33:190-193, 1975.

• OXIDASE TEST

The purpose of the test is to detect in organisms the presence of oxidase enzymes associated with the cytochrome respiratory system. The reagent, a dye, acts as an electron acceptor and changes color in the presence of the oxidase enzymes.

The reagent for the test is a 0.5% aqueous solution of tetramethyl-p-phenylenediamine hydrochloride. The solution is stable 1 week at 4-10°C, or indefinitely when frozen. It is convenient to prepare the oxidase reagent in small amounts: 50-mg amounts of the powder are transferred to 10 or 12, 15- X 125-mm cotton-plugged tubes; these are stored in a brown, screwcap bottle with some calcium sulfate as a drying agent in the bottom. For use, 10-ml of distilled water is added to one tube, the reagent is dissolved, and the solution is transferred to a small dropper bottle.

To test a culture, a drop of oxidase reagent is added to colonies on a plate. The colonies of oxidase-positive organisms become light blue (weak reaction) to blue-black (strong reaction). Because the reagent eventually kills the organisms, colonies must be subcultured as soon as possible after the color begins to change.

Kovacs' modification is a more sensitive procedure for detecting oxidase, and it is used to confirm all negative results obtained by the routine colony procedure. One to two drops of oxidase reagent are placed on a piece of filter paper; a loopful of the organisms from the agar is mixed into the reagent on the paper; the development of a light to dark blue color within 10 sec indicates a positive reaction (a late reaction within 10 sec also is considered weak). The oxidase test performed with commercially-prepared oxidase reagent stabilized with citric acid may give negative results with some *Pasteurella* species. **Caution:** some metals in bacteriological loops produce false positive reactions. Platinum-iridium wire (15%) loops are satisfactory. If another kind of loop is used, it should be tested first by exposing it to the oxidase reagent on filter paper.

References: King EO, Tatum HW: *Actinobacillus actinomycetemcomitans* and *Haemophilus aphrophilus*. J Infect Dis 111:85-94, 1962.

Kovacs N: Identification of *Pseudomonas pyocyanea* by the oxidase reaction. Nature 178:703, 1956.

• PEPTIC DIGEST OF BLOOD

Peptic digest is used as a medium supplement for the cultivation of *Haemophilus* cultures. It is prepared as follows: 150 ml of 0.9% aqueous sodium chloride is autoclaved (121°C for 15 min) in a bottle having a ground glass stopper, but with the stopper separately wrapped in paper and the bottle cotton-plugged. The bottle is placed in a 55°C water bath, and the following are added to it: hydrochloric acid, concentrated, 6 ml; sterile sheep blood, defibrinated, 50 ml; and pepsin, 1 g. The mixture is kept in the water bath at 55°C overnight, with occasional shaking during the first 2 h. Twelve ml of 20% sodium hydroxide is added, then more is added slowly, with intermittent shaking, until the pH reached 7.6. Thereafter, the pH is returned to the range of 7.0 to 7.2 with 1 N hydrochloric acid. Five-tenths of a milliliter of chloroform is added to maintain sterility, and the glass-stoppered bottle is stored in the refrigerator. For the cultivation of *Haemophilus* species, the peptic digest is added to broth and agar media at a concentration of 2% to 5%.

Reference: Fildes P: A new medium for the growth of *B. influenzae*. Brit J Exptl Pathol 1:129-130, 1920.

• PHENYLALANINE AGAR

This medium is used to determine the ability of an organism to deaminate the amino acid phenylalanine to phenylpyruvic acid.

The medium consists of yeast extract (Difco), 3 g; *DL*-phenylalanine, 2 g (or L-phenylalanine, 1 g); disodium phosphate (anydrous), 1 g; sodium chloride, 5 g; agar, 12 g; and distilled water, 1 liter. The medium is tubed in 3-ml amounts into 13- X 100-mm screwcap tubes, autoclaved at 121°C for 10 min, and slanted for cooling.

The medium is inoculated heavily with bacterial growth from an HIA slant or a blood agar plate and incubated up to 24

h at 35°C. Four or five drops of 10% ferric chloride solution are dropped onto the growth on the slant. The formation of phenylpyruvate is indicated by the slant becoming green.

For testing members of the *Enterobacteriaceae*, the ferric chloride sometimes has to be added after only a 4- to 6-h incubation period. Continued incubation results in a false negative reaction.

Reference: Ewing WH, Davis BR, Reaves RW: Phenylalanine and malonate media and their use in enteric bacteriology. Pub Health Lab 15:153-160, 1957.

• PIGMENT PRODUCTION BY PSEUDOMONADS

Frequently, the pseudomonads encountered in the diagnostic laboratory do not produce pigment readily, if at all, in commonly employed laboratory media.

Studies by E. O. King and her associates indicated that the kind of peptone used in the basal medium markedly affects pigment production, and they developed two simple pigment-enhancing media for routine use: medium A (''Tech'') for enhancing the elaboration of pyocyanin and pyorubrin, and medium B (''Flo'') for stimulating the production of pyoverdin (fluorescein).

Medium A (''Tech'') consists of Bacto-peptone (Difco), 20 g; glycerol, 10 ml; magnesium chloride, 1.4 g; potassium sulfate, 10 g; agar, 15 g; and distilled water, 1 liter. Medium B (''Flo'') consists of Proteose peptone #3 (Difco), 20 g; glycerol, 10 ml; dipostassium phosphate (anhydrous), 1.5 g; magnesium sulfate (hydrated), 1.5 g; agar, 15 g; and distilled water, 1 liter. The pH of each medium is adjusted to 7.2. The media are dispensed in 7-ml amounts into 15- X 125-mm tubes, sterilized at 121°C for 15 min, and slanted for cooling.

Each slant is inoculated with one drop of a 24-h HIB culture and incubated at 35°C for up to 7 days. The pigments usually become evident within 3 days.

The pyocyanin-producing cultures produce pigments ranging in color from a faint aqua to a dark blue or green on medium A (''Tech'') and bright green on medium B (''Flo''). *Pseudomonas aeruginosa* is the only known species of bacteria that produces pyocyanin. Pyocyanin is soluble in chloroform and changes from blue in the alkaline pH range to pink in the acid pH range. To confirm the presence of the pigment, 1 to 2 ml of chloroform is added to a slant culture with the suspected pyocyanin, and the agar is mashed to extract the pigment. The chloroform layer becomes blue as it absorbs the pigment. Some of the blue chloroform is pipetted off into a small test tube, and one or two drops of 1 N HCL are added. Pyocyanin turns pink. (Caution: chloroform may be carcinogenic.)

The pyorubrin-producing cultures produce pigments ranging from light pink to dark maroon on the ''Tech'' medium. The pyoverdin-producing cultures produce a pigment on the ''Flo'' medium which fluoresces when excited by ultraviolet light (that is, when a Wood's lamp is used in the dark). Pyoverdin production is often enhanced by incubation at 25°C. Some *Pseudomonas aeruginosa* cultures produce a dark brown pigment on the ''Flo'' medium.

Reference: King EO, Ward MK, Raney DE: Two simple media for the demonstration of pyocyanin and fluorescein. Lab Clin Med 44:301-307, 1954.

• PORPHYRIN TEST FOR *HAEMOPHILUS*

This is a test for rapidly demonstrating porphyrin synthesis in *Haemophilus* strains. It detects the ability of bacteria to use d-aminolaevulinic acid in the biosynthesis of porphobilinogen and porphyrins. The method permits detection of some of the enzyme activities involved in the hemin biosynthetic pathway, and offers a more reproducible alternative to the traditional tests for hemin requirement in *Haemophilus* strains.

The culture is grown on a chocolate agar plate under increased carbon dioxide tension (candle jar) at 35°C for 24-48 h. A heavy suspension of the bacterial growth is made in 0.5 ml of the substrate solution (see below) contained in a 12- X 75-mm tube. The reaction mixture is incubated at 35°C for 4 h. Then the mixture is checked with a Wood's lamp. The mixture fluoresces if the delta-aminolaevulinic acid has been converted to porphyrins (a positive test). If the reaction is negative, the tube is incubated overnight, 18-24 h, and retested. If there is no fluorescence, one volume of Kovacs' modification of Ehrlich's reagent (p-dimethylaminobenzaldehyde, 5 g; amyl alcohol, 75 ml; and concentrated hydrochloric acid, 25 ml) is added. The mixture is shaken vigorously and allowed to stand to permit separation of the water and alcohol phases. A red color in the **lower aqueous** phase indicates the conversion of delta-aminolaevulinic acid to porphobilinogens (a postive test). (A red color develops in the alcohol phase when indole is present.) Either reaction, fluorescence with the Wood's lamp or a red reaction in the aqueous phase with Kovacs' reagent, indicates that the organism does not require hemin (X-factor) for growth.

Substrate solution. Delta-aminolaevulinic acid hydrochloride (Sigma), 2mM (33.5 mg), and magnesium sulfate (hydrated), 0.8 mM (19.72 mg), in 100 ml 0.1 M phosphate buffer, pH 6.9 (Sorensen: 6.3 g $NaH_2PO_4 \cdot H_2O$ and 14.8 g $Na_2HPO_4 \cdot 7H_2O$ [or 19.7 g $Na_2HPO_4 \cdot 12\ H_2O$] in 1 liter H_2O).

Reference: Kilian M: A rapid method for the differentiation of *Haemophilus* strains—the porphyrin test. Acta Pathol Microbiol Scand Sect B 82:835-842, 1974.

• POTASSIUM HYDROXIDE TEST FOR VERIFYING THE GRAM REACTION OF BACTERIA

The purpose of this test is to enable the user to distinguish between gram-positive and gram-negative bacteria when the Gram stain results are dubious.

One to two drops of 3% KOH are placed on a glass slide. A loopful of young bacterial growth from agar is mixed with the reagent. If on raising the loop a thread of slime follows it, the organism is considered to be gram-negative; gram-positive organisms do not form the slime thread.

It has been found that some *Bacillus* strains form the slime thread, particularly older cultures that have lost their gram-positivity.

Reference: Ryu E: On the gram-differentiation of bacteria by the simplest method. J Jpn Soc Vet Sci 17:31, 1938.

Gregersen T: Rapid method for distinction of gram-negative from gram-positive bacteria. Eur J Appl Microbiol Biotechnol 5:123-127, 1978.

• PRESERVATION OF BACTERIA BY FREEZING

Stock cultures ranging from eugonic enteric organisms to the more fastidious organisms, such as *Campylobacter fetus*, *Haemophilus* species, and *Bordetella pertussis*, can be preserved indefinitely by freezing them in defibrinated blood and storing at low temperatures (−40°C). Their survival over many years is excellent, and they can be recultivated with relative ease.

Freezing tubes are made from polypropylene with stoppers attached (#701, Walter Sarstedt, Inc., Princeton, N.J.). These particular kinds of tubes, which withstand the sudden changes in temperature to which they are subjected, must be used. The tubes are autoclaved without rinsing.

Cultures are labeled by using 1/2- X 3/4-in strips of nonwaterproof adhesive tape (we use "White Cross", American White Cross Laboratories, Inc., New Rochelle, N.Y.) on which the name or number of the culture has been typewritten. The tape is affixed near the top of the tube.

Storage boxes are constructed of 3/8-in marine waterproof plywood, with inside dimensions of 15 X 6 X 3 in. A 1- X 6- X 15-in block of styrofoam, prepunched with 3/8-in holes in line each way, is placed in the bottom of the box. (The size of the box and styrofoam insert depends on the inside dimensions of the freezer.) The diameters of the holes are slightly less than the diameters of the tubes to allow a snug fit. A 20-penny nail is used satisfactorily as a punch. Before a new box is used, it is prechilled in a deep freeze.

Cultures to be frozen are transferred to a heart infusion agar slant and incubated 18-24 h, or until growth appears. (Any carbohydrate-free agar medium which supports good growth can be used.) Bacteria are suspended in 7-8 drops of sterile defibrinated rabbit blood added to each slant with a capillary pipette. (Other kinds of antimicrobial-free blood probably can be used.) The suspension is aspirated and delivered to a freezing tube to about two-thirds volume.

The tubes with their contents are cooled to freezing by placing them in the deep freeze.

To recultivate the frozen organism, the chilled tube is held at the meniscus of the blood and rotated, with thumb and forefinger, until a small portion of the culture thaws. A droplet of the suspension is removed from the tube with a Pasteur pipette or other suitable means and inoculated to an appropriate medium for growth. The tube is returned immediately to the freezer to prevent further thawing.

• RAPID SUGAR TEST FOR *NEISSERIA*

Neisseria gonorrhoeae is presumptively identified in the clinical laboratory by growth and colonial morphology on selective media, cell morphology and appearance with the Gram reaction, and oxidase reaction. It is confirmed either by the fluorescent antibody test or by its reaction on various carbohydrate media. The rapid sugar test is a time-saving modification of the standard procedure for the latter. Although it was developed for testing neisseriae, we have found it useful for other fastidious organisms, such as *Haemophilus* species, DF-2, and *Kingella denitrificans*.

Reagents. The buffer-salt solution consists of dipotassium phosphate (anhydrous), 0.04 g; monopotassium phosphate, 0.01 g; potassium chloride, 0.8 g; 1% phenol red solution (aqueous), 0.4 ml; and distilled water, 100 ml. The pH is 7. The solution is filter-sterilized and stored at 4°C in a sterile, screwcap bottle. The desired carbohydrate solutions are made up to 20% concentrations in distilled water or in broth (peptone, 10 g; meat extract, 3 g; sodium chloride, 5 g; distilled water, 1 liter), and the pH is adjusted to 7. These solutions are filter-sterilized.

The test. 0.1 ml of buffer-salt solution is placed in a 10- X 75-mm sterile tube. One drop of the appropriate carbohydrate solution is added to the tube. For a control, one drop of distilled water or broth is added to the tube. Then one drop of a very heavy 18- to 24-h culture suspension (prepared in 0.35 ml of the buffer-salt solution) is added. (Alternatively, a loopful of the suspected organism is added directly into a tube of the buffer-salt-carbohydrate solution.) The tube is incubated in a 35°C water bath for 4 h and read at 30-min intervals. A positive test is indicated by acid formation (yellow color).

References: Kellog DS, Turner EM: Rapid fermentation confirmation of *Neisseria gonorrhoeae*. Appl Microbiol 25:550-552, 1973.

Brown WJ: Stability of working reagents for the modified rapid fermentation test (MRFT). Hlth Lab Sci 14:172-176, 1976.

Hollis DG, Sottnek FO, Brown WJ, Weaver RE: Use of the rapid fermentation test in determining reactions of fastidious bacteria in clinical laboratories. J Clin Microbiol 12:620-623, 1980.

• SALINE, BUFFERED (pH 7.0)

Stock solutions: Na_2HPO_4, 9.5 g per liter; KH_2PO_4, 9.1 g per liter. Working solution: Na_2HPO_4 stock solution, 61.1 ml; KH_2PO_4 stock solution, 38.9 ml; NaCl, 8.5 g; distilled water, 900 ml.

• S S AGAR (DIFCO)

This is a differentially selective medium for the isolation of pathogenic enteric bacilli, especially those belonging to the genera *Shigella* and *Salmonella*.

The medium consists of Bacto-beef extract, 5 g; Proteose peptone (Difco), 5 g; Bacto-lactose, 10 g; Bacto-bile salts No. 3, 8.5 g; sodium citrate, 8.5 g; sodium thiosulfate, 8.5 g; ferric citrate, 1 g; Bacto-agar, 13.5 g; Bacto-brilliant green, 0.00033 g; and Bacto-neutral red, 0.025 g. It is prepared according to the manufacturer's instructions. It is dispensed while hot in 5-ml amounts into 15- X 125-mm tubes and slanted (it is not autoclaved).

Slants are inoculated with one drop of a 24-h HIB culture and incubated at 35°C for up to 7 days.

• SALT TOLERANCE TEST

The purpose of the test is to determine the ability of organisms to grow in different concentrations of sodium ion.

The medium consists of Bacto-Nutrient Broth (Difco), 8 g; distilled water, 1 liter; and various concentrations (0%, 6%, 7%, 8%, 9%, 10%, 11%, and 12%) of sodium chloride. Each quantity of sodium chloride is dissolved in two-thirds of the required amount of nutrient broth in a volumetric flask. The flasks are autoclaved at 121°C for 15 min. The broths are cooled to room temperature, brought to volume with additional sterile nutrient broth, and mixed well. The media are dispensed aseptically in 4-ml amounts into 16- X 125-mm screwcapped tubes.

Each medium is inoculated with one drop of an 18- to 24-h HIB culture and incubated at 35°C for 24 h. The cultures are examined for growth or growth inhibition in the different concentrations of sodium chloride.

Reference: King EO: The Identification of Unusual Pathogenic Gram Negative Bacteria. Atlanta, Communicable Disease Center, 1964.

• SELECTIVE MEDIUM FOR PATHOGENIC *NEISSERIA* (THAYER-MARTIN MEDIUM-MODIFIED)

The medium consists of Mueller-Hinton agar (BBL), 38g (composed of beef infusion, 300 g; Acidicase peptone (BBL), 17.5 g; starch, 1.5 g; and agar, 17 g) and distilled water, 1 liter. Twenty-five-milliliter amounts are dispensed into 2-oz screwcap bottles, autoclaved at 121°C for 15 min, and cooled to 50°C. Twenty-five hundredths of a milliliter of V-C-N Inhibitor (BBL) (vancomycin, colistin, nystatin)* antibiotic mixture is added. (V-C-N in 10-ml vials is reconstituted, then dispensed in 0.25-ml amounts into 13- X 100-mm screwcap tubes, and frozen.) The contents are mixed well and poured in 20-ml amounts into Petri dishes.

*Or the equivalent.

Note: *Neisseria lactamica* and an occasional strain of other *Neisseria* species, as well as some species of Gram-negative rods, for example, *Kingella denitrificans* and Hf, can grow on this medium.

Reference: Thayer JD, Martin JE: Improved medium for cultivation of *Neisseria gonorrhoeae* and *Neisseria meningitidis*. Publ Health Rep 81:559-562, 1966.

• "STRING" TEST FOR VIBRIOS

A small amount of bacterial growth from a 20- to 24-h agar slant culture is taken with a loop and emulsified in one drop of 0.5% sodium deoxycholate solution (aqueous) on a slide. As the loop is lifted from the slide, a mucous-like string from the drop to the loop is observed. Three kinds of results are obtained: 1) + +, a string occurs shortly after the growth is emulsified and can still be obtained 45-60 sec later (all cultures of classical *Vibrio cholerae* and most cultures of *V. cholerae* biotype El Tor produce this reaction); 2) + −, a string occurs initially, but is absent 45-60 sec later (other *Vibrio* species usually give this reaction); and 3) − −, no reaction occurs.

Reference: Smith HL, Jr: A presumptive test for vibrios: the "string" test. Bull WHO 42:817-818, 1970.

• THIOSULFATE CITRATE BILE SALTS SUCROSE (TCBS) AGAR (BBL)

This is a selective medium used to isolate vibrios that cause cholera and food-poisoning.

The medium consists of yeast extract, 5 g; polypeptone peptone (BBL), 10 g; sodium citrate, 10 g; sodium thiosulfate, 10 g; oxgall, 5 g; sodium cholate, 3 g; sucrose, 20 g; and sodium chloride, 10 g. It is prepared according to the manufacturer's directions. It is cooled to 50°C and poured in 20-ml portions into Petri dishes.

Sucrose-fermenting cultures of *Vibrio cholerae* and *V. alginolyticus* form yellow colonies. The sucrose-negative species *V. parahaemolyticus* and *V. mimicus* form colonies with blue to greenish centers. *Pseudomonas* and *Aeromonas* cultures sometimes grow and usually form blue colonies.

Reference: Kobayashi T, Enomoto S, Sakazaki R: A new selective isolation medium for the *Vibrio* group on a modified Nakanishi's medium (TCBS agar medium). Jap J Bacteriol 18:387-392, 1963.

• TRANSFORMATION ASSAY FOR *ACINETOBACTER* AND *MORAXELLA OSLOENSIS*

The identification of these coccobacillary bacteria by biochemical techniques may be inconclusive. Transformation provides a more definitive and critical assessment of the

taxonomic placement of these organisms. Juni has described a transformation procedure for determining genetic relatedness for *Acinetobacter calcoaceticus* and *Moraxella osloensis* cultures. The procedure involves mixing crude DNA extracts of test strains with the cellular mass of an amino acid auxotroph. Genetically related test strains will convert the auxotroph to prototrophy. This conversion is evidenced by the subsequent growth of colonies from the auxotroph-crude DNA test mixture upon a basal mineral salts medium.

Lactate mineral medium (solution A) is prepared as follows: to 800 ml of distilled water the following chemicals are added, one at a time (each chemical is allowed to dissolve before the next one is added): monopotassium phosphate (anhydrous), 1.5 g; disodium phosphate (anhydrous), 13.5 g; magnesium sulfate, 0.1 g; ammonium chloride, 2 g in 100 ml distilled water, added slowly with stirring; calcium chloride (anhydrous), 10 ml of a 0.1% aqueous solution; ferric sulfate (hydrated), 5 ml of a 0.01% solution; lactic acid (reagent grade), 5 ml; and distilled water, added to make 1 liter. The solution is dispensed in 150-ml portions into 8-oz screwcap bottles and autoclaved at 121°C for 15 min.

Basal Medium (solution B) is prepared as follows: 30 g of agar is heated in 1 liter of distilled water to dissolve; this is dispensed in 50-ml amounts into 8-oz screwcap bottles and autoclaved at 121°C for 15 min.

One bottle of solution A is added to the contents of one bottle of solution B (melted and cooled to 50°C). Twenty-milliliter amounts of the medium are poured into 15- X 100-mm Petri plates. If the plates are not used immediately after drying in a 35°C incubator for about 15 h, they are stored in air-tight plastic bags and placed in the refrigerator (4°C). Stored in this manner, they retain their effectiveness for at least 3 weeks.

Preparing crude transforming DNA. The culture to be used as the DNA donor is grown on the surface of any suitable medium, for example, HIA, blood agar, or nutrient agar. The age of the culture is not critical. A loopful of cell growth is suspended in approximately 0.5 ml of a sterile solution of 0.05% sodium dodecyl sulfate in standard saline citrate (0.15 M NaCl, 0.015 M trisodium citrate) contained in a tightly capped test tube. The cell paste is suspended to immerse all of the visible cellular material. The suspension is then heated in a 60°C water bath for 15 to 60 min to lyse the cells and produce a sterile DNA-transforming preparation. This kind of DNA solution may be stored at 3°-5°C for months.

The transformation assay. A small portion of bacterial cell mass from a 24-h culture of the recipient strain to be transformed is applied to a section of an HIA plate (the complete medium).

A loopful of the crude DNA preparation is used to suspend and spread the cell paste in an area about 1 cm in diameter. A portion of the cell mass of the recipient strain is also spread onto another section of the plate to serve as a non-DNA-treated control. A third section of the same plate is streaked with a loopful of the DNA preparation to test its sterility. The plate is then inverted and incubated at 35°C for 4-6 h for *Acinetobacter* assays or 8 h to overnight for *Moraxella osloensis* assays. The incubation period may be prolonged if necessary.

After the incubation period on the complete medium, assuming that the DNA preparation is sterile, cellular growth from each of the growth areas is streaked heavily onto a section of the lactate mineral agar plate (minimal medium), upon which only the prototrophic transformants will grow. If the DNA preparation is not sterile, it is heated for an additional 30 min at 60°C and retested. The minimal medium plates are incubated inverted at 35°C for 24 h.

Transformant colonies are clearly visible, but the non-DNA-treated control cells do not grow on this selective medium. The *M. osloensis* strains are incubated for an additional 24 h if no growth is noted during the initial 24-h incubation period. A small percentage of strains which produce no visible transformant colonies at 24 h are positive at 48 h.

NOTE: Transformation procedures have been described for *Moraxella urethralis* and *Neisseria gonorrhoeae:* Janik A, Juni E, Heym, GA: Genetic transformation as a tool for detection of *Neisseria gonorrhoeae*. J Clin Microbiol 4:71-81, 1976; Juni E: Genetic transformation assays for identification of strains of *Moraxella urethralis*. J Clin Microbiol 5:227-235, 1976.

References: Juni E: Interspecies transformation of *Acinetobacter:* genetic evidence for a ubiquitous genus. J Bacteriol 112:917-931, 1972.

Juni E: Simple genetic transformation assay for rapid diagnosis of *Moraxella osloensis*. Appl Microbiol 27:16-24, 1974.

• TRIPLE SUGAR IRON AGAR (TSI AGAR)

This medium is used to determine an organism's ability to use fermentatively specific carbohydrates incorporated in a basal growth medium, with or without the production of gas, and to test for the production of hydrogen sulfide.

The medium contains beef extract (Difco), 3.0 g; yeast extract (Difco), 3.0 g; Bacto-peptone (Difco), 15.0 g; proteose peptone (Difco), 5.0 g; lactose, 10.0 g; sucrose, 10.0 g; glucose, 1.0 g; ferrous sulfate, 0.2 g; sodium chloride, 5.0 g; sodium thiosulfate, 0.3 g; agar, 15.0 g; phenol red, 0.024 g; and distilled water, 1 liter. After being heated to dissolve the agar, the medium is dispensed in 5-ml portions into 15- X 125-mm tubes and autoclaved at 121°C for 15 min. Tubes are slanted to obtain approximately a 2.5-cm butt and a 4-cm slant.

Growth from an 18- to 24-h 35°C blood agar plate or HIB culture is used to inoculate the slant and stab the butt of the medium. A strip of lead acetate paper is inserted in the tube and held in place with the cotton plug so that it extends about 2 cm below the plug, close to, but not touching the agar. The culture is incubated at 35°C for 7 days. Blackening of the paper is recorded as positive for H_2S production. Hydrogen sulfide formed in the agar butt is also recorded. Acid, alkaline, or neutral reactions, occurring both in the agar butt and on the

slant, and gas formation in the butt are noted as evidence of carbohydrate fermentation or lack of it.

Reference: Sulkin SE, Willett JC: A triple sugar-ferrous sulfate medium for use in identification of enteric organisms. J Lab Clin Med 25:649-653, 1940.

• TRYPTONE GLUCOSE YEAST EXTRACT (TGY) AGAR

This medium is used to determine the range of temperature in which an organism grows.

The medium, tryptone glucose yeast extract (TGY) agar consists of tryptone, 5 g; glucose, 1 g; yeast extract (Difco), 5 g; dipotassium phosphate, 1 g; agar, 15 g; and distilled water, 1 liter. The pH is adjusted to 6.8-7.0. The medium is tubed in 5-ml amounts into 15- X 125-mm tubes, autoclaved at 121°C for 15 min, and slanted for cooling.

For each culture tested, three slants are inoculated with one drop of a 24-h HIB culture. The tubes are incubated at 25°, 35°, and 42°C, respectively, for 18-24 h. The relative amount of growth at each temperature is recorded.

Reference: Haynes WC: *Pseudomonas aeruginosa* - its characterization and identification. J Gen Microbiol 5:939-950, 1951.

• UREA AGAR (CHRISTENSEN'S)

This medium is used to determine an organism's ability to hydrolyze urea, forming ammonia by action of the enzyme urease.

The medium is prepared with Bacto-urea agar base (Difco), 29 g (composed of Bacto-peptone, 1 g; Bacto-dextrose, 1 g; sodium chloride, 5 g; monopotassium phosphate, 2 g; urea (Difco), 20 g; and Bacto-phenol red, 0.012 g) and distilled water, 100 ml; this solution is Seitz-filtered. Bacto-agar, 15 g, and distilled water are autoclaved at 121°C for 15 min. The agar is cooled to 55°C, and the urea solution is added. The medium is tubed in 5-ml amounts into 15- X 125-mm screwcap tubes and slanted. The slants are inoculated with one drop of a 24-h HIB culture and incubated at 35°C for up to 7 days. A positive test is indicated by the development of a pronounced pink color in the agar. If the organism does not grow on the slant, a second slant is inoculated heavily with growth from an 18- to 24-h blood agar plate or an HIA slant.

Reference: Christensen WB: Urea decomposition as a means of differentiating *Proteus* and paracolon cultures from each other and from *Salmonella* and *Shigella* types. J Bacteriol 52:461-466, 1946.

• VOGES-PROSKAUER (VP) TEST (COBLENTZ METHOD)

The purpose of the test is to determine the ability of some organisms to produce a neutral end product, acetylmethylcarbinol (acetoin), from glucose fermentation.

The medium (MR-VP (Difco)) is composed of buffered peptone, 7 g; glucose, 5 g; dipotassium phosphate (anhydrous), 5 g; distilled water, 1 liter. The pH is adjusted to 6.9. The medium is tubed in 2-ml amounts into 18- X 150-mm screwcap tubes and autoclaved at 121°C for 10 min.

The tubes are heavily inoculated with the growth from an 18- to 24-h HIA slant. The broth culture is incubated at 35°C for 6 h.

Test for acetylmethylcarbinol. The reagents are (A) 5% alpha naphthol in 95% ethanol (deteriorates after 30 days at room temperature) and (B) 0.3% creatinine in 40% potassium hydroxide (keeps at least 2 weeks at room temperature).

The test is initiated by adding 0.6 ml of reagent A to the culture broth and shaking gently to disperse the reagent. Then 0.2 ml of reagent B is added, and the mixture is shaken vigorously and observed for 5 to 10 min, with occasional shaking. A positive reaction is indicated by the development of a pink to cherry-red color.

Reference: Coblentz LM: Rapid detection of the production of acetylmethyl-carbinol. Amer J Publ Health 33:815-817, 1943.

• WAYSON'S STAIN

Wayson's stain is used to demonstrate bipolar staining in organisms such as *Yersinia pestis*.

The stain is prepared as follows: solution A consists of basic fuchsin (90% dye content), 0.20 g; methylene blue (90% dye content), 0.75 g; and ethyl alcohol (95%), 20 ml. Solution B consists of 5% phenol, 200 ml. Solution A is poured slowly into solution B, and the mixture is filtered.

Air-dried smears are fixed in absolute methanol for 2-5 min and then stained for 10-20 sec, washed with water, and blotted dry.

Symbols and Abbreviations

SYMBOLS

[ ]	key tests
( )	late reaction, 3 to 7 days
+	positive reaction (90% or more strains tested were positive) within 48 h, except with gelatin, within 14 days
+, gas	with nitrate test, indicates nitrates reduced to nitrites, incomplete reduction of nitrites with gas formation
−	negative reaction (10% or less strains tested were positive); with action on blood, indicates no reaction
+ or (+)	positives and late positives together total 90% or more
>	more than
<	less than
←	from

ABBREVIATIONS

A	acid
AFB	Air Force Base (Brooks)
al or α	alpha hemolysis
amb	amber
ATCC	American Type Culture Collection (Rockville, Md.)
β	beta-like (hemolysis)
BAP	rabbit blood agar plate
BBL	"Baltimore Biological Laboratory", a Division of Bioquest (Becton, Dickinson, and Company, Cockeysville, Md)
beta	beta hemolysis around isolated colonies
B-G	Bordet-Gengou
br	brown (pigment); browning of blood; broad rod
bsrs	bipolar staining short straight rod
C	centigrade
c	coagulation
CA	cysteine agar
cc	coccobacillus
cc,pr	paired coccobacilli
cd	diplococcus
CDC	Centers for Disease Control (Atlanta)
ch	chains
char	chartreuse
chs	short chains
cl	clot
co	coral

CTA	cystine trypticase agar (BBL)
CYE	cysteine yeast extract (agar)
DF	dysgonic (fastidious) fermenter
EF	eugonic (nonfastidious) fermenter
EO	eugonic oxidizer
F	fermentative (glucose); Enteric fermentation base
FA	fluorescent antibody
FB	flagella broth
fc	filaments, curved
F-G	Feeley-Gorman (medium)
G	gas
gr	greening of blood, usually accompanied by lysis; growth
h	hour(s) (incubation)
HB	initials of a patient
HIA	Heart infusion agar (Difco)
HIB	Heart infusion broth (Difco)
H_2S	hydrogen sulfide
inc	incomplete
ins	insoluble
ins gr	insufficient growth
H	"H-forms", pleomorphism where rod-shaped cells often appear thin to very thin in the central region, with thicker ends
IR	indicator reduced
k	alkaline
l	long
L	lateral
lav	lavender
lg	light growth
LG	lavender green coloration under heavy growth, indication of proteolysis
lr	long rod
lrch	long rods in chains
lrs	long, straight rods
ltrt	long, thin, tapered rods
ltsper	long, thin, spindle-shaped, curved rods
ly	diffuse lysis of red cells extending out from heavy growth; no action by individual colonies
m	medium; motile
M	*Moraxella*-like
mbr	medium length broad rod
MM	motility medium
mod	moderate
M-R	methyl red, methyl red test
mrc	medium curved rod
mrs	medium straight rod
msr	medium length slender rod
NaCl	sodium chloride
nc	no change

NCTC	National Collection of Type Cultures (London)
neg	negative
ngr	no growth
nm	nonmotile
n-o	nonoxidative (glucose)
NT	test not done
nut br	nutrient broth
O	oxidative (glucose)
occ	occasional
OF	oxidation-fermentation
ONPG	O-nitrophenyl-beta-D-galactopyranoside (test)
or	orange
p	polar
p,1-2	1-2 polar (flagella)
p,1-4	1-4 polar (flagella)
p,>2	more than 2 polar (flagella)
pb ac	lead acetate
pep	peptonization
pe	peritrichous (flagella)
pk	pink
p,L	polar and lateral (flagella), only one or two flagella on most cells
plr	pleomorphic rod
pr	paired
plrs	pleomorphic straight rod
PPA	phenylpyruvic acid
pt	polar tuft
pu	purple
r	rod
rc	curved rod
rs	straight rod
RTD	routine test dilution
rv	very short rod
SBL	Special Bacteriology Laboratory (CDC)
sbr	short broad rod
sc	comma-shaped, spiral
sl	slight, slightly
s-lr	short to long rods
sol	soluble
sp.	species (singular)
spp.	species (plural)
spr	short pleomorphic rods
srs	short straight rod
SS	*Salmonella-Shigella* (agar)
ss	subspecies
subsp	subspecies
szf	serum zone formation (clear top in milk)
t	thin

T	total
ta	tan
TCBS	Thiosulfate citrate bile salts sucrose agar (BBL)
TGY	tryptone glucose yeast extract medium
Thio	Fluid thioglycollate medium (BBL)
TM	Thayer-Martin
tr	trace
trt	thin tapered rods
TSI	Triple sugar iron (agar)
tsrs	thin, short, straight rods
v	variable (11% – 89% tested positive)
vbr	variable length broad rod
V-P	Voges Proskauer (test)
vps-mr	vacuolated, pale-staining short to medium rod
w	weak, weakly
wh	white
WHO	World Health Organization
WRAMIR	Walter Reed Army Medical Institute of Research (Washington, D
yel	yellow

Symbols for CDC "Groups", with Published Names

DF-1	*Capnocytophaga* spp.
EF-6	*Vibrio fluvialis*
EF-9	*Tatumella ptyseos*
EO-1	*Pseudomonas cepacia*
HB-1	*Eikenella corrodens*
M-1	*Kingella kingae*
M-3	*Moraxella atlantae*
M-4	*Moraxella urethralis*
M-4f (also IVf)	*Flavobacterium odoratum*
TM-1	*Kingella denitrificans*
I	*Pseudomonas maltophilia*
Ia	*P. diminuta*
Ib	*P. putrefaciens*
IIa	*Flavobacterium meningosepticum*
IId	*Cardiobacterium hominis*
IIk-1	*Pseudomonas paucimobilis*
IIk-2	*Flavobacterium multivorum*
IIIa, IIIb	*Achromobacter xylosoxidans*
IVa	*Bordetella bronchiseptica*
IVb	*B. parapertussis*
IVf (also M-4f)	*Flavobacterium odoratum*
Va-1	*Pseudomonas pickettii*
Va-2	*Pseudomonas pickettii*
Vb-1	*P. stutzeri*
Vd-3	*Agrobacterium radiobacter*
VI	*Alcaligenes faecalis*

Keys for Identification of Bacteria

In Miss King's original edition of the "Identification of Unusual Gram Negative Bacteria," a key for identifying aerobes was included. In the Key the various species and unnamed groups of bacteria were placed in categories based on three characteristics: 1) the mechanism by which the organism produced acid from glucose, fermentation or oxidation (organisms not producing acid from glucose were designated as Glucose Nonoxidizers); 2) the ability of the organism to grow on MacConkey agar; and 3) the oxidase reaction of the organism. An organism was to be identified by comparing its biochemical profile to the profiles of known organisms having the same "key" characteristics (referring to the glucose, MacConkey, and oxidase reactions) as those of the unknown organism.

A revision of the Key follows. In it a species or unnamed group has been considered positive for a characteristic if 90% or more of the strains have been positive for the characteristic. If 11% through 89% of the strains have been positive, the organism has been considered variable for the characteristic and has been included in both a positive and negative category. If less than 11% of the strains have been positive, the organism has been considered negative for the characteristic.

The key is only a **guide** for identification. If an organism cannot be identified as one of the species listed in its category on the key, one should consider the possibility that it belongs to a species or group for which less than 11% of the strains have one or more of the "key" characteristics exhibited by the unidentified culture. This is more likely to occur with the oxidase or MacConkey reaction than with the glucose reaction. Such a culture, therefore, should also be compared with species which differ from it in its oxidase or MacConkey reaction, or in both.

If an organism with a negative Gram stain cannot be identified in the schema, one should consider the possibility that it is a gram-positive organism, particularly if it does not grow on MacConkey agar.

There is also the possibility that an organism which cannot be identified with this Key belongs to a species which we have neither studied nor encountered.

KEY I FOR IDENTIFICATION OF GRAM-NEGATIVE AEROBES

GLUCOSE FERMENTERS

MacConkey-positive/oxidase-negative

Chromobacterium violaceum (3)*
Enterobacteriaceae**
Haemophilus aphrophilus (9)
HB-5 (9)
Tatumella ptyseos (3)
Yersinia enterocolitica (4)
Y. pestis (4)
Y. pseudotuberculosis (4)

MacConkey-positive/oxidase-positive

Actinobacillus equuli (8)
A. lignieresii (7)
A. suis (8)
Aeromonas hydrophila (3)
Chromobacterium violaceum (3)
EF-4a (6)
Haemophilus aphrophilus (9)
HB-5 (9)
Kingella kingae (13)
Neisseria mucosa (12,40)
N. sicca (12,40)
N. subflava (11,40)
Pasteurella aerogenes (6)
P. gallinarum (7)
P. haemolytica (7)
P. pneumotropica (6)
Plesiomonas shigelloides (3)
Vibrio alginolyticus (1)
V. anguillarum (2)
V. cholerae (0:1 Group and other O Groups) (1)
V. damsela (2)
V. fluvialis (2)
V. hollisae (2)
V. parahaemolyticus (1)
V. vulnificus (2)

MacConkey-negative/oxidase-negative

Actinobacillus actinomycetemcomitans (9)
Capnocytophaga spp. (DF-1) (9)
DF-3 (10)
Gardnerella vaginalis (10)
Haemophilus aphrophilus (9)
HB-5 (9)
Streptobacillus moniliformis (36)

MacConkey-negative/oxidase-positive

Actinobacillus actinomycetemcomitans (9)
A. equuli (8)
A. lignieresii (7)
A. suis (8)
Cardiobacterium hominis (10)
DF-2 (10)
EF-4a (6)
Haemophilus aphrophilus (9)
HB-5 (9)
Kingella denitrificans (13)
K. indologenes (13)
K. kingae (13)
Neisseria lactamica (11,40)
N. mucosa (12,40)
N. sicca (12,40)
N. subflava (11,40)
Pasteurella gallinarum (7)
P. haemolytica (7)
P. multocida (5)
P. pneumotropica (6)
P. ureae (5)
Pasteurella sp. "n. sp. 1" ("gas") (5)
Vibrio anguillarum (2)
V. hollisae (2)

*( ) = Chart number.
**Not treated in this manual (other than *Yersinia* and *Tatumella*).

GLUCOSE OXIDIZERS

MacConkey-positive/oxidase-negative

Acinetobacter calcoaceticus (*A. anitratus*) (23)
Brucella canis (35,37)
Pseudomonas cepacia (31)
P. mallei (34)
P. maltophilia (31)
P. marginata (33)
P. paucimobilis (32)
∇e-1 (31)
∇e-2 (32)

MacConkey-positive/oxidase-positive

Achromobacter xylosoxidans (23)
Agrobacterium radiobacter (24)
Brucella abortus (35,37)
B. canis (35,37)
B. melitensis (35,37)
B. suis (35,37)
EF-4b (24)
EO-2 (23)
Flavobacterium breve (14)
F. meningosepticum (13)
F. multivorum (32)
F. spiritivorum (32)
Flavobacterium sp. (IIb)(14)
Pseudomonas aeruginosa (27)
P. cepacia (31)
P. diminuta (29)
P. fluorescens (27)
P. mallei (34)
P. mendocina (28)
P. mesophilica (18)
P. paucimobilis (32)
P. pickettii (33)
P. pseudomallei (31)
P. putida (27)
P. putrefaciens biotype 1 (34)
P. stutzeri (28)
P. thomasii (33)
P. vesicularis (29)
∇a-1 (33)
∇b-3 (28)
∇d (*Achromobacter* sp.) (24)

MacConkey-negative/oxidase-negative

Brucella canis (35,37)
Francisella tularensis (36)
Pseudomonas mallei (34)
P. paucimobilis (32)
IIe (15)

MacConkey-negative/oxidase-positive

Brucella abortus (35,37)
B. canis (35,37)
B. melitensis (35,37)
B. suis (35,37)
EF-4b (24)
EO-2 (23)
Flavobacterium meningosepticum (13)
F. multivorum (32)
F. spiritivorum (32)
Flavobacterium sp. (IIb) (14)
Neisseria gonorrhoeae (11,39)
N. lactamica (11,40)
N. meningitidis (11,39)
Pasteurella anatipestifer (22)
Pseudomonas mallei (34)
P. mesophilica (18)
P. paucimobilis (32)
P. vesicularis (29)
IIe (15)
IIh (15)
IIi (16)

GLUCOSE NONOXIDIZERS

MacConkey-positive/oxidase-negative

Acinetobacter calcoaceticus (A. lwoffi) (23)
Bordetella parapertussis (26)
Brucella canis (35,37)
Pseudomonas maltophilia (31)

MacConkey-positive/oxidase-positive

Achromobacter xylosoxidans (23)
Alcaligenes denitrificans (25)
A. faecalis (25)
A. odorans (25)
Bordetella bronchiseptica (26)
Brucella abortus (35,37)
B. canis (35,37)
B. melitensis (35,37)
Campylobacter fetus subsp. fetus (18)
C. fetus subsp. *jejuni* (17)
Flavobacterium odoratum (14)
M-5 (22)
M-6 (22)
Moraxella atlantae (21)
M. osloensis (20)
M. phenylpyruvica (21)
M. urethralis (21)
Neisseria flavescens (12,39)
N. mucosa (12,40)
N. sicca (12,40)
N. subflava (11,40)
Pseudomonas acidovorans (29)
P. alcaligenes (30)
P. diminuta (29)
P. mesophilica (18)
P. pseudoalcaligenes (30)
P. putrefaciens biotype 1 (34)
P. putrefaciens biotype 2 (34)
P. testosteroni (29)
P. vesicularis (29)
Pseudomonas sp. CDC Group 1 (30)
IVc-2 (26)
IVe (25)
Vd (*Achromobacter* sp.) (24)

MacConkey-negative/oxidase-negative

Brucella canis (35,37)
Francisella tularensis (36)

MacConkey-negative/oxidase-positive

Bordetella pertussis (36)
Branhamella catarrhalis (19,39)
Brucella abortus (35,37)
B. canis (35,37)
B. melitensis (35,37)
Campylobacter fetus subsp. *fetus* (18)
C. fetus subsp. *jejuni* (17)
C. fetus subsp. *venerealis* (17)
Eikenella corrodens (17)
M-5 (22)
M-6 (22)
Moraxella atlantae (21)
M. bovis (20)
M. lacunata (19)
M. nonliquefaciens (20)
M. osloensis (20)
M. phenylpyruvica (21)
Neisseria elongata (19,39)
N. flavescens (12,39)
N. mucosa (12,40)
N. sicca (12,40)
N. subflava (11,40)
Pseudomonas mesophilica (18)
P. vesicularis (29)
IIf (15)
IIj (16)
IVe (25)

(Tables for the differentiation of species and biotypes of *Brucella* and *Haemophilus*, and species of *Neisseria* and *Branhamella* follow the "Round Table" charts below.)

KEY II FOR IDENTIFICATION OF GRAM-NEGATIVE AEROBES

[Key II is the same as Key I, with the addition of the percentages of positive MacConkey and oxidase reactions for each organism.]

FERMENTERS

Organism	MacConkey % +	Oxidase % +	Comments
MacConkey-positive/oxidase-negative			
Chromobacterium violaceum (3)*	100	67	
Enterobacteriaceae (other than *Yersinia*)			Usually (>90%) grow on MacConkey agar and are oxidase negative.
Haemophilus aphrophilus (9)	19	28	
HB-5 (9)	34(23)	54	
Tatumella ptyseos (3)	93	7	
Yersinia enterocolitica (4)	100	0	
Y. pestis (4)	100	0	
Y. pseudotuberculosis (4)	100	3	
MacConkey-positive/oxidase-positive			
Actinobacillus equuli (8)	84(5)	100	
A. lignieresii (7)	67(8)	100	
A. suis (8)	82(12)	100	
Aeromonas hydrophila (3)	100	99	
Chromobacterium violaceum (3)	100	67	
EF-4a (6)	44(11)	100	
Haemophilus aphrophilus (9)	19	28	
HB-5 (9)	34(23)	54	
Kingella kingae (13)	33(3)	100	
Neisseria mucosa (12,40)	57(3)	100	
N. sicca (12,40)	68	100	
N. subflava (11,40)	47(2)	100	
Pasteurella aerogenes (6)	100	100	
P. gallinarum (7)	20(10)	90	
P. haemolytica (7)	79(6)	91	
P. pneumotropica (6)	36(17)	99	
Plesiomonas shigelloides (3)	100	96	
Vibrio alginolyticus (1)	100	100	
V. anguillarum (2)	(+)	+	
V. cholerae (0:1 Group and other 0 groups) (1)	100	100	
V. damsela (2)	100	91	
V. fluvialis (2)	92(8)	100	
V. hollisae (2)	64(18)	100	
V. parahaemolyticus (1)	100	100	
V. vulnificus (2)	100	100	

( ) Chart number

FERMENTERS

Organism	MacConkey % +	Oxidase % +	Comments
MacConkey-negative/oxidase-negative			
Actinobacillus actinomycetemcomitans (9)	4(1)	19	
Capnocytophaga spp. (DF-1) (9)	*0*	*7*	
DF-3 (10)	*0*	*0*	
Gardnerella vaginalis (10)	5	6	
Haemophilus aphrophilus (9)	19	28	
HB-5(9)	34(23)	54	
Streptobacillus moniliformis (36)	0	0	
MacConkey-negative/oxidase-positive			
Actinobacillus actinomycetemcomitans (9)	4(1)	19	
A. equuli (8)	84(5)	100	
A. lignieresii (7)	67(8)	100	
A. suis (8)	82(12)	100	
Cardiobacterium hominis (10)	0	100	
DF-2 (10)	0	96	
EF-4a (6)	44(11)	100	
Haemophilus aphrophilus (9)	19	28	
HB-5 (9)	34(23)	54	
Kingella denitrificans (13)	0	100	
K. indologenes (13)	–	+	
K. kingae (13)	33(3)	100	
Neisseria lactamica (11,40)	0	100	
N. mucosa (12,40)	57(3)	100	
N. sicca (12,40)	68	100	
N. subflava (11,40)	47(2)	100	
Pasteurella gallinarum (7)	20(10)	90	
P. haemolytica (7)	79(6)	91	
P. multocida (5)	2(1)	97	
P. pneumotropica (6)	36(17)	99	
P. ureae (5)	0	99	
Pasteurella sp. "n. sp. 1" (P. "gas") (5)	1	98	
Vibrio anguillarum (2)	(+)	+	
V. hollisae (2)	64(18)	100	

GLUCOSE OXIDIZERS

Organism	MacConkey % +	Oxidase % +	Comments
MacConkey-positive/oxidase-negative			
Acinetobacter calcoaceticus (23) *(A. anitratus)*	99(1)	0	
Brucella canis (35,37)	12(29)	72	
Pseudomonas cepacia (31)	100	86	
P. mallei (34)	88	25	<10 strains
P. maltophilia (31)	100	0	glucose 85 (5)% +, frequently weak
P. marginata (33)	+	−	1 strain
P. paucimobilis (32)	10(13)	75	
Ye-1 (31)	100	0	
Ye-2 (32)	100	0	
MacConkey-positive/oxidase-positive			
Achromobacter xylosoxidans (23)	100	100	glucose 78% +
Agrobacterium radiobacter (24)	100	100	
Brucella abortus (35,37)	50(20)	96	glucose 50(25)% +
B. canis (35,37)	12(29)	72	glucose 81(14)% +
B. melitensis (35,37)	62	100	glucose 33% +
B. suis (35,37)	23(15)	95	
EF-4b (24)	65(6)	100	
EO-2 (23)	63(11)	100	
Flavobacterium breve (14)	100	100	3 strains
F. meningosepticum (13)	89(3)	99	
F. multivorum (32)	87(17)	95	
F. spiritivorum (32)	0(55)	100	
Flavobacterium sp. (IIb) (14)	54(9)	96	
Pseudomonas aeruginosa (27)	100	99	
P. cepacia (31)	100	86	
P. diminuta (29)	97(3)	100	glucose 21(9)% +
P. fluorescens (27)	100	97	
P. mallei (34)	88	25	<10 strains
P. mendocina (28)	100	100	
P. mesophilica (18)	15	96	glucose 40% +
P. paucimobilis (32)	10(13)	75	
P. pickettii (33)	100	100	
P. pseudomallei (31)	100	100	
P. putida (27)	100	100	
P. putrefaciens, biotype 1 (34)	100	100	glucose 17(33)% +
P. stutzeri (28)	100	100	
P. thomasii (33)	100	100	
P. vesicularis (29)	81(8)	94	glucose 87(9)% +
Ya-1 (33)	99(1)	100	
Yb-3 (28)	100	100	
Yd (*Achromobacter* sp.) (24)	100	100	glucose 86(13)% +

GLUCOSE OXIDIZERS

Organism	MacConkey % +	Oxidase % +	Comments
MacConkey-negative/oxidase-negative			
Brucella canis (35,37)	12(29)	72	
Francisella tularensis (36)	0	0	
Pseudomonas mallei (34)	88	25	≤10 strains
P. paucimobilis (32)	10(13)	75	
IIe (15)	7	88	
MacConkey-negative/oxidase-positive			
Brucella abortus (35,37)	50(20)	100	glucose 50(25)% +
B. canis (35,37)	12(29)	72	glucose 81(14)% +
B. melitensis (35,37)	62	100	
B. suis (35,37)	23(15)	100	
EF-4b (24)	65(6)	100	
EO-2 (23)	63(11)	100	
Flavobacterium meningosepticum (13)	89(3)	99	
F. multivorum (32)	81(17)	98	
F. spiritivorum (32)	0(55)	100	
Flavobacterium sp. (IIb) (14)	54(9)	96	
Neisseria gonorrhoeae (11,39)	0	100	
N. lactamica (11,40)	0	100	
N. meningitidis (11,39)	0	100	
Pasteurella anatipestifer (22)	0	100	1 strain
Pseudomonas mallei (34)	88	25	
P. mesophilica (18)	15	96	glucose 40% +
P. paucimobilis (32)	10(13)	75	
P. vesicularis (29)	81(8)	94	
IIe (15)	7	88	
IIh (15)	0	100	
IIi (16)	0	100	

GLUCOSE NON-OXIDIZERS

Organism	MacConkey % +	Oxidase % +	Comments
MacConkey-positive/oxidase-negative			
Acinetobacter calcoaceticus (A. lwoffi) (23)	90(7)	0	
Bordetella parapertussis (26)	100	0	<10 strains
Brucella canis (35,37)	12(29)	72	
Pseudomonas maltophilia (31)	100	0	glucose 85(5)% +, frequently weak
MacConkey-positive/oxidase-positive			
Achromobacter xylosoxidans (23)	100	100	glucose 78% +
Alcaligenes denitrificans (25)	100	100	
A. faecalis (25)	100	100	
A. odorans (25)	100	100	
Bordetella bronchiseptica (26)	100	92	
Brucella abortus (35,37)	50(20)	96	
B. canis (35,37)	12(29)	72	glucose 81(14)% +
B. melitensis (35,37)	62	100	glucose 33% +; <10 strains
Campylobacter fetus ss. fetus (18)	83(12)	98	
C. fetus ss. *jejuni* (17)	44(12)	97	
Flavobacterium odoratum (14)	91(5)	99	
M-5 (22)	42(20)	100	
M-6 (22)	22(28)	100	
Moraxella atlantae (21)	87(13)	100	
M. osloensis (20)	70	100	
M. phenylpyruvica (21)	80(6)	100	
M. urethralis (21)	96	100	
Neisseria flavescens (12,39)	67	100	<10 strains
N. mucosa (12,40)	57(3)	100	
N. sicca (12,40)	68	100	
N. subflava (11,40)	47(2)	100	
Pseudomonas acidovorans (29)	100	100	
P. alcaligenes (30)	96	96	
P. diminuta (29)	97(3)	100	glucose 21(9)% +
P. mesophilica (18)	15	96	glucose 40% +
P. pseudoalcaligenes (30)	100	100	
P. putrefaciens, biotype 1 (34)	100	100	glucose 17(33)% +
P. putrefaciens, biotype 2 (34)	100	100	
P. testosteroni (29)	96(4)	100	
P. vesicularis (29)	81(8)	94	glucose 87(9)% +
Pseudomonas sp. CDC Group 1 (30)	97(3)	100	
IVc-2 (26)	94(6)	100	
IVe (25)	62(27)	100	
Vd (*Achromobacter* sp.) (24)	100	100	glucose 86(13)% +

GLUCOSE NONOXIDIZERS

Organism	MacConkey % +	Oxidase % +	Comments
MacConkey-negative/oxidase-negative			
Brucella canis (35,37)	12(29)	72	glucose 81(14)% +
Francisella tularensis (36)	0	0	
MacConkey-negative/oxidase-positive			
Bordetella pertussis (36)	0	94	
Branhamella catarrhalis (19,39)	5	100	
Brucella abortus (35,37)	50(20)	96	glucose 50(25)% +
B. canis (35,37)	12(29)	72	glucose 81(14)% +
B. melitensis (35,37)	62	100	glucose 33% +; <10 strains
Campylobacter fetus ss. *fetus* (18)	83(12)	98	
C. fetus ss. *jejuni* (17)	44(12)	97	
C. fetus ss. *veneralis* (17)	0	100	2 strains
Eikenella corrodens (17)	0	100	
M-5 (22)	42(20)	100	
M-6 (22)	22(28)	100	
Moraxella atlantae (21)	87(13)	100	
M. bovis (20)	0	100	<10 strains
M. lacunata (19)	4	100	
M. nonliquefaciens (20)	8(2)	100	
M. osloensis (20)	70	100	
M. phenylpyruvica (21)	80(6)	100	
Neisseria elongata (19,39)	0	100	<10 strains
N. flavescens (12,39)	67	100	<10 strains
N. mucosa (12,40)	57(3)	100	
N. sicca (12,40)	68	100	
N. subflava (11,40)	47(2)	100	
Pseudomonas mesophilica (18)	15	96	glucose 40% +
P. vesicularis (29)	81(8)	94	glucose 87(9)% +
IIf (15)	0(10)	100	
IIj (16)	2	100	
IVe (25)	62(27)	100	

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** Validly published name as of January 1, 1980 (see Approved Lists of Bacterial Names, Int J Syst Bacteriol 30:225-240, 1980)

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Ve-1	
Ve-2	

CHART 1	*Vibrio cholerae* (O 1 Group)		*Vibrio cholerae* (Other groups than O 1)[b]		*Vibrio parahaemolyticus*		*Vibrio alginolyticus*	
Number of strains	9		73		160		58	
Test Performed	Sign	% +	Sign	% +	Sign	% +	Sign	% +
Morphology	rrc		rrc		rrc		rrc	
Motility, flagella	[m],p,1-2		[m],p,1-2		[m],p,1-2		[m],p,1-2	
Gas from glucose		0	–	0		0	–	0
Action on blood	v	80B>	.	96B	v	32 LG	v	46 LG
Fermentative or oxidative	[F]		[F]		[F]		[F]	
Carbohydrate base	F		F		F		F	
Acid from: Glucose	+	100	+	100	+	100	+	100
Xylose		0		0	–	0		0
Mannitol	[+]	100	[+]	96(3)	[+]	98(1)	+	100
Lactose	v	11(33)	v	11(27)	[–]	0	[–]	0
Sucrose	+	100	v	89	[–]	2	[+]	100
Maltose	+	100	+	97(3)	+	96	+	100
10% Lactose	v	12	v	9(24)		0		0
Catalase	+	90	+	98	+	93	+	97
Oxidase	[+]	100	[+]	100	[+]	100	[+]	100
Growth on: MacConkey	[+]	100	[+]	100	[+]	100	[+]	100
SS	+	100	v	84	+	94	v	77(4)
Simmons citrate	+	100	+	96(1)	v	76(1)	v	66
Urea, Christensen's		0		4(3)		8		4
Nitrate reduction	+	90	+	100	+	100	+	100
Indole	+	100	+	100	v	100	v	90
TSI slant, acid	+	90	v	36	–	3	+	100
TSI butt, acid	+	100	+	99	+	97	+	100
H_2S (TSI butt)		0	–	0		0		0
H_2S (Pb ac paper)	+	100	v	64	v	35(1)	v	66
MR		10	v	30	v	57	v	19
V-P	+	90	v	84	[–v]	1	[+v]	93
Gelatin hydrolysis[a]	+	100	+	100	+	98	+	94
Litmus milk	pep	90	v	75 pep	v	86 pep	v	72 pep
Growth in nutrient broth								
0% NaCl	[+]	100	[+]	100	[–]	0	[–]	0
6% NaCl	+	90	v	84	[+]	100	[+]	100
8% NaCl					+	98	+	100
10% NaCl					[–]	3	[+]	100
Growth at 25 C	+	100	+	100	v[d]	98	v[d]	94
35 C	+	100	+	100	v[d]	91	v[d]	87
42 C	+	90	v	88	v[d]	43	v[d]	27
Esculin hydrolysis	v	20	–	0	v	10(3)	v	84
Lysine decarboxylase	[+]	100	[+]	100	[+v]	100	[+v]	100
Arginine dihydrolase	[–]	0	[–]	0	[–v]	0	[–v]	0
Ornithine decarboxylase	[+]	90	[+]	100	[+v]	98(1)	v	57
ONPG						0		0

[a] 7-14 days' incubation.
[b] Commonly called "non-cholera" vibrios; recently the sucrose negative strains have been classified and named *Vibrio mimicus*.
[c] Add 0.15 ml of 20% NaCl per ml of medium.
[d] Higher % may grow if HIA used.
[e] Less than 10 strains tested.
[f] Suggested "working type" strain, ATCC 19264.
[g] Grows in 0.5-5.0% NaCl.

[ ]: Indicate Key Tests

CHART 2	*Vibrio vulnificus*		*Vibrio anguillarum*[1]	*Vibrio damsela*		*Vibrio fluvialis*		*Vibrio hollisae*	
Number of strains	47		1	23		25		12	
Test Performed	Sign	% +	Sign	Sign	% +	Sign	% +	Sign	% +
Morphology	mrc		mrc	mrs,mrc		mrs,mrc		mrc	
Motility; flagella	[m];p,1-2		[m];p,1-2	[m];p,1-2		[m];p,1-2		[m];p,1-2	
Gas from glucose	[–]	0		v	9(4)	v	48		0
Action on blood	v	77β	β	v	87β	v	44β	v	50β
Fermentative or oxidative	[F]		[F]	[F]		[F]		[F]	
Carbohydrate base	F		F	F		F		F	
Acid from Glucose	+	100	+	+	100	+	100	+	100
Xylose		0	–		0		0		0
Mannitol	v	62	+	[–]	0	[+]	100	–	0
Lactose	[+ or (+)]	87(11)		[–]	0		0(8)		0
Sucrose	[ ]	2	+	–	0(9)	[+]	100	–	0
Maltose	+	100	+	[+]	100	+	100	[–]	0
10% Lactose	v	16(35)			0	v	0(14)		0
Catalase	+	100	+	v	71	v	64	+	100
Oxidase	[+]	100	[+]	[+]	91	[+]	100	[+]	100
Growth on MacConkey	[+]	100	[+]	[+]	100	[+]	92(8)	v	64(18)
SS	v	42(6)	–	v	39(4)	v	64		0
Simmons citrate	v	74(11)	–		0	v	84		9
Urea, Christensen's		0	–	[+]	100	–	0	[–]	0
Nitrate reduction	+	100	+	+	100	+	92	+	100
Indole	+[c]	100	+[c]	[–[c]]	0	v[c]	24	[+]	100
TSI slant; acid	v	21	+	v	26(4)	+	100	v	18
TSI butt; acid	+	100	+	+	91	+	100	+	100
H_2S (TSI butt)	–	0			0	–	0	–	0
H_2S (Pb ac paper)	+	91	+	v	83	v	84	+	91
MR	v[c]	25(2)	+[c]	v[c]	50	v[c]	44	–	0
V-P	[–[c]]	0	+[c]	v[c]	85	v[c]	16	–	0
Gelatin hydrolysis[d]	+	97			0[e]	+	100[e]	(v)	0(20)
Litmus milk	v	32(11) A	–	v	22 A	pep	92	v	25 Alk
Growth in nutrient broth:									
0% NaCl	[–]	0	[–]	[–]	0	[–]	0	[–]	0
6% NaCl	[+]	95	[...[g]]	[+]	100	[+]	100	[+]	100
8% NaCl	[ ]	5			10	v	65	–	0
10% NaCl		0					0		
Growth at: 25 C	+	100	+	+[d]	95	+	100	+	100
35°C	+	100	+	+[d]	95	+	100	+	100
42°C	v[d]	36	–	v[d]	23	v[d]	40	+	100
Esculin hydrolysis	v	9(2)		v	14	v	16		0
Lysine decarboxylase	[+[c]]	100	[–[c]]	[v[c]]	27(4)	[–[c]]	0	[–]	0
Arginine dihydrolase	[–[c]]	0	[+[c]]	[+[c]]	100	[+[c]]	96	[–]	8
Ornithine decarboxylase	+ or (+)[c]	89(8)	v	[–[c]]	0	v	0	[–]	0
ONPG	+	100							

See footnotes on Chart 1

CHART 3	*Aeromonas hydrophila*		*Plesiomonas shigelloides*		*Chromobacterium violaceum*[b]		*Tatumella ptyseos*	
Number of strains	115		28		37		41	
Test Performed	Sign	% +	Sign	% +	Sign	% +	Sign	% +
Morphology	mrs		mrs		mrs		cocci	
Motility, flagella	[m],p,1-2		[m],p,1-4		[m],p,1		[m],p,1[f]	
Gas from glucose	[v]	72		0	−[c]	0		0
Action on blood	v	76β	v	41 [γ]	v	48β	v	18 αl
Fermentative or oxidative	[F]		[F]		[F]		[F]	
Carbohydrate base	F		F		F		F	
Acid from Glucose	+	97(3)	+	96(4)	+	100	[+]	100
Xylose	−	1		0	−	0	v	24(51)
Mannitol	[+]	100	[−]	0	[−]	0		0
Lactose	v	20(9)	+	92(4)	[−]	0		0
Sucrose	v	87(3)		0	v	20(6)	[+]	100
Maltose	+	99	+	96(4)	[−]	0(3)	[−]	0
10% Lactose	v	25	v	38(8)		5	v[c]	0(44)[g]
Catalase	+	97	+	93	+	97	+	100
Oxidase	[+]	99	[+]	96	v	67	[−]	7
Growth on MacConkey	[+]	100	[+]	100	[+]	100	[+]	93
SS	+	97	+	96	[+ or (+)]	71(23)	[−]	2
Simmons citrate	v	52(7)		0	v	68(9)	v	10(7)
Urea, Christensen's		2	−	0	v	5(14)	[−]	0
Nitrate reduction	+	97	+	100	+	97	+	90
Indole	+	92	+	96	v[d]	21	[−]	0
TSI slant, acid	v	53	v	11		8	v	39
TSI butt, acid	+	100	+	100	+	94	+ or (+)	88(2)
H_2S (TSI butt)		0		0		0		0
H_2S (Pb ac. paper)	+	92	v	86	v	41	+	93
MR	v	55	v	82	v	37	v	28
V-P	v	78		4	[−]	0	[v]	81
Gelatin hydrolysis[a]	+	96	−	0	v	86	[−]	0
Litmus milk	v	85 pep	v	63 A	pep	92	v	11(6) A
Growth in nutrient broth								
0% NaCl	[+]	100	[+]	100	[+]	100	+	98
6% NaCl	v	14	v	50(8)		8	v	32
8% NaCl								
10% NaCl								
Growth at 25 C	+	100	+	100	+	100	+	95
35 C	+	100	+	100	+	100	+	98
42 C	v	84	v	89	v	85		7
Esculin hydrolysis	v	74	−	0		58	v	88
Lysine decarboxylase	[v]	17	[+]	100	[−]	0	[−]	0
Arginine dihydrolase	[+]	99	[+]	100	[+]	100	v	12
Ornithine decarboxylase	[−]	0	[+]	100	[−]	0	[−]	0

[a] 7-14 days' incubation
[b] 91% produced violet pigment
[c] Gas-producing strains have been described
[d] Non-pigmented colonies may be indole +
[e] The 2 esculin-positive strains were not pigmented
[f] Motile at 25°C, non-motile at 35°C
[g] Less than 10 strains tested
[h] Some of the strains identified as *E. enterocolitica* now would be identified as *E. kristensenii* (sucrose −, D-trehalose +, L-rhamnose −, D-melibiose +), or *E. intermedia* (sucrose +, D-trehalose +, L-rhamnose +, D-melibiose +)
[i] Incubated at 25°C.

CHART 4	*Yersinia enterocolitica*[h]		*Yersinia pseudotuberculosis*		*Yersinia pestis*	
Number of strains	83		34		37	
Test Performed	Sign	% +	Sign	% +	Sign	% +
Morphology	mrs		srs		srs	
Motility; flagella	[m];pe[f]		[m];pe[f]		[um]	
Gas from glucose	v	36		0	–	0
Action on blood	v	24 ly	v	47 ly	v	19 ly
Fermentative or oxidative	[F]		[F]		[F]	
Carbohydrate base	F		F		F	
Acid from: Glucose	[+]	100	[+]	100	[+]	100
Xylose	+	97(2)	+	100	+	100
Mannitol	+	100	+	100	+	100
Lactose	v	18(18)	[–]	0	[–]	0
Sucrose	[+]	98	[–]	0	[–]	0
Maltose	+	92(7)	+	97(3)	v	89
10% Lactose	v	30(22)	–	0	–	0
Catalase	+	100	+	100	+	100
Oxidase	[–]	0	[–]	3	[–]	0
Growth on MacConkey	[+]	100	[+]	100	[+]	100
SS	+	90(6)	v	17(41)	–	3(3)
Simmons citrate	–	0	–	0	–	0
Urea, Christensen's	[+ or(+)]	70(30)	[+]	100	[–]	0
Nitrate reduction	+	100	[+]	100	[+]	100
Indole	v	79(1)	[–]	0	[–]	0
TSI slant, acid	+	100	–	7	v	50
TSI butt, acid	+	100	+	93	+	97
H_2S (TSI butt)	–	0	–	0		0
H_2S (Pb ac paper)	+	99	v	81	+	100
MR	v	63(10)	+	100	+ or(+)	86(11)
V-P	[+[i]]	98	[–]	0	–	0
Gelatin hydrolysis[d]	–	0	–	0	–	0
Litmus milk	A	96	v	25 k	v	11 A
Growth in nutrient broth:						
0% NaCl	+	100	+	100[e]	+	100
6% NaCl	v	35	–	0[e]	–	4
8% NaCl						
10% NaCl						
Growth at: 25°C	+	100	+	100	+	100
35°C	+	100	+	100	+	96
42°C	v	81	v	15(31)	–	0
Esculin hydrolysis	v	28	+	100	+	100
Lysine decarboxylase	[–]	0	[–]	0	[–]	0
Arginine dihydrolase	[–]	0	[–]	0	[–]	0
Ornithine decarboxylase	[+]	100	[–]	0	[–]	0

See footnotes on Chart 3.

CHART 5	*Pasteurella multocida*						*Pasteurella* sp "n sp. 1" (*Pasteurella* "gas")		*Pasteurella ureae*	
Number of strains	306						91		97	
Test Performed	Sign					% +	Sign	% +	Sign	% +
Morphology	srs						srs		plr	
Motility	[nm]						[nm]		[nm]	
Gas from glucose	[ ]					0	v	16[b]		0
Action on blood	v					60 al	v	48 al	v	16 al
Fermentative or oxidative	[F]						[F]		[F]	
Carbohydrate base	F						F		F	
Number of strains	168	66	32	24	7					
Acid from Glucose	+	+	+	+	+	100	+	100	+	100
Xylose	+			+	–	67	[–]	0	[–]	0
Mannitol	+		+	+	+	78	[–]	0	[+]	99(1)
Lactose				+	–	8		3		0
Sucrose	+	+	+	+	+	100	+	99	+	99(1)
Maltose	[–]	[–]	[–]	[–]	[+]	2	[+]	100	+	91(5)
Trehalose										
Melibiose										
Raffinose										
Mannose										
10% Lactose						8	v	18		0
Catalase			+			98	+	96	v	63
Oxidase			[+]			97	[+]	98	[+]	99
Growth on MacConkey	[–]					2(1)	[–]	1	[–]	0
SS	–					0	–	0	–	0
Simmons citrate	–					0	–	0		0
Urea, Christensen's	[–]					0	[vs]	78(3)	[+s]	100
Nitrate reduction	[+]					99	[+]	100	[+]	99
Gas from nitrate	-					0	–	0		0
Indole	[+]					99	[+]	100	[–]	0
TSI slant, acid	+					98(1)	+	100	+	100
TSI butt, acid	+					99	+	100	+	99
H_2S (TSI butt)	-					0		0		0
H_2S (Pb ac paper)	v					76	+	98	+	91(1)
MR	-					1	v	15	–	0
V-P	–					0	v	28	v	21
Gelatin hydrolysis[a]	–					0	v	13	–	0
Litmus milk	–					3A	v	4A	v	9(1)k
Growth at 25 C	+					91	v	89	v	88
35 C	+					99	+	99	v	82
42°C	v					32	v	27	-	5
Esculin hydrolysis	–					0	-	0	–	0
Lysine decarboxylase	–					0	-	2	–	0
Arginine dihydrolase	–					0	-	0	-	0
Ornithine decarboxylase	[+]					94	[–]	0	–	0
Nutrient broth, 0% NaCl	v					68	v	56(1)	–	0
Nutrient broth, 6% NaCl	–					2	–	1	–	0

[a] 7-14 days' incubation.
[b] 37% (15/43) of strains negative for gas in glucose broth were positive for gas in thioglycollate broth when tested with a hot needle
[c] May require a drop of serum on slant or a heavy inoculum.
[d] Less than 10 strains tested.
[e] Yellow to amber pigment, soluble or insoluble, produced by some strains.
[f] 13 strains (13%) reduced NO_3 and NO_2 without gas formation.

CHART 6	*Pasteurella pneumotropica*		*Pasteurella aerogenes*		EF-4a[c]	
Number of strains	107		16		97	
Test Performed	Sign	% +	Sign	% +	Sign	% +
Morphology	srs		cc,srs		cc,srs	
Motility	[nm]		[nm]		[nm]	
Gas from glucose	[−]	0	[+]	100	[−]	0
Action on blood	v	32 al	v	56 al	v	34 ly
Fermentative or oxidative	[F]		[F]		[F]	
Carbohydrate base	F		F		F	
Number of strains						
Acid from: Glucose	+	97(3)	+	100	+	100
Xylose	[+ or (+)]	76(19)	v	81		0
Mannitol	[−]	2(1)	−	6	−	0
Lactose	v	14(39)	v	19(38)	[−]	0
Sucrose	+	97(3)	+	94		0
Maltose	+	97(3)	+	100	[−]	0
Trehalose						
Melibiose						
Raffinose						
Mannose						
10% Lactose	v	25(3)	v	38	−	0
Catalase	+	100	+	100	[+]	100
Oxidase	[+]	99	[+]	100	[+]	100
Growth on: MacConkey	[v]	36(17)	[+]	100	[v]	42(8)
SS		0	−	6		1
Simmons citrate	−	0	−	0	−	3(1)
Urea, Christensen's	[+[c]]	95(1)	[+]	100	[−]	0
Nitrate reduction	[+]	100	[+]	100	[+[c]]	97
Gas from nitrate		0	−	0	[v]	62
Indole	[+]	90	[−]	0	[−]	0
TSI slant, acid	+	100	+	94		3
TSI butt, acid	+	97	+	100	v	73
H_2S (TSI butt)	−	0	−	0		0
H_2S (Pb ac paper)	+	100	+	100	+	95(2)
MR	−	3	−	0	v	26
V-P	v	13	v	29	−	2
Gelatin hydrolysis[d]	−	0	−	0[d]	v	60
Litmus milk	−	9 A	v	56 A	−	2(1) k
Growth at: 25°C	v	74	+	94	v	89
35°C	+	98	+	100	+	100
42°C	−	8	+	94	v	70
Esculin hydrolysis	−	0	−	0		0
Lysine decarboxylase	v	33		0	−	0
Arginine dihydrolase	−	0	−	0	[v]	77(2)
Ornithine decarboxylase	[+]	100	v	88		0
Nutrient broth, 0% NaCl	v	84	+	100	+	94(2)
Nutrient broth, 6% NaCl	−	2		0		6(2)

See footnotes on Chart 5

CHART 7	*Pasteurella haemolytica*			*Pasteurella gallinarum*		*Actinobacillus lignieresii*	
Number of strains	67			10		30	
Test Performed	Sign		%	Sign	%	Sign	%
Morphology	srs			srs		srs	
Motility	[nm]			[nm]		[nm]	
Gas from glucose			0		0		0
Action on blood	v		72B	v	30 al	v	17 al
Fermentative or oxidative	[F]			[F]		[F]	
Carbohydrate base	F			F		F	
Number of strains	46[b]	21[c]					
Acid from Glucose	+	+	98(2) 90(10)	+	90(10)	+	96(4)
Xylose	[+]	[−]	96(4) 0	v	33[d]	+ or (+)	87(13)
Mannitol	v	+ or (+)	9(2) 76(24)		0	+	91(9)
Lactose	v	−	11(50) 0	−	0	v	17(61)
Sucrose	+	+	98(2) 95(5)	+	100	+	96(4)
Maltose	+	+ or (+)	91(6) 72(28)	+	100	+ or (+)	83(17)
Trehalose		v	0 80			[−]	0
Melibiose						[−]	0
Raffinose						v	25(46)
Mannose	[−]	[+]	3 95(5)				
10% Lactose			7	v	17[d]	v	47
Catalase	[v]		84(2)	[+]	100	[v]	89
Oxidase	[+]		95	[+]	90	[+]	100
Growth on MacConkey	[v]		79(6)	v	20(10)	v	67(8)
SS			0		0		0
Simmons citrate	−		0	−	0		0
Urea, Christensen's	[−]		0	[−]	0	[+[e]]	100
Nitrate reduction	[+]		100	[+]	100	[+]	100
Gas from nitrate			0	−	0		0
Indole	[−]		0	[−]	0		0
TSI slant, acid	+		100	+	100	+	100
TSI butt, acid	+		100	+	100	+	100
H-S (TSI butt)	−		0	−	0		0
H-S (Pb ac paper)	+		100	+	90	+	100
MR	−		2		0[d]	+	100
V-P	v		71	v	50[d]		0
Gelatin hydrolysis[a]	v		12		0		0
Litmus milk	v		11(2) A		10 k	v	29 A
Growth at 25 C	v		73	v	88[d]	v	82
35 C	+		100	+	100[d]	+	100
42 C	v		50	v	67[d]	v	14
Esculin hydrolysis	−	v	0 83		0[d]	[−]	0
Lysine decarboxylase	[−]		3		0[d]		0[d]
Arginine dihydrolase	[−]		0		0[d]		0[d]
Ornithine decarboxylase	−		10	v	25[d]		0[d]
Nutrient broth, 0% NaCl	v		65	v	25[d]	+	100[d]
Nutrient broth, 6% NaCl	−		3		0[d]		10[d]

[a] 7-14 days' incubation
[b] Biotype A
[c] Biotype T
[d] Less than 10 strains tested.
[e] May require a drop of serum on slant or a heavy inoculum

CHART 8	*Actinobacillus equuli*		*Actinobacillus suis*	
Number of strains	19		33	
Test Performed	Sign	% +	Sign	% +
Morphology	srs		srs	
Motility	[nm]		[nm]	
Gas from glucose	–	0	–	0
Action on blood	v	11 g:	[v]	76β
Fermentative or oxidative	[F]		[F]	
Carbohydrate base	F		F	
Number of strains				
Acid from: Glucose	+	100	+	94(6)
Xylose	+	100	+	94(6)
Mannitol	+	100	v	54
Lactose	+	95	+ or (+)	79(18)
Sucrose	+	100	+	94(6)
Maltose	+	95	+	94(6)
Trehalose	[+]	100	+	95
Melibiose	[+ or (+)]	74(26)	+ or (+)	82(13)
Raffinose	+	100	+	100
Mannose			+	91(9)
10% Lactose	v	35	+	94
Catalase	[v]	68(5)	[v]	85
Oxidase	[+]	100	[+]	100
Growth on: MacConkey	v	84(5)	[+ or (+)]	82(12)
SS	–	0	–	3
Simmons citrate	–	0	–	0
Urea, Christensen's	[+[c]]	100	[+[c]]	97(3)
Nitrate reduction	[+]	100	[+]	100
Gas from nitrate	–	0	–	0
Indole	–	0	[–]	0
TSI slant, acid	+	100	+	100
TSI butt, acid	+	100	+	100
H_2S (TSI butt)	–	0		0
H_2S (Pb ac paper)	+	100	+	100
MR	v	87	v	24
V-P	v	12	v	50
Gelatin hydrolysis[a]	v	47	–	6
Litmus milk	v	83 A	v	82 A
Growth at: 25°C	v	89	+	94
35°C	+	100	+	97
42°C	v	31	v	31
Esculin hydrolysis	[–]	0	[+]	100
Lysine decarboxylase	–	0[d]	–	0[d]
Arginine dihydrolase	–	0[d]	–	0[d]
Ornithine decarboxylase	–	0[d]	–	0[d]
Nutrient broth, 0% NaCl	v	89	v	88
Nutrient broth, 6% NaCl	–	5	–	0

See footnotes on chart 7.

CHART 9	*Capnocytophaga* spp[b] (DF-1)		*Haemophilus aphrophilus*		*Actinobacillus actinomycetemcomitans*								HB-5	
Number of strains	155		203		120								44	
Test Performed	Sign	% +	Sign	% +	Sign							% +	Sign	% +
Morphology	[rt]		[rv to plr]		rv								(srs	
Motility, flagella	v[c]		nm		[nm]								[nm]	
Gas from glucose	[–]	0	[+[e]]	98	v							28	[+[e]]	100
Action on blood	v	34 al or gr	v	43 al	v							44 al	v	32 al
Fermentative or oxidative	[F[d]]		[F]		[F]								[F]	
Carbohydrate base	F		F		FF								F	
Number of strains					63	30	15	6	4	1	1			
Acid from Glucose	[+]	90(10)	[+]	98(2)	[+]	[+]	[+]	[+]	[+]	[+]	[–]	83(16)	[+]	100
Xylose		0		0		+	+	–	+	–	+	33(9)	–	0
Mannitol		0		0	+	+		–	+	+		66(16)	[–]	0
Lactose	v	25(11)	[+]	98(3)	[–]	[–]	[–]	[–]	[–]	[–]	[–]	0		0
Sucrose	[+]	90(9)	+	99(1)	[–]	[–]	[–]	[–]	[–]	[–]	[–]	0	[–]	0
Maltose	[+ or (+)]	86(14)	[+]	99(1)	+	+	+	+	–			80(15)		0
Fructose													+	100
10% Lactose			ngr				ngr							0
Catalase	[–]	7	[–]	5			[+]					99	[–]	2
Oxidase	[–]	7	v	28			v					19	v	54
Growth on MacConkey	[–]	0	v	19			[–]					4(1)	v	34(23)
SS		0		0			–					0		0
Simmons citrate		0		0			–					0		0
Urea, Christensen's		0		0			[–]					0	[–]	0
Nitrate reduction	v	63	[+]	100			[+]					100	[+]	100
Gas from nitrate		0		0			–					0		0
Indole		0	[–]	0			[–]					0	[+]	100
TSI slant, acid	v	73	+	100			+					100	+	100
TSI butt, acid	v	55	+	100			[+]					100	[+]	98
H_2S (TSI butt)		0		0			–					0		0
H_2S (Pb ac paper)	v	50	+	100			+					91	+	91
MR		8	v	12								8		0
V-P				0			–					0	–	7
Gelatin hydrolysis[a]		0	–	0								0		0
Litmus milk	v	49(7) A	v	30(7) A								4 IR		0
Growth at 25 C	v	21	v	55			v					24	v	74
35 C	+	100	+	99			+					100	+	100
42 C	v	16	v	26								4	v	12
Esculin hydrolysis	[v]	81(2)	[–]	0			–					0	–	0[f]
Lysine decarboxylase	–	0	–	0[f]										0[f]
Arginine dihydrolase	–	8		0[f]										0[f]
Ornithine decarboxylase		0	–	0[f]									–	0
Nutrient broth, 0% NaCl	v	21(1)	v	56			–					?	v	83
Nutrient broth, 6% NaCl	–	1	–	1								0	–	0

[a] 7-14 days' incubation

[b] Yellow non-soluble pigment produced by 61 of 155 strains

[c] Gliding motility has been reported, we have observed delayed spreading in motility medium and an occasional single polar or lateral "flagellum"

[d] TSI reaction not always indicative of fermentative activity

[e] Volume of gas is frequently small.

[f] Less than 10 strains tested

[g] May need to add 1-2 drops of rabbit serum per 3 ml of medium; reactions may be obtained within 4 h using the Rapid Sugar Test

[h] 64% of strains tested did not grow on TSI

[i] After 2-7 days' incubation.

CHART 10	*Gardnerella vaginalis*		*Cardiobacterium hominis*		DF-2		DF-3	
Number of strains	126		65		27		21	
Test Performed	Sign	% +	Sign	% +	Sign	% +	Sign	% +
Morphology	srs (gram variable)		mrs		[m-ltrt]		cc,srs	
Motility; flagella	nm		[nm]		[nm]		nm	
Gas from glucose	–	0	–	0	–	0		0
Action on blood	[incß[1]]	93	v	12 gr	v	33 al	–	10 al
Fermentative or oxidative	F[d]		[F[d]]		[F[d]]		[F]	
Carbohydrate base	F[g]		F[g]		F[g]		F[g]	
Number of strains								
Acid from Glucose	[+ or(+)]	81(13)	+ or(+)	80(20)	[v]	67(18)	[+ or(+)]	86(14)
Xylose	v	36(9)	[–]	0	–	0	+ or(+)	86(14)
Mannitol	[–]	0	[+ or(+)]	52(43)	[–]	0	–	0
Lactose		0(1)	[–]	0	[+ or(+)]	81(19)	[+ or(+)]	52(43)
Sucrose	v	11(4)	+ or(+)	63(35)	–	0	+ or(+)	62(33)
Maltose	[+ or(+)]	87(13)	+ or(+)	69(31)	[+ or(+)]	81(19)	[+ or(+)]	81(19)
Fructose	v	60(23)						
10% Lactose	ngr		–	0			+	100
Catalase	[–]	2	[–]	1	[+]	100	[–]	0
Oxidase	[–]	6	[+]	100	[+]	96	[–]	0
Growth on: MacConkey	[–]	5	[–]	0	[–]	0	[–]	0
SS		0	–	0	–	0	–	0
Simmons citrate	–	0	–	0	–	0	–	0
Urea, Christensen's	–	0(5)	[–]	0	–	0	–	0(4)
Nitrate reduction	–	0	[–]	0	[–]	0	[–]	0
Gas from nitrate	–	0			–	0		
Indole	–	0	[+]	100	[–]	0	[v]	71(14)
TSI slant, acid	v	14[h]	+	92(1)	v	17	+	95
TSI butt, acid	v	14[h]	v	81(3)	v	15	[+]	100
H_2S (TSI butt)	–	0	–	0	–	0[f]	–	0
H_2S (Pb ac paper)	v	31	+	90	v	17	+	90
MR	v	17	v	50			v	62[f]
V-P			–	0			–	0[f]
Gelatin hydrolysis[d]	–	0	–	0	–	0[f]	–	0[f]
Litmus milk	v	32(16) A	v	12(5) Aw	–	4 IR	v	76 A
Growth at 25°C	–	5	v	35	v	30	v	50
35 C	+	100	+	100	+	100	v	85
42 C	v	14	v	20	v	18	v	20
Esculin hydrolysis	–	0	–	2	v	77	[+]	100
Lysine decarboxylase					–	0	–	0[f]
Arginine dihydrolase					v	61	–	0[f]
Ornithine decarboxylase					–	0	–	0[f]
Nutrient broth, 0% NaCl	v	24	v	62	–	0(4)	v	38
Nutrient broth, 6% NaCl	–	0	–	8	–	0	–	10

See footnotes on Chart 9.

CHART 11	*Neisseria gonorrhoeae*		*Neisseria meningitidis*		*Neisseria lactamica*		*Neisseria subflava*	
Number of strains	197		375		287		153	
Test Performed	Sign	% +	Sign	% +	Sign	% +	Sign	% +
Morphology	[cd]		[cd]		[cd]		[cd]	
Motility: flagella	[nm]		[nm]		[nm]		[nm]	
Action on blood		0	–	2 ly		0	v	56 ly
Fermentative or oxidative	O		O		F		F	
Carbohydrate base	CTA		CTA		CTA		CTA	
Acid from: Glucose	[+[b]]	90(3)	[+[b]]	99	[+]	96(3)	v	68(6)
Mannitol		0	–	0	-	0		0
Lactose		0	[–]	0	[+]	95(5)	[–]	0
Sucrose	-	0	[–]	0	–	0	[v]	56(5)
Maltose	[–]	0	[+[b]]	99	[+]	98(2)	[+]	99(1)
Fructose		0		0		0	[v]	77(5)
10% Lactose					+	92	–	0
ONPG		0	–	0	[+]	100		0
Catalase					+	100	v	80
Oxidase	[+]	100	[+]	100	[+]	100	[+]	100
Growth on: MacConkey		0	-	0	–	0	v	47(2)
SS						0		0
Simmons citrate						0		0
Urea, Christensen's					–	0		0
Nitrate reduction	[–]	0	–	0	[–]	0	[–]	0
Gas from nitrate								
Nitrite reduction	[–]	0	v	29[c]	+	100[c]	v	80[c]
TSI slant, acid					v	30	v	68
TSI butt, acid					v	19	v	56(4)
H_2S (TSI butt)					–	0		0
H_2S (Pb ac paper)					v	88	+	100
Gelatin hydrolysis[a]								0
Litmus milk							v	14 IR
Pigment on Loeffler	-	10 sl yel	–	7 sl yel	v	38 sl yel	[yel]	99
Phenylalanine		0	–	0	-	0[c]	v	32
Growth on nutrient agar								
25 C	[–]	0	[–]	0	v	22	[+]	95
35 C	-	0	–	1	v	74	+	95
Esculin hydrolysis								
Amylosucrase	–	0	–	0		0	v	73
Growth on Thayer-Martin	[+]	96	[+]	100	[+]	100	[–]	8

[a]7-14 days' incubation.
[b]Occasional negative reaction if caps on CTA tubes are not tightened before incubation.
[c]Less than 10 strains tested.

CHART 12	*Neisseria sicca*		*Neisseria mucosa*		*Neisseria flavescens*	
Number of strains	43		30		10	
Test Performed	Sign	% +	Sign	% +	Sign	% +
Morphology	[cd]		[cd]		[cd]	
Motility; flagella	[nm]		[nm]		[nm]	
Action on blood	v	22 ly	v	25 ly	v	33 ly[c]
Fermentative or oxidative	F		F		n-o	
Carbohydrate base	CTA		CTA		CTA	
Acid from: Glucose	[+ or (+)]	78(14)	[+ or (+)]	83(10)	[−]	0
Mannitol	−	0	−	0	−	0
Lactose	[−]	0	[−]	0	−	0
Sucrose	[+]	92(8)	[+]	90(10)	−	0
Maltose	[+]	95(5)	+	90(10)	[ ]	0
Fructose	[+]	100	[+]	90(10)	·	0
10% Lactose		5	−	0[c]	−	0
ONPG	−	0[c]				
Catalase	v	80	+	100[c]	+	100[c]
Oxidase	[+]	100	[+]	100	[+]	100
Growth on MacConkey	v	68	v	57(3)[c]	v	67[c]
SS	−	0	−	0[c]	−	0[c]
Simmons citrate	−	0	−	0[c]	−	0[c]
Urea, Christensen's	−	0	−	0[c]	−	0[c]
Nitrate reduction	[−]	0	[+]	100	[−]	0
Gas from nitrate			[+]	100		
Nitrite reduction	+	100[c]	[+]	100	+	100[c]
TSI slant, acid	+	100	+	100[c]	−	0[c]
TSI butt, acid	+ or (+)	82(9)	v	88[c]	−	0[c]
H_2S (TSI butt)	−	0	−	0[c]	−	0[c]
H_2S (Pb ac paper)	+	100	+	100[c]	+	100[c]
Gelatin hydrolysis[a]	−	0	−	0[c]	−	0[c]
Litmus milk	−	0	−	0[c]	v	50 k[c]
Pigment on Loeffler	−	0	v	50 sl yel	[yel]	90
Phenylalanine	v	50[c]	v	88[c]		
Growth on nutrient agar:						
25°C	[+]	100	[+]	96	[v]	86[c]
35°C	+	90(10)	+	100	+	100[c]
Esculin hydrolysis	−	0	−	0[c]		
Amylosucrase	+	100[c]	+	100[c]	[+]	100[c]
Growth on Thayer-Martin	[−]	0[c]	−	0	[−]	0[c]

See footnotes on Chart 11.

CHART 13	*Kingella kingae*		*Kingella denitrificans*		*Kingella indologenes*	*Flavobacterium meningosepticum*	
Number of strains	33		60		1	148	
Test Performed	Sign	% +	Sign	% +	Sign	Sign	% +
Morphology	cc,pr,chs		cc,pr,chs		vbr	lrs,fl	
Motility; flagella	[nm]		[nm]		[nm]	[nm[d]]	
Action on blood	[v]	84β	–	0	–	v	75 LG
Fermentative or oxidative	F[b]		[F[b]]		F	[O]	
Carbohydrate base	F[c]		F[c]		F	OF	
Acid from: Glucose	[+ or(+)]	42(48)	[+ or(+)]	30(62)	[+]	[+]	95(4)
Xylose	–	0	–	0	–	–	2(1)
Mannitol	–	0	–	0	[–]	[+]	91(8)
Lactose	–	0	–	0	–	v	42(15)
Sucrose	[–]	0	–	0	[+]	–	0
Maltose	[+ or(+)]	76(24)	[–]	0	[(+)]	+	93(7)
Starch						–	0
Trehalose						+	100
ONPG						+	100
Catalase	[–]	0	[–]	10	[–]	+	100
Oxidase	[+]	100	[+]	100	[+]	[+]	99
Growth on: MacConkey	v	33(3)	[–]	0	[–]	[+ or(+)]	89(3)
SS	–	0	–	0	–	–	1
Simmons citrate	–	0	–	0	–	v	9(3)
Urea, Christensen's	–	0	–	0	–	–	3(5)
Nitrate reduction	–	3(3)	[+]	93	–	[–]	0
Gas from nitrate	–	0	[v]	88			
Nitrite reduction							
Indole	[–]	0	–	0	[+]	[+]	100
TSI slant, acid	v	28(4)	–	2	+	–	0
TSI butt, acid	v	12	–	0	+	–	0(3)
H_2S (TSI butt)	–	0	–	0	–	–	3
H_2S (Pb ac paper)	v	59	v	73	+	+	98
Gelatin hydrolysis[a]	–	0	–	0	–	+	91
Litmus milk	v	82 pep	–	6 IR	–	[pep]	98
Pigment, insoluble			–	2 yel	–	e	
Pigment, soluble	v	18 yel-br	–	2 yel	–	v	0(37) ta-br
Growth at: 25°C	v	32	v	40	+	+	100
35°C	+	96	+	100	+	+	100
42°C	–	4	v	48	–	v	45
Esculin hydrolysis	–	0	–	0	–	[+]	99
Lysine decarboxylase					–		
Arginine dihydrolase					–		
Ornithine decarboxylase					–		
Nutrient broth, 0% NaCl	v	38	v	78(4)	–	+	100
Nutrient broth, 6% NaCl	–	0	–	0	+	–	7

[a]7-14 days' incubation.
[b]TSI reaction not always indicative of fermentative activity.
[c]May need to add 1-2 drops of rabbit serum per 3 ml of medium.
[d]Polar and lateral flagella have been demonstrated on some strains.
[e]Some strains exhibit slight yellow pigment.

CHART 14	*Flavobacterium* sp. (IIb)		*Flavobacterium breve*		*Flavobacterium odoratum*	
Number of strains	155		3		74	
Test Performed	Sign	% +	Sign	% +	Sign	% +
Morphology	mrs. II		s-lr, fc		lr	
Motility, flagella	[nm]		[nm]		[nm]	
Action on blood	v	26 LG	sl hy	100	v	77 LG
Fermentative or oxidative	[O]		[O]		[n-o]	
Carbohydrate base	OF		OF		OF	
Acid from: Glucose	[+]	92(6)	[+]	100	[−]	0
Xylose	v	30(1)	−	0	[−]	0
Mannitol	[−]	10	[−]	0	−	0
Lactose	−	0	−	0	−	0
Sucrose	v	13(1)	−	0	−	0
Maltose	+	92(6)	+	100	−	0
Starch	+	100	+	100		
Trehalose	[+]	100	[−]	0		
ONPG	v	57	[−]	0		
Catalase	+	99	+	100	+	100
Oxidase	[+]	96	[+]	100	[+]	99
Growth on: MacConkey	v	54(9)	[+]	100	[+]	91(5)
SS		0	−	0	v	30(11)
Simmons citrate		2(1)	−	0	−	0
Urea, Christensen's	v	14(28)	−	0	[+]	100
Nitrate reduction	v	22	−	0	[−]	0
Gas from nitrate		0				
Nitrite reduction	v	20			v	83
Indole	[+]	98	[+]	100	[−]	0
TSI slant, acid	−	1	−	0	−	0
TSI butt, acid	−	10	−	0		0
H_2S (TSI butt)	−	1	−	0	[−]	0
H_2S (Pb ac paper)	+	99	+ or(+)	33(67)	v	26
Gelatin hydrolysis[a]	v	78	+	100	[+]	96
Litmus milk	[pep]	93	v	67 k	pep	93
Pigment, insoluble	[yel]	99	sl yel	100	v	85 yel
Pigment, soluble	v	11 yel-br	(br)	0(100)	v	28 ta-br
Growth at 25 C	+	100	+	100	+	100
35 C	+	100	+	100	+	100
42 C	v	42	−	0	v	31
Esculin hydrolysis	v	70	[−]	0	−	0
Lysine decarboxylase			−	0	−	0
Arginine dihydrolase			−	0	−	0(9)
Ornithine decarboxylase			−	0	−	0
Nutrient broth, 0% NaCl	+	100	+	100	+	100
Nutrient broth, 6% NaCl	−	0	−	0	v	20(5)

See footnotes on Chart 13

CHART 15	Ⅱe		Ⅱi		Ⅱh	
Number of strains	18		87		21	
Test Performed	Sign	% +	Sign	% +	Sign	% +
Morphology	srs,Ⅱ		srs,Ⅱ		srs, Ⅱ	
Motility, flagella	[nm]		[nm]		[nm]	
Action on blood	v	13 LG	v	76 LG	v	32 LG
Fermentative or oxidative	[O]		[n-o]		[O]	
Carbohydrate base	OF		OF		OF	
Acid from: Glucose	[+]	100	[−]	0	[+ or(+)]	85(15)
Xylose		0	[−]	0	−	5
Mannitol	[−]	0	−	0	[−]	0
Lactose	[−]	0		0	[−]	0
Sucrose		0	−	0		0
Maltose	[+]	100	−	0	[+]	95
Starch						
Trehalose						
ONPG						
Catalase	v	88	+	98	+	100
Oxidase	v	88	[+]	100	[+]	100
Growth on: MacConkey	[−]	7	[−]	0(10)	[−]	0
SS		0	−	0		0
Simmons citrate		0		0		0
Urea, Christensen's	−	0	[−[b]]	0		0
Nitrate reduction	[−]	0	−	0	[−]	0
Gas from nitrate						
Nitrite reduction						
Indole	[+]	100	[+]	100	[+]	100
TSI slant, acid		0	−	0		0
TSI butt, acid		0		0		5
H_2S (TSI butt)		0	−	0		0
H_2S (Pb ac paper)	v	78	+	95	+	100
Gelatin hydrolysis[a]	−	0	[+]	100	[−]	7
Litmus milk	v	12 IR	v	39 pep		7 IR
Pigment, insoluble	[−]	0		0		0
Pigment, soluble	br-yel	82	br-ta	98	br	100
Growth at: 25 C	+	100	v	58	+	100
35 C	+	100	+	100	+	100
42 C		0	v	70		5
Esculin hydrolysis	[−]	0	−	0	[+]	100
Lysine decarboxylase			−	0[c]		
Arginine dihydrolase				0[c]		
Ornithine decarboxylase			−	0[c]		
Nutrient broth, 0% NaCl	v	88	+	99	v	86
Nutrient broth, 6% NaCl	−	0	−	7		5

[a]7-14 days' incubation.
[b]In 3-7 days, 27 of 86 strains produced pink reactions which might be interpreted as weakly positive
[c]Less than 10 strains tested.

CHART 16	IIIi		IIIj	
Number of strains	23		41	
Test Performed	Sign	% +	Sign	% +
Morphology	srs, II		srs, II	
Motility, flagella	[nm]		[nm]	
Action on blood	v	39 LG	v	36 LG
Fermentative or oxidative	[O]		[n-o]	
Carbohydrate base	OF		OF	
Acid from: Glucose	[+]	91(93)	[−]	0
Xylose	+ or(+)	87(13)	[−]	0
Mannitol	[−]	0	−	0
Lactose	+	91(93)	−	0
Sucrose	[+]	91(93)		0
Maltose	+	91(93)	−	0
Starch				
Trehalose				
ONPG				
Catalase	[+]	100	+	100
Oxidase	[+]	100	[+]	100
Growth on: MacConkey	[−]	0	[−]	2
SS	−	0	−	0
Simmons citrate	−	0	−	0
Urea, Christensen's	v	14(18)	[+]	100
Nitrate reduction	−	0	[−]	0
Gas from nitrate				
Nitrite reduction				
Indole	[+]	100	[+]	98
TSI slant, acid	−	0	−	0
TSI butt, acid	[−]	0		0
H_2S (TSI butt)	−	0	−	0
H_2S (Pb ac paper)	v	70	v	59
Gelatin hydrolysis[a]	[−]	0	+	98
Litmus milk	−	4 A	v	18 pep
Pigment, insoluble	v	22 yel	−	0
Pigment, soluble	v	48 yel-ta	ta-yel	100
Growth at: 25 C	+	100	v	30
35 C	+	100	+	95
42 C	v	36	−	10
Esculin hydrolysis	+	96	−	0
Lysine decarboxylase	−	0[c]	−	0[c]
Arginine dihydrolase	−	0[c]	+	100[c]
Ornithine decarboxylase	−	0[c]	−	0[c]
Nutrient broth, 0% NaCl	+	100	v	15
Nutrient broth, 6% NaCl	−	9	−	0

See footnotes on Chart 15.

CHART 17	*Eikenella corrodens*[b]		*Campylobacter fetus* subsp. *venerealis*[c]		*Campylobacter fetus* subsp. *jejuni*[c,d]	
Number of strains	506		2		41	
Test Performed	Sign	% +	Sign	% +	Sign	% +
Morphology	nrs		[sc]		[sc]	
Motility, flagella	[nm]		[m];p,1-2		[m];p,1-2	
Action on blood	v	17 [y		0		6 al
Fermentative or oxidative	[n-o]		[n-o]		[n-o]	
Carbohydrate base	OF		OF		OF	
Acid from: Glucose	[−]	0	[−]	0	[−]	0
Xylose	[−]	0	−	0		0
Mannitol		0		0		0
Lactose		0	−	0	-	0
Sucrose		0	−	0		0
Maltose		0	-	0		0
Catalase	[−]	8	[+]	100	[+]	100
Oxidase	[+]	100	[+]	100	[+]	97
Growth on: MacConkey	[−]	0	[−]	0	v	44(12)
SS		0	-	0		0
Simmons citrate	-	0	-	0		0
Urea, Christensen's	[−]	0	−	0		0
Nitrate reduction	[+]	99	[+]	100	[+]	100
Gas from nitrate	[−]	0	-	0	-	0
Indole	−	0	−	0		0
TSI slant, acid	−	0	−	0		0
TSI butt, acid	-	0	−	0		0
H_2S (TSI butt)	−	0		0	-	0
H_2S (Pb ac paper)	v	73	−	0	v	83
Gelatin hydrolysis[a]	−	0				4
Litmus milk	−	1 k		0	−	8 k
Pigment	v	0(70) sl yel				
Growth at: 25 C	v	15	[+]	100	[ ]	0
35 C	+	99	+	100	+	100
42 C	v	52	[−]	0	[+]	100
Esculin hydrolysis	−	0		0		0
Lysine decarboxylase	v	82				
Arginine dihydrolase	−	0				
Ornithine decarboxylase	[+]	98				
Growth in: Nutrient broth, 0% NaCl	v	71		0		0
Nutrient broth, 6% NaCl	-	1	-	0		0
Brucella broth, 3.5% NaCl			[−]	0	[−]	0
Brucella broth, 1% glycine			[−]	0	[+]	93
H_2S, Brucella broth (Pb ac paper)			[−]	0	[+]	97

[a]7-14 days' incubation.
[b]Candle jar (CO_2) required, or enhances growth.
[c]Microaerophilic – may grow in candle jar, but O_2 requirement for optimal growth is 5-6%.
[d]This group of organisms probably includes both *C. coli* and *C. jejuni* as referred to in the Approved Lists of Bacterial Names.

CHART 18	*Campylobacter fetus subsp. fetus*[c]		*Pseudomonas mesophilica*	
Number of strains	113		90	
Test Performed	Sign	% +	Sign	% +
Morphology	[sc]		[vps-mr]	
Motility; flagella	[m];p,1-2		[m];p,1-2	
Action on blood	–	10 al	–	8 ly
Fermentative or oxidative	[n-o]		[O]	
Carbohydrate base	OF		OF	
Acid from: Glucose	[ ]	0	v	40
Xylose		0	[+]	94
Mannitol		0	–	2
Lactose	–	0	–	0
Sucrose	–	0		0
Maltose	–	0	–	2
Catalase	[+]	99(1)	+	100
Oxidase	[+]	100	[+]	96
Growth on: MacConkey	[+ or (+)]	83(12)	v	15
SS		0	–	0
Simmons citrate	–	0	–	2(3)
Urea, Christensen's	–	0	v	29(26)
Nitrate reduction	[+]	100	v	25
Gas from nitrate	–	0	–	0
Indole	–	0	[–]	0
TSI slant, acid	–	0	–	0
TSI butt, acid	–	0	–	0
H_2S (TSI butt)	–	0	–	0
H_2S (Pb ac paper)	v	49(2)	v	47
Gelatin hydrolysis[d]	–	0	–	0
Litmus milk	–	10 IR	v	49 k
Pigment	–	0	[co-pk]	99
Growth at: 25°C	[+]	99	+	98
35°C	+	100	+	93
42°C	[–]	10	v	12
Esculin hydrolysis	–	0	–	0
Lysine decarboxylase				
Arginine dihydrolase				
Ornithine decarboxylase				
Growth in: Nutrient broth, 0% NaCl	v	14	+	93
Nutrient broth, 6% NaCl	–	0	–	0
Brucella broth, 3.5% NaCl	[–]	0		
Brucella broth, 1% glycine	[+]	97(3)		
H_2S, Brucella broth (Pb ac paper)	[+]	96		

See footnotes on Chart 17.

CHART 19	*Neisseria elongata*	*Branhamella catarrhalis*[c]		*Moraxella lacunata*	
Number of strains	1	74		25	
Test Performed	Sign	Sign	% +	Sign	% +
Morphology	[cc,srs]	[cd]		cc	
Motility: flagella	[nm]	[nm]		[nm]	
Action on blood		v	32 hy	v	24 al
Fermentative or oxidative	n-o	n-o		n-o	
Carbohydrate base	CTA	CTA		OF[d]	
Acid from: Glucose	[−]	[−]	0	[−]	0
Xylose			0	−	0
Mannitol			0	−	0
Lactose	−	−	0	−	0
Sucrose	−	−	0	−	0
Maltose		[−]	0	−	0
Catalase	[−]	+	100	+	100
Oxidase	[+]	[+]	100	[+]	100
Growth on: MacConkey	[−]	[−]	5	−	4
SS	−		0	−	0
Simmons citrate	−	−	0		0
Sodium acetate	+			[−]	7
Urea, Christensen's	−	−	0	[−]	0
Phenylalanine deaminase	−	v	68	v	31
Nitrate reduction	[−]	[+]	92	[+]	100
Nitrite reduction	−	+	91		
Indole	−	−	0	−	0
TSI slant, acid			0		0
TSI butt, acid	−	−	0		0
H_2S (TSI butt)	−	−	0		0
H_2S (Pb ac paper)	+	v	73		0
Gelatin hydrolysis[a]	−	−	0	v	74
Loeffler slant, digestion				[+]	100
Litmus milk	−	−	6 k	v	58 pep
Pigment	−	v	25 sl yel		4 br sol
Growth at: 25 C	+	v	85	v	47
35 C	+	+	97	v	87
42°C	+	v	23	−	0
Esculin hydrolysis	−	−	0		0
Nutrient broth, 0% NaCl	+	v	47	[−]	0
Nutrient broth, 6% NaCl	−				0
Penicillin sensitivity[b]	+			+	100

[a]7-14 days' incubation

[b]Based on results obtained by streaking a blood agar plate with growth from an 18-36 h culture and then placing a 10 unit penicillin disc on the streaked area. A positive reaction is indicated by the appearance of a zone of inhibition.

[c]See chart "Differentiation of Species of *Neisseria* and *Branhamella*" (Charts 39-40) for distinguishing *B. catarrhalis* and *Neisseria* species.

[d]Reactions neutral (does not grow or grows poorly) in King's OF medium.

CHART 20	*Moraxella nonliquefaciens*		*Moraxella osloensis*		*Moraxella bovis*	
Number of strains	243		163		7	
Test Performed	Sign	% +	Sign	% +	Sign	% +
Morphology	cc,sbr		cc,sbr		cc,sbr	
Motility, flagella	[nm]		[nm]		[nm]	
Action on blood	v	10 hy	v	7 al	[β]	100
Fermentative or oxidative	n-o		n-o		n-o	
Carbohydrate base	OF[d]		OF		OF	
Acid from Glucose	[–]	0	[–]	0	[–]	0
Xylose		0	–	0	–	0
Mannitol		0	–	0	–	0
Lactose		0	–	0		0
Sucrose		0	–	0	–	0
Maltose	–	0	–	0	–	0
Catalase	+	95	+	95	v	14
Oxidase	[+]	100	[+]	100	[+]	100
Growth on MacConkey	[–]	8(2)	v	70	[–]	0
SS	–	0	–	0	–	0
Simmons citrate	–	0	–	0	–	0
Sodium acetate	[–]	0	[+]	100		
Urea, Christensen's	[–]	0	[–]	0	[–]	0
Phenylalanine deaminase			v	14	–	0
Nitrate reduction	+	95	v	24	v	14
Nitrite reduction			[–]	0		
Indole	–	0	–	0	–	0
TSI slant, acid		0	–	0	–	0
TSI butt, acid	–	0	–	0		0
H_2S (TSI butt)	–	0	–	0	–	0
H_2S (Pb ac paper)	v	83	v	74	+	100
Gelatin hydrolysis[a]		0	–	0	[+]	100
Loeffler slant, digestion	[–]	0				
Litmus milk	–	2(1) k	–	9 k	pep	100
Pigment		3 yel sol	–	6 yel-br sol		
Growth at 25°C	+	93	+	96	+	100
35°C	v	88	+	98	+	100
42°C	v	15	v	51	–	0
Esculin hydrolysis	–	0	–	0		
Nutrient broth, 0% NaCl	v	22	[+]	98		
Nutrient broth, 6% NaCl	–	0	v	12		
Penicillin sensitivity[b]	+	99	+	92	+	100

See footnotes on Chart 19

CHART 21	*Moraxella phenylpyruvica*		*Moraxella urethralis*		*Moraxella atlantae*	
Number of strains	50		22		23	
Test Performed	Sign	% +	Sign	% +	Sign	% +
Morphology	[cc,br]		[cc]		sbr,cc	
Motility; flagella	[nm]		[nm]		[nm]	
Action on blood	v	16 ly	v	18 ly	-	9 al or ly
Fermentative or oxidative	n-o		n-o		[n-o]	
Carbohydrate base	OF		OF		OF	
Acid from: Glucose	[−]	0	[−]	0	−[b]	0
Xylose		0	[−]	0	−	0
Mannitol		0	-	0	-	0
Lactose		0	−	0		0
Sucrose		0	−	0	−	0
Maltose	−	0	−	0	−	0
Catalase	+	90	+	100	+	91
Oxidase	[+]	100	[+]	100	[+]	100
Growth on: MacConkey	v	80(6)	[+]	96	[+ or(+)]	87(13)
SS		0	−	9	−[c]	0
Simmons citrate	−	0	v	46	−	0
Sodium acetate	v	43	v	60	v	21(21)
Urea, Christensen's	[+]	100	[−]	0	[−]	0
Phenylalanine deaminase	[+]	97	[+]	100	−	08
Nitrate reduction	v	68	[−]	0	[−]	5
Nitrite reduction		0	[+]	100	v	20
Indole		0	−	0	−	0
TSI slant, acid		0	−	0		0
TSI butt, acid	−	0	−	0		0
H_2S (TSI butt)	−	0	−	0	-	0
H_2S (Pb ac paper)	v	47	−	9	v	71
Gelatin hydrolysis[a]	−	0	−	0	-	0
Loeffler slant, digestion						
Litmus milk	v	22(20) k	v	36 k		9 IR
Pigment	−	8 yel-br sol	−	4 amb sol		
Growth at: 25 C	v	85	v	50	v	68
35 C	+	100	+	100	+	100
42 C	v	29	v	59	v	50
Esculin hydrolysis	−	0	-	0		0
Nutrient broth, 0% NaCl	v	53	+	96	v	36
Nutrient broth, 6% NaCl	v	19	v	59	−	0
Penicillin sensitivity[c]	v	73	+	100		

[a]7-14 days' incubation.
[b]Usually does not grow in King's OF medium; if it grows, it usually does not change the pH of the medium.
[c]Less than 10 strains tested.
[d]Weak reaction.
[e]Based on results obtained by streaking a blood agar plate with growth from an 18-36 h culture and then placing a 10-unit penicillin disc on the streaked area. A positive reaction is indicated by the appearance of a zone of inhibition.

CHART 22	M-5		M-6		*Pasteurella anatipestifer*
Number of strains	59		40		1
Test Performed	Sign	% +	Sign	% +	Sign
Morphology	[mrs]		mbr		srs
Motility, flagella	[nm]		[nm]		[nm]
Action on blood	v	44 ly	-	5 al or gr	ly
Fermentative or oxidative	n-o		n-o		[O]
Carbohydrate base	OF		OF		OF
Acid from: Glucose	[–]	0	[–]	0	[+[d]]
Xylose	[–]	0	[–]	0	–
Mannitol	–	0	-	0	–
Lactose	-	0	–	0	[–]
Sucrose	–	0	–	0	
Maltose	–	0	–	0	[+[d]]
Catalase	+	100	[–]	8	+
Oxidase	[+]	100	[+]	100	[+]
Growth on: MacConkey	v	42(20)	v	22(28)	[–]
SS	–	0	–	0	–
Simmons citrate	-	0	-	0	-
Sodium acetate	v	25[c]	v	83[c]	
Urea, Christensen's	-	0	[–]	0	+
Phenylalanine deaminase	v	73		0[c]	
Nitrate reduction	[–]	0	[+]	100	[–]
Nitrite reduction	[v]	84	[+]	100	
Indole	–	0	–	0	[–]
TSI slant, acid	-	0	–	0	
TSI butt, acid		0	-	0	–
H_2S (TSI butt)	–	0	–	0	
H_2S (Pb ac paper)	v	86	v	88	+
Gelatin hydrolysis[a]	–	0		0	+
Loeffler slant, digestion					
Litmus milk	–	3 k	-	0	–
Pigment	v	48 yel ta sol	v	18 yel-ta sol	yel-br sol
Growth at: 25 C	+	95	v	78	+
35 C	+	100	+	100	+
42 C	v	63	v	47	-
Esculin hydrolysis	-	0		0	
Nutrient broth, 0% NaCl	+ or (+)	65(29)	+	93	
Nutrient broth, 6% NaCl	–	8	-	3	
Penicillin sensitivity[e]	+	100	v	88	

See footnotes on Chart 21.

CHART 23	*Acinetobacter calcoaceticus (A. anitratus)*		*Acinetobacter calcoaceticus (A. lwoffi)*		EO-2[b]		*Achromobacter xylosoxidans*	
Number of strains	501		253		93		135	
Test Performed	Sign	% +	Sign	% +	Sign	% +	Sign	% +
Morphology	cc		cc		cc		mrs	
Motility; flagella	[nm]		[nm]		[nm]		[m];pe	
Action on blood	v	45 ly	v	48 ly	v	23 al or gr	v	42 ly
Fermentative or oxidative	[O]		[n-o]		[O]		[O]	
Carbohydrate base	OF		OF		OF		OF	
Acid from: Glucose	[+]	100	[−]	0	[+]	100	[v]	78
Xylose	+	99	−	0	[+]	99(1)	[+]	99
Mannitol	[−]	2	−	0	v	10(10)	−	0
Lactose	[+]	97(2)	−	0	+	91(6)		0
Sucrose	−	0		0	[−]	0	−	0
Maltose	v	63(6)	−	0	v	20(1)	−	0
Catalase	+	100	+	100	+	98	+	98
Oxidase	[−]	0	[−]	0	[+]	100	[+]	100
Growth on: MacConkey	[+]	99(1)	[+]	90(7)	v	63(11)	[+]	100
SS	v	20(1)	v	7(5)		4(1)	[+]	98
Cetrimide		3	−	0		0	+	95(1)
Simmons citrate	+	93	v	42	v	22(3)	+	95
Urea, Christensen's	v	28(18)	−	5(4)	v	74(3)	[−]	0
Nitrate reduction	[−]	1	−	3	v	85	[+]	100
Gas from nitrate	−	0		0	[−]	2	[v]	60
Indole		0	−	0	[−]	0	−	0
TSI slant, acid		5	−	0	−	0		0
TSI butt, acid	−	0	−	0	−	0	−	0
H_2S (TSI butt)	−	0	−	0	−	0	[−]	0
H_2S (Pb ac paper)	v	32	v	58	v	75	−	0
Gelatin hydrolysis[a]	−	9	−	4	−	0	−	0
Litmus milk	v	37(10) A	v	86 k	v	32 k	k	95
Pigment	−	7 yel-ta sol	v	13 yel-br sol	v	17 yel sol	−	5 br sol
Growth at: 25°C	+	97	+	100	v	83	+	98
35°C	+	97	+	100	+	95	+	100
42°C	v	87	v	63	v	35	v	84
Esculin hydrolysis	−	0	−	0	−	0	−	0
Lysine decarboxylase					[−]	0[f]	−	0
Arginine dihydrolase					[−]	0[f]	v	13
Ornithine decarboxylase					−	0[f]	−	0
Nutrient broth, 0% NaCl	+	100	[+]	97	v	66	+	100
Nutrient broth, 6% NaCl	v	22	−	9	v	34	v	69
3-ketolactonate								

[a]7-14 days' incubation
[b]Colonies frequently are mucoid.
[c]Less than 10 strains tested.
[d]Frequently individual cells have only a single flagellum, either polar, subpolar, or lateral.
[e]Some strains reduced both nitrate and nitrite without gas formation
[f]Weak reaction.

CHART 24	Σd *(Achromobacter* sp.)		*Agrobacterium radiobacter*		EF-4b	
Number of strains	71		38		34	
Test Performed	Sign	% +	Sign	% +	Sign	% +
Morphology	mrs		spr		cc,srs	
Motility; flagella	[m;pe[d]]		[m]pe[d]		[nm]	
Action on blood	v	23 I,G	v	18 ly	v	41 ly
Fermentative or oxidative	[O]		[O]		[O]	
Carbohydrate base	OF		OF		OF	
Acid from Glucose	[+ or(+)]	86(13)	[+]	100	+ or(+)	7(0,26)
Xylose	+	96(4)	[+]	100	-	0
Mannitol	v	46(34)	+	100	-	0
Lactose	[-]	0	[+]	100	[-]	0
Sucrose	v	28(25)	+	100	-	0
Maltose	v	32(25)	[+]	100	[-]	0
Catalase	+	100	+	100	[+]	100
Oxidase	[+]	100	[+]	100	+	100
Growth on: MacConkey	[+]	100	[+]	100	[v]	65(6)
SS	[+]	96(1)	v	26(3)	-	0
Cetrimide	-	0(3)	-	0		
Simmons citrate	v	54(13)	+	100	v	14(6)
Urea, Christensen's	[+]	92(8)	[+ or(+)]	89(11)	[-]	0
Nitrate reduction	+	100	v[e]	87	[+[e]]	97
Gas from nitrate	[+]	99	[-]	5	[-]	0
Indole	[-]	0	[-]	0	[-]	0
TSI slant, acid		0	-	0	-	0
TSI butt, acid	-	0	-	0	-	6[f]
H_2S (TSI butt)	v	49	v	8(5)	-	0
H_2S (Pb ac paper)	+	100	+	100	v	88
Gelatin hydrolysis[a]	-	0	-	3	-	9
Litmus milk	v	85 k	szl	100	-	6 k
Pigment	v	11 br-yel sol	-	0		
Growth at: 25°C	+	100	+	100	v	88
35°C	+	100	+	100	+	100
42°C	v	56	v	34	v	69
Esculin hydrolysis	v	38(11)	+	100	-	0
Lysine decarboxylase	-	0	[-]	0	-	0
Arginine dihydrolase	v	68	[-]	3	[-]	0
Ornithine decarboxylase	-	0	-	0	-	0
Nutrient broth, 0% NaCl	+	100	+	100	+ or(+)	89(7)
Nutrient broth, 6% NaCl	v	45	-	0	-	0
3-ketolactonate	-	0[g]	[+]	100		

See footnotes on Chart 23

CHART 25	*Alcaligenes faecalis*[a]		*Alcaligenes odorans*[b,c]		*Alcaligenes denitrificans*[b]		Group IVe	
Number of strains	69		49		34		37	
Test Performed	Sign	% +	Sign	% +	Sign	% +	Sign	% +
Morphology	mrs		mrs		mrs		srs	
Motility; flagella	[m;pe]		[m;pe]		[m;pe]		[m;pe]	
Action on blood	v	48 ly	v	48 br & gr	v	35 ly	v	71 ly
Fermentative or oxidative	[n-o]		[n-o]		[n-o]		[n-o]	
Carbohydrate base	OF		OF		OF		OF	
Acid from: Glucose	[–]	0	[–]	0	[–]	0	[–]	0
Xylose	[–]	0	[–]	0	[–]	0	[–]	0
Mannitol	–	0	–	0	–	0	–	0
Lactose	–	0	–	0	–	0	–	0
Sucrose	–	0	–	0	–	0	–	0
Maltose	–	0	–	0	–	0		0
Catalase	+	100	+	98	+	100	+	100
Oxidase	[+]	100	[+]	100	[+]	100	[+]	100
Growth on: MacConkey	[+]	100	[+]	100	[+]	100	[v]	62(27)
SS	[v]	78(2)	[+]	100	[v]	65	[–]	5
Cetrimide	v	28(1)	v	59	v	26(12)	–	0(3)
Simmons citrate	+	98(1)	+	100	v	85(3)	v	14(16)
Urea, Christensen's	[–]	0(1)	[–]	2	v	12(3)	[+]	97
Nitrate reduction	[v]	45	[–]	0	[+]	100	[+]	100
Gas from nitrate	[–]	0	–	0	[+]	100	[v]	60
Nitrite reduction	[–]	0	[+]	100				
Indole	–	0	–	0	–	0	–	0
TSI slant, acid	–	0	–	0	–	0	–	0
TSI butt, acid	–	0	–	0	–	0		0
H_2S (TSI butt)	[–]	0	–	0	[–]	0	[–]	0
H_2S (Pb ac paper)	v	52	–	8	v	32	v	38
Gelatin hydrolysis[a]	–	0	v	22	–	0	–	0
Litmus milk	k	98	k	96	k or (k)	88(12)	v	65 k
Pigment	v	13 yel,br sol	v	22 yel sol	–	9 br sol	–	3 yel sol
Growth at: 25°C	+	100	+	100	+	100	v	67
35°C	+	100	+	100	+	100	v	88
42°C	v	75	v	18	v	56	v	18
Esculin hydrolysis	–	0	–	0	–	0	–	0
Lysine decarboxylase	–	0	–	0[d]	–	0[d]		
Arginine dihydrolase	–	0	–	0[d]	–	0[d]		
Ornithine decarboxylase	–	0	–	0[d]	–	0[d]		
Nutrient broth, 0% NaCl	+	100	+	100	+	100	v	19(3)
Nutrient broth, 6% NaCl	v	54(6)	+	98(2)	v	29	v	14(5)

[a]7-14 days' incubation.
[b]Listed in Bergey's Manual (8th Ed.) as a synonym of *Alcaligenes faecalis*.
[c]The type strain of *A. faecalis* has the characteristics listed for *A. odorans*.
[d]Less than 10 strains tested.
[e]Reaction is neutral compared to the alkaline reaction of the control.

CHART 26	*Bordetella bronchiseptica*		Group IXc-2		*Bordetella parapertussis*	
Number of strains	85		36		7	
Test Performed	Sign	% +	Sign	% +	Sign	% +
Morphology	mrs		srs		srs	
Motility, flagella	[m;pe]		[m;pe]		[nm]	
Action on blood	v	46 ly	v	53 ly	[β]	100
Fermentative or oxidative	[n-o]		[n-o]		[n-o]	
Carbohydrate base	OF		OF		OF	
Acid from: Glucose	[−]	0	[−]	0	[−]	0
Xylose	[−]	7c	[−]	0	−	0
Mannitol		1	−	0		0
Lactose	−	0	−	0	−	0
Sucrose		0	−	0	−	0
Maltose		0	−	0	[−]	0
Catalase	+	100	+	100	+	100
Oxidase	[+]	100	[+]	100	[ ]	0
Growth on: MacConkey	[+]	100	[+]	94(6)	[+]	100
SS	[+]	99	[−]	3(6)	−	0
Cetrimide	−	0	−	0(3)		0
Simmons citrate	+	98(1)	+	100	v	71(14)
Urea, Christensen's	[+]	99	[+]	100	[+]	100
Nitrate reduction	[+]	92	[v]	11	[−]	0
Gas from nitrate	[−]	0	[−]	0	[−]	0
Nitrite reduction						
Indole	−	0	−	0		0
TSI slant, acid		0	−	0		0
TSI butt, acid		0	−	0	−	0
H_2S (TSI butt)	[−]	0	[−]	0	[−]	0
H_2S (Pb ac paper)	v	74	v	51	v	28
Gelatin hydrolysis[d]	−	0	−	0	[−]	0
Litmus milk	k	98	k	97	v	80 k
Pigment	−	0	v	22 yel-ta sol	[br sol]	100
Growth at: 25°C	+	99	+	94	v	50
35°C	+	100	+	100	+	100
42°C	v	78	v	86	v	17
Esculin hydrolysis		0	−	0		
Lysine decarboxylase						
Arginine dihydrolase						
Ornithine decarboxylase						
Nutrient broth, 0% NaCl	+	100	+	100	+	100
Nutrient broth, 6% NaCl	v	82(5)	v	11	−	0

See footnotes on Chart 25

CHART 27	*Pseudomonas aeruginosa*		*Pseudomonas fluorescens*		*Pseudomonas putida*	
Number of strains	201		155		16	
Test Performed	Sign	% +	Sign	% +	Sign	% +
Morphology	mrs		mrs		mrs	
Motility, flagella	[m];p,1-2		[m];p,>2		[m];p,>2	
Action on blood	v	42 LG	v	50 ly	v	38 gr
Fermentative or oxidative	[0]		[0]		[0]	
Carbohydrate base	OF		OF		OF	
Acid from Glucose	[+]	97(1)	[+]	100	[+]	100
Xylose	+	90(1)	+	100	+	100
Mannitol	v	70(3)	v	53(2)	v	25
Lactose	−	1	v	24(3)	v	25(13)
Sucrose	[−]	0	[v]	48	[−]	0
Maltose	[−]	0(<1)	[−]	2	[v]	31
Starch						
Catalase	+	100	+	99(1)	+	100
Oxidase	[+]	99	[+]	97	[+]	100
Growth on MacConkey	[+]	100	[+]	100	[+]	100
SS	+	96	[+ or (+)]	86(7)	+	100
Cetrimide	[+]	94(2)	+	89	v	81(6)
Simmons citrate	+	95(1)	+	93(3)	+	94(6)
Urea, Christensen's	v	48(9)	v	21(31)	v	13(44)
Nitrate reduction	+	98	v	19	[−]	0
Gas from nitrate	[+]	93	[−]	3		0
Indole	[−]	0	[−]	0		0
TSI slant, acid	−	0	−	0		0
TSI butt, acid		0	−	0		0
H_2S (TSI, butt)		0	−	0	−	0
H_2S (Pb ac paper)	−	4	v	12		6
Gelatin hydrolysis[a]	[v]	82	[+[b]]	100	[−[b]]	0
Litmus milk	[v]	89 pep	[pep]	95	v	62 k
Pigment Pyoverdin	[v]	65	[+]	96	[+]	93
Pyocyanin	[v]	46	[−]	0		0
Pyorubrin	v	25	−	0		0
Other	v	23 pyomelanin		4 br sol		
Growth at 5 C			−	95(1)		
25 C	+	100	+	100	+	100
35 C	+	100	+	90	+	100
42 C	[+]	100	[−]	0	[−]	0
Esculin hydrolysis		0	−	0		0
Lysine decarboxylase	[−]	0	[−]	0	[−]	0
Arginine dihydrolase	[+]	100	[+]	97	[+]	100
Ornithine decarboxylase	−	0	−	0	−	0
Nutrient broth, 0% NaCl	+	100	+	99	+	100
Nutrient broth, 6% NaCl	v	65	v	43	v	44
Acetamide	[+]	100	v	6(12)	[−]	0
2-ketogluconate	+	96				

[a]7-14 days' incubation.
[b]Incubated at 25°C.
[c]Less than 10 strains tested.

CHART 28	*Pseudomonas stutzeri*		∑b 3		*Pseudomonas mendocina*	
Number of strains	28		65		3	
Test Performed	Sign	% +	Sign	% +	Sign	% +
Morphology	mrs		mrs		mrs	
Motility, flagella	[m][p,L 2		[m][p,L 2		[m][p,L 2	
Action on blood	v	26 lv	v	32 gr	lv	100
Fermentative or oxidative	[O]		[O]		[O]	
Carbohydrate base	OF		OF		OF	
Acid from Glucose	[+]	96(4)	[+]	100	[+]	100
Xylose	+	93(7)	+	97(3)	+	100
Mannitol	+ or v	89(4)	v	65(9)		0
Lactose	[−]	0	[−]	0	[−]	0
Sucrose		0	−	0		0
Maltose	[+]	100	[+]	95(5)	[−]	0
Starch	+	100	+	100		0
Catalase	+	100	+	98	+	100
Oxidase	[+]	100	[+]	100	[+]	100
Growth on MacConkey	[+]	100	[+]	100	[+]	100
SS	v	84	+	100	[+]	100
Cetrimide		4	v	18(2)	+	100
Simmons citrate	+ or v	82(14)	+	92(6)	+	100
Urea, Christensen's	v	33(22)	v	12(45)	v	33
Nitrate reduction	+	100	+	100	+	100
Gas from nitrate	[+]	100	[+]	100	[+]	100
Indole	[−]	0		0		0
TSI slant, acid		0	−	0		0
TSI butt, acid		0		0		0
H_2S (TSI butt)	[−]	0	[−]	0	[−]	0
H_2S (Pb ac paper)	v	36	v	5(14)	+	100
Gelatin hydrolysis[d]	[−]	0	[−]	4	[−]	0
Litmus milk	v	57 k	v	69 k	0k	0(100)
Pigment Pyoverdin						
Pyocyanin						
Pyorubrin						
Other	v	86 sl yel ins	yel ins	98	sl yel ins	100
Growth at 5 C						
25 C	+	100	+	98	+	100
35 C	+	100	+	100	+	100
42 C	v	69	+	91	+	100
Esculin hydrolysis		0		0		0
Lysine decarboxylase	[−]	0	[−]	0	[−]	0
Arginine dihydrolase	[−]	0	[+]	100	[+]	100
Ornithine decarboxylase		0	−	0	−	0
Nutrient broth, 0% NaCl	+	96	+	100	+	100
Nutrient broth, 6% NaCl	+ or v	80(16)	v	85(3)	+	100
Acetamide		08	−	08	[−]	0
2-ketogluconate	+	08		10	[−]	0

See footnotes on Chart 27

CHART 29	*Pseudomonas dominuta*		*Pseudomonas vesicularis*		*Pseudomonas acidovorans*		*Pseudomonas testosteroni*	
Number of strains	68		47		64		28	
Test Performed	Sign	% +	Sign	% +	Sign	% +	Sign	% +
Morphology	rs		mrs		mrs		pr f	
Motility, flagella	[m;p,1-2[b]]		[m;p,1-2[b]]		[m;p, -2]		[m;p, -2]	
Action on blood	v	69 I G or gr	v	46 I G	v	38 ly	v	82 ly
Fermentative or oxidative	n-o or O		O		n-o		[n-o]	
Carbohydrate base	OF		OF		OF		OF	
Acid from Glucose	[v]	21(9)	[+ or(+)]	87(9)	[-]	0	[-]	0
Xylose	[-]	0	[v]	21(2)	[-]	0	[-]	0
Mannitol		0	-	0	[+[c]]	100	[-]	0
Lactose		0		0		0		0
Sucrose		0		0		0		0
Maltose	[-]	0	[+]	98(2)		0		0
Fructose					+	100	[-]	64[d]
Catalase	+	98	v	74(11)	+	100	+	96
Oxidase	[+]	100	[+]	94	[+]	100	[+]	100
Growth on MacConkey	[+]	97(3)	[v]	81(8)	[+]	100	[+]	96(4)
SS		1(1)		6(2)	v	67(19)	v	21(11)
Cetrimide		0(1)	-	0		5		0(4)
Simmons citrate		1		2(2)	+	94(5)	v	42(3)
Urea, Christensen's	v	0(13)		0(8)	[-]	0(3)	v	7(14)
Nitrate reduction	[-]	3	[-]	6	+	98	+	96
Gas from nitrate	[-]	0	[-]	0	[-]	0	[-]	0
Indole	[-]	0		0		0		0
TSI slant, acid		0		0		0		0
TSI butt, acid		0		0	-	0		0
H_2S (TSI butt)		0	-	0	[-]	0	[-]	0
H_2S (Pb ac paper)	v	34	v	68	v	56	v	61
Gelatin hydrolysis[a]	v	68	v	40	v	17		0
Litmus milk	v	35 k	v	33 IR	k	97	v	86 k
Pigment Fluorescent					v	23		
Insoluble			v	15 yel				
Soluble	[br-ta]	96	v	66 br ta	v	47 yel ta	v	27 yel-br
Growth at 25 C	+	100	+	96	+	100	+	100
35 C	+	100	+	98	+	100	+	100
42 C	v	38	v	38	v	31(11)	v	68
Esculin hydrolysis	-	5(1)	v	79(5)		0		0
Lysine decarboxylase		0	-	0		0		0
Arginine dihydrolase	-	0	-	0		0		0
Ornithine decarboxylase		0	-	0		0		0
Nutrient broth, 0% NaCl	+	100	+	98	+	100	+	100
Nutrient broth, 6% NaCl	v	21(2)	v	17		7	v	14

[a]7-14 days' incubation
[b]Flagella have a short wavelength with low amplitude
[c]Mannitol reaction weak
[d]Fructose reaction weak.
[e]Less than 10 strains tested
[f]Referred to as *P. denitrificans* in 1972 revision of The Identification of Unusual Pathogenic Gram Negative Bacteria

CHART 30	*Pseudomonas alcaligenes*		*Pseudomonas pseudoalcaligenes*		*Pseudomonas* sp. CDC Group I[1]	
Number of strains	26		19		31	
Test Performed	Sign	% +	Sign	% +	Sign	% +
Morphology	mrs		mrs		mrs	
Motility; flagella	[m;p,1-2]		[m;p,1-2]		[m;p,1-2]	
Action on blood	v	31 ly	v	26 ly	v	33 ly or gr
Fermentative or oxidative	[n-o]		[n-o]		[n-o]	
Carbohydrate base	OF		OF		OF	
Acid from Glucose	[–]	0	[–]	0	[–]	0
Xylose	[–]	0	v	32	[–]	0
Mannitol	–	0	–	0	–	0
Lactose	–	0	–	0	–	0
Sucrose	–	0	–	0	–	0
Maltose	[–]	0	[–]	0	[–]	0
Fructose	[–]	0	[v]	82		
Catalase	+	92	+	95	v	81
Oxidase	[+]	96	[+]	100	[+]	100
Growth on: MacConkey	[+]	96	[+]	100	[+]	97(3)
SS	v	38(8)	v	84	v	30(6)
Cetrimide	v	15(4)	v	37(10)	v	13(6)
Simmons citrate	v	57(8)	v	43(10)	v	42(6)
Urea, Christensen's	[–]	0	[–]	5	[–]	3(7)
Nitrate reduction	v	54	+	90	+	100
Gas from nitrate	[–]	0	[–]	0	[+]	100
Indole	–	0	–	0	–	0
TSI slant, acid	–	0	–	0	–	0
TSI butt, acid	–	0	–	0	–	0
H_2S (TSI butt)	–	0	[–]	0	[–]	0
H_2S (Pb ac paper)	v	65	+	95	v	68
Gelatin hydrolysis[a]	–	0	–	0		4
Litmus milk	v	46 k	v	37 k	v	39 k
Pigment: Fluorescent						
Insoluble						
Soluble	v	32 ta	v	26 yel-br	v	60 yel-br
Growth at 25 C	+	100	+	100	+	97
35 C	+	100	+	100	+	97
42 C	–	0	[+]	100	v	48
Esculin hydrolysis	–	0	–	0	–	0
Lysine decarboxylase	–	0[c]	–	0[c]	–	0[c]
Arginine dihydrolase	v	12[c]	+	100[c]	v	33[c]
Ornithine decarboxylase	–	0[c]	–	0[c]	–	0[c]
Nutrient broth, 0% NaCl	+	95	+	100	+	93
Nutrient broth, 6% NaCl	v	41	v	68	v	14

See footnotes on Chart 29.

CHART 31	*Pseudomonas cepacia*		*Pseudomonas pseudomallei*		*Pseudomonas maltophilia*		Group Xe 1	
Number of strains	189		70		228		18	
Test Performed	Sign	% +	Sign	% +	Sign	% +	Sign	% +
Morphology	nurs		nurs		nurs		nurs	
Motility, flagella	[m] f p, >2		[m] f p, >2		[m] f p, >2		[m, p, >2]	
Action on blood	v	47 hy	v	40 gr	v	81 LG	v	29 hy
Fermentative or oxidative	[O]		[O]		O		[O]	
Carbohydrate base	OF		OF		OF		OF	
Acid from: Glucose	[+]	100	[+]	100	[+ or + l]	85(5)	[+]	100
Xylose	+	100	+ or + l	86(14)	v	35(1)	+	100
Mannitol	[+]	100	+	94(6)	[–]	0	[v]	82(6)
Lactose	[+]	99(1)	[+]	99(1)	v	60(1)	v	6(28)
Sucrose	v	86(1)	v	66(4)	v	63(1)		6
Maltose	[+]	99(1)	[+]	99(1)	[+[e]]	100	+	100
Catalase	+	99	+	100	[+]	100	+	100
Oxidase	v	86	[+]	100	–[f]	0	[–]	0[g]
Growth on MacConkey	[+]	100	[+]	100	[+]	100	[+]	100
SS	v	6(18)	v	8(31)	v	22(21)	v	67
Cetrimide	v	44(22)		7(3)		2(7)		0
Simmons citrate	+	94(5)	v	77(4)	v	34(12)	+	100
Urea, Christensen's	v	60(18)	v	13(8)	v	3(12)	v	39(44)
Nitrate reduction	v	57	[+]	100	v	39	v	67
Gas from nitrate	[–]	0	[+[c]]	100	[–]	0		0
Indole		0		0		0		0
TSI slant, acid		1	v	72		0		0
TSI butt, acid		0	–	0	–	0		0
H_2S (TSI butt)		0	–	0	[–]	0		0
H_2S (Pb as paper)	–	0	v	26	+	95		6
Gelatin hydrolysis[d]	v	20	v	79	[+]	93	v	53
Litmus milk	pep	94	pep	96	pep	96	v	61 k
Pigment: Soluble					br ta	98		
Insoluble	v	60 yel/TSI[b]	v	51 wh			[yel]	94
Growth at 25 C	+	98	+	100	+	99	+	100
35 C	+	100	+	100	+	99	+	100
42 C	v	83	+	100	v	48	+	94
Esculin hydrolysis	v	63(6)	v	59	v	39	[+]	100
Lysine decarboxylase	[v]	80	[–]	0[d]	[+]	93	[–]	0
Arginine dihydrolase	[–]	0	[+]	100[d]		0	[+]	100
Ornithine decarboxylase	v	48	–	0[d]		0		0
Nutrient broth, 0% NaCl	+	100	+	100	+	100	+	100
Nutrient broth, 6% NaCl	+	?	v	12	v	22	v	83

[a]7-14 days' incubation

[b]60% of the *P. cepacia* strains produced a yellow pigment which occurs only on iron containing media, e.g., TSI, usually not evident until 42-48 h. Sometimes a chartreuse pigment occurs on ordinary media

[c]The volume of gas may be small. Gas may not be detected unless incubated at 25 C

[d]Less than 10 strains tested

[e]Acid reaction in maltose usually stronger than acid reactions in the other carbohydrates listed

[f]14 of 73 strains which were negative when the reagent was applied directly to growth on a blood agar plate were positive when tested by Kovacs' method. 10 of the 14 were recorded as only weakly positive

[g]A few strains were weakly positive by the Kovacs' method

[h]Usually appears nonmotile in motility medium.

CHART 32	Group Ye-2		*Pseudomonas paucimobilis*		*Flavobacterium multivorum*		*Flavobacterium spirituvorum*	
Number of strains	36		134		22		11	
Test Performed	Sign	% +	Sign	% +	Sign	% +	Sign	% +
Morphology	srs		mrs		srs		srs	
Motility; flagella	[m;p,1-2]		[m];p,1-2[h]		[nm]		nm	
Action on blood	v	43 ly	v	32 gr	v	14 gr		0
Fermentative or oxidative	[O]		[O]		O		O	
Carbohydrate base	OF		OF		OF		OF	
Acid from: Glucose	[+]	100	[+]	93(7)	[+]	100	[+]	100
Xylose	+	100	+	96(4)	+	100	+	91(9)
Mannitol	[+]	100	[−]	0	[−]	0	[+]	100
Lactose	v	14(22)	[+]	93(7)	+	100	+	100
Sucrose	v	25	+	93(7)	+	100	+	100
Maltose	+	97(3)	[+]	97(3)	[+]	100	+	100
Catalase	+	94	+	95	+	100	+	100
Oxidase	[−]	0[g]	v	75	[+]	100	[+]	100
Growth on: MacConkey	[+]	100	v	10(13)	[+]	100	[v]	0(55)
SS	v	22	−	0	−	0	−	0
Cetrimide	v	25(28)	−	0		0	−	0
Simmons citrate	+	97	−	6(1)	−	0	−	0
Urea, Christensen's	v	77	[−]	6(3)	[+]	95	[+ or(+)]	55(36)
Nitrate reduction	[−]	6	−	3	−	0	−	0
Gas from nitrate	−	0	−	0	−	0	−	0
Indole		0	−	0	[−]	0	[−]	0
TSI slant, acid		0	v	20(3)	v	57(5)	−	0[d]
TSI butt, acid	−	0	v	6(31)	v	5(76)		0[d]
H_2S (TSI butt)	−	0	−	0	−	0	−	0[d]
H_2S (Pb ac paper)	+	97	v	76	+	86(5)	v	56[d]
Gelatin hydrolysis[d]	v	17	−	2	−	0	v	11[d]
Litmus milk	v	57 k	v	60 IR	−	0(10) Aw	−	0[d]
Pigment, Soluble					−	5 ta		
Insoluble	[yel]	100	[yel]	95	v	57 sl yel	v	36 pale yel
Growth at: 25°C	+	100	+	100	+	100	+	100[d]
35°C	+	100	+	100	+	100	+	100[d]
42°C	v	33	v	24	−	0	v	11
Esculin hydrolysis	[−]	0	+	91	[+]	100	+	100
Lysine decarboxylase	[−]	7	[−]	0	[−]	0	−	0[d]
Arginine dihydrolase	v	14	[−]	8	[−]	0	v	25[d]
Ornithine decarboxylase	−	3	−	0	−	0	−	0[d]
Nutrient broth, 0% NaCl	+	100	+	93	+	100	+	100[d]
Nutrient broth, 6% NaCl	v	62	−	4	v	25	−	0[d]

See footnotes on Chart 31.

CHART 33	*Pseudomonas pickettii*		∇a-1[d]		*Pseudomonas thomasii*[e]		*Pseudomonas marginata*
Number of strains	54		70		31		type strain, ATCC 10248
Test Performed	Sign	% +	Sign	% +	Sign	% +	Sign
Morphology	mrs		mrs		mrs		mrs
Motility, flagella	[m],p,1-2[b]		[m],p,1-2[b]		[m],p,1-2		[m],p,1-2[f]
Action on blood	v	21 ly	v	56 ly	v	24 gr	
Fermentative or oxidative	[O]		[O]		[O]		[O]
Carbohydrate base	OF		OF		OF		OF
Acid from: Glucose	[+]	100	[+]	100	[+]	100	[+]
Xylose	[+]	100	[+]	100	+	100	+
Mannitol		0	–	0	[+]	100	[+]
Lactose	[–]	0	[+]	100	[+]	100	–[g]
Sucrose	–	0		0	[–]	0	–
Maltose	[–]	0	[+]	100	[+]	100	[–]
Catalase	+	100	+	100	v	87	+
Oxidase	[+]	100	[+]	100	[+]	100	[–[h]]
Growth on: MacConkey	[+]	100	[+]	99(1)	[+]	100	[+]
SS	–	0	–	0	–	0	–
Cetrimide	–	0	–	1	–	0(3)	(+)
Simmons citrate	+	100	+	99	+	100	+
Urea, Christensen's	[+]	100	[+]	100	+ or (+)	81(19)	+
Nitrate reduction	+	100	+	100	v	13	–
Gas from nitrate	[+[c]]	100	[v]	86	[–]	0	–
Indole	–	0	–	0	–	0	
TSI slant, acid	–	0	–	0	–	0	
TSI butt, acid		0	–	0	–	0	–
H_2S (TSI butt)	–	0	–	0	–	0	
H_2S (Pb ac paper)	v	38	v	67	v	23	
Gelatin hydrolysis[a]	–	33	v	12	v	30	–
Litmus milk	k	90	v	83 k	v	52 k	(pep)
Pigment: Soluble			v	36 yel-ta	v	33 yel-ta	
Insoluble							
Growth at 25 C	+	100	+	100	+	97	+
35 C	+	100	+	100	+	100	+
42 C	+	94	v	83	v	84	–
Esculin hydrolysis	–	0	–	0	–	0	
Lysine decarboxylase	[–]	0	[–]	0	[–]	0	[–]
Arginine dihydrolase	[–]	0	[–]	6	[–]	3	[–]
Ornithine decarboxylase		0	–	0	–	0	
Nutrient broth, 0% NaCl	[+]	100	+	100	+	100	+
Nutrient broth, 6% NaCl	–	3	–	4	–	0	

[a]7-14 days' incubation.
[b]Weakly motile, may be difficult to demonstrate.
[c]May be negative if not tested in semi-aerobic nitrate broth.
[d]Shown by DNA hybridization to be a biovar of *P. pickettii*.
[e]Possibly a biovar of *P. pickettii*.
[f]Polar multitrichous flagellation according to Bergey's Manual (8th ed.).
[g]OF lactose held at room temperature after the 7th day, became acid between 8th and 21st day.
[h]Oxidase is positive but very weak in some strains according to Bergey's Manual (8th ed.)
[i]Less than 10 strains tested.
[j]Some delayed carbohydrate reactions required 8-14 days' incubation.

CHART 34	*Pseudomonas putrefaciens* (biotype 1)		*Pseudomonas putrefaciens* (biotype 2)		*Pseudomonas mallei*	
Number of strains	24		26		8	
Test Performed	Sign	% +	Sign	% +	Sign	% +
Morphology	mrs		mrs		cc	
Motility; flagella	[m];p,1-2		[m];p,1-2		[nm]	
Action on blood	v	75 LG	LG	92	ly	100
Fermentative or oxidative	O or n-o		[n-o]		[O]	
Carbohydrate base	OF		OF		OF	
Acid from: Glucose	[v]	17(33)	[−]	0	[+]	100
Xylose	[−]	0	[−]	0	v	12(50)[p]
Mannitol	−	0	−	0	v	62(14)[p]
Lactose		0	−	0	v	12(62)[p]
Sucrose	[+]	96(4)	−	0	[−]	0
Maltose	+	92(8)	−	0	v	0(75)[p]
Catalase	+	100	+	100	+	100
Oxidase	[+]	100	[+]	100	v	25
Growth on: MacConkey	[+]	100	[+]	100	v	88
SS	−	0(8)	+	92(4)	−	0
Cetrimide	−	4	−	0	−	0
Simmons citrate	−	4(4)	−	8	[−]	0
Urea, Christensen's	v	4(8)	v	42	v	12
Nitrate reduction	[+]	100	[+]	100	[+]	100
Gas from nitrate	[−]	0	[−]	0	[−]	0
Indole	−	0	−	0	−	0
TSI slant, acid	−	0	−	0	−	0
TSI butt, acid		0	−	0	−	0
H_2S (TSI butt)	[+]	96	[+]	100	−	0
H_2S (Pb ac paper)	+	100	+	100	+	100
Gelatin hydrolysis[a]	v	65	+	100	−	0
Litmus milk	v	61 pep	v	50 IR&pep	−	0
Pigment: Soluble	v	71 br-ta	br-yel	100	−	0
Insoluble						
Growth at: 25°C	+	100	+	100	+	100
35°C	+	96	+	100	+	100
42°C	v	38	v	23	[−]	0
Esculin hydrolysis	−	0	−	0	−	0
Lysine decarboxylase	−	0	−	0[l]	[−]	0
Arginine dihydrolase	−	0	−	0[l]	[+]	100
Ornithine decarboxylase	[+]	100	[+]	100[l]	−	0
Nutrient broth, 0% NaCl	[+]	100	[−]	0	+	100
Nutrient broth, 6% NaCl	v	43	[+]	100	−	0

See footnotes on Chart 33.

CHART 35	*Brucella abortus*		*Brucella melitensis*		*Brucella suis*		*Brucella canis*[c]	
Number of strains	81		48		190		28	
Test Performed	Sign	% +	Sign	% +	Sign	% +	Sign	% +
Morphology	[rv]		[rv]		[rv]		[rv]	
Motility	[nm]		[nm]		[nm]		[nm]	
Action on blood	v	25 hly	–	0[8]	v	17 al	v	20 sl hly
Fermentative or oxidative	[O]		[O]		[O]		[O]	
Carbohydrate base	OF		OF		OF		OF	
Acid from: Glucose	[v]	50(25)[9]	[v]	33[8]	[+]	100	[+ or +]	81(14)
Xylose	[+]	100[8]	[+]	100[8]	[+]	100	[+ or +]	81(19)
Mannitol		0[8]		0[8]	–	0		0
Lactose	–	0[8]	–	0[8]	–	0	–	0
Sucrose	[–]	0[8]	[–]	0[8]	[–]	0	[–]	0
Maltose		0[8]	–	0[8]	–	0	–	0
Glycerol								
Catalase	+	100	+	100[8]	+	100	+	100
Oxidase	[+]	96	[+]	100	[+]	95	[v]	72
Growth on: MacConkey	v	50(20)	v	62[8]	v	23(15)	v	12(29)
SS	–	0	–	0[8]	–	0		0
Simmons citrate	[–]	0	[–]	0[8]	[–]	0	[–]	0
Urea, Christensen's	[+]	98	[+]	100	[+]	100	[+]	100
Nitrate reduction	[+]	100	[+]	100[8]	[+]	100	[+]	100
Gas from nitrate	–	0	–	0[8]	[v]	52	[v]	77
Indole	[–]	0	[–]	0[8]	[–]	0	[–]	0
TSI slant, acid	–	0[8]	–	0[8]	–	0[8]	–	0
TSI butt, acid	–	0[8]	–	0[8]	–	0[8]	–	0
H_2S (TSI butt)	–	0[8]	–	0[8]	–	0[8]	–	0
H_2S (Pb ac paper)[a]	+	89(4)	v	39	+	95	v	82
Gelatin hydrolysis[b]	–	0[8]	–	0[8]	–	0[8]	–	0[8]
Growth at 25 C	v	43[8]	v	17[8]	v	70	v	42
35 C	+	100	+	100	+	100	+	100
42 C	v	14[8]	v	50[8]	v	50	v	33
Esculin hydrolysis	–	0[8]	–	0[8]	–	0[8]	–	0
Nutrient broth, 0% NaCl	+	100[8]	+	100[8]	+	100	+	100
Nutrient broth, 6% NaCl	–	0[8]	–	0[8]	–	0	–	0
Requires CO_2 atmosphere	v	67	–[d]	0[8]				

[a]Generally, *Brucella* strains are tested with heart infusion agar

[b]7–14 days' incubation

[c]Less than 10 strains tested

[d]Growth of 4 of 20 strains tested was enhanced in a candle jar

[e]A heavy suspension in 0.5% phenolized saline usually forms a "gel" within 30 minutes.

[f]Generally does not grow on the usual laboratory media; Bordet-Gengou is the medium of choice

[g]Heavy inoculum

[h]Requires or is stimulated by cystine or cysteine; grows on glucose-cystine-blood agar, Thayer-Martin medium containing hemoglobin, and may grow lightly on heart infusion agar with rabbit blood. Identification confirmed by slide agglutination or by direct FA staining

[i]The indole reaction was obtained in heart infusion broth supplemented with rabbit serum and was determined after 7 days' incubation

[j]Usually requires 20% serum or ascitic fluid

CHART 36	*Bordetella pertussis*[1]		*Francisella tularensis*[h]		*Streptobacillus moniliformis*	
Number of strains	51		53		16	
Test Performed	Sign	% +	Sign	% +	Sign	% +
Morphology	srs		[rv]		[plr,ch]	
Motility	nm		nm		nm	
Action on blood	[v]	81β	−	0		0
Fermentative or oxidative						
Carbohydrate base			CA		F[i]	
Acid from: Glucose			+	86(14)	[+ or(+)]	67(27)
Xylose					−	0(7)
Mannitol					[−]	0
Lactose					[−]	0
Sucrose						0
Maltose					[+ or(+)]	67(33)
Glycerol			v	46(7)		
Catalase	+	100	+	93	[−]	0
Oxidase	[+]	94	[−]	2	[−]	0
Growth on: MacConkey			[−]	0	[−]	0
SS			−	0	−	0
Simmons citrate			−	0		0
Urea, Christensen's	[−[g]]	0	[−]	0		0
Nitrate reduction			[−]	4	[−]	0
Gas from nitrate			−	0		
Indole			−	0(3)[j]		0
TSI slant, acid			−	0	v	71
TSI butt, acid				0	v	57
H_2S (TSI butt)			−	0	−	0
H_2S (Pb ac paper)[a]			v	42	v	71
Gelatin hydrolysis[b]			−	0	−	0
Growth at: 25 C			v	67	+	100
35 C			+	100	+	100
42 C			−	0	v	40
Esculin hydrolysis			−	0		
Nutrient broth, 0% NaCl			−	6		0
Nutrient broth, 6% NaCl			−	0		0
Requires CO_2 atmosphere						

See footnotes on Chart 35.

SPECIES AND BIOTYPES OF *BRUCELLA* ASSOCIATED WITH HUMAN DISEASE[a]

CHART 37						Growth on dye media					Agglutination in Mono-specific sera[d]				
						Thionin			Basic fuchsin						
Species	Biotype	CO_2 required	H_2S production[b]	Urease[c] < 5 min	Urease[c] < 5 min	1:25,000	1:50,000	1:100,000	1:50,000	1:100,000	*B. abortus*	*B. melitensis*	Acriflavin	Lysis by phage TB[d,e] RTD	Lysis by phage TB[d,e] RTDX10[f]
B. melitensis	1			v	+	−	+	+	+	+	−	+	−		
	2			v	+		+	+	+	+	+	−		−	
	3			v	+		+	+	+	+	+	+	−		
B. abortus	1	v	+		+[f]		−	−	+	+	+	−	−	+	+
	2	+	+		+			−	−	−	+			+	+
	3	v	+		+	+	+	+	+	+	+	−	−	+	+
	4	v	+		+				+	+		+		+	+
	5				+		+	−	+	+	−	+		+	+
	6		v		+		+	−	+	+	+		−	+	+
	7		v		+		+	+	+	+	+	+		+	+
	8	+			+		+	+	+	+	−	+		+	+
	9	v	+		+		+	+	+	+		+		+	+
B. suis	1		+	+		+	+	+		−	+	−	+		+
	2			+		−	+	+			+		−		+
	3			+		+	+	+	+	+	+				+
	4			+		+	+	+	+	+	+	+	+		+
B. canis	1			+		+	+	+	−[h]	v			+[i]		−

[a]Reactions based on those obtained in the SBL, CDC, and those given in Laboratory Techniques in Brucellosis, 2nd ed., Geneva: World Health Organization, 1975

[b]Heart infusion agar with lead acetate paper.

[c]Warm urea slant inoculated heavily.

[d]Agglutination and phage procedures given in reference cited in a above.

[e]Tbilisi, a *Brucella* phage originally isolated in the USSR, has been designated as the reference phage.

[f]Rare strain urease negative.

[g]Usually immediate or instant reaction

[h]Some strains studied in SBL, CDC, grew at this concentration

[i]*B. canis* forms a stringy mass or "gel" of increased viscosity when suspended in phenolized saline and agglutinates in specific antiserum.

SPECIES AND BIOTYPES OF *HAEMOPHILUS* ISOLATED FROM HUMANS AND TWO ANIMAL SPECIES

CHART 38 Species and Biotypes	*No. strains*	*X factor required*	*V factor required*	*Porphyrin test*	*Hemolysis*	*Glucose, acid*	*Lactose, acid*	*Urease*	*Indole*	*Ornithine decarboxylase*
H. influenzae	154									
biotype I	34	+	+		−	+		+	+	+
biotype II	62	+	+		−	+	−	+	+	−
biotype III[a]	36	+	+		−	+	−	+		−
biotype IV	14	+	+			+	−	+	−	+
biotype V	5	+	+			+			+	+
biotype VI[b]	1	+	+		−	+	−		+	
unclassified	2	+	+	−		+	−		−	−
H. haemolyticus	18	+	+	−	+	+		+	v	−
H. parainfluenzae	41									
biotype I	9		+	+	−	+	−	−	−	+
biotype II	14		+	+	v	+		+		+
biotype III	18	−	+	+	v	+	−	+	−	−
H. paraphrophilus	16		+	+		+	+			−
H. segnis[c]			+	+		w	−	−		
H. ducreyi[d]	31	+	−	−	−	−			−	−
H. haemoglobinophilus (*H. canis*)[c]	2	+	−			+			+	−
H. equigenitalis	2				−		−			

[a] *H. aegyptius* has the characteristics of *H. influenzae* biotype III but according to Mazloum et al. (Acta Path. Microbiol. Immunol. Scand. Sect. B, 90:109-112, 1982), *H. aegyptius* can be differentiated from strains of *H. influenzae* by the following characteristics: No growth on tryptic soy agar in the presence of X and V factors, inhibition by trooleandomycin, distinct bacillary morphology (vs. coccobacillary morphology), and no acid production from xylose (some biotype III strains, of *H. influenzae* also are negative).

[b] As defined by Kilian (Manual of Clinical Microbiology, 3rd ed., p. 334). Oberhofer and Back (J. Clin. Microbiol. 10: 168-174, 1979) described an *H. influenzae* biotype VI which is urease −, indole −, and ornithine decarboxylase +.

[c] Based on Kilian's description, (J. Gen. Microbiol. 93:9-62, 1976). Oberhofer's *H. parainfluenzae* biotype IV has these same characteristics (J. Clin. Microbiol. 10:168-174, 1979).

[d] Alkaline phosphatase: *H. ducreyi* positive; *H. haemoglobinophilus* negative.

DIFFERENTIATION OF SPECIES OF *NEISSERIA* AND *BRANHAMELLA*

CHART 39	*Branhamella catarrhalis*		*Neisseria elongata*[a]	*Neisseria flavescens*		*Neisseria gonorrhoeae*		*Neisseria meningitidis*	
Number of strains	74		1	10		197		375	
Test Performed	Sign	% +	Sign	Sign	% +	Sign	% +	Sign	% +
Oxidase	+	100	+	+	100	+	100	+	100
Acid from carbohydrates in CTA base									
Glucose		0	−		0	+	90(3)	+	99
Lactose		0			0		0		0
Sucrose		0		−	0	−	0		0
Maltose		0	−	−	0		0	+	99
Fructose		0	−	−	0		0		0
Growth on:									
Nutrient agar, 0% NaCl, 35 C	+	97		+	100[b]	−	0		1
Nutrient agar, 0% NaCl, 25 C	v	86		v	86[b]		0	−	0
Thayer Martin Medium-Modified (>10 colonies)	v	17		−	0[b]	+	96	+	100
Nitrate reduction	+	92	−	−	0		0		0
Gas from nitrate		0		−	0		0		0
Nitrite reduction	+	91		+	100[b]	−	0	v	29[b]
Pigmentation on Loeffler slant	v	25 sl yel	−	yel	90	−	10 sl yel		2 sl yel
Synthesis of polysaccharide from sucrose		0		+	100	−	0		0

[a]Coccobacillus, short straight rod
[b]Less than 10 strains tested
[c]ONPG + (+, 100%)

DIFFERENTIATION OF SPECIES OF *NEISSERIA* AND *BRANHAMELLA*

CHART 40	*Neisseria lactamica*		*Neisseria subflava*		*Neisseria sicca*		*Neisseria mucosa*	
Number of strains	287		153		43		30	
Test Performed	Sign	% +	Sign	% +	Sign	% +	Sign	% +
Oxidase	+	100	+	100	+	100	+	100
Acid from carbohydrates in CTA base								
Glucose	+	96(3)	v	68(6)	+ or(+)	78(14)	+ or(+)	83(10)
Lactose	(+)	95(5)		0	–	0	–	0
Sucrose		0	v	56(5)	+	92(8)	+	90(10)
Maltose	+	98(2)	+	99(1)	+	95(5)	+	90(10)
Fructose		0	v	77(5)	+	100	+	90(10)
Growth on:								
Nutrient agar, 0% NaCl, 35 C	v	74	+	95	+	98(10)	+	100
Nutrient agar, 0% NaCl, 25 C	v	22	+	95	+	100	+	96
Thayer-Martin Medium-Modified (>10 colonies)	+	100	–	8	–	0[b]	–	0
Nitrate reduction	–	0	–	0		0	+	100
Gas from nitrate	–	0	–	0		0	+	100
Nitrite reduction	+	100[b]	v	80[b]	+	100[b]	+	100
Pigmentation on Loeffler slant	v	38 sl yel	yel	99	–	0	v	50 sl yel
Synthesis of polysaccharide from sucrose	–	0[b]	v	73	+	100	+	100[b]

See footnotes on Chart 39

DESCRIPTIONS OF SPECIES

Achromobacter xylosoxidans

Gram-negative short to medium straight rod, motile (peritrichous flagella); oxidase-positive; oxidizes glucose (weakly) and xylose; grows on MacConkey and SS agars; citrate-positive, urease-negative; nitrate-positive, sometimes with gas; H_2S-negative (TSI).

Sources[1] (135 strains)

Blood (14%), cerebrospinal fluid (11%), urine (10%), sputum (6%), wound (5%), ear and bubo (3% each), saline, water, skin, throat, mouth, and stool (2% each), other (15%), unknown (21%).

Reference strain[2]

KC1064 < E. Yabuuchi, KM453 (type strain), from ear discharge.

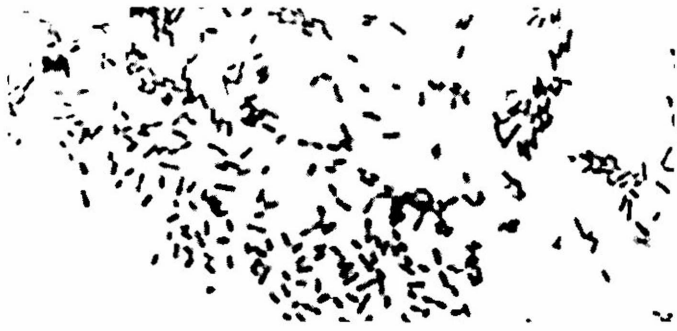
Literature

Jpn J Microbiol 15:477-481, 1971; Int J Syst Bacteriol 24:470-477, 1971.

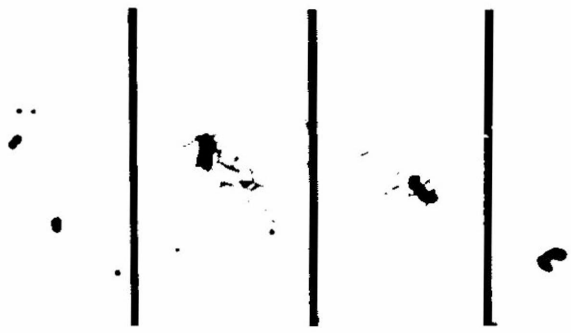
Comment

Appears barred in Gram stain of heart infusion broth culture.

[1]Sources listed are human unless otherwise stated.
[2]Culture(s) photographed.



Gram stain BAP 35°C 24 h ×1700.



Flagella stain FB 25°C 24 h ×1700.

Achromobacter xylosoxidans

	NUMBER OF STRAINS 135		
TEST PERFORMED	**SIGN**	**%**	**# + /T***
Morphology	mrs		
Motility; flagella	[m]; pe		
Action on blood	v	42 ly	51 122,24 LG,21 gr,5 lav,2 al
Fermentative or oxidative	[0]		
Carbohydrate base	OF		
Acid from			
Glucose	[v]	78	2,97w 127
Xylose	[+]	99	123,lw 125
Mannitol		0	0 129
Lactose		0	0 129
Sucrose	-	0	0 130
Maltose	-	0	0 128
Catalase	+	98	126 128
Oxidase	[+]	100	133 133
Growth on:			
MacConkey	[+]	100	133 133
SS	[+]	98	130 133
Cetrimide	+	95(1)	119,4w(1) 129
Simmons Citrate	+	95	128 130
Urea, Christensen's	[ ]	0	0 128,2 pk
Nitrate reduction	[+]	100	133 133
Gas from nitrate	[v]	60	80 133
Indole	-	0	0 134
TSI slant, acid	-	0	0 132
TSI butt, acid		0	0 132
H_2S (TSI butt)	[ ]	0	0 133
H_2S (Pb ac paper)		0	0 133
Gelatin hydrolysis[d]		0	0 112
Litmus milk	k	95	122 128
Pigment		5 br sol	6 112
Growth at:			
25 C	+	98	120 122
35 C	+	100	122 122
42 C	v	84	102 122
Esculin hydrolysis		0	0 118
Lysine decarboxylase		0	0 16
Arginine dihydrolase	v	13	2 16
Ornithine decarboxylase		0	0 16
Nutrient broth, 0% NaCl	+	100	118 118
Nutrient broth, 6% NaCl	v	69	82 118

[d]=14 days' incubation
*T = total strains tested.

Acinetobacter calcoaceticus

Gram-negative coccoid thick rod occurring in pairs and short chains, with some filaments and pleomorphism, nonflagellated (some strains show a twitching motility under special conditions); capsules and fimbriae variable; oxidase-negative; usually grows on MacConkey agar; may or may not produce acid from glucose, xylose, lactose, and maltose, usually does not reduce nitrate.

Sources (499 acid-producing strains)

Urine (14%), blood (10%), sputum (8%), wound (7%), throat (6%), cerebrospinal fluid (5%), ear (4%), dialysis fluid (4%), genital (2%), eye (2%), other (23%), unknown (15%).

Sources (253 non-acid-producing strains)

Blood (10%), sputum (8%), cerebrospinal fluid (7%), wound (7%), urine (6%), genital (5%), eye (4%), throat and trachea (4%), lung and bronchial washing (4%), water (3%), ear, brain, nose, peritoneal fluid, environment, abscess, bile, food (2% each), other (16%), unknown (10%).

Reference strains

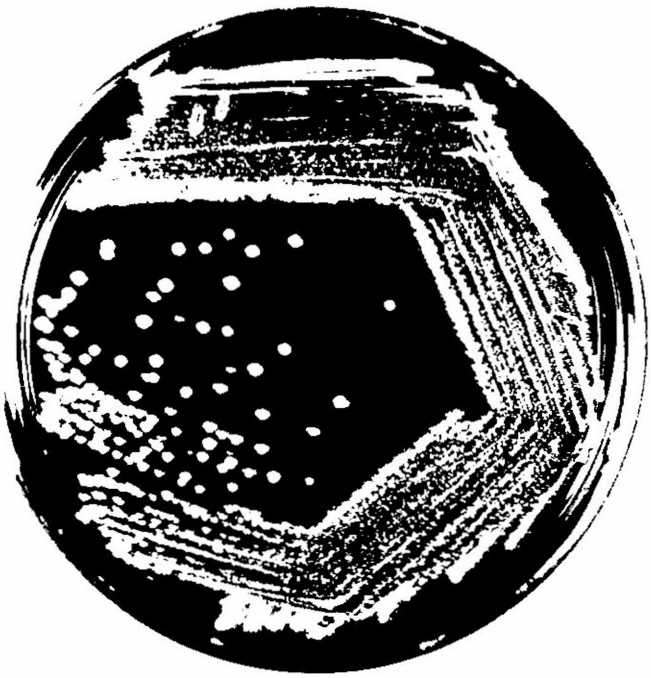
KC1125 ← W. B. Cherry ← ATCC, 19638; KC1127 ← W. B. Cherry ← ATCC, 23055.

Literature

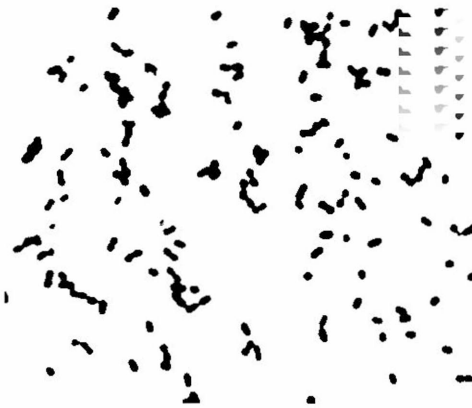
J Bacteriol 95:1520-1541, 1968; J Bacteriol 112:917-931, 1972.

Comments

One of the more frequent non-fermenters encountered in the clinical laboratory. Synonyms for acid-producing strains: *Herellea vaginicola, A. anitratus, Bacterium anitratum;* synonyms for non-acid-producing strains: *Mima polymorpha, Acinetobacter lwoffi*. Usually resistant to penicillin. Some strains are beta-hemolytic; these are usually proteolytic. Both biotypes react positively in the *Acinetobacter* transformation test. A fish-like odor may be detected with some strains. In some strains rod forms may be rare.



KC1127 BAP 35°C 24 h ×1 (incident light).



KC1125 Gram stain BAP 35°C 24 h ×

Acinetobacter calcoaceticus (A. anitratus)

NUMBER OF STRAINS 501			
TEST PERFORMED	**SIGN**	**%**	**# / T**
Morphology	cc		
Motility, flagella	[nm]		
Action on blood	v	45 hy	209/461,50 β,44 gr,21 LG,18 br
Fermentative or oxidative	[O]		
Carbohydrate base	OF		
Acid from:			
Glucose	[+]	100	359/359
Xylose	+	99	337/338
Mannitol		2	6w/348
Lactose	+	97(2)	315/28w(7)/355
Sucrose		0	0/347
Maltose	v	63(6)	62/158w(7)/350
Catalase	+	100	491/491
Oxidase	[ ]	0	0/488
Growth on:			
MacConkey	[+]	99(1)	492(3)/497
SS	v	20(1)	101(4)/492
Cetrimide		3	14/483
Simmons Citrate	+	93	454/488
Urea, Christensen's	v	28(18)	133(86)/479,36 pk
Nitrate reduction	[+]	1	4/488
Gas from nitrate	+	0	0/488
Indole		0	0/500
TSI slant, acid		5	24/494
TSI butt, acid	+	0	0/494
H_2S (TSI butt)	+	0	0/492
H_2S (Pb ac paper)	v	32	61,96w/492
Gelatin hydrolysis[a]		9	30/351
Litmus milk	v	37(10) A	180/491,183 IR,17 k,10 pep
Pigment	+	7 yel-ta sol	22/312
Growth at:			
25 C	+	97	470/486
35 C	+	97	470/486
42 C	v	87	425/486
Esculin hydrolysis		0	1/488
Nutrient broth, 0% NaCl	+	100	463/463
Nutrient broth, 6% NaCl	v	22	103/463

[a]7-14 days' incubation.

Acinetobacter calcoaceticus (A. lwoffi)

	NUMBER OF STRAINS 253		
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	cc		
Motility: flagella	[nm]		
Action on blood	v	48 ly	49/101;29 β,15 gr,4 br,4 al
Fermentative or oxidative	[n-o]		
Carbohydrate base	[OF]		
Acid from:			
Glucose	[-]	0	0/253
Xylose		0	0/253
Mannitol	-	0	0/253
Lactose	-	0	0/253
Sucrose	-	0	0/253
Maltose	-	0	0/253
Catalase	+	100	253/253
Oxidase	[-]	0	0/253
Growth on:			
MacConkey	[+]	90(7)	229(17)/253
SS	v	7(5)	18(14)/253
Cetrimide		0	0/253
Simmons Citrate	v	42	105/253
Urea, Christensen's		5(4)	12(10)/253
Nitrate reduction		3	7/253
Gas from nitrate		0	0/253
Indole		0	0/253
TSI slant, acid		0	0/229
TSI butt, acid	-	0	0/229
H_2S (TSI butt)	-	0	0/253
H_2S (Pb ac paper)	v	58	146/253
Gelatin hydrolysis[a]	-	4	8/223
Litmus milk	v	86 k	74/86;8 IR,4 pep
Pigment	v	13 yel-br sol	34/253
Growth at:			
25°C	+	100	250/250
35°C	+	100	250/250
42°C	v	63	157/250
Esculin hydrolysis	-	0	0/241
Nutrient broth, 0% NaCl	[+]	97	241/248
Nutrient broth, 6% NaCl	--	9	22/248

[a]7-14 days' incubation.

Actinobacillus actinomycetemcomitans

Gram-negative very small rod, nonmotile; fastidious; requires or is enhanced by candle-jar atmosphere; ferments glucose, sometimes with slight gas, not lactose and sucrose (biotypes are based on other carbohydrates used) (xylose, mannitol and maltose); catalase- and nitrate-positive; MacConkey-negative, urease-negative, indole-negative.

Sources (120 strains)

Blood (56%), neck (7%), lung tissue (5%), mandibular and submandibular abscesses (4%), abscess-site unknown (4%), wound, rib, and facial sinus (2% each), other (15%), unknown (3%).

Reference strain

KC517 < NCTC, 9710, from abscess.

Literature

Topley WWC, Wilson GS: Principles of Bacteriology and Immunity. 1st ed. London, Edward Arnold, 1929, p 256; J Infect Dis 111: 85-94, 1962; N Engl J Med 275:181-188, 1966.

Comments

Growth in broth is granular, with colonies adhering to sides of tube; star-like colonies may be observed on prolonged incubation. Often found in association with *Actinomyces*, especially *A. israelii*.



BAP 35°C 24 h ×1 (incident light).



BAP 35°C 24 h ×6 (axial light).

Gram stain BAP 35°C 24 h ×1700.

Actinobacillus actinomycetemcomitans

TEST PERFORMED	SIGN	NUMBER OF STRAINS 120							% +	# + /T
Morphology	rx									
Motility, flagella	[mm]									
Gas from glucose	x								28	33/120
Action on blood	x								44 al	32/72/1 st day
Fermentative or oxidative	[F]									
Carbohydrate base	F[a]									
Number of strains	63	30	15	6	4	1	1			
Acid from:										
Glucose	+	+	+	+	+	+	+	+	83(16)	100/120
Xylose	-	-	-	-	-	-	-	-	33(9)	39/120
Mannitol	-	-	-	-	-	-	-	-	66(16)	79/120
Lactose	-	-	-	-	-	-	-	-	0	0/120
Sucrose	-	-	-	-	-	-	-	-	0	0/120
Maltose	-	-	-	-	-	-	-	-	80(15)	96/120
10% Lactose	ngt									
Catalase	[+]								99	110/w 120
Oxidase	x								19	6/15w 111
Growth on:										
MacConkey	[-]								4(1)	5w/D 120
SS									0	0/120
Simmons Citrate									0	0/120
Urea, Christensen's	[-]								0	0/120
Nitrate reduction	[+]								100	118/w 119
Gas from nitrate									0	0/120
Indole	[-]								0	0/120
TSI slant, acid	+								100	101/19w 120
TSI butt, acid	[+]								100	101/19w 120
H_2S (TSI butt)									0	0/119
H_2S (Pb ac paper)	+								91	88/20w 119
MR									8	4/1w 60
VP									0	0/55
Gelatin hydrolysis[b]									0	0/45
Litmus milk									4 HR	4/118/1 A
Growth at:										
25 C	x								24	24/98
35 C	+								100	112/112
42 C									4	4/98
Esculin hydrolysis									0	0/120
Nutrient broth, 0% NaCl										4/3w 98
Nutrient broth, 6% NaCl									0	0/98

[a] 1-2 drops of rabbit serum may need to be added per 3 ml of medium; reactions may be obtained within 4h using the Rapid Sugar Tes
[b] 7-14 days' incubation.

Actinobacillus equuli

Gram-negative coccobacillary to rod form, nonmotile; ferments glucose, xylose, mannitol, lactose, sucrose, maltose, raffinose, melibiose, and trehalose; oxidase- and urease-positive; indole- and esculin-negative; usually produces catalase; nitrate-positive.

Sources (27 strains)

Horse (27%), man (animal bite or wound) (19%), pig (12%), other animals (11%), unknown (31%).

Reference strain

KC601 < NCTC, 3365, from foal, pyemic nephritis.

Literature

Ann Inst Pasteur, Paris 104:108-114, 1963; Int J Syst Bacteriol 16:18-19, 1966.

Comments

Pathogenic for horses and pigs. Colonies may be sticky.



BAP 35°C 24 h ×1 (axial light).



Gram stain BAP 35°C 24 h ×1700.

Actinobacillus equuli

TEST PERFORMED	SIGN	NUMBER OF STRAINS 19 % +	# + /T
Morphology	sts		
Motility	[nm]		
Gas from glucose		0	0/19
Action on blood	v	11 gr	2/19;2 al;1 hy
Fermentative or oxidative	[F]		
Carbohydrate base	F		
Acid from			
Glucose	+	100	19/19
Xylose	+	100	19/19
Mannitol	+	100	19/19
Lactose	+	95	18/19
Sucrose	+	100	18/19
Maltose	+	95	18/19
Trehalose	[+]	100	19/19
Melibiose	[+ or(+)]	74(26)	15(4)/19
Raffinose	+	100	19/19
10% Lactose	v	35	6w/17
Catalase	[v]	68(5)	10,3w(1)/19
Oxidase	[+]	100	17,2w/19
Growth on:			
MacConkey	v	84(5)	16(1)/19
SS	-	0	0/19
Simmons Citrate		0	0/19
Urea, Christensen's	[+][a]	100	19/19
Nitrate reduction	[+]	100	19/19
Gas from nitrate	-	0	0/19
Indole	-	0	0/19
TSI slant, acid	+	100	0/19
TSI butt, acid	+	100	0/19
H_2S (TSI butt)		0	0/19
H_2S (Pb ac paper)	+	100	0/19
MR	v	87	0/19
VP	v	12	0/19
Gelatin hydrolysis[b]	v	47	0/19
Litmus milk	v	83 A	0/19
Growth at:			0/19
25 C	v	89	0/19
35 C	+	100	0/19
42 C	v	31	0/19
Esculin hydrolysis	[+]	0	0/19
Lysine decarboxylase	-	0	0/9
Arginine dihydrolase	-	0	0/9
Ornithine decarboxylase		0	0/9
Nutrient broth, 0% NaCl	v	89	17/19
Nutrient broth, 6% NaCl	-	5	1/19

[a]May require a drop of serum on slant or a heavy inoculum.
[b]7-14 days' incubation.

Actinobacillus lignieresii

Gram-negative coccobacillary to rod form, nonmotile; ferments glucose, xylose, mannitol, lactose (late), sucrose, and maltose, not trehalose or melibiose; oxidase- and urease-positive; indole- and esculin-negative; usually produces catalase; nitrate-positive.

Sources (23 strains)

Bovine (17%), human wound (9%), horse bite (9%), human finger (4%), other animals (dog, sheep, rat) (13%), unknown (48%).

Reference strain

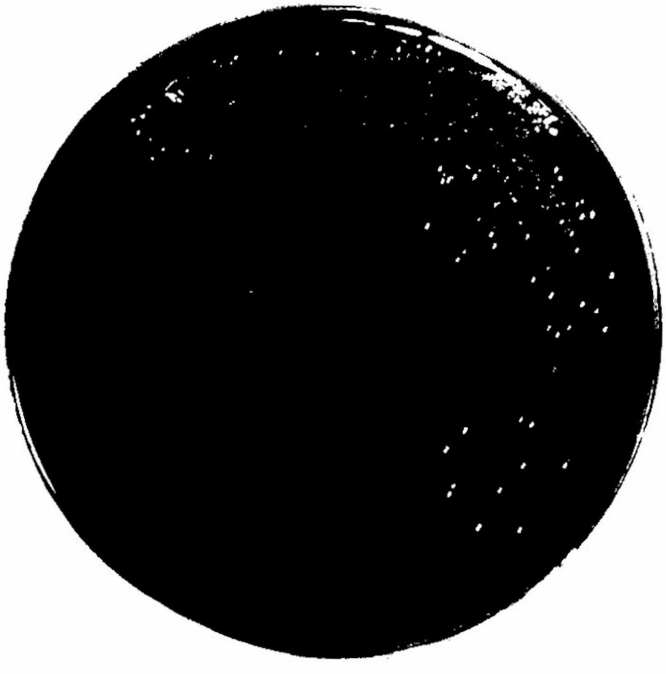
KC208 < NCTC, 4975, from bovine lesions.

Literature

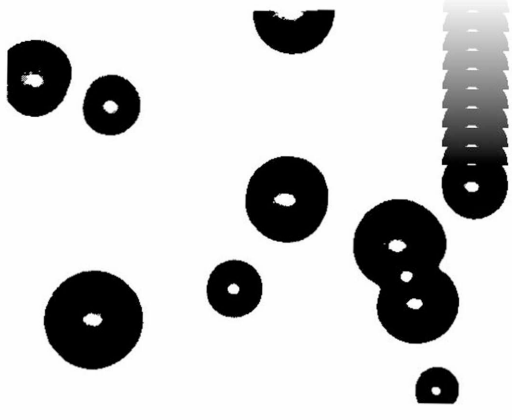
J Gen Microbiol 19:555, 1958; Ann Inst Pasteur (Paris), 104:108-114, 1963; Int J Syst Bacteriol 16:18-19, 1966.

Comment

Pathogenic for sheep and cattle.



BAP 35°C 24 h ×1.



BAP 35°C 24 h ×6.



Gram stain BAP 35°C 24 h ×1700.

Actinobacillus lignieresii

NUMBER OF STRAINS 30			
TEST PERFORMED	**SIGN**	**%**	**# + / T**
Morphology	sts		
Motility	[nm]		
Gas from glucose		0	0/23
Action on blood	v	17 al	4/23:1β
Fermentative or oxidative	[F]		
Carbohydrate base	F		
Acid from			
Glucose	+	96(4)	22/F/23
Xylose	+ or (+)	87(13)	20/F/23
Mannitol	+	91(9)	19/Fw/21/22
Lactose	v	17(61)	3/Fw/14/23
Sucrose	+	96(4)	23/F/24
Maltose	+ or (+)	83(17)	19/F/23
Trehalose	[F]	0	0/24
Melibiose	[F]	0	0/24
Raffinose	v	25(46)	6/F/24
10% Lactose	v	47	6/3w/19
Catalase	[v]	89	16/8w/22
Oxidase	[+]	100	19/3w/22
Growth on:			
MacConkey	v	67(8)	16/2/24
SS		0	0/22
Simmons Citrate		0	0/23
Urea, Christensen's	[+][a]	100	22/22
Nitrate reduction	[+]	100	23/23
Gas from nitrate		0	0/23
Indole		0	0/23
TSI slant, acid	+	100	23/23
TSI butt, acid	+	100	23/23
H_2S (TSI butt)		0	0/23
H_2S (Pb ac paper)	+	100	23/23
MR	+	100	9/6w/15
VP		0	0/21
Gelatin hydrolysis[b]		0	0/23
Litmus milk	v	29 A	7/24/3 IR/2 w
Growth at:			
25 C	v	82	18/22
35 C	+	100	22/22
42 C	v	14	3/22
Esculin hydrolysis	[F]	0	0/23
Lysine decarboxylase		0	0/7
Arginine dihydrolase		0	0/7
Ornithine decarboxylase		0	0/7
Nutrient broth, 0% NaCl	+	100	9/9
Nutrient broth, 6% NaCl		10	1/9

[a]May require a drop of serum on slant or a heavy inoculum
[b]7-14 days' incubation.

Actinobacillus suis

Gram-negative coccobacillary to rod form, nonmotile; ferments glucose, xylose, lactose, sucrose, maltose, and sometimes mannitol; oxidase-, urease-, and esculin-positive; indole-negative, usually produces a clear zone of hemolysis; usually grows on MacConkey agar; reduces nitrate, usually produces catalase.

Sources (41 strains)

Horse (22%), human wound (15%), sputum and upper respiratory (15%), cattle (10%), animal bite (donkey, hamster, zebra) (7%), pig (7%), human blood (5%), finger lesion (2%), other animals (12%), unknown (5%).

Reference strain

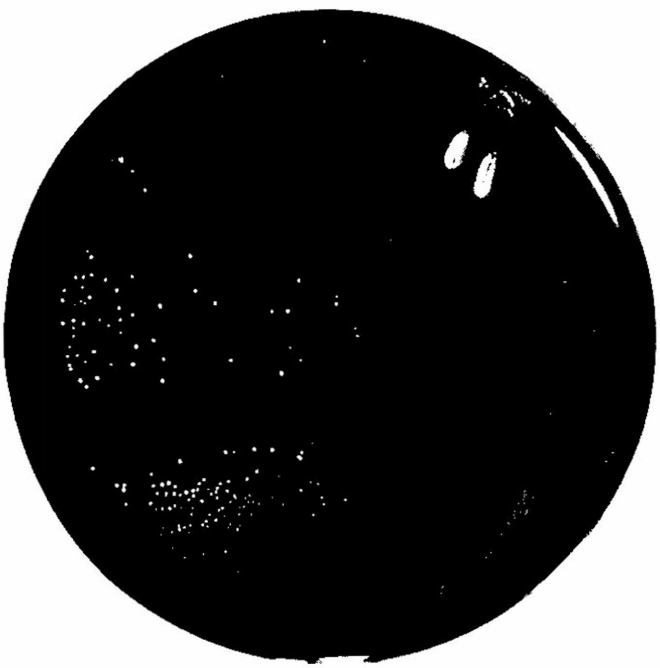
KC560 < P.W. Wetmore, 1627, from hock of piglet.

Literature

Tijdschr Diergeneesk 87:450-458, 1962; Deut Tieraerztl Wochenschr 71:457-461, 1964; Kilian M, Frederiksen W, Biberstein E L (eds): *Haemophilus, Pasteurella* and *Actinobacillus*. New York, N.Y. Academic Press, 1981, pp 286-287.

Comments

Van Dorssen and Jaartsveld (1962), Zimmerman (1964), and Kilian and Frederiksen (1981) have variously described *Actinobacillus suis*. Strains we have encountered and considered to be *A. suis* may represent a heterogeneous group and should be studied further.



BAP 35°C 24 h ×1 (incident and transmitted light)

Gram stain BAP 35°C 24 h ×1700.

Actinobacillus suis

TEST PERFORMED	SIGN	% +	# + /T
	NUMBER OF STRAINS 33		
Morphology	srs		
Motility	[nm]		
Gas from glucose		0	0/33
Action on blood	[v]	76 β	25,33;2 al,1 ly,1 LG
Fermentative or oxidative	[F]		
Carbohydrate base	F		
Acid from			
Glucose	+	94(6)	30,1w(2)/33
Xylose	+	94(6)	30,1w(2)/33
Mannitol	v	54	18/33
Lactose	+ or(+)	79(18)	25,1w(6)/33
Sucrose	+	94(6)	30,1w(2)/33
Maltose	+	94(6)	30,1w(2)/33
Trehalose	+	95	21/22
Melibiose	+ or(+)	82(13)	18(3)/22
Raffinose	+	100	22/22
Mannose	+	91(9)	20(2)/22
10% Lactose	+	94	15/16
Catalase	[v]	85	14,14w/33
Oxidase	[+]	100	30,3w/33
Growth on:			
MacConkey	[+ or(+)]	82(12)	20,7w(4)/33
SS	−	3	1/33
Simmons Citrate	−	0	0/33
Urea, Christensen's	[+][a]	97(3)	31,1w(1)/33
Nitrate reduction	[+]	100	33/33
Gas from nitrate		0	0/33
Indole	[−]	0	0/33
TSI slant, acid	+	100	32,1w/33
TSI butt, acid	+	100	32,1w/33
H_2S (TSI butt)		0	0/33
H_2S (Pb ac paper)	+	100	29,4w/33
MR	v	24	7w/33
VP	v	50	6,8w/28
Gelatin hydrolysis[b]	−	6	2/31
Litmus milk	v	82/A	14,13w/33,1 kw,1 IR
Growth at			
25°C	+	94	31/33
35°C	+	97	32/33
42°C	v	31	10/32
Esculin hydrolysis	[+]	100	33/33
Lysine decarboxylase		0	0/7
Arginine dihydrolase	−	0	0/7
Ornithine decarboxylase	−	0	0/7
Nutrient broth, 0% NaCl	v	88	22/25
Nutrient broth, 6% NaCl	−	0	0/25

[a]May require a drop of serum on slant or a heavy inoculum.
[b]7-14 days' incubation.

Aeromonas hydrophila

Gram-negative rod, motile (single polar flagellum). Ferments glucose, mannitol, and other carbohydrates; grows on MacConkey agar; oxidase-positive, arginine dihydrolase- and sometimes lysine decarboxylase-positive, ornithine decarboxylase-negative; does not require NaCl.

Sources (115 strains)

Wounds (20%), blood (15%), stool (9%), sputum (8%), gall bladder (4%), intraabdominal (4%), appendix (3%), urine, placenta, knee drain, mosquito larva, horse rectum, water, trachea (2% each), other (16%), unknown (6%).

Reference strain

KC1343 < J. L. Mitchell, Brooks AFB <- ATCC, 7966 (neotype strain), from milk.

Literature

J Bacteriol 46:213-214, 1943; Int J Syst Bacteriol 17:27, 1967.

Comments

Some strains prefer 25°C; 2- to 4-h cultures have lateral flagella with short wavelength. We have made no attempt to differentiate between *Aeromonas hydrophila*, *A. punctata*, and *A. sobria*. For further information concerning species and subspecies of *Aeromonas*, see Lennette EH, Balows A, Hausler WJ, Truant JP (eds): Manual of Clinical Microbiology, 3rd ed. Washington, D.C., American Society for Microbiology, 1980, pp 220-225.



BAP 35°C 24 h ×1 (incident and transmitted light).



Gram stain BAP 35°C 24 h ×1700.



Flagella stain BAP 35°C 24 h ×1700.

Aeromonas hydrophila

TEST PERFORMED	SIGN	% +	# + /T
	NUMBER OF STRAINS 115		
Morphology	mrs		
Motility; flagella	[m],p,1-2		
Gas from glucose	[v]	72	83/115
Action on blood	v	76 β	85/111;26 ly or LG
Fermentative or oxidative	[F]		
Carbohydrate base	F		
Acid from:			
Glucose	+	97(3)	112(3)/115
Xylose		1	1/115
Mannitol	[+]	100	109,6w/115
Lactose	v	20(9)	18,5w(10)/113
Sucrose	v	87(1)	99,1w(1)/115
Maltose	+	99	112,2w/115
10% Lactose	v	25	24/95
Catalase	+	97	79,30w/112
Oxidase	[+]	99	110,4w/115
Growth on:			
MacConkey	[+]	100	115/115
SS	+	97	109/112
Simmons Citrate	v	52(7)	58(8)/112
Urea, Christensen's		2	2/113
Nitrate reduction	+	97	112/115
Indole	+	92	106/115
TSI slant, acid	v	53	60/114
TSI butt, acid	+	100	115/115
H_2S (TSI butt)		0	0/109
H_2S (Pb ac paper)	+	92	77,29w/115
MR	v	55	46,16w/112
VP	v	78	74/95
Gelatin hydrolysis[a]	+	96	83/86
Litmus milk	v	85 pep	97/114;13 A, 4 IR
Growth in nutrient broth:			
0% NaCl	[+]	100	93/93
6% NaCl	v	14	13/90
Growth at			
25 C	+	100	110/110
35 C	+	100	110/110
42 C	v	84	92/110
Esculin hydrolysis	v	74	79/106
Lysine decarboxylase	[v]	17	18/104
Arginine dihydrolase	[+]	99	103/104
Ornithine decarboxylase	[-]	0	0/104

[a]7-14 days' incubation

Agrobacterium radiobacter

Gram-negative rod, motile (peritrichous flagella; if only one flagellum is observed, it is usually polarly, subpolarly, or laterally located); oxidizes glucose, xylose, mannitol, lactose, sucrose, and maltose; grows on MacConkey agar; oxidase and urease-positive, 3-ketolactonate- and nitrate-positive (most strains also reduce nitrite, usually without gas production); does not produce indole; does not produce lysine decarboxylase or arginine dihydrolase.

Sources (38 strains)

Sputum (21%), genitourinary (10%), blood (8%), wound (8%), eye (8%), bronchial washing (5%), other (37%), unknown (3%).

Reference strain

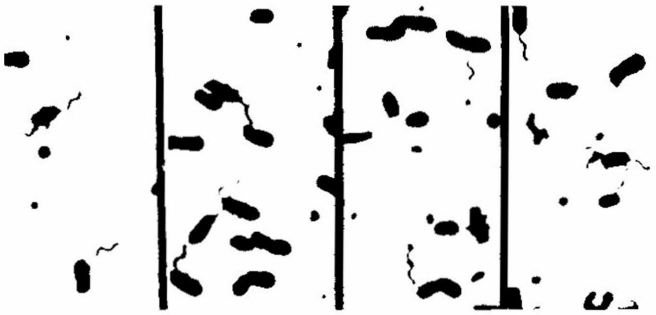
KC1283 < ATCC, 19358.

Literature

J Clin Microbiol 5:172-177, 1977.

Comments

Most of the strains in the group formerly designated ΣId-3 have been identified as *A. radiobacter*. Distinctive clearing zone is produced at the surface of litmus milk in 24-48 h; a strongly positive H_2S test results with lead acetate paper placed over a TSI agar slant.



Flagella stain FB 25°C 24 h ×1700.

Agrobacterium radiobacter

	NUMBER OF STRAINS 38		
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	spr		
Motility: flagella	[m]-pe[a]		
Action on blood	v	18 hy	7,38;5 gr,4 LG,1 β
Fermentative or oxidative	[O]		
Carbohydrate base	OF		
Acid from			
Glucose	[+]	100	38/38
Xylose	[+]	100	38/38
Mannitol	+	100	35,3w/38
Lactose	[+]	100	31,7w/38
Sucrose	+	100	37,1w/38
Maltose	[+]	100	37,1w/38
Catalase	+	100	36,2w/38
Oxidase	[+]	100	34,4w/38
Growth on:			
MacConkey	[+]	100	38/38
SS	v	26(3)	10w(1)/38
Cetrimide		0	0/38
Simmons Citrate	+	100	38/38
Urea, Christensen's	[+ or (+)]	89(11)	34(4)/38
Nitrate reduction	v	87	33/38
Gas from nitrate	[ ]	5	2/38
Indole	[ ]	0	0/38
TSI slant, acid		0	0/38
TSI butt, acid		0	0/38
H_2S (TSI butt)	v	8(5)	3w(2)/38
H_2S (Pb ac paper)	+	100	38/38
Gelatin hydrolysis[b]		3	1/37
Litmus milk	s/f	100	38/38
Pigment	-	0	0/38
Growth at:			
25 C	+	100	28,10w/38
35 C	+	100	36,2w/38
42 C	v	34	13w/38
Esculin hydrolysis	+	100	38/38
Lysine decarboxylase	[-]	0	0/37
Arginine dihydrolase	[-]	3	1/37
Ornithine decarboxylase		0	0/37
Nutrient broth, 0% NaCl	+	100	31,7w/38
Nutrient broth, 6% NaCl	-	0	0/38
3-Ketolactonate	[+]	100	38/38

[a]Frequently individual cells have only a single flagellum, either polar, subpolar, or lateral.
[b]7-14 days' incubation.

Alcaligenes denitrificans

[Synonym for *A. faecalis*. Buchanan, RE, Gibbons NE (eds): Bergey's Manual of Determinative Bacteriology. 8th ed. Baltimore, Williams and Wilkins Co., 1974.]

Gram-negative rod, motile (peritrichous flagella); does not utilize carbohydrates *; grows on MacConkey agar and sometimes on SS agar; oxidase-positive, urease-variable; reduces both nitrate and nitrite to gas; does not produce H_2S on TSI agar.

Sources (34 strains)

Sputum (12%), cerebrospinal fluid (12%), blood (9%), urine (9%), finger (6%), water and soil (9%), other (37%), unknown (6%).

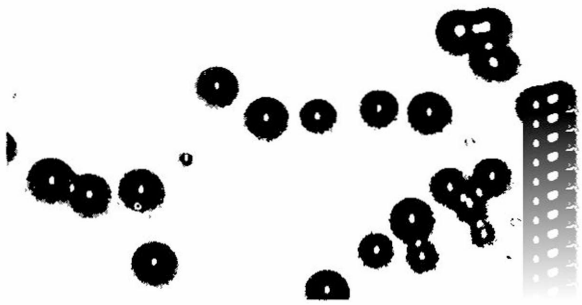
Reference strain

KC367 < E. Leifson, 412.

*Refers here and throughout the text only to those tested (see charts)



BAP 35°C 24 h ×1.



BAP 35°C 24 h ×6 (axial light).



Gram stain BAP 35°C 24 h ×1700.



B3500 Flagella stain FB 25°C 24 h ×1700.

Literature

J Gen Microbiol 11:512-513, 1954.

Comment

Evidence supporting species status was published in Int J Syst Bacteriol 33: 85-89, 1983.

Alcaligenes denitrificans[a]

	NUMBER OF STRAINS 34		
TEST PERFORMED	SIGN	% +	# + T
Morphology	mrs		
Motility, flagella	[m;pe]		
Action on blood	v	35 ly	11,31,8 gr,2 lav,1 br,1 gr & br
Fermentative or oxidative	[n-o]		
Carbohydrate base	OF		
Acid from			
Glucose	[ ]	0	0,34
Xylose	[ ]	0	0,34
Mannitol		0	0,34
Lactose		0	0,34
Sucrose		0	0,34
Maltose		0	0,34
Catalase	+	100	34,34
Oxidase	[+]	100	31,3w,34
Growth on			
MacConkey	[+]	100	33,1w,34
SS	[v]	65	18,4w,34
Cetrimide	v	26(12)	6,3w(4),34
Simmons Citrate	v	85(3)	27,2w(1),34
Urea, Christensen's	v	12(3)	4(1),34,7 pk
Nitrate reduction	[+]	100	34,34
Gas from nitrate	[+]	100	34,34
Indole		0	0,34
TSI slant, acid		0	0,34
TSI butt, acid		0	0,34
H_2S (TSI butt)	[ ]	0	0,34
H_2S (Pb ac paper)	v	32	7,4w,34
Gelatin hydrolysis[b]		0	0,30
Litmus milk	k or (k)	88(12)	28,1w(4),33
Pigment		9 br sol	3,34
Growth at			
25 C	+	100	28,6w,34
35 C	+	100	34,34
42 C	v	56	3,16w,34
Esculin hydrolysis	-	0	0,34
Lysine decarboxylase	-	0	0,5
Arginine dihydrolase	-	0	0,5
Ornithine decarboxylase		0	0,5
Nutrient broth, 0% NaCl	+	100	34,34
Nutrient broth, 6% NaCl	v	29	3,7w,34

[a]Listed in Bergey's Manual (8th ed.) as a synonym of *Alcaligenes faecalis*.
[b]7-14 days' incubation.

Alcaligenes faecalis

Gram-negative coccoid to medium-length or long rod, motile (peritrichous flagella); does not use carbohydrates; grows on MacConkey agar and sometimes on SS agar; oxidase-positive, urease-negative; may or may not reduce nitrate, does not reduce nitrite; does not form H_2S on TSI agar.

Sources (69 strains)

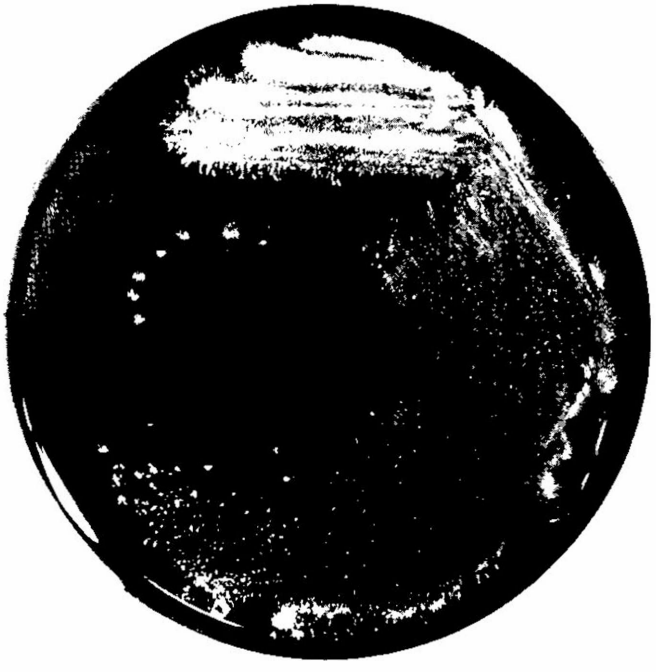
Ear (14%), urine (9%), blood (7%), mouth-wash solution (7%), wound (6%), sputum (6%), pleural fluid, chest, lung (6%), leg (4%), cerebrospinal fluid (4%), feces (3%), other (24%), unknown (10%).

Reference strains

KC111 < NCTC, 415; KC675 < ATCC, 8750; B6353, from nasopharynx.

Literature

Int J Syst Bacteriol 24:534-550, 1974; J Gen Microbiol 40:343-348, 1965.



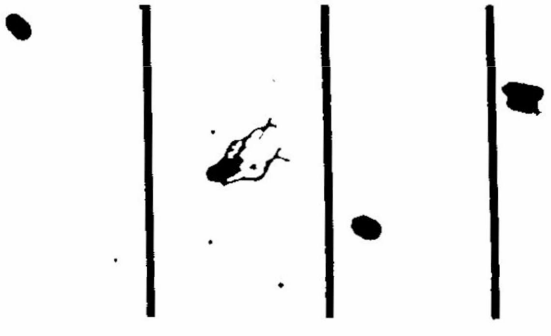
KC111 BAP 35°C 24 h ×1.



KC111 BAP 35°C 24 h ×6 (axial light).



KC675 Gram stain BAP 35°C 24 h ×1700.



B6353 Flagella stain BAP 35°C 24 h ×1700.

Comments

Three biotypes have been observed at CDC: I, nitrate +, SS −; II, nitrate −, SS −; III, nitrate −, SS +. May show large, spreading, dull colonies. The type strain of *A. faecalis* has the characteristics listed in this manual for *A. odorans*. *Alcaligenes odorans* and *A. denitrificans* are listed as synonyms of *A. faecalis* in Bergey's Manual (8th ed.).

Alcaligenes faecalis[a]

	NUMBER OF STRAINS 69		
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	mrs		
Motility; flagella	[m; pe]		
Action on blood	v	48 ly	24/50;7 al,6 gr,3 LG,2 br
Fermentative or oxidative	n-o		
Carbohydrate base	OF		
Acid from:			
Glucose	[−]	0	0/69
Xylose	[−]	0	0/69
Mannitol	−	0	0/69
Lactose	−	0	0/69
Sucrose	−	0	0/69
Maltose	−	0	0/69
Catalase	+	100	68,1w/69
Oxidase	[+]	100	51,17w/68
Growth on:			
MacConkey	[+]	100	69/69
SS	[v]	78(2)	40,11w(1)/65
Cetrimide	v	28(1)	11,8w(1)/69
Simmons Citrate	+	98(1)	67(1)/69
Urea, Christensen's	[−]	0(1)	(1)/69
Nitrate reduction	[v]	45	31/69
Gas from nitrate	[−]	0	0/69
Nitrite reduction	[−]	0	0/44
Indole	−	0	0/69
TSI slant, acid	−	0	0/34
TSI butt, acid	−	0	0/34
H_2S (TSI butt)	[−]	0	0/69
H_2S (Pb ac paper)	v	52	15,21w/69
Gelatin hydrolysis[b]	−	0	0/57
Litmus milk	k	98	68/69
Pigment	v	13 yel, br sol	9/69
Growth at:			
25°C	+	100	63,5w/68
35°C	+	100	67,1w/68
42°C	v	75	38,13w/68
Esculin hydrolysis	−	0	0/69
Lysine decarboxylase	−	0	0/11
Arginine dihydrolase	−	0	0/11
Ornithine decarboxylase	−	0	0/11
Nutrient broth, 0% NaCl	+	100	68,1w/69
Nutrient broth, 6% NaCl	v	54(6)	22,14w(4)/67

[a]The type strain of *A. faecalis* has the characteristics listed for *A. odorans*.
[b]7-14 days' incubation.

Alcaligenes odorans

[Synonym for *A. faecalis*, Bergey's Manual, 8th ed.]

Gram-negative coccoid to medium-length rod, motile (peritrichous flagella); does not use carbohydrates; grows on MacConkey and SS agars; oxidase-positive, urease-negative; does not reduce nitrate; reduces nitrite to gas.

Sources (49 strains)

Urine (43%), ear (16%), wound (8%), blood (4%), toe (4%), leg ulcer (4%), other (21%).

Reference strain

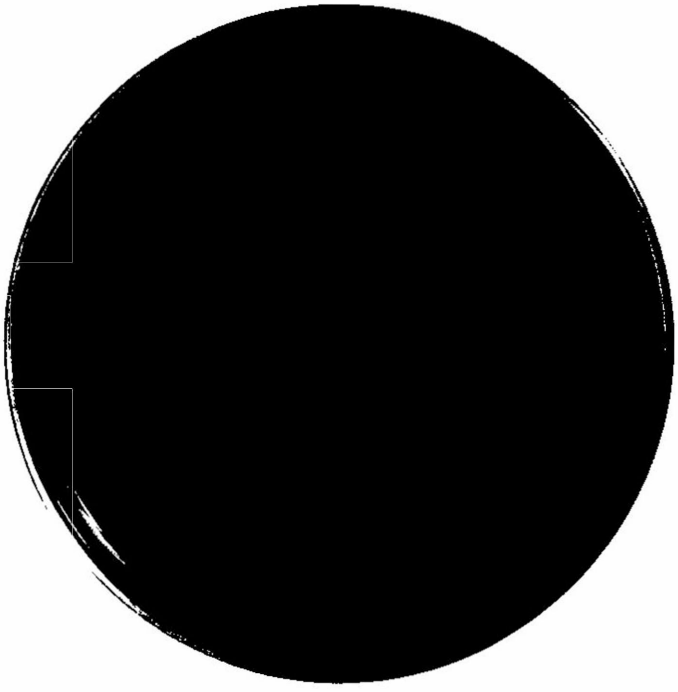
KC1281 ← NCTC, 10416.

Literature

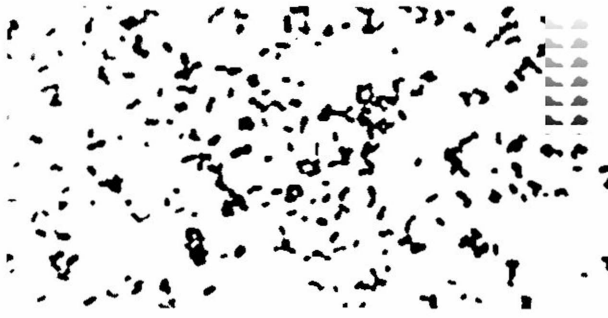
J Gen Microbiol 33:354, 1963.

Comments

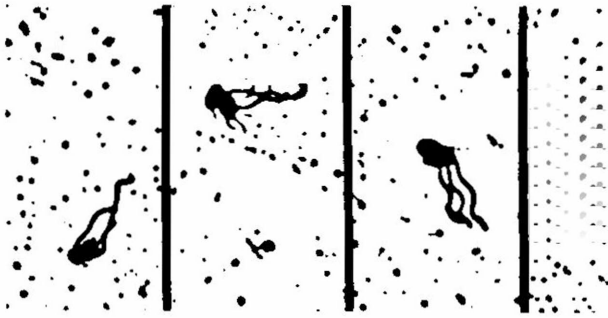
Produces a distinctive fruity odor. Growth is dull, spreading. The type strain of *A. faecalis* has the characteristisces listed for *A. odorans* in this manual.



BAP 35°C 24 h ×1.



Gram stain BAP 35°C 24 h ×1700.



Flagella stain BAP 35°C 24 h ×1700.

Alcaligenes odorans[a]

NUMBER OF STRAINS 49			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	mrs		
Motility, flagella	[m, pe]		
Action on blood	v	48 br & gr	23,48;8 gr,3 ly,3 br
Fermentative or oxidative	[n-o]		
Carbohydrate base	OF		
Acid from			
Glucose	[ ]	0	0/49
Xylose	[ ]	0	0/49
Mannitol		0	0/49
Lactose		0	0/49
Sucrose		0	0/49
Maltose		0	0/49
Catalase	+	98	48/49
Oxidase	[+]	100	48,1w/49
Growth on:			
MacConkey	[+]	100	49/49
SS	[+]	100	47,2w/49
Cetrimide	v	59	25,4w/49
Simmons Citrate	+	100	49/49
Urea, Christensen's	[+]	2	1w/49;13 pk
Nitrate reduction	[ ]	0	0/49
Gas from nitrate		0	0/49
Nitrite reduction	[+]	100	49/49
Indole	-	0	0/49
TSI slant, acid	-	0	0/49
TSI butt, acid	-	0	0/49
H_2S (TSI butt)	-	0	0/49
H_2S (Pb ac. paper)	-	8	4w/49
Gelatin hydrolysis[b]	v	22	9/41
Litmus milk	k	96	47/49
Pigment	v	22 yel sol	11/49
Growth at			
25 C	+	100	49/49
35 C	+	100	49/49
42 C	v	18	6,3w/49
Esculin hydrolysis		0	0/49
Lysine decarboxylase	-	0	0/9
Arginine dihydrolase	-	0	0/9
Ornithine decarboxylase	-	0	0/9
Nutrient broth, 0% NaCl	+	100	49/49
Nutrient broth, 6% NaCl	+	98(2)	45,3w(1)/49

[a]Listed in Bergey's Manual (8th ed.) as a synonym of *Alcaligenes faecalis*; the type strain of *A. faecalis* has the characteristics listed above.
[b]7-14 days' incubation.

Bordetella bronchiseptica

Gram-negative rod, motile (peritrichous flagella); does not use carbohydrates; grows well on MacConkey and SS agars; oxidase-positive, usually urease- and nitrate-positive without gas formation; does not produce H_2S on TSI agar.

Sources (85 strains)

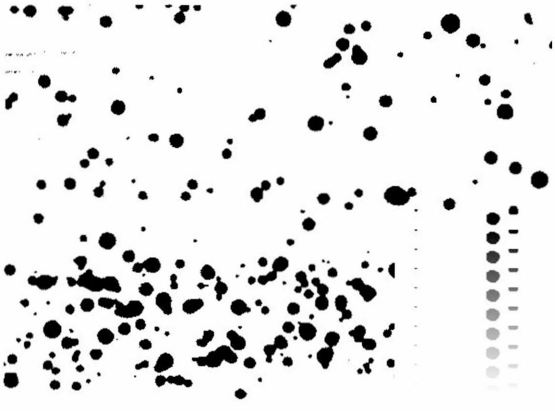
Guinea pig respiratory tract (14%), sputum (12%), human respiratory tract (12%), rabbit respiratory tract (7%), throat (6%), dog respiratory tract (5%), horse respiratory tract (4%), cerebrospinal fluid (2%), cat respiratory tract (2%), rat respiratory tract (2%), other (18%), unknown (16%).

Reference strain

KC1390 ← R. Hugh, 549 (ATCC 10580) (type strain).



BAP 35°C 24 h ×1.



BAP 35°C 24 h ×6 (axial light).



Gram stain BAP 35°C 24 h ×1700.



Flagella stain BAP 35°C 24 h ×1700.

Literature

Microbiol Esp 5:117-181, 1952; Int J Syst Bacteriol 16:38-39, 1966.

Comment

Sometimes referred to as *B. bronchicanis*.

Bordetella bronchiseptica

	NUMBER OF STRAINS 85		
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	mrs		
Motility; flagella	[m; pe]		
Action on blood	v	46 ly	33 72;10 β,8 gr,7 LG,2 br
Fermentative or oxidative	[n-o]		
Carbohydrate base	OF		
Acid from:			
Glucose	[ ]	0	0 79
Xylose	[-]	7	5w[a] 70
Mannitol	-	1	1w 71
Lactose	-	0	0 71
Sucrose	-	0	0 71
Maltose	-	0	0 71
Catalase	+	100	70 70
Oxidase	[+]	100	84,1w 85
Growth on:			
MacConkey	[+]	100	71 71
SS	[+]	99	78 79
Cetrimide	-	0	0 70
Simmons Citrate	+	98(1)	83(1) 85
Urea, Christensen's	[+]	99	84 85
Nitrate reduction	[+]	92	78 85
Gas from nitrate	[-]	0	0 85
Indole	-	0	0 70
TSI slant, acid	-	0	0 79
TSI butt, acid	-	0	0 79
H_2S (TSI butt)	[-]	0	0 79
H_2S (Pb ac paper)	v	74	8,49w 77
Gelatin hydrolysis[b]	-	0	0 55
Litmus milk	k	98	83 85
Pigment	-	0	0 50
Growth at:			
25°C	+	99	69 70
35°C	+	100	70 70
42°C	v	78	54 69
Esculin hydrolysis	-	0	0 62
Nutrient broth, 0% NaCl	+	100	61 61
Nutrient broth, 6% NaCl	v	82(5)	53(3) 65

[a]Reaction is neutral compared with the alkline of the control.
[b]7-14 days' incubation.

Bordetella parapertussis

Gram-negative small rod, nonmotile; does not use carbohydrates; MacConkey-positive, oxidase-negative, urease-positive, nitrate-negative; grows slowly on blood agar, producing miniscule colonies, beta hemolysis, and a brown, water-soluble pigment (attributed to tyrosine); does not produce H_2S on TSI agar; does not hydrolyze gelatin.

Sources (8 strains)

Nasopharynx (4), cough plate (1), throat (1), unknown (2).

Reference strains

KC431 ← E. Updyke, 13; C8872, from nasopharynx.

Literature

Microbiol Esp 5:117-181, 1952; J Bacteriol 35:561-572, 1938.

Comments

Causes a pertussis-like syndrome; sometimes isolated when not expected; may be confused with fastidious *Acinetobacter (Mima polymorpha)*. Fluorescent antibody test and serology are useful for confirmation.



C8872 BAP 35°C 24 h ×1 (axial light).



KC431 Gram stain BAP 35°C 24 h ×1700.

Bordetella parapertussis

NUMBER OF STRAINS 7			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	sts		
Motility; flagella	nm		
Action on blood	[β]	100	7/7
Fermentative or oxidative	[n-o]		
Carbohydrate base	OF		
Acid from:			
Glucose	[-]	0	0/4
Xylose	-	0	0/4
Mannitol	-	0	0/4
Lactose	-	0	0/4
Sucrose	-	0	0/4
Maltose	[-]	0	0/4
Catalase	+	100	6/6
Oxidase	[-]	0	0/6
Growth on:			
MacConkey	[+]	100	7/7
SS	-	0	0/7
Cetrimide	-	0	0/7
Simmons Citrate	v	71(14)	5(1)/7
Urea, Christensen's	[+]	100	7/7
Nitrate reduction	[-]	0	0/7
Gas from nitrate	-	0	0/7
Indole	-	0	0/7
TSI slant, acid	-	0	0/7
TSI butt, acid	-	0	0/7
H_2S (TSI butt)	[-]	0	0/7
H_2S (Pb ac paper)	v	28	2w/7
Gelatin hydrolysis[a]	[-]	0	0/3
Litmus milk	v	80 k	4/5
Pigment	[br sol]	100	7/7
Growth at:			
25°C	v	50	3/6
35°C	+	100	6/6
42°C	v	17	1/7
Nutrient broth, 0% NaCl	+	100	5/5
Nutrient broth, 6% NaCl	-	0	0/5

[a]7-14 days' incubation.

Bordetella pertussis

Gram-negative small rod, nonmotile; does not grow on usual laboratory media, although it may adapt by repeated transfer; grows well on Bordet-Gengou medium with 15% (v/v) blood in 48-72 h (addition of penicillin, 0.3 unit per ml and diamidinodiphenylamine, 2 μg per ml is recommended for isolation); usually produces beta-like action on blood, but not as distinct as that produced by *B. parapertussis*; urease-negative with heavy inoculum; produces no pigment; oxidase-positive.

Sources (38 strains)

Nasopharynx (32%), cough plate (18%), throat (18%), nasal (5%), lung (3%), unknown (24%).

Reference strain

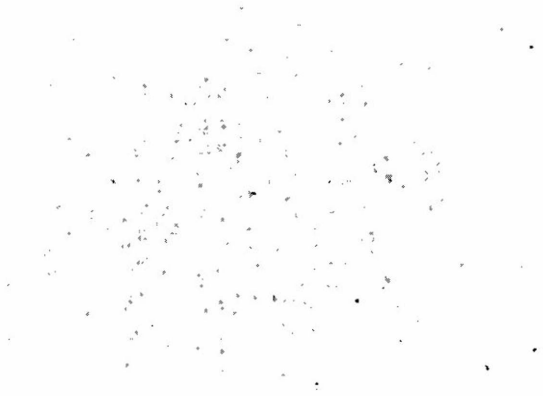
KC437 ⇐ M. Pittman, 18323 (ATCC 9797).

Literature

Microbiol Esp 5:117-181, 1952; Acta Pathol Microbiol Scand 33:202, 1953; J Bacteriol 74:133, 1957; Bull WHO 23:15-35, 1960; Lennette EH, Spaulding EH, Truant JP (eds): Manual of Clinical Microbiology, 2nd ed. Washington, D.C. American Society for Microbiology, 1974, pp 308-315.

Comment

Agglutination and fluorescent antibody testing are particularly useful for identification.



KC437 Gram stain BG 35°C 48 h ×1700.

Bordetella pertussis[a]

NUMBER OF STRAINS 51			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	srs		
Motility	nm		
Action on blood	[v]	81 β	35/43;6 ly
Fermentative or oxidative			
Carbohydrate base			
Acid from:			
Glucose			
Xylose			
Mannitol			
Lactose			
Sucrose			
Maltose			
Glycerol			
Catalase	+	100	2 2
Oxidase	[+]	94	8,8w-17
Growth on:			
MacConkey			
SS			
Simmons Citrate			
Urea, Christensen's	[−][b]	0	0/37
Nitrate reduction			
Gas from nitrate			
Indole			
TSI slant, acid			
TSI butt, acid			
H_2S (TSI butt)			
H_2S (Pb ac paper)			
Gelatin hydrolysis			
Growth at:			
25°C			
35°C			
42°C			
Esculin hydrolysis			
Nutrient broth, 0% NaCl			
Nutrient broth, 6% NaCl			
Requires CO_2 atmosphere			

[a]Generally does not grow on the usual laboratory media; Bordet-Gengou is the medium of choice.
[b]Heavy inoculum.

Branhamella catarrhalis

Gram-negative coccus, nonmotile; asaccharolytic; catalase-positive, strongly oxidase-positive; usually reduces nitrate and nitrite without gas; usually grows on nutrient agar at 35° and 25°C; grows only rarely on MacConkey agar; amylosucrase-negative.

Sources (74 strains)

Eye (26%), sputum (7%), blood (7%), ear (5%), throat (5%), nose (4%), other (15%), unknown (31%).

Reference strain

KC917 ← S. Branham, N-9.

Literature

J Gen Microbiol 37:341-352, 1964; J Bacteriol 95:58-73, 1968; Int J Syst Bacteriol 20:155-159, 1970.

Comments

Formerly *Neisseria catarrhalis*; single species in the genus *Branhamella*. Usually no growth pigment occurs on Loeffler blood serum medium. A few strains which have been identified as *B. catarrhalis* have grown on Thayer-Martin media. Deoxyribonuclease-positive. There is some evidence for *B. catarrhalis* as a respiratory pathogen (J Clin Microbiol 13:1066-1069, 1981).



Gram stain BAP 35°C 24 h ×1700.

Branhamella catarrhalis[a]

NUMBER OF STRAINS 74			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	[cd]		
Motility: flagella	[nm]		
Action of blood	v	32 [y	7 32
Fermentative or oxidative	n-o		
Carbohydrate base	CTA		
Acid from:			
Glucose	[-]	0	0 69
Xylose		0	0 15
Mannitol		0	0 66
Lactose	−	0	0 66
Sucrose	−	0	0 70
Maltose	[−]	0	0 69
Catalase	+	100	37 37
Oxidase	[+]	100	73 73
Growth on:			
MacConkey		5	2 39
SS		0	0 39
Simmons Citrate	−	0	0 38
Urea, Christensen's	-	0	0 40
Phenylalanine deaminase	v	68	19,5w/34
Nitrate reduction	[+]	92	68 74
Nitrite reduction	+	91	10 11
Indole		0	0 37
TSI slant, acid	-	0	0 29
TSI butt, acid		0	0 29
H_2S (TSI butt)		0	0 31
H_2S (Pb ac paper)	v	73	3,21w/33
Gelatin hydrolysis[b]	−	0	0 31
Litmus milk		6k	2w/36;2 IR
Pigment	v	25	15 sl yel/60
Growth at:			
25 C	v	85	28 33
35 C	+	97	30 31
42 C	v	23	5 22
Esculin hydrolysis	−	0	0 14
Nutrient broth, 0% NaCl	v	47	8 17

[a]See chart "Differentiation of species of *Neisseria* and *Branhamella*" for distinguishing *B. catarrhalis* and *Neisseria* species (pp. 84-85).
[b]7-14 days' incubation.

Brucella abortus

Gram-negative small coccoid rod, nonmotile; microaerophilic to aerobic; oxidizes xylose and usually glucose; growth on MacConkey agar variable; catalase-positive, urease-positive (but not immediately); oxidase-positive, reduces nitrate, citrate-negative, indole-negative; usually resistant to basic fuchsin dye and sensitive to thionin dye (1:50,000 and 1:100,000 dilutions of the dyes); *Brucella* phage strain Tbilisi lyses smooth strains at routine test dilution (RTD) and 10,000 × RTD.

Sources (81 strains)

Blood (63%), bone marrow (5%), bovine tissue (2%), abscess (2%), other (4%), unknown (24%).

Reference strain

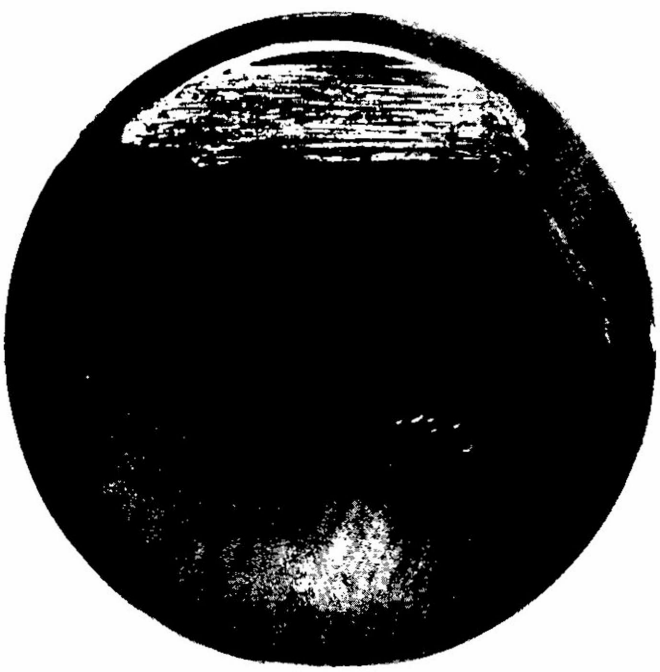
KC797 ← L. Jones, R19.

Literature

J Infect Dis 27:173-184, 1920; Nature 188:74-75, 1960; Int Bull Bacterial Nomencl Taxon 13:145-148, 1963; J Gen Microbiol 30:437-443, 1963; Int J Syst Bacteriol 21:127, 1971; Alton GG, Jones LM, Pietz DE: Laboratory Techniques in Burcellosis. 2nd ed. WHO, 1975.

Comments

Pathogenic for cattle and other species, including man. Growth on agar plate after 18-24 h incubation is scant and usually occurs only in areas where inoculum is heaviest; discrete colonies usually develop after further incubation. An occasional strain may give a "fuzzy" appearance in motility medium, suggesting motility; however, no flagella have been demonstrated. Nine biotypes are recognized (see Alton et al, 1975, or Bergey's Manual, 8th ed).



BAP 35°C 24 h ×1.

Gram stain BAP 35°C 24 h ×1700.

Brucella abortus

NUMBER OF STRAINS 81			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	[rv]		
Motility	[nm]		
Action on blood	v	25 ly	1 4
Fermentative or oxidative	[0]		
Carbohydrate base	OF		
Acid from:			
Glucose	[v]	50(25)	2w(1) 4
Xylose	[+]	100	4 4
Mannitol	-	0	0 4
Lactose	-	0	0 4
Sucrose	[-]	0	0 4
Maltose		0	0 4
Catalase	+	100	14,2w 16
Oxidase	[+]	96	22 23
Growth on:			
MacConkey	v	50(20)	2,3w(2) 10
SS	-	0	0/10
Simmons Citrate	[-]	0	0/10
Urea, Christensen's	[+]	98	79/81
Nitrate reduction	[+]	100	13/13
Gas from nitrate	-	0	0/13
Indole	[-]	0	0/13
TSI slant, acid	-	0	0/5
TSI butt, acid	-	0	0/5
H_2S (TSI butt)	-	0	0/6
H_2S (Pb ac paper) [a]	+	89(4)	50,20w(3)/79
Gelatin hydrolysis [b]	-	0	0/3
Growth at:			
25°C	v	43	1,2w/7
35°C	+	100	81/81
42°C	v	14	1w/7
Esculin hydrolysis	-	0	0/6
Nutrient broth, 0% NaCl	+	100	7/7
Nutrient broth, 6% NaCl	-	0	0/7
Requires CO_2 atmosphere	v	67	41/61

[a]Generally *Brucella* strains are tested with heart infusion agar.
[b]7-14 days' incubation.

Brucella canis

Gram-negative small coccoid rod, nonmotile; biochemically similar to *Brucella suis*, but on primary isolation cultures are in the rough phase and show strong agglutination in acriflavine; oxidase test is variable; gives positive string test and forms "gel" in phenolized saline; agglutinates in specific antiserum.

Sources (28 strains)

Human blood (43%), canine blood (11%), canine cervix (7%), other canine sources (39%).

Reference strain

D2773, from dog placenta.

Literature

Cornell Vet 58:578-592, 1968; J Bacteriol 95:618-624, 1968; J Bacteriol 625-630, 1968; Alton GG, Jones LM, Pietz DE: Laboratory Techniques in Brucellosis. 2nd ed. WHO, 1975; J Clin Microbiol 14:111-112, 1981.

Comments

Pathogenic for dogs and humans. Growth on agar plates after 18-24 h is scant and usually occurs only in areas where inoculum is heaviest; discrete colonies usually develop after further incubation. Cellular fatty acid profile is useful in distinguishing *B. canis* from other antigenically rough *Brucella* strains.



Gram stain BAP 35°C 24 h ×1700.

Brucella canis [a]

NUMBER OF STRAINS 28			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	[rv]		
Motility	[nm]		
Action on blood	v	20 sl hy	3 15;1 gr
Fermentative or oxidative	[0]		
Carbohydrate base	OF		
Acid from:			
Glucose	[+ or (+)]	81(14)	13,4w(3) 21
Xylose	[+ or (+)]	81(19)	15,2w(4) 21
Mannitol	–	0	0 21
Lactose	–	0	0 21
Sucrose	[ ]	0	0 21
Maltose		0	0 21
Catalase	+	100	15,2w 17
Oxidase	[v]	72	4,9w 18
Growth on:			
MacConkey	v	12(29)	2w(5) 17
SS	–	0	0 17
Simmons Citrate	[–]	0	0 17
Urea, Christensen's	[+]	100	28 28
Nitrate reduction	[+]	100	22 22
Gas from nitrate	[v]	77	17 22
Indole	[–]	0	0 18
TSI slant acid	–	0	0 20
TSI butt, acid	–	0	0 20
H_2S (TSI butt)	–	0	0 19
H_2S (Pb ac paper) [b]	v	82	9,9w 22
Gelatin hydrolysis [c]	–	0	0 7
Growth at:			
25°C	v	42	5w 12
35°C	+	100	28 28
42°C	v	33	4w 12
Esculin hydrolysis	–	0	0 15
Nutrient broth, 0% NaCl	+	100	13,1w 14
Nutrient broth, 6% NaCl	–	0	0 14

[a] A heavy suspension in 0.5% phenolized saline usually forms a "gel" within 30 min.
[b] Generally *Brucella* strains are tested with heart infusion agar.
[c] 7-14 days' incubation.

Brucella melitensis

Gram-negative small coccoid rod, nonmotile; oxidizes xylose and usually glucose; growth on MacConkey agar variable; urease-positive (immediately or delayed), oxidase-positive; reduces nitrate; indole-and citrate-negative; resistant to basic fuchsin and thionin dyes (1:50,000 and 1:100,000 dilutions); *Brucella* phage strain Tbilisi does not lyse *B. melitensis* at routine test dilution and 10,000 × RTD.

Sources (48 strains)

Blood (69%), bone marrow (6%), liver (4%), spleen (2%), unknown (19%).

Reference strain

KC341 ⇐ J. Schubert, M-1, ⇐ H. Bauer.

Literature

J Infect Dis 27:173-184, 1920; J Gen Microbiol 19:555, 1958; Int J Syst Bacteriol 16:40-41, 1966, and 21:127, 1971; Alton GG, Jones LM, Pietz DE: Laboratory Techniques in Brucellosis. 2nd ed. WHO, 1975.

Comments

Pathogenic for goats, sheep, and other species, including man. Growth on agar plate after 18-24 h is scant and usually occurs only in areas where inoculum is heaviest; discrete colonies usually develop after further incubation. Three biotypes are recognized (see Alton et al, 1975, or Bergey's Manual, 8th ed.).



Gram stain BAP 35°C 24 h ×1700.

Brucella melitensis

NUMBER OF STRAINS 48			
TEST PERFORMED	**SIGN**	**% +**	**# +/T**
Morphology	[rv]		
Motility	[nm]		
Action on blood		0	0/1
Fermentative or oxidative	[0]		
Carbohydrate base	OF		
Acid from:			
Glucose	[v]	33	1/3
Xylose	[+]	100	3/3
Mannitol		0	0/3
Lactose		0	0/3
Sucrose	[ ]	0	0/3
Maltose	-	0	0/3
Catalase	+	100	7/7
Oxidase	[+]	100	13,2w/15
Growth on:			
MacConkey	v	62	1w/4/8
SS	−	0	0/8
Simmons Citrate	[−]	0	0/8
Urea, Christensen's	[+]	100	48/48
Nitrate reduction	[+]	100	8/8
Gas from nitrate		0	0/8
Indole	[-]	0	0/8
TSI slant, acid		0	0/3
TSI butt, acid	-	0	0/3
H_2S (TSI butt)	-	0	0/3
H_2S (Pb ac paper)[a]	v	39	6,11w/16/44
Gelatin hydrolysis[b]		0	0/2
Growth at:			
25°C	v	17	1/6
35°C	+	100	48/48
42°C	v	50	3w/6
Esculin hydrolysis	-	0	0/2
Nutrient broth, 0% NaCl	+	100	7,1w/8
Nutrient broth, 6% NaCl		0	0/8
Requires CO_2 atmosphere	-[c]	0	0/20

[a]Generally *Brucella* strains are tested with heart infusion agar.
[b]7-14 days' incubation.
[c]Growth of 4 of 20 strains tested was enhanced in a candle jar.

Brucella suis

Gram-negative small rod, nonmotile; oxidizes xylose and glucose; growth on MacConkey agar variable; urease-positive immediately with heavy inoculum, oxidase-positive; reduces nitrate, frequently with slight gas production, does not utilize citrate; does not produce indole; usually sensitive to basic fuchsin and resistant to thionin dyes (1:50,000 and 1:100,000 dilutions); *Brucella* phage strain Tbilisi lyses smooth *B. suis* strains at 10,000 × RTD, but not at RTD.

Sources (190 strains)

Blood (74%), bone marrow (5%), lung tissue (2%), spleen (1%), other (6%), unknown (12%).

Reference strain

KC322 ← J. Schubert, S-13 ← G. Eldridge.

Literature

Mich State Coll Agric Expt St Tech Bull 100:1-16, 1929; Int J Syst Bacteriol 16:40-41, 1966; Am J Vet Res 27:353-358, 1966; Alton GG, Jones LM, Pietz DE: Laboratory Techniques in Brucellosis. 2nd ed. WHO, 1975.

Comments

Pathogenic for pig, hare, reindeer, and other species, including man. Growth on agar plate after 18-24 h incubation is scant and usually occurs only in areas where inoculum is heaviest; discrete colonies usually develop after further incubation. Four biotypes are recognized (see Alton et al, 1975, or Bergey's Manual, 8th ed.).



Gram stain BAP 35°C 24 h ×1700.

Brucella suis

NUMBER OF STRAINS 190			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	[rx]		
Motility	[nm]		
Action on blood	v	17 al	2/12
Fermentative or oxidative	[0]		
Carbohydrate base	OF		
Acid from:			
Glucose	[+]	100	8,4w/12
Xylose	[+]	100	11,1w/12
Mannitol	−	0	0/12
Lactose		0	0/12
Sucrose	[ ]	0	0/12
Maltose		0	0/12
Catalase	+	100	37,2w/39
Oxidase	[+]	95	40,13w/56
Growth on:			
MacConkey	v	23(15)	1,2w(2)/13
SS		0	0/13
Simmons Citrate	[ ]	0	0/13
Urea, Christensen's	[+]	100	190/190
Nitrate reduction	[+]	100	26,1w/27
Gas from nitrate	[v]	52	14/27
Indole	[ ]	0	0/16
TSI slant, acid		0	0/7
TSI butt, acid		0	0/7
H_2S (TSI butt)		0	0/7
H_2S (Pb ac paper)[a]	+	95	142,25w/176
Gelatin hydrolysis[b]		0	0/2
Growth at:			
25 C	v	70	2,5w/10
35 C	+	100	190/190
42 C	v	50	1,4w/10
Esculin hydrolysis	−	0	0/2
Nutrient broth, 0% NaCl	+	100	12,1w/13
Nutrient broth, 6% NaCl	−	0	0/13

[a]Generally *Brucella* strains are tested with heart infusion agar.
[b]7-14 days' incubation.

Campylobacter fetus subsp. *fetus*

Gram-negative curved or spiral rod (may be S- or gull-shaped), motile (1-2 polar flagella); microaerophilic, does not use carbohydrates; grows at 25° and 35°C, usually not at 42°C; oxidase-, catalase-, and nitrate-positive; usually light or delayed (3-7 days) growth on MacConkey agar; no growth in Brucella broth with 3.5% NaCl, grows in Brucella broth with 1% glycine; produces H_2S with lead acetate paper.

Sources (111 strains)

Blood (76%), cerebrospinal fluid (3%), pleural fluid (2%), other (13%), unknown (6%).

Reference strains

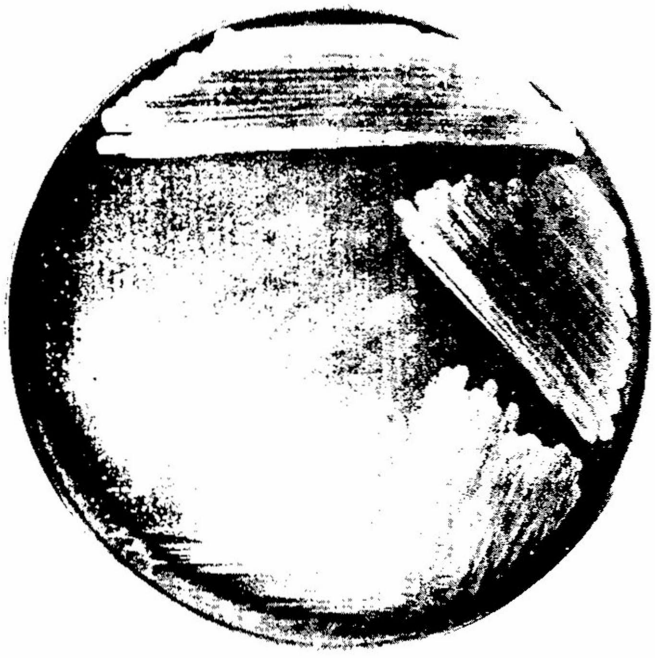
D4533, pleural fluid; D6611, source unknown.

Literature

J Infect Dis 101:119-128, 1957; Int J Syst Bacteriol 23:122-134, 1973; Buchanan RE, Gibbons NE (eds): Bergey's Manual, 8th ed. pp 208-210. Manual of Clinical Microbiology, 3rd ed. pp 235-241.

Comments

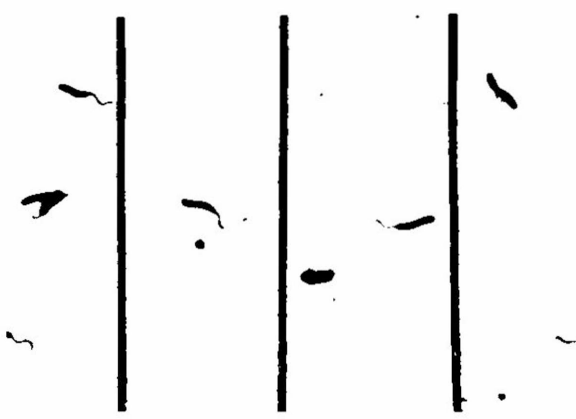
Formerly "*Vibrio fetus*." Also this organism was referred to as *C. fetus* subsp. *intestinalis* in Bergey's Manual, 8th ed. Spirals loosely wound (medium to long wavelength); usually resistant to nalidixic acid. A cause of infection in humans and abortion in sheep and cattle.



D6611 BAP 35°C 48 h ×1.



D4533 Gram stain BAP 35°C 48 h ×1700.



D6611 Flagella stain BAP 35°C 48 h ×1700.

Campylobacter fetus subsp. *fetus*[a]

TEST PERFORMED	SIGN	% +	# + /T
	NUMBER OF STRAINS 113		
Morphology	[sc]		
Motility: flagella	[m];p,1-2		
Action on blood	–	10 al	9/85;3 ly,2 gr,1 β
Fermentative or oxidative	[n-o]		
Carbohydrate base	OF		
Acid from:			
Glucose	[-]	0	0/101
Xylose	–	0	0/100
Mannitol	–	0	0/101
Lactose	–	0	0/100
Sucrose	–	0	0/100
Maltose	–	0	0/100
Catalase	[+]	99(1)	101,6w(1)/108
Oxidase	[+]	100	105,3w/108
Growth on:			
MacConkey	[+ or(+)]	83(12)	91(13)/109
SS	–	0	0/110
Simmons Citrate	–	0	0/110
Urea, Christensen's	–	0	0/84
Nitrate reduction	[+]	100	111/111
Gas from nitrate	–	0	0/111
Indole	–	0	0/106
TSI slant, acid	–	0	0/111
TSI butt, acid	–	0	0/110
H_2S (TSI butt)	–	0	0/107
H_2S (Pb ac paper)	v	49(2)	1,52w(2)/109
Gelatin hydrolysis[b]	–	0	0/58
Litmus milk	–	10 IR	12/110;6 k
Pigment	–	0	0/44
Growth at:			
25°C	[+]	99	105/106
35°C	+	100	106/106
42°C	[–]	10	10/95
Esculin hydrolysis	–	0	0/76
Growth in:			
Nutrient broth, 0% NaCl	v	14	12/88
Nutrient broth, 6% NaCl	–	0	0/86
Brucella broth, 3.5% NaCl	[–]	0	0/88
Brucella broth, 1% glycine	[+]	97(3)	56(2)/58
H_2S, Brucella broth (Pb ac paper)	[+]	96	14,37w/53

[a]Microaerophilic; may grow in a candle jar, but 0_2 requirement for optional growth is 5-6%.
[b]7-14 days' incubation.

Campylobacter fetus subsp. *jejuni*

Gram-negative curved or spiral rod (may be S- or gull-shaped), motile (1-2 polar flagella); microaerophilic; does not use carbohydrates; grows at 35° and 42°C, not at 25°C; both discrete and mucoid colonies may be observed with some strains; oxidase-, catalase-, and nitrate-positive; frequently light or delayed (3-7 days) growth on MacConkey agar; no growth in Brucella broth with 3.5% NaCl; grows in Burcella broth with 1% glycine; produces H_2S with lead acetate paper.

Sources (41 strains)

Blood (63%), stool (5%), cerebrospinal fluid (5%), other (7%), unknown (20%).

Reference strain

D7023, human blood.

Literature

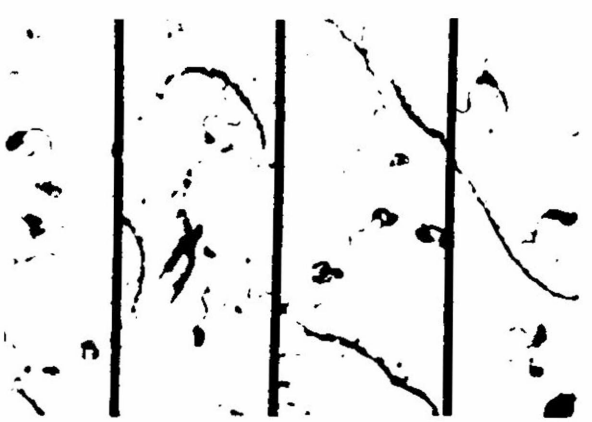
J Infect Dis 101:119-128, 1957; Int J Syst Bacteriol 23:122-134, 1973; Br Med J 2:9-11, 1977; Bergey's Manual, 8th ed. pp 208-210. Manual of Clinical Microbiology, 3rd ed. pp 235-241.



Strain number not identified, BAP, 35°C, 48 h ×1.



D7023 Gram stain BAP 35°C 48 h ×1700.



D7023 Flagella stain BAP 35°C 48 h ×1700.

Comments

Formerly referred to as "related vibrio." Classification and nomenclature according to Bergey's Manual, 8th ed. The name *C. fetus* subsp. *jejuni* is not included in the Approved Lists of Bacterial Names. This group of organisms probably includes both *C. coli* and *C. jejuni* as referred to in the Approved Lists. Further studies should resolve the taxonomic and nomenclature problems associated with these bacteria.

Spiral forms less loosely wound (shorter wavelength) and more delicate than with *C. fetus* subspp. *venerealis* and *fetus;* coccoid forms frequently seen in older (3 days or more) cultures; usually sensitive to nalidixic acid. Recently determined to be a cause of acute enteritis in man.

Campylobacter fetus subsp. *jejuni*[a]

NUMBER OF STRAINS 41			
TEST PERFORMED	**SIGN**	**% –**	**# + / T**
Morphology	[sc]		
Motility: flagella	[m],p,1-2		
Action on blood		6 al	2,34,1 gr,1 ly
Fermentative or oxidative	[n-o]		
Carbohydrate base	OF		
Acid from:			
Glucose	[-]	0	0/32
Xylose	-	0	0/32
Mannitol		0	0/32
Lactose	-	0	0/32
Sucrose	-	0	0/32
Maltose	-	0	0/32
Catalase	[+]	100	36/36
Oxidase	[+]	97	38/39
Growth on:			
MacConkey	v	44(12)	3,15w(5)/41
SS		0	0/40
Simmons Citrate	-	0	0/40
Urea, Christensen's	–	0	0/33
Nitrate reduction	[+]	100	40/40
Gas from nitrate	-	0	0/40
Indole	-	0	0/35
TSI slant, acid	–	0	0/37
TSI butt, acid		0	0/32
H_2S (TSI butt)	-	0	0/35
H_2S (Pb ac paper)	v	83	16,14w/36
Gelatin hydrolysis[b]	–	4	1/21
Litmus milk	–	8 k	3/39
Growth at:			
25 C	[-]	0	0/35
35 C	+	100	35/35
42 C	[+]	100	35/35
Esculin hydrolysis	-	0	0/38
Growth in:			
Nutrient broth, 0% NaCl	-	0	0/25
Nutrient broth, 6% NaCl	-	0	0/25
Brucella broth, 3.5% NaCl	[-]	0	0/30
Brucella broth, 1% glycine	[+]	93	28/30
H_2S, Brucella broth (Pb ac paper)	[+]	97	29/30

[a]Microaerophilic; may grow in a candle jar, but O_2 requirement for optimal growth is 5-6%.
[b]7-14 days' incubation.

Campylobacter fetus subsp. *venerealis*

Gram-negative curved or spiral rod (may be S- or gull- shaped), motile (single polar flagellum); microaerophilic, does not use carbohydrates; does not grow on MacConkey agar; grows at 25° and 35°C, usually not at 42°C; oxidase-, catalase-, and nitrate-positive; does not grow in Brucella broth with either 3.5% NaCl or with 1% glycine; does not produce H_2S with lead acetate paper.

Source

Bovine fetus - one culture.

Reference strain

D6378, bovine fetus.

Literature

Ann Inst Pasteur, Paris 105:897-910, 1963; Int J Syst Bacteriol 23:122-134, 1973; Bergey's Manual, 8th ed. pp 208-210; Manual of Clinical Microbiology, 3rd ed. pp 235-241.

Comments

This organism was referred to as *C. fetus subsp. fetus* in Bergey's Manual, 8th ed. Spiral forms loosely wound (medium to long wavelength). A cause of abortion and infertility in cattle.



D6378 Gram stain HIA 35°C 48 h ×1700.

Campylobacter fetus subsp. *venerealis*[a]

NUMBER OF STRAINS 2			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	[sc]		
Motility; flagella	[m]; p,1-2		
Action on blood	–	0	0/2
Fermentative or oxidative	[n-o]		
Carbohydrate base	OF		
Acid from:			
Glucose	[–]	0	0/2
Xylose	–	0	0/2
Mannitol	–	0	0/2
Lactose	–	0	0/2
Sucrose	–	0	0/2
Maltose	–	0	0/2
Catalase	[+]	100	2/2
Oxidase	[+]	100	2/2
Growth on:			
MacConkey	[–]	0	0/2
SS	–	0	0/2
Simmons Citrate	–	0	0/2
Urea, Christensen's	–	0	0/1
Nitrate reduction	[+]	100	2/2
Gas from nitrate	–	0	0/2
Indole	–	0	0/1
TSI slant, acid	–	0	0/2
TSI butt, acid	–	0	0/2
H_2S (TSI butt)	–	0	0/2
H_2S (Pb ac paper)	–	0	0/2
Gelatin hydrolysis[b]	–	0	0/2
Litmus milk	–	0	0/2
Growth at:			
25°C	[+]	100	2/2
35°C	+	100	2/2
42°C	[–]	0	0/2
Esculin hydrolysis	–	0	0/2
Growth in:			
Nutrient broth, 0% NaCl	–	0	0/2
Nutrient broth, 6% NaCl	–	0	0/2
Brucella broth, 3.5% NaCl	[–]	0	0/2
Brucella broth, 1% glycine	[–]	0	0/2
H_2S, Brucella broth (Pb ac paper)	[–]	0	0/2

[a] Microaerophilic; may grow in a candle jar, but O_2 requirement for optimal growth is 5-6%.
[b] 7-14 day's incubation.

Capnocytophaga spp. (DF-1)

Gram-negative thin rod with tapered ends; gliding motility has been observed; some strains appear motile late in motility medium, with only an occasional very delicate single polar or lateral "flagellum" detectable (in our laboratory); facultatively anaerobic; fastidious; requires or is enhanced by candle-jar atmosphere; ferments glucose (without gas), sucrose, maltose, and usually lactose; frequently produces yellow to orange growth pigment; catalase-negative, oxidase-negative; no growth on MacConkey agar; esculin hydrolysis is variable; arginine dihydrolase is usually negative.

Sources (172 strains)

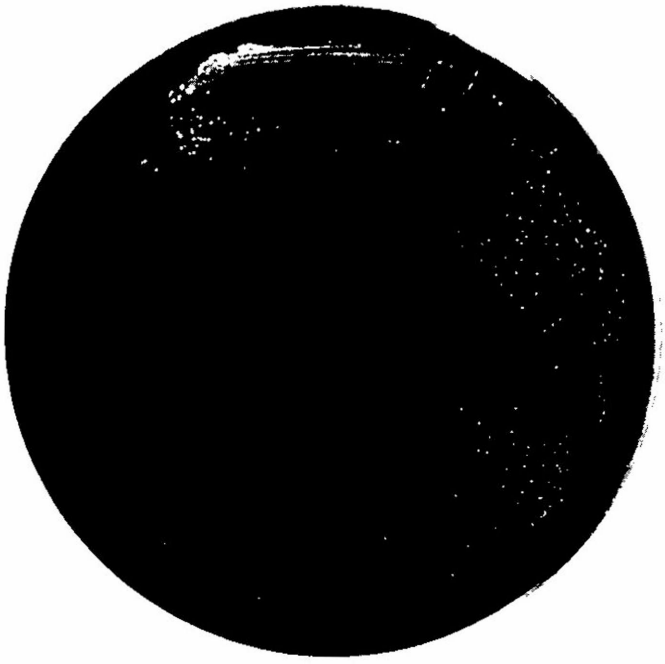
Blood (23%), sputum (18%), throat (16%), vaginal and cervical (6%), bronchial (4%), cerebrospinal fluid (4%), tracheal (2%), pleural fluid (2%), thoracentesis (2%), eye (2%), amniotic fluid (2%), other (14%), unknown (5%).

Reference strains

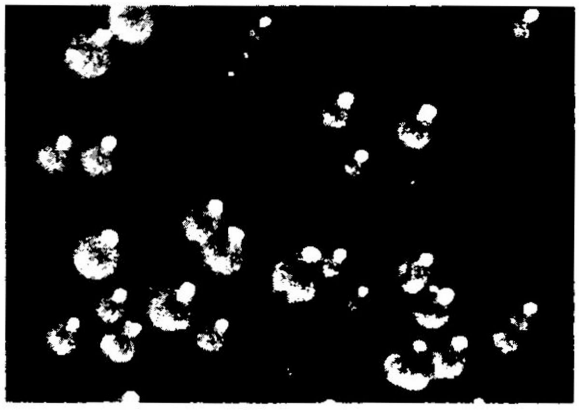
B2906, from blood; B4267, from blood; B7310, from throat.

Literature

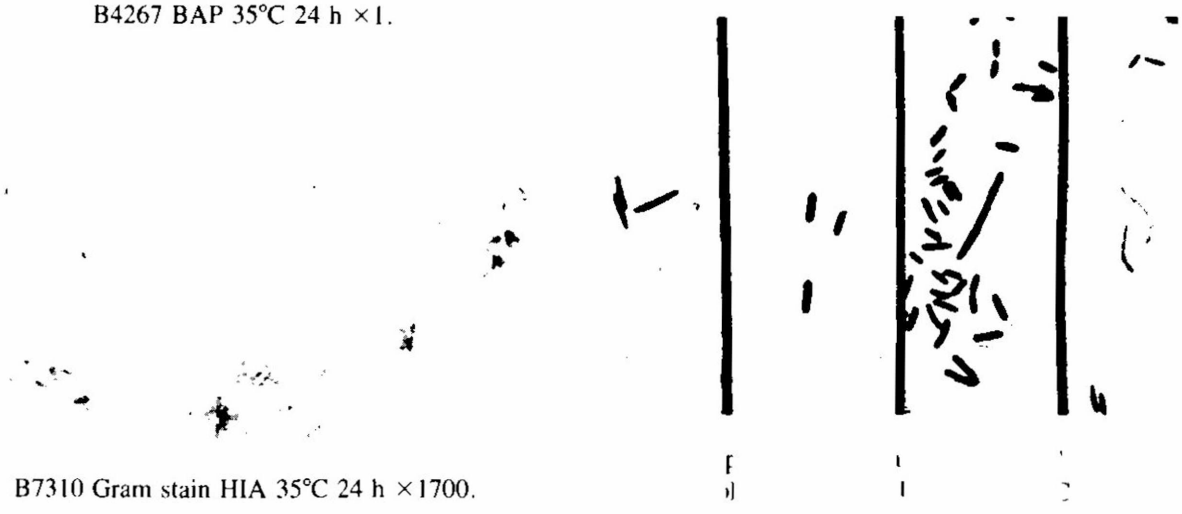
Arch Microbiol 122:9-39, 1979; J Clin Microbiol 10:550-556; 557-562, 1979.



B4267 BAP 35°C 24 h ×1.



B4267 BAP 35°C 24 h ×6.



B7310 Gram stain HIA 35°C 24 h ×1700.

Comments

Distinctive fringe-like or pseudo-pitting colonies frequently grow into the agar. Usually (17 of 28) grow on Thayer-Martin selective medium. On the basis of results of deoxyribonucleic acid homology studies (J Clin Microbiol 10:550-556, 1979), the DF-1 organisms belong in the genus *Capnocytophaga*. Three species of *Capnocytophaga* have been described: *C. ochracea*, *C. gingivalis*, and *C. sputigena* (Arch Microbiol 122: 9-16, 1979). The reactions listed for *Capnocytophaga* spp. (DF-1) in this manual probably represent the combined reactions of all three species.

Capnocytophaga spp.[a] (DF-1)

	NUMBER OF STRAINS 155		
TEST PERFORMED	**SIGN**	**% +**	**# +/T**
Morphology	[trt]		
Motility, flagella	v[b]		
Gas from glucose	[−]	0	0/155
Action on blood	v	34 al or gr	40/116;1 ly
Fermentative or oxidative	[F][c]		
Carbohydrate base	F		
Acid from:			
Glucose	[+]	90(10)	134,5w(16)/155
Xylose		0	0/155
Mannitol		0	0/155
Lactose	v	75(11)	113,4w(17)/155
Sucrose	[+]	90(9)	134,6w(14)/155
Maltose	[+ or (+)]	86(14)	128,6w(21)/155
Catalase	[−]	7	3,9w/155
Oxidase	[−]	7	10,1w/155
Growth on:			
MacConkey	[−]	0	0/155
SS		0	0/155
Simmons Citrate	−	0	0/155
Urea, Christensen's	−	0	0/120
Nitrate reduction	v	63	98/155
Gas from nitrate	−	0	0/98
Indole	−	0	0/155
TSI slant, acid	v	73	112/154
TSI butt, acid	v	55	85/154
H_2S (TSI butt)	−	0	0/155
H_2S (Pb ac paper)	v	50	39,38w/155
MR	−	8	1w 13
Gelatin hydrolysis[d]	−	0	0 87
Litmus milk	v	49(7) A	44,30w(10) 152;13 IR
Growth at:			
25°C	v	21	5,20w 119
35°C	+	100	34,85w 119
42°C	v	16	8,11w 119
Esculin hydrolysis	[v]	81(2)	125(3) 155
Lysine decarboxylase	−	0	0 11
Arginine dihydrolase	−	8	2w 26
Ornithine decarboxylase	−	0	0 11
Nutrient broth, 0% NaCl	v	21(1)	3,28w(2) 145
Nutrient broth, 6% NaCl	−	1	2w 145

[a]Yellow nonsoluble pigment produced by 61 of 155 strains.
[b]Gliding motility has been reported; we have observed delayed spreading in motility medium and an occasional single polar or lateral "flagellum".
[c]TSI reaction is not always indicative of fermentative activity.
[d]7-14 days' incubation.

Cardiobacterium hominis

Gram-negative, pleomorphic rod which may form rosettes and may retain crystal violet stain, nonmotile; fastidious, and growth may be enhanced by candle-jar atmosphere; ferments glucose, mannitol, sucrose, and maltose, not xylose or lactose; does not grow on MacConkey agar; oxidase-positive; catalase-, urease-, and nitrate-negative; indole-positive (may be weak).

Sources (32 strains)

Blood (88%), other (12%).

Reference strain

KC1408 ← ATCC, 29308.

Literature

Antonie van Leuwenhoek J Microbiol Serol 30:261-272, 1964; J Med Microbiol 3:91-98; 1970; J Clin Microbiol 5:75-80, 1977.

Comments

Colonies frequently pit the agar substrate. Addition of yeast extract to the medium reported to render cells uniform in appearance. The rare mannitol-negative strain would be difficult to differentiate from *Kingella indologenes*. Snell and Lapage reported (Int J Syst Bacteriol *26*:451-458, 1976) that casein digestion, phosphatase production, and Tween 20 and Tween 40 hydrolysis are negative for *C. hominis*, but positive for *Kingella indologenes*. Found in human nose and throat; a cause of endocarditis in man.



Gram stain BAP 35°C 24 h ×1700.

Cardiobacterium hominis

	NUMBER OF STRAINS 65		
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	mrs		
Motility; flagella	[nm]		
Gas from glucose		0	0/65
Action on blood	v	12 gr	8/65
Fermentative or oxidative	[F][a]		
Carbohydrate base	F[b]		
Acid from:			
Glucose	+ or(+)	80(20)	43,9w(13)/65
Xylose	[-]	0	0/65
Mannitol	[+ or(+)]	52(43)	29,5w(28)/65
Lactose	[-]	0	0/65
Sucrose	+ or(+)	63(35)	35,6w(23)/65
Maltose	+ or(+)	69(31)	37,8w(20)/65
10% Lactose	-	0	0/11
Catalase	[-]	1	1w/65
Oxidase	[+]	100	65/65
Growth on:			
MacConkey	[-]	0	0/65
SS	-	0	0/65
Simmons Citrate	-	0	0/65
Urea, Christensen's	[-]	0	0/58
Nitrate reduction	[-]	0	0/65
Indole	[+]	100	64/64
TSI slant, acid	+	92(1)	58(1)/63
TSI butt, acid	v	81(3)	51(2)/61
H_2S (TSI butt)		0	0/61
H_2S (Pb ac paper)	+	90	55/61
MR	v	50	5/10
VP	-	0	0/6
Gelatin hydrolysis[c]	-	0	0/38
Litmus milk	v	12(5)AW	7w(3)/58;6 IR
Growth at:			
25°C	v	35	15/43
35°C	+	100	55/55
42°C	v	20	8/40
Esculin hydrolysis	-	2	1/52
Nutrient broth, 0% NaCl	v	62	39/63
Nutrient broth, 6% NaCl	-	8	5/62

[a]TSI reaction is not always indicative of fermentative activity.
[b]1-2 drops of rabbit serum per 3 ml of medium may be required; reactions may be obtained within 4 h using the rapid sugar test.
[c]7-14 days' incubation.

Chromobacterium violaceum

Gram-negative, usually straight rod, motile (both 1 polar and 1-4 lateral flagella); ferments glucose, usually without gas, and sometimes sucrose, but not mannitol, or maltose; grows on MacConkey and SS agars; usually produces ethanol-soluble violet pigment; growth best at 30°-35°C, no growth at 4°C; nonpigmented colonies frequently indole-positive; does not require NaCl for growth; lysine and ornithine decarboxylase-negative, arginine dihydrolase-positive.

Sources (38 strains)

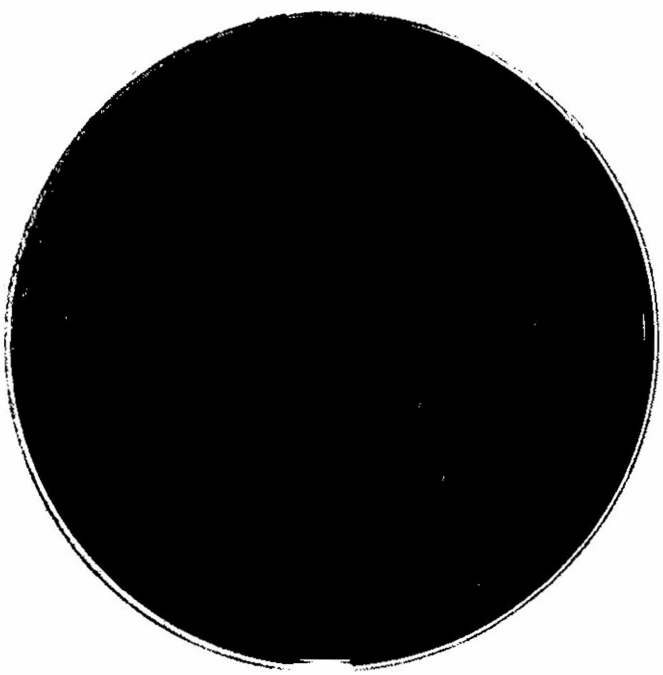
Water (18%), blood (13%), wound (11%), skin lesion (10%), pine shavings (8%), urine (6%), ear (5%), autopsy material (5%), other (16%), unknown (8%).

Reference strains

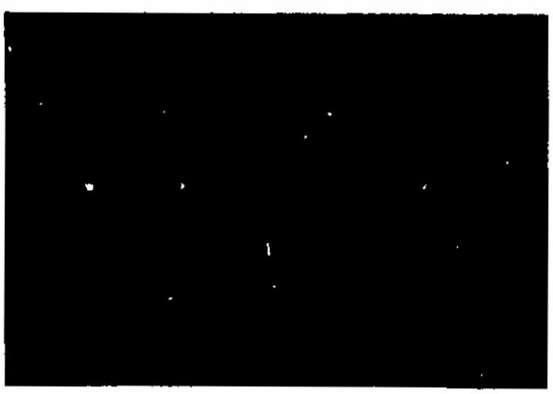
KC531 ← ATCC, 12472 (neotype strain); F4417, from leg abscess.

Literature

AMA Arch Intern Med 88:461-466, 1951; J Bacteriol 71:393-400, 1956; J Gen Microbiol 15:70-98, 1956; Int Bull Bacteriol Nomencl Taxon 8:151-152, 1958; J Clin Microbiol 3:70-71, 1976.



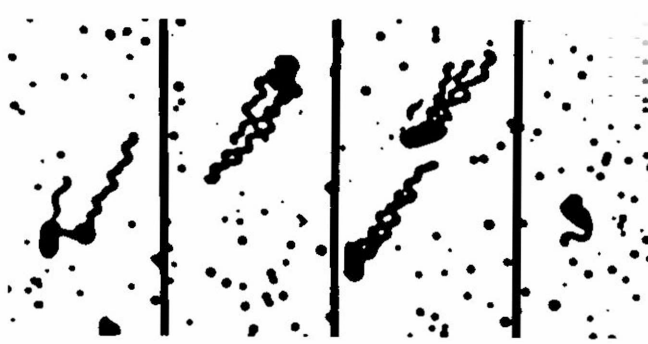
KC531 BAP 35°C 24 h ×1.



KC531 BAP 35°C 24 h ×6.



KC531 Gram stain BAP 35°C 24 h ×1700.



F4417 Flagella stain HIA 25°C 24 h ×1700.

Comments

Common in tropics; soil and water organism; sometimes causes serious pyogenic or septicemic infections of man and other animals.

Chromobacterium violaceum[a]

NUMBER OF STRAINS 37			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	mrs		
Motility: flagella	[m]: p.l.		
Gas from glucose	–[b]	0	0/36
Action on blood	v	48β	14/29/9 ly,1 br
Fermentative or oxidative	[F]		
Carbohydrate base	F		
Acid from:			
Glucose	+	100	36/36
Xylose		0	0/36
Mannitol	[–]	0	0/36
Lactose	[–]	0	0/36
Sucrose	v	20(6)	4/3w(2)/35
Maltose	[–]	0(3)	(1)/36
10% Lactose	-	5	1/21
Catalase	+	97	30/5w/36
Oxidase	v	67	19/3w/33
Growth on:			
MacConkey	[+]	100	35/1w/36
SS	[+ or(+)]	71(23)	25(8)/35
Simmons Citrate	v	68(9)	23(3)/34
Urea, Christensen's	v	5(14)	2(5)/36
Nitrate reduction	+	97	34/35
Indole	v[c]	21	7/33
TSI slant, acid	–	8	3/35
TSI butt, acid	+	94	33/35
H_2S (TSI butt)	–	0	0/34
H_2S (Pb ac paper)	v	41	3/11w/34
MR	v	37	11/30
VP	[–]	0	0/31
Gelatin hydrolysis[d]	v	86	8/11w/22
Litmus milk	pep	97	34/35
Growth in nutrient broth:			
0% NaCl	[+]	100	13/13
6% NaCl	–	8	1/13
Growth at:			
25°C	+	100	34/34
35°C	+	100	34/34
42°C	v	85	29/34
Esculin hydrolysis	–	5[e]	2/37
Lysine decarboxylase	[–]	0	0/22
Arginine dihydrolase	[+]	100	22/22
Ornithine decarboxylase	[–]	0	0/25

[a]91% of strains tested produced a violet pigment.
[b]Gas-producing strains have been described.
[c]Non-pigmented colonies may be indole +.
[d]7-14 days' incubation.
[e]The two esculin-positive strains were not pigmented.

DF-2

Gram-negative medium to long thin rod, frequently slightly tapered, and with occasional spindle forms; nonmotile; fastidious; requires or is enhanced by candle-jar atmosphere; ferments glucose, lactose, and maltose (serum may enhance reaction); no growth on MacConkey agar; oxidase-positive (may be weak); catalase-positive; urease-negative; esculin-positive; nitrate- and indole-negative; arginine dihydrolase-positive.

Sources (27 strains)

Blood (25, at least 10 with histories of dog bite), cerebrospinal fluid (1), ear (1).

Reference strain

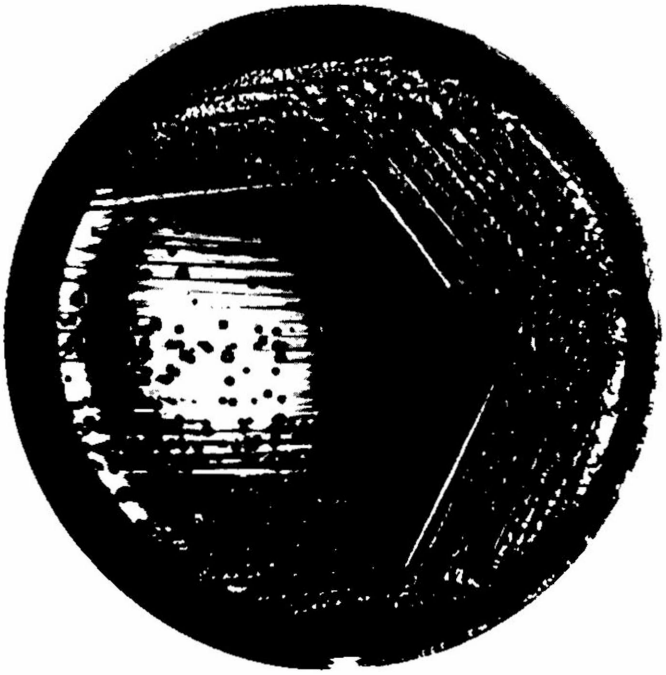
A3565, from blood.

Literature

Ann Int Med 86:1-5, 1977; J Clin Microbiol 7:223-231, 1978.

Comments

In some cases these organisms have been seen in peripheral blood smears. They have been associated with dog bites and have been isolated from canine oral cavities; several patients have had histories of splenectomy.



BAP 35°C 48 h ×1.

DF-2

NUMBER OF STRAINS 27			
TEST PERFORMED	**SIGN**	**% +**	**# + / T**
Morphology	[m,frt]		
Motility; flagella	[nm]		
Gas from glucose		0	0/27
Action on blood	v	33 al	9/27(1 ß,1 hy,1 lav
Fermentative or oxidative	[F][a]		
Carbohydrate base	F[b]		
Acid from:			
Glucose	[v]	67(18)	18(5)/27
Xylose	–	0	0/27
Mannitol	[–]	0	0/27
Lactose	[+ ort + d]	81(19)	22(5)/27
Sucrose	–	0	0/27
Maltose	[+ ort + d]	81(19)	22(5)/27
Catalase	[+]	100	27/27
Oxidase	[+]	96	20,6w/27
Growth on:			
MacConkey	[–]	0	0/27
SS		0	0/27
Simmons Citrate	–	0	0/27
Urea, Christensen's	–	0	0/27
Nitrate reduction	[–]	0	0/27
Gas from nitrate	–	0	0/27
Indole	[–]	0	0/27
TSI slant, acid	v	17	3w/18
TSI butt, acid	v	15	4w/18
H_2S (TSI butt)	–	0	0/5
H_2S (Pb ac paper)	v	17	1,2w/18
Gelatin hydrolysis[c]	–	0	0/9
Litmus milk	–	4 IR	1/27
Growth at:			
25 C	v	30	8/27
35 C	+	100	27/27
42 C	v	18	5/27
Esculin hydrolysis	v	77	15,2w/22
Lysine decarboxylase	–	0	0/18
Arginine dihydrolase	v	61	11/18
Ornithine decarboxylase	–	0	0/18
Nutrient broth, 0% NaCl	+	(4)	(1)/27
Nutrient broth, 6% NaCl	–	0	0/27

[a]TSI reaction is not always indicative of fermentative activity.
[b]1-2 drops of rabbit serum per 3 ml of medium may be required; reactions may be obtained within 4 h using the rapid sugar test.
[c]7-14 days' incubation.

DF-3

Gram-negative small coccoid to short rod; nonmotile; facultatively anaerobic; fastidious; ferments glucose, xylose, lactose, sucrose, and maltose; usually produces indole weakly; catalase- and oxidase-negative; produces no growth on MacConkey agar; does not reduce nitrate; hydrolyzes esculin; produces acid on slant and in butt of TSI agar.

Sources (21 strains)

Blood (26%), wound (15%), urine (9%), peritoneal fluid (6%), umbilicus (6%), abscess (4%), abdominal (4%), stool (4%), genital (4%), other (22%).

Reference strain

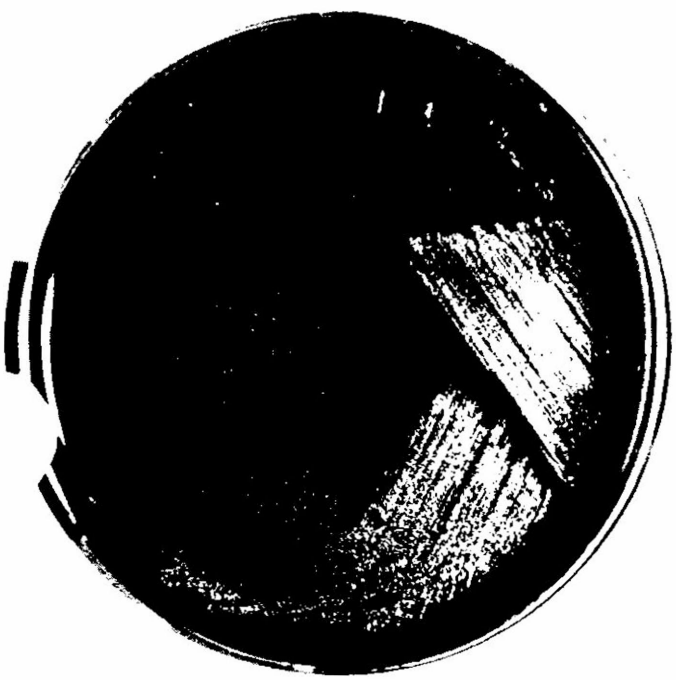
D7608, from shunt.

Literature

None

Comment

Usually two drops of serum must be added to each carbohydrate tube for reaction, which is frequently delayed.



D7608 BAP 35°C 24 h ×1.

DF-3

NUMBER OF STRAINS 21			
TEST PERFORMED	**SIGN**	**% +**	**# +/T**
Morphology	cc,srs		
Motility; flagella	nm		
Gas from glucose		0	0/21
Action on blood		10 al	2/21;1 B
Fermentative or oxidative	[F]		
Carbohydrate base	F[a]		
Acid from:			
Glucose	[+ or (+)]	86(14)	18(3) 21
Xylose	+ or (+)	86(14)	18(3) 21
Mannitol		0	0 21
Lactose	[+ or (+)]	52(43)	11(9) 21
Sucrose	+ or (+)	62(33)	13(7) 21
Maltose	[+ or (+)]	81(19)	17(4) 21
10% Lactose	+	100	5 5
Catalase	[ ]	0	0 21
Oxidase	[ ]	0	0 21
Growth on:			
MacConkey	[-]	0	0 21
SS	-	0	0 21
Simmons Citrate	-	0	0 21
Urea, Christensen's	-	0(4)	0(1) 21
Nitrate reduction	[ ]	0	0 21
Indole	[v]	71(14)	15(3) 21
TSI slant, acid	+	95	20 21
TSI butt, acid	[+]	100	21 21
H_2S (TSI butt)	-	0	0 21
H_2S (Pb ac paper)	+	90	19 21
MR	v	62	5/8
VP	-	0	0/7
Gelatin hydrolysis[b]	-	0	0/5
Litmus milk	v	76 A	16 21;3 IR
Growth at:			
25°C	v	50	10 20
35°C	v	85	17 20
42°C	v	20	4 20
Esculin hydrolysis	[-]	100	21 21
Lysine decarboxylase	-	0	0 4
Arginine dihydrolase	-	0	0 4
Ornithine decarboxylase	-	0	0 4
Nutrient broth, 0% NaCl	v	38	8 21
Nutrient broth, 6% NaCl	-	10	2 21

[a]1-2 drops of rabbit serum per 3 ml of medium may be required; reactions may be obtained within 4h by using the rapid sugar test.
[b]7-14 days' incubation.

EF-4a

Gram-negative coccoid to short rod which stains deeply; nonmotile; ferments glucose only without gas; oxidase- and catalase-positive; sometimes grows on MacConkey agar; does not hydrolyze urea or produce indole; reduces nitrate, accompanied frequently by nitrite reduction with the formation of gas; most strains hydrolyze arginine and digest gelatin; usually phenylalanine-positive.

Sources (97 strains)

Dog bite (32%), wound (22%), cat bite (6%), gum or tonsil of dog or cat (5%), hand (3%), leg (3%), finger (3%), canine wound (3%), cat pulmonary (2%), eye (2%), skin (2%), dog (2%), wallaby mandible, pet monkey mass, and dog teeth (1% each), other (10%), unknown (2%).

Reference strain

D6567, from finger wound.

Literature

Manual of Clinical Microbiology, 2nd ed., pp 289-290 and 291.

Comments

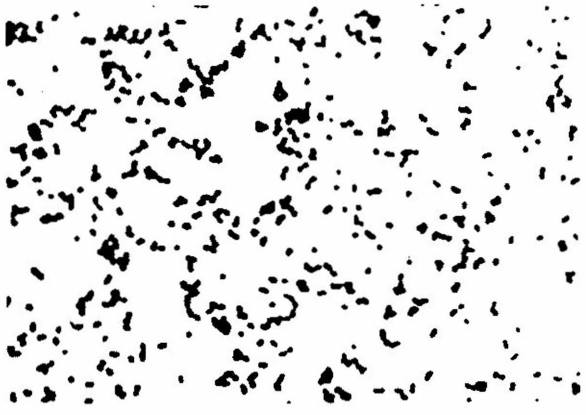
Of 27 substrates, only glucose is fermented. Cultures smell like popcorn. Mostly associated with dog bites. This group, with EF-4b, was originally designated EF-4; recently, it became evident that it contained two groups.



BAP 35°C 24 h ×1.



BAP 35°C 24 h ×6.



Gram stain BAP 35°C 24 h ×1700.

EF-4a[a]

NUMBER OF STRAINS 97			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	cc,srs		
Motility	[nm]		
Gas from glucose	[−]	0	0/97
Action on blood	v	34 ly	30.87;10 gr,8 al,3 gr,1 ß
Fermentative or oxidative	[F]		
Carbohydrate base	F		
Acid from:			
Glucose	+	100	97/97
Xylose		0	0/97
Mannitol		0	0/97
Lactose	[−]	0	0/97
Sucrose	−	0	0/97
Maltose	[−]	0	0/97
10% Lactose	−	0	0/47
Catalase	[+]	100	97/97
Oxidase	[+]	100	97 97
Growth on:			
MacConkey	[v]	42(8)	17,23w(8)/96
SS	−	1	1/96
Simmons Citrate	−	3(1)	3(1) 97
Urea, Christensen's	[−]	0	0/97
Nitrate reduction	[+]	97[b]	94/97
Gas from nitrate	[v]	62	60/97
Indole	[−]	0	0/97
TSI slant, acid	−	3	2,1w/96
TSI butt, acid	v	73	54,16w/96
H_2S (TSI butt)	−	0	0/96
H_2S (Pb ac paper)	+	95(2)	77,15w(2)/97
MR	v	26	5,10w/57
VP		2	1/51
Gelatin hydrolysis[c]	v	60	21/35
Litmus milk		2(1)k	2(1)/93
Growth at:			
25 C	v	89	43,39w/92
35°C	+	100	92 92
42 C	v	70	22,42w 92
Esculin hydrolysis	−	0	0 97
Lysine decarboxylase	−	0	0 57
Arginine dihydrolase	[v]	77(2)	43,1w(1) 57
Ornithine decarboxylase	−	0	0 57
Nutrient broth, 0% NaCl	+	94(2)	73,12w(2)/90
Nutrient broth, 6% NaCl	−	6(2)	3,2w(2) 90

[a]Yellow to amber pigment, soluble or insoluble, produced by some strains.
[b]13 strains (13%) completely reduced nitrate and nitrite without gas formation.
[c]7-14 days' incubation.

EF-4b

Gram-negative coccoid to short rod which stains deeply; nonmotile; oxidizes glucose only; oxidase- and catalase-positive; may grow on MacConkey agar; does not hydrolyze urea or produce indole; reduces nitrate (and sometimes nitrite) without producing gas; does not hydrolyze arginine; usually does not digest gelatin; generally phenylalanine-positive.

Sources (34 strains)

Dog bite (29%), cat bite (21%), cat scratch (9%), hand (12%), wound (6%), arm (6%), finger (6%), other (8%), unknown (3%).

Reference strain

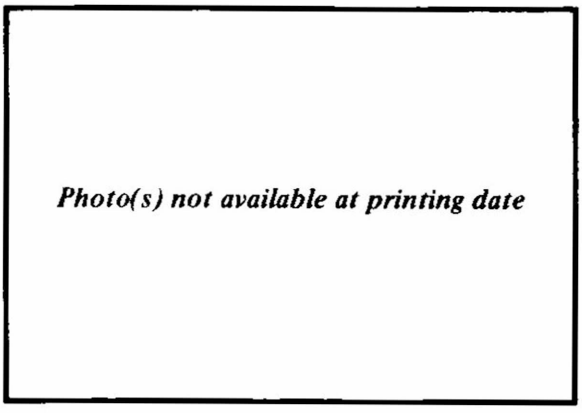
A2617, from cat-bite.

Literature

Manual of Clinical Microbiology, 2nd ed., pp 289-290 and 291.

Comments

This group, with EF-4a, originally was designated EF-4; recently, it became evident that it contained two groups. Group EF-4b does not ferment glucose, does not hydrolyze arginine, and does not produce gas from nitrate during reduction. More of EF-4b strains (than EF-4a) are associated with cats. Cultures smell like popcorn.



EF-4b

NUMBER OF STRAINS 34			
TEST PERFORMED	**SIGN**	% +	# + /T
Morphology	cc,srs		
Motility: flagella	[nm]		
Action on blood	v	41 ly	13/32;4 al,1 gr,1 β
Fermentative or oxidative	[0]		
Carbohydrate base	OF		
Acid from:			
Glucose	+ or(+)	70(26)	4,12w(6)/23
Xylose	-	0	0/23
Mannitol	−	0	0/23
Lactose	[−]	0	0/23
Sucrose		0	0/23
Maltose	[ ]	0	0/23
Catalase	[+]	100	34/34
Oxidase	+	100	34/34
Growth on:			
MacConkey	[v]	65(6)	16,6w(2)/34
SS	−	0	0/34
Simmons Citrate	v	14(6)	4,1w(2)/34
Urea, Christensen's	[−]	0	0/34
Nitrate reduction	[+][a]	97	32,1w/34
Gas from nitrate	[−]	0	0/34
Indole	[−]	0	0/34
TSI slant, acid	−	0	0/34
TSI butt, acid	−	6	2w/34
H_2S (TSI butt)	−	0	0/34
H_2S (Pb ac paper)	v	88	19,11w/34
Gelatin hydrolysis[b]	-	9	3/33
Litmus milk	-	6 k	2/34
Growth at:			
25 C	v	88	19,9w/32
35 C	+	100	29,4w/33
42 C	v	69	5,15w/29
Esculin hydrolysis	-	0	0/34
Lysine decarboxylase	-	0	0/16
Arginine dihydrolase	[−]	0	0/16
Ornithine decarboxylase	-	0	0/16
Nutrient broth, 0% NaCl	+ or(+)	89(7)	18,6w(2)/27
Nutrient broth, 6% NaCl	−	0	0/27

[a] 16 strains (46%) reduced nitrate and nitrite without gas formation.
[b] 7-14 days' incubation.

Eikenella corrodens

Gram-negative slender straight rod, nonmotile; fastidious; does not produce acid from carbohydrates; oxidase- and nitrate-positive (no gas), no growth on MacConkey agar, catalase- and urease-negative; lysine and ornithine decarboxylase-positive.

Sources (506 strains)

Wound (15%), abscess (14%), sputum (6%), abdominal cavity (5%), peritoneal fluid (4%), throat (4%), appendix (4%), blood (4%), pleural fluid (4%), sinus (2%), pus (2%), eye (2%), bronchi (2%), other (26%), unknown (6%).

Reference strain

KC972 ← Henriksen, 333-54-55 (type strain).

Literature

Acta Pathol Microbiol Scand 43:404-416, 1958, and 75:91-96, 1969; J Med Microbiol 3:483-491, 1970, and 4:171-184, 1971; J Clin Pathol 24:117-119, 1971; Int J Syst Bacteriol 22:73-77, 1972; Int J Syst Bacteriol 23:75-76, 1973; Mayo Clin Proc 49:950-953, 1974.

Comments

Formerly "HB-1". Growth enhanced by CO_2; usually pits or corrodes agar; colonial variation may occur; distinctive odor (hypochlorite-like), growth better in peptone nitrate broth than in infusion nitrate broth; frequently shows a slight yellowish growth at 3-7 days, particularly when accumulated on a loop.



Gram stain BAP 35°C 24 h ×1700.

Eikenella corrodens[a]

	NUMBER OF STRAINS 506		
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	mrs		
Motility; flagella	[nm]		
Action on blood	v	17 ly	67/400;24 gr,4 al, 2 β
Fermentative or oxidative	[n-o]		
Carbohydrate base	OF		
Acid from:			
Glucose	[-]	0	0/67
Xylose	[-]	0	0/67
Mannitol	-	0	0/67
Lactose	-	0	0/67
Sucrose		0	0/67
Maltose	-	0	0/67
Catalase	[-]	8	20,20w/482
Oxidase	[+]	100	502/502
Growth on:			
MacConkey	[-]	0	2/484
SS	-	0	0/481
Simmons Citrate	-	0	0/484
Urea, Christensen's	[-]	0	0/450;4 pk
Nitrate reduction	[+]	99	502/504
Gas from nitrate	[-]	0	0/504
Indole	-	0	0/489
TSI slant, acid	-	0	0/446
TSI butt, acid	-	0	0/446
H_2S (TSI butt)	-	0	0/479
H_2S (Pb ac paper)	v	73	131,225w/489
Gelatin hydrolysis[b]	-	0	0/258
Litmus milk	-	1 k	4/484;3 IR
Pigment	v	0(70) sl yel	(160)/227
Growth at:			
25°C	v	15	50/337
35°C	+	99	339/342
42°C	v	52	174/337
Esculin hydrolysis	-	0	0/456
Lysine decarboxylase	v	82	49/59
Arginine dihydrolase	-	0	0/59
Ornithine decarboxylase	[+]	98	58/59
Growth in:			
Nutrient broth, 0% NaCl	v	71	284/399
Nutrient broth, 6% NaCl	-	1	5/427

[a]Candle jar (CO_2) required or enhances growth.
[b]7-14 days' incubation.

EO-2

Gram-negative coccoid to short thick rod, frequently appearing vacuolated or peripherally stained ("O"-shaped), in pairs and short chains or packets; nonmotile; aerobic; uses glucose, xylose, lactose, sometimes mannitol, and maltose, not sucrose; growth on MacConkey agar variable; oxidase-positive; may reduce nitrate, without gas; does not produce indole; lysine decarboxylase- and arginine dihydrolase-negative.

Sources (93 strains)

Urine (15%), eye (14%), blood (7%), wound (6%), pleural fluid (5%), cerebrospinal fluid (5%), urogential (4%), leg (3%), lung (3%), finger (2%), exudate (2%), isolette (2%), foot (2%), nonhuman (poultry, horse) (2%), other (25%), unknown (3%).

Reference strain

D5834, from throat.

Literature

None.

Comments

Growth frequently is mucoid. Biochemical reactions are similar to those of *Acinetobacter calcoaceticus* (glucose-oxidizing); major differences are the oxidase reaction and the *Acinetobacter* transformation test (negative for this organism). Possibly the same as, or similar to, "Other *Moraxella*-like Taxa" cited in Bergey's Manual, 8th ed., p 436.



Gram stain BAP 35°C 24 h ×1700.

EO-2[a]

TEST PERFORMED	SIGN	% +	# + /T
	NUMBER OF STRAINS 93		
Morphology	cc		
Motility; flagella	[nm]		
Action on blood	v	23 al or gr	19/82;11 ly,2 β,1 br,1 LG
Fermentative or oxidative	[0]		
Carbohydrate base	OF		
Acid from:			
Glucose	[+]	100	89,4w/93
Xylose	[+]	99(1)	91,1w(1)/93
Mannitol	v	10(10)	9w(9)/93
Lactose	+	91(6)	72,13w(6)/93
Sucrose	[-]	0	0/93
Maltose	v	20(1)	6,13w(1)/93
Catalase	+	98	81,10w/93
Oxidase	[+]	100	92,1w/93
Growth on:			
MacConkey	v	63(11)	40,19w(10)/93
SS	-	4(1)	2,2w(1)/93
Cetrimide	-	0	0/93
Simmons Citrate	v	22(3)	19,2w(3)/93
Urea, Christensen's	v	74(3)	68,1w(3)/93
Nitrate reduction	v	85	77,2w/93
Gas from nitrate	[-]	2	2w/93
Indole	[-]	0	0/93
TSI slant, acid	--	0	0/92
TSI butt, acid	--	0	0/92
H_2S (TSI butt)	-	0	0/93
H_2S (Pb ac paper)	v	75	53,17w/93
Gelatin hydrolysis[b]	-	0	0/58
Litmus milk	v	32 k	28/87;3 A,1 pep
Pigment	v	17 yel sol	16/93;13 ta-br;9 yel ins
Growth at:			
25°C	v	83	74,3w/93
35°C	+	95	88/93
42°C	v	35	9,24w/93
Esculin hydrolysis	-	0	0/93
Lysine decarboxylase	[-]	0	0/6
Arginine dihydrolase	[-]	0	0/6
Ornithine decarboxylase	-	0	0/6
Nutrient broth, 0% NaCl	v	66	37,22w/90
Nutrient broth, 6% NaCl	v	34	21,10w/91

[a]Colonies frequently are mucoid.
[b]7-14 days' incubation.

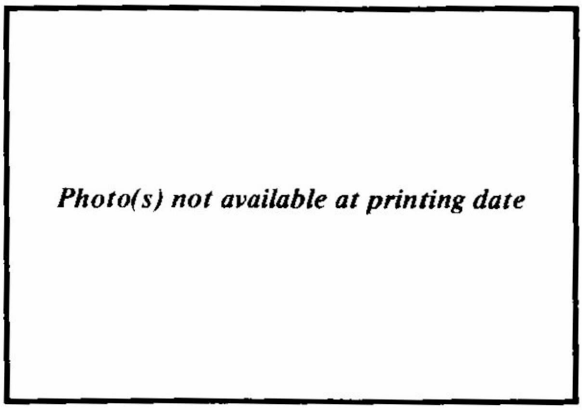
Flavobacterium breve

Gram-negative short rod, nonmotile; uses glucose, maltose, and starch, not mannitol or trehalose, ONPG-negative; grows on MacConkey agar; indole-, oxidase-positive; nitrate- and urea-negative; proteolytic; produces a light yellow pigment; does not hydrolyze esculin.

Source (1 strain)
Bronchial section.

Reference strain
KC1428← NCTC, 11099 (proposed neotype).

Literature
Int J Syst Bacteriol 28:201-208, 1978.



Flavobacterium breve

	NUMBER OF STRAINS 3		
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	s-lr,fc		
Motility: flagella	[nm]		
Action on blood	sl hy	100	3/3
Fermentative or oxidative	[O]		
Carbohydrate base	OF		
Acid from:			
Glucose	[+]	100	3/3
Xylose		0	0/3
Mannitol	[ ]	0	0/3
Lactose		0	0/3
Sucrose	–	0	0/3
Maltose	+	100	3/3
Starch	+	100	2/2
Trehalose	[ ]	0	0/2
ONPG	[ ]	0	0/3
Catalase	+	100	3/3
Oxidase	[+]	100	3/3
Growth on:			
MacConkey	[+]	100	3/3
SS	-	0	0/3
Simmons Citrate	–	0	0/3
Urea, Christensen's	–	0	0/3
Nitrate reduction	–	0	0/3
Indole	[+]	100	3/3
TSI slant, acid	-	0	0/3
TSI butt, acid	-	0	0/3
H_2S (TSI butt)		0	0/3
H_2S (Pb ac paper)	+ or(+)	33(67)	1(2)/3
Gelatin hydrolysis[a]	+	100	3/3
Litmus milk	v	67 k	2/3(1) IR
Pigment, insoluble	sl yel	100	3/3
Pigment, soluble	(br)	0(100)	(3)/3
Growth at:			
25°C	+	100	3/3
35°C	+	100	3/3
42°C	-	0	0/3
Esculin hydrolysis	[–]	0	0/3
Lysine decarboxylase	–	0	0/3
Arginine dihydrolase	–	0	0/3
Ornithine decarboxylase	–	0	0/3
Nutrient broth, 0% NaCl	+	100	3/3
Nutrient broth, 6% NaCl	–	0	0/3

[a]7-14 days' incubation.

Flavobacterium meningosepticum

Gram-negative slightly pleomorphic rod (cells often appear thin to very thin in the central region, with thicker ends, "II-forms", so-called because these forms occurred in King's Group II organisms, which included *Flavobacterium meningosepticum* and Group IIb); motility not demonstrated in motility medium; however, Webster and Hugh demonstrated flagella and reported indirect evidence of motility (Int J Syst Bacteriol 29:333-337, 1979); grows well aerobically; uses glucose, mannitol, maltose, and lactose (the latter, slowly); starch hydrolysis-negative; ONPG-positive, weakly indole-positive, oxidase-positive, nitrate-negative; usually grows on MacConkey agar; pigment, if present, only slightly yellow; proteolytic; hydrolyzes esculin.

Sources (148 strains)

Cerebrospinal fluid (24%), blood (19%), urine (7%), sputum (7%), trachea (3%), eye (3%), nose (2%), other (18%), unknown (17%).

Reference strain

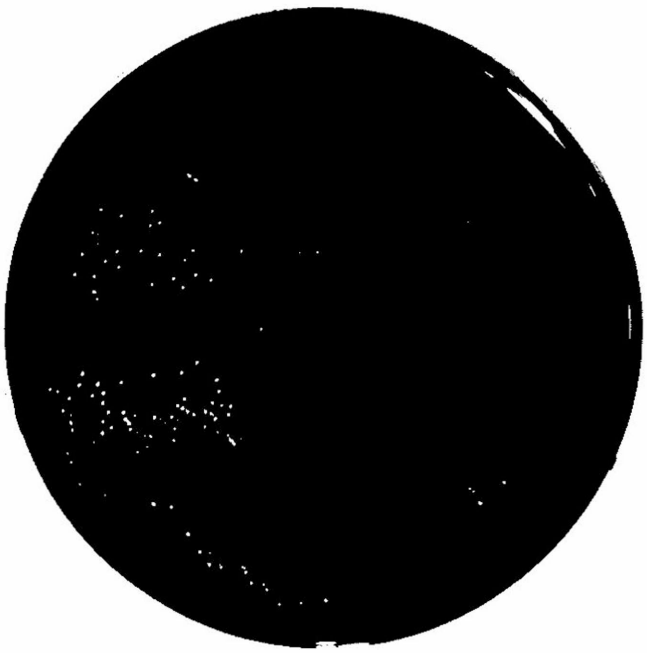
KC1378 ← ATCC, 13253 (NCTC 10016) (type culture).

Literature

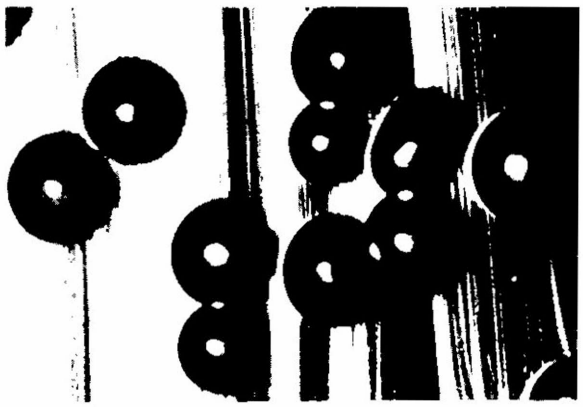
Ann Soc Belg Med Trop 38:563-570, 1958; Am J Clin Pathol 31:241-247, 1959; Belg Tijdschr Geneeskd 21:244-256, 1965.

Comments

Appears as an oxidizer at 48 h incubation; usually acidifies the butt of TSI in 3-7 days. Pathogenic for man, especially premature infants; associated with meningitis and septicemia.



BAP 35°C 24 h ×1.



BAP 35°C 24 h ×6 (axial light).



Gram stain BAP 35°C 24 h ×1700.

Flavobacterium meningosepticum

	NUMBER OF STRAINS 148		
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	lrs,II		
Motility: flagella	[nm][a]		
Action on blood	v	75 LG	103/138;22 ly,4 al
Fermentative or oxidative	[O]		
Carbohydrate base	OF		
Acid from:			
Glucose	[+]	95(4)	123,12w(6) 142
Xylose	-	2(1)	1,2w(2) 142
Mannitol	[+]	91(8)	101,30w(11) 143
Lactose	v	42(15)	34,23w(20) 134
Sucrose	-	0	0 143
Maltose	+	93(7)	122,9w(10) 141
Starch	-	0	0 15
Trehalose	+	100	15 15
ONPG	+	100	15/15
Catalase	+	100	146/146
Oxidase	[+]	99	147/148
Growth on:			
MacConkey	[+ or(+)]	89(3)	130(5)/146
SS	-	1	2/146
Simmons Citrate	v	9(3)	10,3w(4)/146
Urea, Christensen's		3(5)	4(8)/147
Nitrate reduction	[-]	0	0/146
Indole	[+]	100	55,45w/100
TSI slant, acid		0	0/148
TSI butt, acid		0(3)	(5)/148
H_2S (TSI butt)	-	3	4w/148
H_2S (Pb ac paper)	+	98	145/148
Gelatin hydrolysis[b]	+	91	111/121
Litmus milk	[pep]	98	141/144
Pigment, insoluble	c		
Pigment, soluble	v	0(37)ta-br	(61) 106
Growth at:			
25°C	+	100	144/144
35°C	+	100	144/144
42°C	v	45	65 143
Esculin hydrolysis	[+]	99	140/141
Nutrient broth, 0% NaCl	+	100	135 135
Nutrient broth, 6% NaCl	-	7	10 135

[a]Polar and lateral flagella have been demonstrated on some strains.
[b]7-14 days' incubation.
[c]Some strains exhibit slight yellow pigment.

Flavobacterium multivorum

Gram-negative coccoid to short rod; nonmotile; aerobic; grows on MacConkey agar; oxidizes glucose, xylose, lactose, sucrose, and maltose, not mannitol; hydrolyzes urea; usually does not reduce nitrate; oxidase-positive; indole-negative; hydrolyzes esculin; lysine- and arginine-negative.

Sources (22 strains)

Blood (36%), urine (14%), cerebrospinal fluid (9%), other [41% - 1 each: serous cavity fluid, wound, eye discharge, abdominal fluid, throat, skin, spleen, abdominal hematoma, catheter tip (kidney transplant)].

Reference strain

B9570, from whirlpool tank.

Literature

Manual of Clinical Microbiology, 2nd ed. p 281; Int J Syst Bacteriol 27:133-146, 1977; Int J Syst Bacteriol 31:21-34, 1981.

Comment

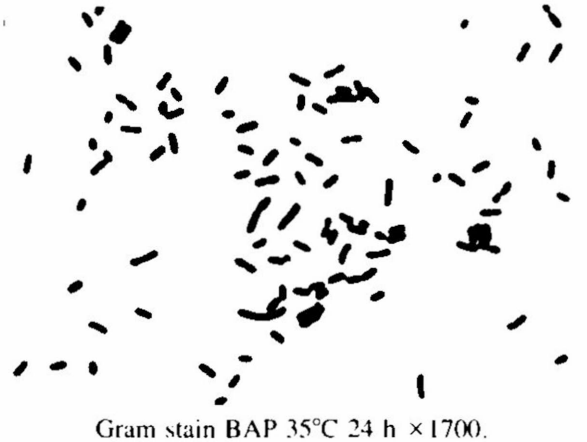
Formerly called IIk-2.



BAP 35°C 24 h ×1.



BAP 35°C 24 h ×6.



Gram stain BAP 35°C 24 h ×1700.

Flavobacterium multivorum

NUMBER OF STRAINS 22			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	srs		
Motility: flagella	[nm]		
Action on blood	v	14 gr	3/21; 3 lav, 2 LG, 1 ly, 1 al (stab)
Fermentative or oxidative	O		
Carbohydrate base	OF		
Acid from:			
Glucose	[+]	100	22/22
Xylose	+	100	22/22
Mannitol	[−]	0	0/22
Lactose	+	100	22/22
Sucrose	+	100	22/22
Maltose	[+]	100	22/22
Catalase	+	100	16,5w/21
Oxidase	[+]	100	22/22
Growth on:			
MacConkey	[+]	100	19,3w/22
SS	−	0	0/22
Cetrimide	−	0	0/22
Simmons Citrate	−	0	0/22
Urea, Christensen's	[+]	95	21/22
Nitrate reduction	−	0	0/21
Gas from nitrate	−	0	0/21
Indole	[−]	0	0/22
TSI slant, acid	v	57(5)	10,2w(1)/22
TSI butt, acid	v	5(76)	1(16)/21
H_2S (TSI butt)	−	0	0/21
H_2S (Pb ac paper)	+	86(5)	19(1)/22
Gelatin hydrolysis[a]	−	0	0/18
Litmus milk	−	0(10) Aw	(2)/20; 1 k, 1 Aw
Pigment:			
Soluble	−	5 ta	1/21
Insoluble	v	57 sl yel	12/21
Growth at:			
25°C	+	100	20/20
35°C	+	100	20/20
42°C	−	0	0/20
Esculin hydrolysis	[+]	100	22/22
Lysine decarboxylase	[−]	0	0/4
Arginine dihydrolase	[−]	0	0/4
Ornithine decarboxylase	−	0	0/4
Nutrient broth, 0% NaCl	+	100	20/20
Nutrient broth, 6% NaCl	v	25	5/20

[a] 7-14 days' incubation.

Flavobacterium odoratum

Gram-negative straight to slightly curved rod, nonmotile; aerobic; does not use carbohydrates; oxidase- and strongly urease-positive, indole- and nitrate-negative; usually grows on MacConkey agar; proteolytic; usually produces a yellow growth pigment; H_2S-negative on TSI agar.

Sources (74 strains)

Urine (62%), foot (5%), leg (5%), animal (5%), toe wound (4%), other wounds (4%), sputum (3%), other (11%).

Reference strain

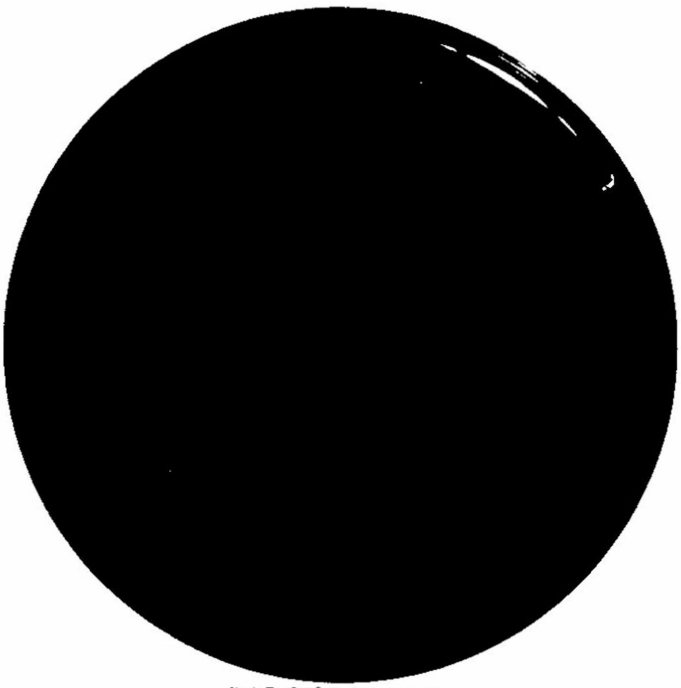
D7703, from urine.

Literature

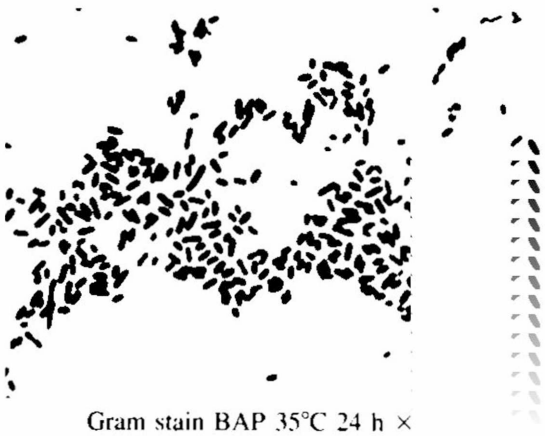
Zentralbl Bakteriol Parasitenkd Infektionskr Hyg Abt Orig 102:113, 1929; Int J Syst Bacteriol 27:330-336, 1977.

Comments

Formerly called M-4f. Usually exhibits fruity odor; colonies are usually dull and spreading.



BAP 35°C 24 h ×1.



Gram stain BAP 35°C 24 h ×

Flavobacterium odoratum

	NUMBER OF STRAINS 74		
TEST PERFORMED	**SIGN**	**% +**	**# +/T**
Morphology	plr		
Motility: flagella	[nm]		74
Action on blood	v	77 LG	57/74;4 lav,4 gr,2 ly,2 ß
Fermentative or oxidative	[n-o]		
Carbohydrate base	OF		
Acid from:			
Glucose	[ ]	0	0/74
Xylose	[ ]	0	0/74
Mannitol	–	0	0/74
Lactose	–	0	0/74
Sucrose	–	0	0/74
Maltose	–	0	0/74
Catalase	+	100	74/74
Oxidase	[+]	99	73/74
Growth on:			
MacConkey	[+]	91(5)	67(4)/74
SS	v	30(11)	22(8)/74
Simmons Citrate	–	0	0/74
Urea, Christensen's	[+]	100	74/74
Nitrate reduction	[-]	0	0/74
Nitrite reduction	v	83	20/24
Indole	[–]	0	0/74
TSI slant, acid	–	0	0/74
TSI butt, acid	–	0	0/74
H_2S (TSI butt)	[–]	0	0/74
H_2S (Pb ac paper)	v	16	12/74
Gelatin hydrolysis[a]	[+]	96	53/55
Litmus milk	pep	93	69/74;5 IR,1 k
Pigment:			
Insoluble	v	85 vel	63/74
Soluble	v	28 ta-br	21/74
Growth at:			
25°C	+	100	74/74
35°C	+	100	74/74
42°C	v	31	23/74
Esculin hydrolysis	–	0	0/70
Lysine decarboxylase	–	0	0/11
Arginine dihydrolase	–	0(9)	(1)/11
Ornithine decarboxylase	–	0	0/11
Nutrient broth, 0% NaCl	+	100	66/66
Nutrient broth, 6% NaCl	v	20(5)	13(3)/66

[a]7-14 days' incubation.

Flavobacterium sp. (IIb)

Gram-negative slightly pleomorphic rod (cells often appear thin to very thin in the central region, with thicker ends, the so-called "II forms"), motility not demonstrated; grows well aerobically; all strains use glucose, maltose, and trehalose, not mannitol; ONPG-negative, usually starch hydrolysis-positive; oxidase-positive; proteolytic, weakly indole-positive; produces a yellow-orange growth pigment.

Sources (155 strains)

Blood (16%), sputum (10%), throat (9%), urine (8%), lung (6%), spinal fluid (4%), brain (4%), nose (2%), trachea (2%), other (29%), unknown (10%).

Reference strain

B7755, from a water fountain.

Literature

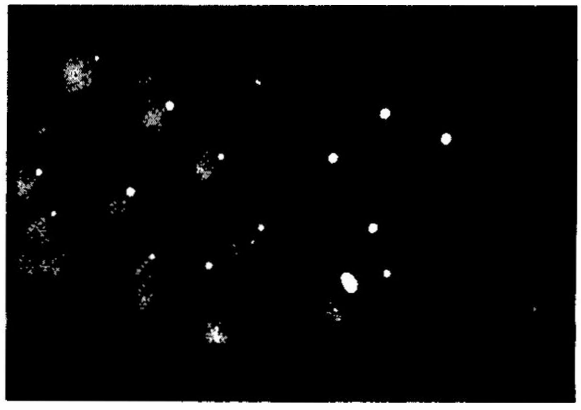
Am J Clin Pathol 31:241-247, 1959; Manual of Clinical Microbiology, 2nd ed. pp 285-287.

Comments

Relationship to *F. meningosepticum* is not fully established; usually nitrate-negative, although some strains reduce nitrate and nitrite completely without gas formation. Some strains produce a fruity odor. It appears that IIb is a heterogeneous group.



BAP 35°C 24 h ×1.



BAP 35°C 24 h ×6.



Gram stain BAP 35°C 24 h ×1700.

Flavobacterium sp. (IIb)

NUMBER OF STRAINS 155			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	mrs.II		
Motility; flagella	[nm]		
Action on blood	v	26 LG	36 144;35 ly,32 β,22 gr
Fermentative or oxidative	[0]		
Carbohydrate base	OF		
Acid from:			
Glucose	[+]	92(6)	82,58w(10)/153
Xylose	v	30(1)	2,44w(1) 153
Mannitol	[−]	10	6,10w 153
Lactose	−	0	0 153
Sucrose	v	13(1)	14,16w(2) 153
Maltose	+	92(6)	85,55w(9) 153
Starch	+	100	10,4w 14
Trehalose	[+]	100	14 14
ONPG	v	57	8 14
Catalase	+	99	149,5w 155
Oxidase	[+]	96	133,15w 154
Growth on:			
MacConkey	v	54(9)	52,32w(14)/155
SS	−	0	0 155
Simmons Citrate	-	2(1)	4(2)/155
Urea, Christensen's	v	14(28)	15,7w(43)/154
Nitrate reduction	v	22	34 155
Gas from nitrate	-	0	0 155
Nitrite reduction	v	20	31 155
Indole	[+]	98	109,43w/155
TSI slant, acid		1	1w/155
TSI butt, acid	-	10	1,7w(7)/153
H_2S (TSI butt)	−	1	1,1w/155
H_2S (Pb ac paper)	+	99	135,18w/155
Gelatin hydrolysis[a]	v	78	120 153
Litmus milk	[pep]	93	144 155
Pigment			
Insoluble	[yel]	99	154 155
Soluble	v	11 yel-br	17 155
Growth at:			
25°C	+	100	155 155
35°C	+	100	155 155
42°C	v	42	17,48w 155
Esculin hydrolysis	v	70	105,4w 149
Nutrient broth, 0% NaCl	+	100	143 143
Nutrient broth, 6% NaCl	−	0	0 142

[a]7-14 days' incubation.

Flavobacterium spiritivorum

Gram-negative rod, non-motile; aerobic; oxidizes glucose, mannitol, and other carbohydrates; oxidase-positive, indole-negative, urea-positive (sometimes delayed), and nitrate-negative; may grow on MacConkey agar.

Sources (11 strains)

Urine (2), vagina (2), and one each from peritoneal fluid, sputum, bone marrow, uterus, humidifier, and mediastinal fluid.

Literature

Int J Syst Bacteriol *32*:157-165, 1982.

Comment

The ability of *F. spiritovorum* to produce acid from mannitol (and also ethanol) distinguishes it from other *Flavobacterium* species.

Photo(s) not available at printing date

Flavobacterium spiritivorum

TEST PERFORMED	SIGN	% +	# + /T
	NUMBER OF STRAINS 11		
Morphology	srs		
Motility: flagella	nm		
Action on blood		0	0/11
Fermentative or oxidative	O		
Carbohydrate base	OF		
Acid from:			
Glucose	[+]	100	11/11
Xylose	+	91(9)	9,1w(1)/11
Mannitol	[+]	100	11/11
Lactose	+	100	11/11
Sucrose	+	100	10,1w/11
Maltose	+	100	11/11
Catalase	+	100	11/11
Oxidase	[+]	100	11/11
Growth on:			
MacConkey	[v]	0(55)	(6)/11
SS	−	0	0/11
Cetrimide	−	0	0/11
Simmons Citrate	−	0	0/11
Urea, Christensen's	[+ or(+)]	55(36)	6(4)/11
Nitrate reduction	−	0	0/11
Gas from nitrate	−	0	0/11
Indole	[−]	0	0/11
TSI slant, acid	−	0	0/9
TSI butt, acid	−	0	0/9
H_2S (TSI butt)	−	0	0/9
H_2S (Pb ac paper)	v	56	5/9
Gelatin hydrolisis[a]	v	11	1/9
Litmus milk	−	0	0/9
Pigment:			
Insoluble	v	36 pale yel	4/11
Growth at:			
25°C	+	100	9/9
35°C	+	100	9/9
42°C	v	11	1/11
Esculin hydrolysis	+	100	11/11
Lysine decarboxylase	−	0	0/8
Arginine dihydrolase	v	25	2/8
Ornithine decarboxylase	−	0	0/8
Nutrient broth, 0% NaCl	+	100	9/9
Nutrient broth, 6% NaCl	−	0	0/9

[a]7-14 days' incubation.

Francisella tularensis

Gram-negative, very small coccoid form, nonmotile; fastidious, requires or is stimulated by cystine or cysteine; glucose-cysteine-peptone agar with blood, chocolate agar with IsoVitaleX, and Thayer-Martin medium support growth (the latter medium is useful for isolating *F. tularensis* from a mixed bacterial population); heart infusion agar with rabbit blood and charcoal yeast extract agar containing ketoglutarate enable some strains to grow; slide agglutination and/or direct fluorescent antibody tests are used to confirm identification. Carbohydrate reactions are determined on cysteine agar slants. Oxidase-negative; does not grow on MacConkey agar; urea- and nitrate-negative.

Sources (40 strains)

Blood (25%), dog tick (12%), lymph node (12%), small animals (muskrat, marmoset, beaver, rabbit) (10%), superficial lesion (10%), pleural fluid (5%), cat bite (5%), other (eye, lung, bone marrow) (8%), unknown (12%).

Reference strain

KC619 ← Biegeleisen, P-35.

Literature

Symp Res Works Inst Epidemiol Mikrobiol Chita 1:170-180, 1947; Tularemia. Philadelphia, W. B. Saunders Co., 1973.

Comments

Very infectious in the laboratory; causes tularemia in man; usually is transmitted from wild animals to man. Acid production from glycerol and presence of citrulline ureidase help distinguish strains fully virulent for humans and rabbits from those of lower virulence (J Hyg Epidemiol Microbiol Immunol (Prague) 3:138-149, 1959; J Bacteriol 82:26-32, 1961). The very small size of *F. tularensis* stained with the Gram stain should help point to its identity.



Gram stain CA 35°C 48 h ×1700.

Francisella tularensis

NUMBER OF STRAINS 53			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	[rv]		
Motility	nm		
Action on blood	–	0	0/13
Fermentative or oxidative			
Carbohydrate base	CA		
Acid from:			
Glucose	+ or(+)	86(14)	20,4w(4) 28
Xylose			
Mannitol			
Lactose			
Sucrose			
Maltose			
Glycerol	v	46(7)	11,2w(2) 28
Catalase	+	93	20,7w 29
Oxidase	[–]	2	1w 41
Growth on:			
MacConkey	[–]	0	0/27
SS	–	0	0/27
Simmons Citrate	–	0	0/27
Urea, Christensen's	[–]	0	0/44
Nitrate reduction	[–]	4	1w/25
Gas from nitrate	–	0	0/25
Indole	–	0(3)	(1)/32[a]
TSI slant, acid	–	0	0/19
TSI butt, acid	–	0	0/19
H_2S (TSI butt)	–	0	0/19
H_2S (Pb ac paper)	v	42	3,5w, 19
Gelatin hydrolysis[b]	–	0	0/5
Growth at:			
25°C	v	67	2/3
35°C	+	100	53/53
42°C	–	0	0/3
Esculin hydrolysis	–	0	0/14
Nutrient broth, 0% NaCl	–	6	1/17
Nutrient broth, 6% NaCl	–	0	0/19

[a]The indole reaction was obtained in Heart Infusion Broth supplemented with rabbit serum and was determined after 7 days' incubation.
[b]7-14 days' incubation.

Gardnerella vaginalis

Gram-negative or gram-variable, delicate "diphtheroid" rod, nonmotile; fastidious, but X and V factors are not required; ferments glucose, maltose, starch, and sometimes xylose, fructose, and sucrose, but not mannitol; serum usually is added to the carbohydrate tubes, and growth generally occurs only in positive tubes; in 2-7 days a wide (sometimes double) zone of incomplete beta-like hemolysis usually is seen on heart infusion agar with 5% rabbit blood (this action may occur before colonies are visible); incubation in CO_2 or candle jar is required. Oxidase- and catalase-negative; no growth occurs on MacConkey agar.

Sources (126 strains)

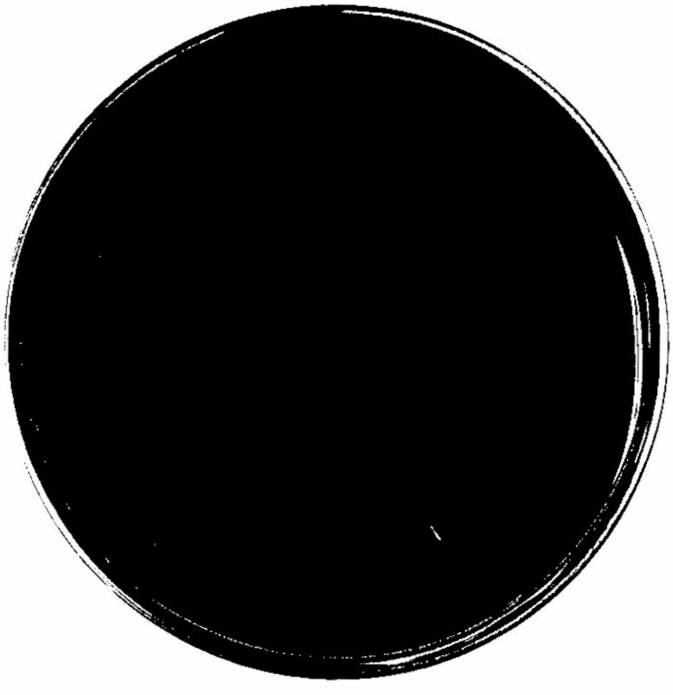
Vagina (23%), urine (19%), blood (19%), cervix (8%), miscellaneous urogenital (penis, placenta, Bartholin gland, labium lesion, ovarian abscess, Skene's gland) (6%), amniotic fluid (3%), uterus (3%), urethra (2%), other (stool, arthrocentesis fluid, wound, cerebrospinal fluid, abscess, pilonidal cyst, throat, breast) (8%), unknown (9%).

Reference strains

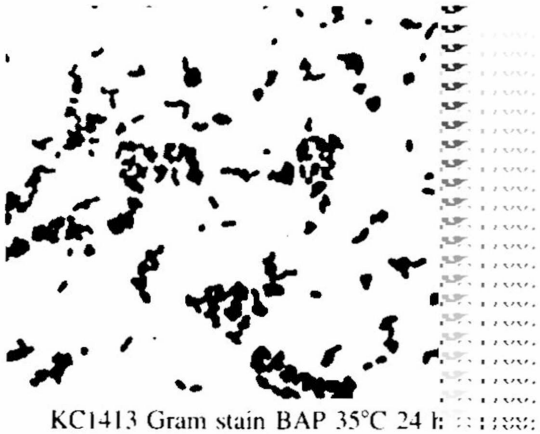
KC1412 ← Skaggs, 594 (type strain, dark colony variant); KC1413 ← Skaggs, 594 (type strain, gold colony variant).

Literature

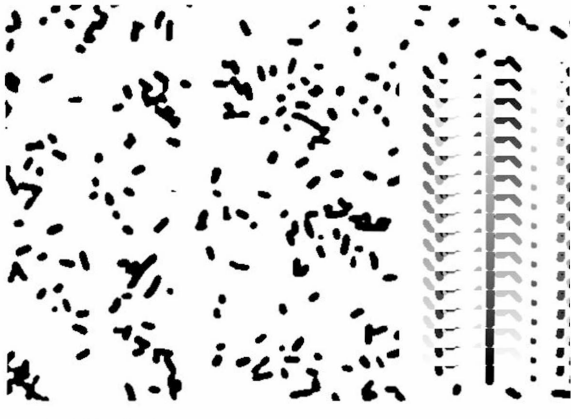
J Pathol Bacteriol 85:213-219, 1963; Antonie van Leeuwenhoek J Microbiol Serol 35:129-145, 1969; Am J Clin Pathol 53:370-377, 1970; Can J Microbiol 17:868-869, 1971; J Clin Microbiol 2:272-275, 1975; J Clin Microbiol 9:200-204, 1979; Int J Syst Bacteriol 30:170-178, 1980; N Eng J Med 303:601-607, 1980.



KC1412 BAP 35°C 48 h ×1 (transmitted light).



KC1413 Gram stain BAP 35°C 24 h



KC1412 Gram stain BAP 35°C 24 h ×1700.

Comments

G. vaginalis formerly was named *Haemophilus vaginalis* and *Corynebacterium vaginale*. This organism has been reported also to hydrolyze hippurate and to hemolyze human (not sheep) blood. A cause of "nonspecific" vaginitis and urethritis; in the discharge, the epithelial cells may be covered with masses of bacteria, "clue cells".

Gardnerella vaginalis

NUMBER OF STRAINS 126			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	srs (gram variable)		
Motility; flagella	nm		
Gas from glucose	–	0	0/122
Action on blood	[inc β]	93	98/105;3 al
Fermentative or oxidative	F[a]		
Carbohydrate base	I[b]		
Acid from:			
Glucose	[+ or(+)]	81(13)	93,6w(16)/122
Xylose	v	36(9)	40,2w(10)/116
Mannitol	[ ]	0	0/116
Lactose		0(1)	(1)/114
Sucrose	v	11(4)	11,2w(5)/114
Maltose	[+ or(+)]	87(13)	109(16)/125
Fructose	v	60(23)	53,13w(25)/100
10% Lactose	ngr		
Catalase	[–]	2	2w/81
Oxidase	[–]	6	4w/73
Growth on:			
MacConkey	[–]	5	2w/42
SS	–	0	0/43
Simmons Citrate	–	0	0/41
Urea, Christensen's	–	0(5)	(2)/43
Nitrate reduction	–	0	0/85
Gas from nitrate	–	0	0/85
Indole	–	0	0/61
TSI slant, acid	v	14	2,4w/44,28 ngr
TSI butt, acid	v	14	2,4w/44,28 ngr
H_2S (TSI butt)	–	0	0/16
H_2S (Pb ac paper)	v	31	5/16
MR	v	17	2/12
Gelatin hydrolysis[c]	–	0	0/43
Litmus milk	v	32(16) A	4,8w(6)/38
Growth at:			
25°C		5	1/20
35°C	+	100	20/20
42°C	v	14	3/21
Esculin hydrolysis	–	0	0/37
Nutrient broth, 0% NaCl	v	24	8/33
Nutrient broth, 6% NaCl	–	0	0/36

[a]TSI reaction not always indicative of fermentative activity.
[b]1-2 drops of rabbit serum per 3 ml of medium may be required.
[c]7-14 days' incubation.

Haemophilus aegyptius

Gram-negative small to medium rod, sometimes filamentous, nonmotile; requires X and V factors; grows in air or in air with increased CO_2; uses glucose but not xylose; oxidase-variable; indole- and ornithine-negative; primary isolates usually agglutinate human erythrocytes.

Source

Conjunctiva

Reference strain

KC1018 ← ATCC 11116 (NCTC 8502). Suggested "working type culture".

Literature

J Bacteriol 59:413-426, 1950.

Comments

M. Kilian (J Gen Microbiol 93:9-62, 1976) has proposed including *H. aegyptius* in biotype III of *H. influenzae*. Common name: Koch-Weeks bacillus. A cause of acute or subacute infectious conjunctivitis.



BAP 35°C 24 h ×1 (axial light).

Gram stain BAP 35°C 24 h

Haemophilus aphrophilus

Gram-negative small rod or filament, nonmotile; fastidious, requires or is enhanced by a candle-jar atmosphere; ferments glucose with slight gas formation, lactose, sucrose, and maltose; catalase-negative, nitrate-positive; indole-negative; esculin hydrolysis-negative.

Sources (203 strains)

Blood (25%), brain abscess (8%), other abscesses (6%), pleural fluid (4%), wound (4%), mouth (4%), eye (4%), sputum (3%), sinus (2%), mandible (2%), brain (2%), cerebrospinal fluid (2%), bone fragment, throat, trachea, nose, cyst, neck, tooth, skin, lung, pus, bronchus, abdominal drainage, urogenital, lymph node (1% each), other (11%), unknown (5%).

Reference strain

KC425 ← Margaret Pittman, 5908.

Literature

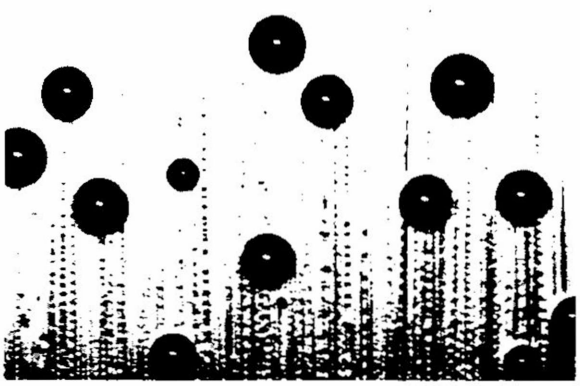
J Pathol Bacteriol 50:497-505, 1940; J Infect Dis 111:85-94, 1962; Int J Syst Bacteriol 16:60-61, 1966; N Engl J Med 275:181-188, 1966; Ann NY Acad Sci 174:468-487, 1970.

Comments:

Usually granular growth in broth, with colonies adhering to sides of tube. Originally described as requiring X factor; however, none of the strains received in our laboratory or the reference strain have required X factor. Genus status questionable. After repeated subcultures, *H. aphrophilus* strains may become elongated and may exhibit some curved filaments.



BAP 35°C 24 h ×1.



BAP 35°C 24 h ×6 (axial light).



Gram stain BAP 35°C 24 h ×1700.

Haemophilus aphrophilus

	NUMBER OF STRAINS 203		
TEST PERFORMED	**SIGN**	**% +**	**# +/T**
Morphology	[rv or plr]		
Motility: flagella	nm		
Gas from glucose	[+][a]	98	168/172
Action on blood	v	41 al	56/135;15 gr;1 I,G
Fermentative or oxidative	[F]		
Carbohydrate base	F		
Acid from:			
Glucose	[+]	98(2)	187,12w(4) 203
Xylose		0	0 202
Mannitol		0	0 202
Lactose	[+]	96(4)	180,13w(9) 202
Sucrose	+	99(1)	191,9w(3) 203
Maltose	[+]	99(1)	190,10w(3) 203
10% Lactose	ngr		
Catalase	[−]	5	1,10w/203
Oxidase	v	28	46,9w/199
Growth on:			
MacConkey	v	19	5,28w/171
SS	−	0	0/169
Simmons Citrate	−	0	0/170
Urea, Christensen's	−	0	0/150
Nitrate reduction	[+]	100	202 202
Gas from nitrate	−	0	0/202
Indole	[-]	0	0/192
TSI slant, acid	+	100	166,4w/170
TSI butt, acid	+	100	163,5w/168
H_2S (TSI butt)	-	0	0/169
H_2S (Pb ac paper)	+	100	126,36w/162
MR	v	12	2,9w/91
VP	−	0	0 63
Gelatin hydrolysis[b]	−	0	0 121
Litmus milk	v	30(7) A	53(12) 179;12IR
Growth at:			
25°C	v	55	86/156
35°C	+	99	157/158
42°C	v	26	40/155
Esculin hydrolysis	[-]	0	0 200
Lysine decarboxylase	−	0	0 9
Arginine dihydrolase	−	0	0 9
Ornithine decarboxylase	−	0	0 9
Nutrient broth, 0% NaCl	v	56	91 162
Nutrient broth, 6% NaCl	-	1	1 165

[a]Volume of gas is frequently small.
[b]7-14 days' incubation.

Haemophilus ducreyi

Gram-negative slender rod or coccoid, sometimes occurring in pairs, chains, or filaments, nonmotile; requires X factor; grows in air or in air with increased CO_2 (growth is enhanced by a moist atmosphere); shows alpha hemolysis in stabs at 48 h; most strains reduce nitrate; alkaline phosphatase positive; indole- and catalase-negative; porphyrin test-negative; oxidase-positive. (For further biochemical reactions see table entitled "Species and biotypes of *Haemophilus* isolated from humans and two animal species", p. 83.)

Sources (31 strains)

Genital lesion (55%), chancroid ulcer (32%), penile lesion (10%), infected lymph node (3%).

Reference strains

KC1449 < G. Hammond < Pasteur Institute A77.

Literature

J Gen Microbiol 93:9-62, 1976; J Clin Microbiol 7:39-43, 1978.

Comments

Growth usually is difficult to remove from agar with an inoculating needle; the colonies can be pushed intact across the surface of the plate. Overall appearance may be that of a mixed culture. Difficulties in isolating this organism have been reported. Of 29 *H. ducreyi* isolates, 19 were beta-lactamase positive.



Gram stain BAP 35°C 48 h ×1700.

Haemophilus equigenitalis

Gram-negative small rod, nonmotile; does not require X or V factors, but growth is enhanced by X factor; glucose not used; urea-, indole-,and ornithine-negative. (For further biochemical reactions see table entitled "Species and biotypes of *Haemophilus* isolated from humans and two animal species," p. 83.)

Source

Cervical swab of mare.

Reference strain

KC1457 ← NCTC, 11184.

Literature

Equine Vet J 10:136-144; 1977.

Comments

The causative organism of contagious equine metritis. This organism could not be identified with any known species and could not be readily placed in an established genus. Although it does not require either X or V factor, it has been proposed as a new species of the genus *Haemophilus* (See reference cited above).



KC1457 Gram stain BAP 35°C 48 h ×1700.

Haemophilus haemoglobinophilus

Gram-negative small pleomorphic rod; sometimes forms filaments; nonmotile; requires X factor only; grows in air or air with increased CO_2; nonhemolytic; uses glucose, xylose, mannitol, and sucrose; oxidase-positive; indole- and nitrate-positive; urea- and ornithine-negative; alkaline phosphatase-negative (all other *Haemophilus* species are positive, with the exception of *H. piscium*). (For further biochemical reactions see table entitled "Species and biotypes of *Haemophilus* isolated from humans and two animal species," p. 83.)

Sources

One from dog prepuce and one from source unknown.

Reference strains

KC613 ← NCTC, 1659.

Literature

J Gen Microbiol 93:9-62, 1976.

Comments

Usually isolated from prepuce or vagina of dog and considered a commensal. We have a group of at least 21 *Haemophilus*-like organisms which exhibited a heavy mucoid growth and required X factor (but not V factor) after one passage from blood agar. Most of these cultures were from sputum. We are not certain of their relationship to *H. haemoglobinophilus*.



KC613 Gram stain BAP 35°C 24 h ×1700.

Haemophilus haemolyticus

Gram-negative coccoid rod; may form tangled filaments; nonmotile; requires X and V factors; grows in air or in air with increased CO_2; uses glucose, usually with gas production; usually urease-, catalase-, oxidase-, and nitrate positive; indole-variable; ornithine-negative; hemolytic on horse and rabbit blood. (For further biochemical reactions, see table entitled "Species and biotypes of *Haemophilus* isolated from humans and two animal species," p. 83.)

Sources (18 strains)

Sputum (28%), throat (22%), blood (17%), other (33%).

Reference strain

D8243, from blood.

Literature

J Exper Med 32:665-682, 1920; J Gen Microbiol 93:9-62, 1976.



Gram stain BAP 35°C 24 h ×1700.

Haemophilus influenzae

Gram-negative small coccoid to slightly pleomorphic rod, sometimes with tendency to filament-formation; nonmotile; some strains are encapsulated; requires X and V factors; grows in air or in air with increased CO_2; uses glucose and usually xylose; oxidase and ornithine- variable, usually, nitrate- and urea- positive, and most strains indole-positive. (For further biochemical reactions see table entitled "Species and biotypes of *Haemophilus* isolated from humans and two animal species," p. 83.)

Sources (154 strains)

Blood (25%), cerebrospinal fluid (22%), eye (10%), sputum (9%), throat (7%), nose and nasopharynx (5%), bronchial washing (3%), cervix and vagina (3%), lung (2%), amniotic fluid (1%), transtracheal (1%), cyst (1%), other (6%), unknown (5%).

Reference strain

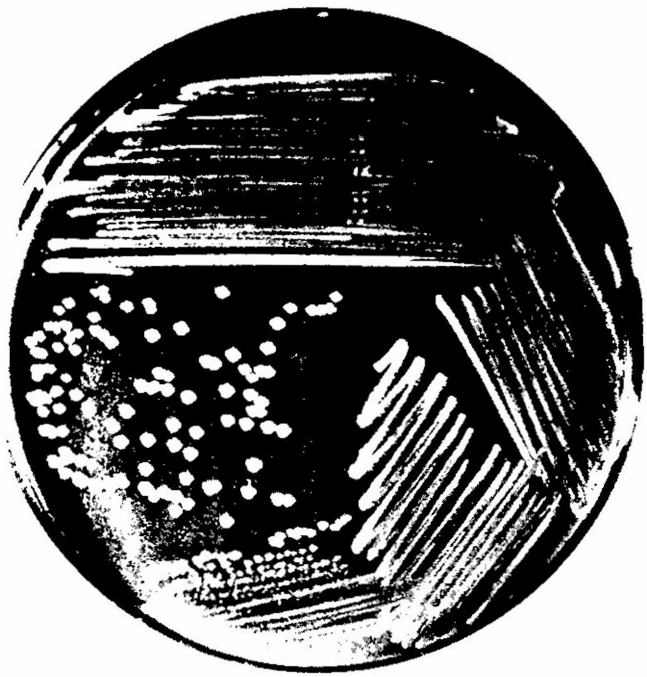
KC1318 ← ATCC, 19418 (NCTC 4560).

Literature

J Bacteriol 2:505-566, 1917; Int J Syst Bacteriol 16:60-61, 1966.

Comments

Biotypes have been proposed (J Gen Microbiol 93:9-62, 1976; J. Clin. Microbiol. 10:168-174, 1979, and Manual of Clinical Microbiology, 3rd ed., p. 334). Six serological types (a-f) are recognized; most isolates from cerebrospinal fluid and blood are serotype b; usually strains from the respiratory tract are not type-specific.



BAP 35°C 24 h ×1 (axial light).



Gram stain BAP 35°C 24 h ×1700.

Haemophilus parainfluenzae

Gram-negative small pleomorphic rod or long filament, nonmotile; requires V factor only, synthesizes porphyrins from d-aminolevulinic acid; aerobic; growth not improved by increased CO_2 except for some biotype III strains; uses glucose, often with gas formation, and sucrose; most strains produce beta-galactosidase; oxidase-variable; indole-negative and nitrate-positive. (For further biochemical reactions, see table entitled "Species and biotypes of *Haemophilus* isolated from humans and two animal species," p. 83.)

Sources (41 strains)

Blood (24%), throat (22%), sputum (15%), eye (5%), nasopharynx (5%), urine (5%), other (24%).

Reference strain

KC363 ← ATCC, 7901 ← Margaret Pittman, 429.

Literature

Public Health Rep 55:915, 1940; J Am Med Assoc 198:1221, 1966.

Comments

Biotypes I, II, and III have been proposed by M. Kilian (J Gen Microbiol 93:9-62, 1976); he has included *H. parahaemolyticus* and *H. paraphrohaemolyticus* in biotypes II and III of *H. parainfluenzae*. Biotype IV of *H. parainfluenzae* has been proposed by Oberhofer and Back (J Clin Microbiol 10:168-174, 1979); it has the same characteristics as Kilian's *H. segnis*.



BAP 35°C 24 h ×1 (axial light).



Gram stain BAP 35°C 24 h ×1700.

Haemophilus paraphrophilus

Gram-negative small rod or filament; nonmotile; requires V factor only; improvement of growth in CO_2 is variable; uses glucose and lactose; urea-, indole-, and ornithine-negative; nitrate reduction is positive. (For further biochemical reactions, see table entitled "Species and biotypes of *Haemophilus* isolated from humans and two animal species," p. 83.)

Sources (16 strains)

Blood (44%), vagina (13%), and 6% each of cheek abscess, liver abscess, ear, pleural fluid, neck wound, sinus, and dental abscess.

Reference strain

KC977 < NCTC, 10556 (co-type culture).

Literature

J Pathol Bacteriol 96:413-419, 1968.



Gram stain BAP 35°C 24 h ×1700.

Haemophilus segnis

Gram-negative small rod or filament; nonmotile; requires V factor; nonhemolytic; uses glucose, usually weakly; oxidase-, lactose-, urea-, indole- and ornithine-negative. (For further biochemical reactions, see table entitled "Species and biotypes of *Haemophilus* isolated from humans and two animal sources," p. 83.)

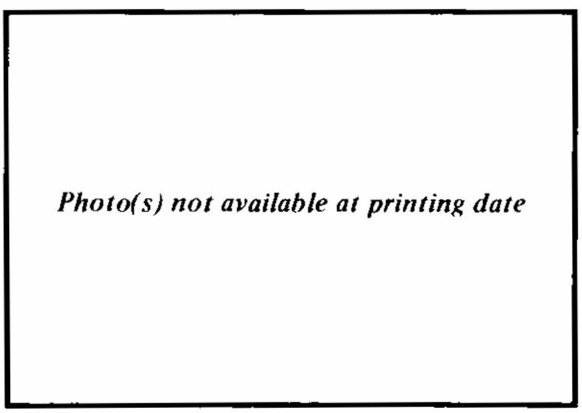
Literature

J Gen Microbiol 93:9-62, 1976.

Comments

M. Kilian proposed *H. segnis* as a new species. The strains studied were isolated primarily from the human oral cavity. We have not studied a reference strain, but we have received 12 strains with characteristics similar to those described for the species. Sources of these stains were blood (4 strains), sputum (2 strains), and 1 strain each from stool, throat, chest fluid, eye, peritoneal cavity, and brain aspirate.

Biotype IV of *H. parainfluenzae* has been proposed by Oberhofer and Back (J Clin Microbiol 10:168-174, 1979); it has the same characteristics as Kilian's *H. segnis*.



HB-5

Gram-negative rod; nonmotile; fastidious, ferments glucose with a small amount of gas but not mannitol or sucrose; growth on MacConkey agar variable; oxidase-variable; catalase- and urease-negative; indole- and nitrate-positive.

Sources (44 strains)

Urine (16%), vagina (9%), penis (9%), finger (9%), blood (9%), placenta (7%), cervix (5%), rectal (5%), perianal (5%), other urogenital (11%), other (15%).

Reference strain

C7682, from blood.

Literature

Manual of Clinical Microbiology, 2nd ed., p. 289.

Comments

Most isolates are from genitourinary tract specimens, particularly those of females.



Gram stain BAP 35°C 24 h ×1700.

HB-5

	NUMBER OF STRAINS 44		
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	tsrs		
Motility: flagella	[nm]		
Gas from glucose	[+][a]	100	44/44
Action on blood	v	32 al	14/43; ly,1 lav
Fermentative or oxidative	[F]		
Carbohydrate base	F		
Acid from:			
Glucose	[+]	100	44/44
Xylose	−	0	0/44
Mannitol	[−]	0	0/44
Lactose	−	0	0/44
Sucrose	[−]	0	0/44
Maltose	-	0	0/44
Fructose	+	100	28/28
10% Lactose	−	0	0/25
Catalase	[−]	2	1w/44
Oxidase	v	54	6,18w/44
Growth on:			
MacConkey	v	34(23)	15w(10)/44
SS	−	0	0/44
Simmons Citrate	−	0	0/44
Urea, Christensen's	[−]	0	0/42
Nitrate reduction	[+]	100	44/44
Gas from nitrate	−	0	0/44
Indole	[+]	100	22,22w/44
TSI slant, acid	+	100	40,4w/44
TSI butt, acid	[+]	98	41,2w/44
H_2S (TSI butt)	−	0	0/44
H_2S (Pb ac paper)	+	91	36,4w/44
MR	−	0	0/23
VP	−	7	1/14
Gelatin hydrolysis[b]	−	0	0/21
Litmus milk	−	0	0/44
Growth at:			
25°C	v	74	5,26w/42
35°C	+	100	11,31w/42
42°C	v	12	5w/42
Esculin hydrolysis	−	0	0/44
Lysine decarboxylase	−	0	0/7
Arginine dihydrolase	−	0	0/7
Ornithine decarboxylase	−	0	0/7
Nutrient broth, 0% NaCl	v	83	17,18w/42
Nutrient broth, 6% NaCl	−	0	0/42

[a]Volume of gas is frequently small.
[b]7-14 days' incubation.

Kingella denitrificans

Gram-negative coccoid or short rod occurring in pairs or short chains, nonmotile; faculatively anaerobic; produces acid fermentatively from glucose (but not from maltose as stated in 1976 reference cited below); oxidase-positive, catalase-negative; usually reduces nitrate and nitrate with gas formation; does not grow on MacConkey agar.

Sources (60 strains)

Throat (68%), urogential (7%), chimpanzee throat (7%), mouth (3%), sputum (3%), nasopharynx (3%), other (1 strain each: epiglottis, mandibular abscess, blood) (5%), unknown (3%).

Reference strain

C1080, from throat.

Literature

Appl Microbiol 24:772-777, 1972; Int J Syst Bacteriol 26:451-458, 1976.

Comments

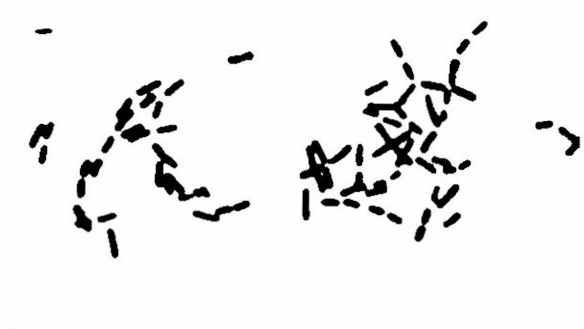
Formerly designated TM-1. Frequently pits the agar. Grows on Thayer-Martin selective media; nonpitting colonies are similar to those of *Neisseria gonorrhoeae*.



BAP 35°C 24 h ×1.



BAP 35°C 24 h ×6.



Gram stain BAP 35°C 24 h ×1700.

Kingella denitrificans

NUMBER OF STRAINS 60			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	cc,pr,chs		
Motility: flagella	[nm]		
Action on blood		0	0/38
Fermentative or oxidative	[F][a]		
Carbohydrate base	I[b]		
Acid from:			
Glucose	[+ or(+)]	30(62)	7,11w(37)/60
Xylose		0	0/60
Mannitol		0	0/60
Lactose		0	0/60
Sucrose		0	0/60
Maltose	[ ]	0	0/60
Catalase	[ ]	10	5,1w/60
Oxidase	[+]	100	60/60
Growth on:			
MacConkey	[ ]	0	0/50
SS	–	0	0/50
Simmons Citrate	–	0	0/50
Urea, Christensen's	–	0	0/29
Nitrate reduction	[+]	93	53/57
Gas from nitrate	[v]	88	50/57
Indole		0	0/57
TSI slant, acid		2	1w/52
TSI butt, acid	–	0	0/52
H_2S (TSI butt)	–	0	0/44
H_2S (Pb ac paper)	v	73	22,10w/44
Gelatin hydrolysis[c]	–	0	0/12
Litmus milk	–	6 IR	2/47;1 k
Pigment:			
Insoluble	–	2 yel	1/60
Soluble	–	2 yel	1/60
Growth at:			
25°C	v	40	19w/48
35°C	+	100	48w/48
42°C	v	48	23w/48
Esculin hydrolysis	–	0	0/48
Nutrient broth, 0% NaCl	v	78(4)	9,26w(2)/45
Nutrient broth, 6% NaCl	–	0	0/45

[a] TSI reaction not always indicative of fermentative activity.
[b] 1-2 drops of rabbit serum per 3 ml of medium may be required.
[c] 7-14 days' incubation.

Kingella indologenes

Gram-negative plump rod which may stain irregularly; nonmotile; facultatively anaerobic; produces acid fermentatively from glucose, sucrose, and maltose, but not mannitol or xylose; catalase-negative, oxidase- and indole-positive; no growth on MacConkey; nitrate- and nitrite-negative.

Source (one strain)

Eye infection.

Reference strain

KC1142 ← NCTC, 10717.

Literature

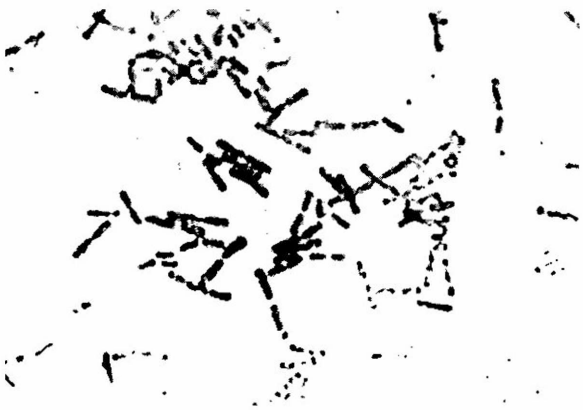
Int J Syst Bacteriol 26:451-458, 1976.

Comments

Pitting of agar medium or spreading edges of colonies may occur, especially in freshly isolated strains. Strains of this organism have been referred to as "Bijsterveld/Sutton" strains.

The rare mannitol-negative strains of *Cardiobacterium hominis* would be difficult to differentiate from *Kingella indolgenes*. Snell and Lapage reported (Int J Syst Bacteriol 26:451-458, 1976) that casein digestion, phosphatase production, and tween 20 and tween 40 hydrolysis are negative for *C. hominis*, but positive for *K. indologenes*.

The group IIc, which has some similarity to *K. indolgenes*, was included in previous editions of the "Round Table"; however, it has been omitted from this manual, because there is some question concerning homogeneity of this group.



Gram stain BAP 35°C 24 h ×1700.

Kingella indologenes

	NUMBER OF STRAINS 1		
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	vbr		
Motility; flagella	[nm]		
Action on blood			
Fermentative or oxidative	F		
Carbohydrate base	F		
Acid from:			
Glucose	[+]		
Xylose	−		
Mannitol	[−]		
Lactose	−		
Sucrose	[+]		
Maltose	[+]		
Catalase	[−]		
Oxidase	[+]		
Growth on:			
MacConkey	[ ]		
SS			
Simmons Citrate			
Urea, Christensen's			
Nitrate reduction			
Indole	[+]		
TSI slant, acid	+		
TSI butt, acid	+		
H_2S (TSI butt)			
H_2S (Pb ac paper)	+		
Gelatin hydrolysis[a]	−		
Litmus milk	−		
Pigment:			
Insoluble	−		
Soluble	−		
Growth at:			
25 C	+		
35 C	+		
42 C			
Esculin hydrolysis			
Lysine decarboxylase	−		
Arginine dihydrolase	−		
Ornithine decarboxylase	−		
Nutrient broth, 0% NaCl	−		
Nutrient broth, 6% NaCl	+		

[a] 7-14 days' incubation.

Kingella kingae

Gram-negative coccoid to short rod occurring in pairs and chains, nonmotile; produces acid fermentatively from glucose and maltose, not sucrose; oxidase-positive, catalase-negative, indole-negative, usually nitrate-negative; proteolytic; usually beta-hemolytic.

Sources (75 strains)

Blood (46%), bone or joint fluid (28%), throat (19%), pustule, wound, urine (1% each), unknown (3%).

Reference strain

A1702, from knee joint.

Literature

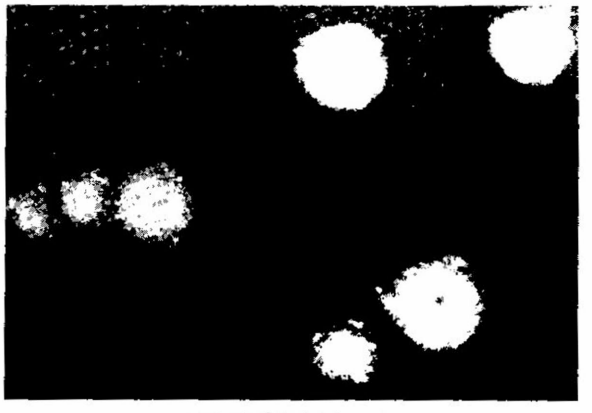
J Gen Microbiol 51:377-385, 1968; Int J Syst Bacteriol 26:447-450, 1976.

Comments

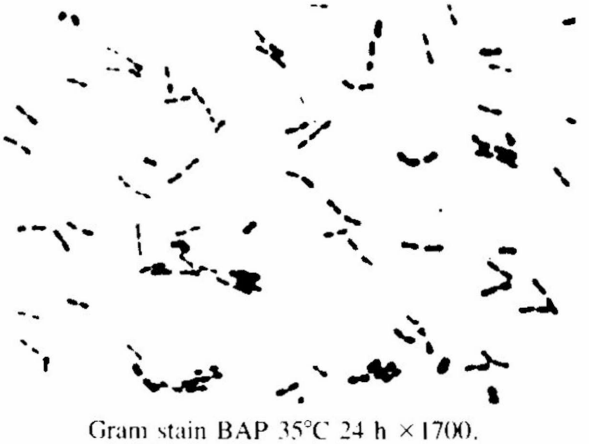
Formerly *Moraxella kingae, M. kingii*. May be confused with beta *Streptococcus*. Shows some tendency to resist decolorization. Maltose reaction frequently occurs earlier and is stronger than that of glucose. Of 32 patients of known age, 22 (69%) were 5 years of age or younger.



BAP 35°C 24 h ×1.



BAP 35°C 24 h ×6.



Gram stain BAP 35°C 24 h ×1700.

Kingella kingae

NUMBER OF STRAINS 33			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	cc,pr,chs		
Motility, flagella	[nm]		
Action on blood	[v]	84 β	26/31;1 al
Fermentative or oxidative	F[a]		
Carbohydrate base	F[b]		
Acid from:			
Glucose	[+ or(+)]	42(48)	7,7w(16) 33
Xylose	–	0	0/33
Mannitol	–	0	0/33
Lactose	–	0	0/33
Sucrose	[–]	0	0/33
Maltose	[+ or(+)]	76(24)	17,8w(8) 33
Catalase	[–]	0	0/32
Oxidase	[+]	100	32/32
Growth on:			
MacConkey	v	33(3)	8,3w(1)/33
SS	–	0	0/33
Simmons Citrate	–	0	0/33
Urea, Christensen's	–	0	0/24
Nitrate reduction	–	3(3)	1w(1)/30
Gas from nitrate	-	0	0/30
Indole	[ ]	0	0/29
TSI slant, acid	v	28(4)	2,5w(1)/25
TSI butt, acid	v	12	2,1w/26
H_2S (TSI butt)	-	0	0/31
H_2S (Pb ac. paper)	v	59	13,4w/29
Gelatin hydrolysis[c]	-	0	0/23
Litmus milk	v	82 pep	27/33
Pigment:			
Soluble	v	18 yel-br	2/11
Growth at:			
25 C	v	32	9/28
35 C	+	96	25/26
42 C		4	1/26
Esculin hydrolysis	-	0	0/30
Nutrient broth, 0% NaCl	v	38	9/24
Nutrient broth, 6% NaCl	-	0	0/24

[a]TSI reaction is not always indicative of fermentative activity.
[b]1-2 drops of rabbit serum per 3 ml of medium may be required.
[c]7-24 days' incubation.

Legionella pneumophila

Gram-negative rod of variable length (2-20 or more μm); motile (polar and lateral flagella, rarely more than one flagellum per cell); aerobic; fastidious, requires L-cysteine HCl for growth (grows on complete CYE agar but not on CYE and blood agar, which do not contain L-cysteine HCl); produces no acid from carbohydrates; does not reduce nitrate; oxidase test weakly positive, or negative; produces gelatinase; produces brown soluble pigment in media containing tyrosine; does not fluoresce on CYE agar under long-wavelength ultraviolet excitation; produces a beta-lactamase. (For methodology, see "Legionnaires' ", 1979 cited below.)

Sources (119 strains)

Lung (45%), trachea (11%), pleural fluid (8%), sputum (6%), blood (2%), bronchial washing (2%), cooling tower water (2%), water (2%), mud, lake water, stream water (1% each), unknown (18%).

Reference strain

Philadelphia 1 (ATCC 33152, type strain).

Literature

N Engl J Med 297:1189-1196, 1977; Ann Intern Med 90:656-658, 1979; "Legionnaires' ": the disease, the bacterium, and methodology. Jones GL, Hébert GA (eds), Atlanta, Center for Disease Control, 1979.

Comments

Formerly called Legionnaires' disease bacterium. There are at least six serogroups. On initial culture, cells may appear slightly swollen in regions having vacuoles that are stainable with the Sudan black B fat stain. Cells stain lightly with safranin, and readily with carbol fuchsin. Causes pneumonia and Legionnaires' disease in humans.



Legionella pneumophila strain Knoxville 1.
Gram stain CYE 35°C 24 h × 1700.

Legionella species other than *L. pneumophila**

At present, three species of *Legionella* other than *L. pneumophila* have been recognized: *L. bozemanii*, *L. micdadei* (*L. pittsburgensis*), and *L. dumoffii*. All three species grow on complete CYE agar but not on CYE or blood agar which do not contain L-cysteine HCl. For each species only one serogroup has been recognized. By the direct fluorescent antibody (FA) technique, when the FA reagents are used at their working dilutions, there are no cross-reactions among these species or between them and the recognized serogroups of *L. pneumophila*. Other reactions which are useful in distinguishing between the four species of *Legionella* are blue-white fluorescence of growth under a Wood's lamp and beta-lactamase detected by the rapid chromogenic cephalosporin technique (Current Microbiology 1:51-54, 1978).

Differentiation of *Legionella* species

	Blue-white fluorescence	Beta-lactamase
L. pneumophila	−	+
L. bozemanii	+	w
L. micdadei	−	−
L. dumoffii	+	+

Sources

L. bozemanii (of 3 strains): lung (3).
L. micdadei (of 4 strains): lung (1), chest tube (1), blood (2).
L. dumoffii (of 2 strains): lung (1), cooling tower water (1).

Reference strains

L. bozemanii WIGA (ATCC 33217, type strain)
L. micdadei TATLOCK (ATCC 33218, type strain)
L. dumoffii NY23 (ATCC 33279, type strain)

Literature

L. bozemanii: Acta Virol (Praha) 12:87-93, 1968; Ann Intern Med 91:673-676, 1979; Curr Microbiol 4:111-116, 1980.
L. micdadei: Proc Soc Exp Biol Med 57:95-99, 1944; Ann Intern Med 92:45-52, 1980; Curr Microbiol 3:255-258, 1980.
L. dumoffii: Lancet 2:927-930, 1979; Curr Microbiol 4:111-116, 1980.

Comment

L. bozemanii, *L. micdadei*, and *L. dumoffii* produce pneumonia in humans.

Photo(s) not available at printing date

*At the time of publication, the following additional species of *Legionella* had been named:
L. gormanii (J Clin Microbiol 12:718-721, 1980), *L. jordanis* (J Clin Microbiol 15:290-297, 1982), *L. longbeachae* (Ann Intern Med 94:739-743, 1981), *L. oakridgensis* (Appl Environ Microbiol 45:536-545, 1983), and *L. wadsworthii* (Ann Intern Med 97:809-813, 1982).

M-5

Gram-negative thin to medium-width rod; nonmotile; aerobic; does not use carbohydrates; strongly oxidase-positive; urease-negative, nitrate- and indole-negative, nitrite reduction variable.

Sources (59 strains)

Dog bite (68%), wound (12%), dog's mouth (7%), other (13%).

Reference strain

D6680, from dog bite.

Literature

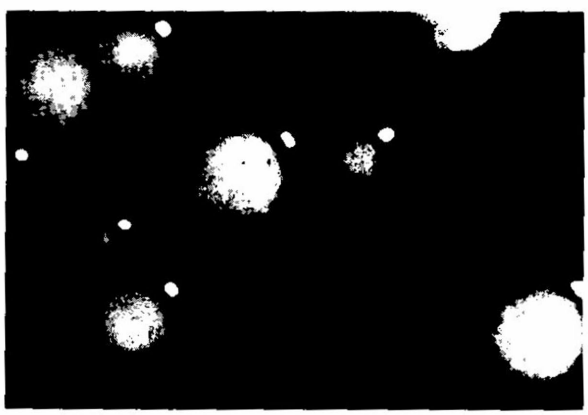
Manual of Clinical Microbiology, 2nd ed., pp 293-294; J Clin Microbiol 7:223-231, 1978.

Comments

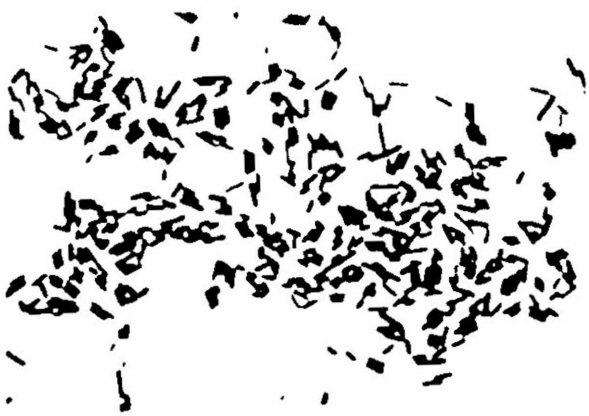
Usually sensitive to penicillin; the *Moraxella osloensis* transformation test is negative. Associated with animal-bite wounds.



BAP 35°C 24 h ×1.



BAP 35°C 24 h ×6.



Gram stain BAP 35°C 24 h ×1700.

M-5

NUMBER OF STRAINS 59			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	[mrs]		
Motility, flagella	[nm]		
Action on blood	v	44 ly	24/54;12 gr, 2 al, 2 LG, 1 br
Fermentative or oxidative	n-o		
Carbohydrate base	OF		
Acid from:			
Glucose	[−]	0	0/50
Xylose	[−]	0	0/52
Mannitol	−	0	0/57
Lactose		0	0/57
Sucrose		0	0/54
Maltose		0	0/57
Catalase	+	100	59/59
Oxidase	[+]	100	59/59
Growth on:			
MacConkey	v	42(20)	25(12)/59
SS		0	0/59
Simmons Citrate		0	0/59
Sodium acetate	v	25	1/4
Urea, Christensen's	−	0	0/58
Phenylalanine deaminase	v	73	22/30
Nitrate reduction	[−]	0	0/59
Nitrite reduction	[v]	84	27/32
Indole	−	0	0/59
TSI slant, acid	−	0	0/59
TSI butt, acid	−	0	0/59
H_2S (TSI butt)	−	0	0/59
H_2S (Pb ac paper)	v	86	22,29w/59
Gelatin hydrolysis[a]	−	0	0/48
Litmus milk	−	3k	2/59
Pigment	v	48 yel-ta sol	19/40
Growth at:			
25°C	+	95	54/57
35°C	+	100	57/57
42°C	v	63	36/57
Esculin hydrolysis		0	0/47
Nutrient broth, 0% NaCl	+ or (+)	65(29)	32(14)/49
Nutrient broth, 6% NaCl	−	8	4/48
Penicillin sensitivity	+	100	33/33

[a]7-14 days' incubation.

M-6

Gram-negative rod; nonmotile; aerobic; does not use carbohydrates; strongly oxidase-positive; catalase-, urease-, and indole-negative; weakly positive nitrate reaction in routine nitrate broth (peptone base), complete reduction of nitrate without gas formation in special nitrate broth (Heart Infusion Broth base), reduces nitrite.

Sources (40 strains)

Throat (35%), sputum (18%), blood (8%), feces (8%), lung (5%), urine (5%), peritoneal fluid (5%), wound (5%), other (11%).

Reference strain

D3205, from lung autopsy.

Literature

Manual of Clinical Microbiology, 2nd ed., p 294.

Comments

Usually sensitive to penicillin. These organisms differ from *Moraxella* in that they are catalase-negative and reduce nitrate to completion. Since our source data were tabulated, several isolates were received which were associated with endocarditis.



BAP 35°C 24 h ×1.



Gram stain BAP 35°C 24 h ×1700.

M-6

NUMBER OF STRAINS 40			
TEST PERFORMED	**SIGN**	**% +**	**# +/T**
Morphology	mbr		
Motility: flagella	[nm]		
Action on blood		5 al or gr	2 38
Fermentative or oxidative	n-o		
Carbohydrate base	OF		
Acid from:			
Glucose	[ ]	0	0 36
Xylose	[ ]	0	0 36
Mannitol		0	0 38
Lactose		0	0 37
Sucrose	-	0	0 38
Maltose	-	0	0 36
Catalase	[ ]	8	2w 24
Oxidase	[+]	100	40 40
Growth on:			
MacConkey	v	22(28)	5,4w(41) 40
SS	-	0	0 40
Simmons Citrate		0	0 40
Sodium acetate	v	83	5 6
Urea, Christensen's	[ ]	0	0 39
Phenylalanine deaminase		0	0/5
Nitrate reduction	[+]	100	39/39
Nitrite reduction	[+]	100	39/39
Indole	-	0	0/39
TSI slant, acid	-	0	0/40
TSI butt, acid		0	0/40
H_2S (TSI butt)	-	0	0/40
H_2S (Pb ac paper)	v	88	26,9w 40
Gelatin hydrolysis[a]	-	0	0/32
Litmus milk	-	0	0/40
Pigment	v	18 yel-ta sol	5/28
Growth at:			
25°C	v	78	28/36
35°C	+	100	37/37
42°C	v	47	17 36
Esculin hydrolysis	-	0	0 33
Nutrient broth, 0% NaCl	+	93	37 40
Nutrient broth, 6% NaCl	-	3	1 36
Penicillin sensitivity	v	88	15 17

[a]7-14 days' incubation.

Moraxella atlantae

Gram-negative coccoid to medium-length thick rod (may tend to retain crystal violet stain), nonmotile; usually does not grow in OF medium or use carbohydrates; grows on MacConkey agar, otherwise fastidious; aerobic; strongly oxidase-positive, nitrate-negative, urea-negative.

Sources (23 strains)

Blood (52%), cerebrospinal fluid (9%), abscess (9%), other (1 each: ulcer, wound, urine, urethra, hamster) (21%), unknown (9%).

Reference strain

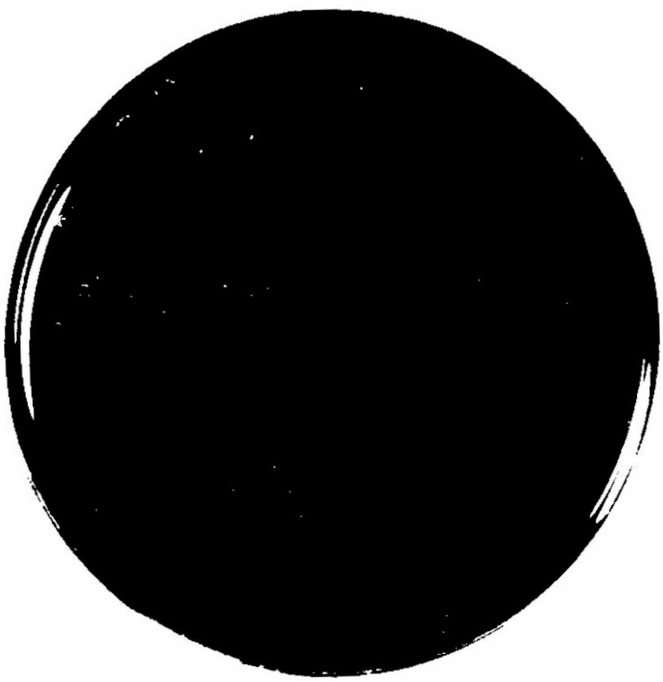
B7333, from urine.

Literature

Int J Syst Bacteriol 26:511-521, 1976.

Comments

Formerly CDC Group M-3. Usually two colony variants can be observed, one convex with entire edges, the other flatter, with irregular edges and a tendency to spread; may pit the agar.



BAP 35°C 24 h ×1.



Gram stain BAP 35°C 24 h ×1700.

Moraxella atlantae

NUMBER OF STRAINS 23			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	sbr,cc		
Motility; flagella	[nm]		
Action on blood		9 al or ly	2/21
Fermentative or oxidative	[n-o]		
Carbohydrate base	OF		
Acid from:			
Glucose	[a]	0	0/10
Xylose		0	0/10
Mannitol		0	0/10
Lactose		0	0/10
Sucrose		0	0/10
Maltose		0	0/10
Catalase	+	91	13,8w/23
Oxidase	[+]	100	23/23
Growth on:			
MacConkey	[+ or(+)]	87(13)	20(3)/23
SS	-	0	0/23
Simmons Citrate	-	0	0/23
Sodium acetate	v	21(21)	3(3)w/14
Urea, Christensen's	[-]	0	0/23
Phenylalanine deaminase	-	0	0/6
Nitrate reduction	[-]	5	1/21
Nitrite reduction	v	20	1/5
Indole	-	0	0/20
TSI slant, acid	-	0	0/17
TSI butt, acid	-	0	0/16
H_2S (TSI butt)	-	0	0/17
H_2S (Pb ac paper)	v	71	6,6w/17
Gelatin hydrolysis[b]	-	0	0/12
Litmus milk	-	9 IR	2/23;1c
Growth at:			
25°C	v	68	15/22
35°C	+	100	22/22
42°C	v	50	11/22
Esculin hydrolysis	-	0	0/14
Nutrient broth, 0% NaCl	v	36	4/11
Nutrient broth, 6% NaCl	-	0	0/11

[a]Usually does not grow in OF Medium; if it grows, it usually does not change the pH of the medium.
[b]7-14 days' incubation.

Moraxella bovis

Gram-negative coccoid to filamentous thick rod (may tend to retain crystal violet stain), nonmotile; fastidious; aerobic; asaccharolytic, hemolytic, strongly oxidase-positive; does not grow on MacConkey agar; nitrate-negative, urea-negative, gelatin-positive (serum may need to be added).

Sources (8 strains)

Bovine eye (5), bovine nose (1), human blood (bovine artery used in arm shunt) (1), unknown (1).

Reference strain

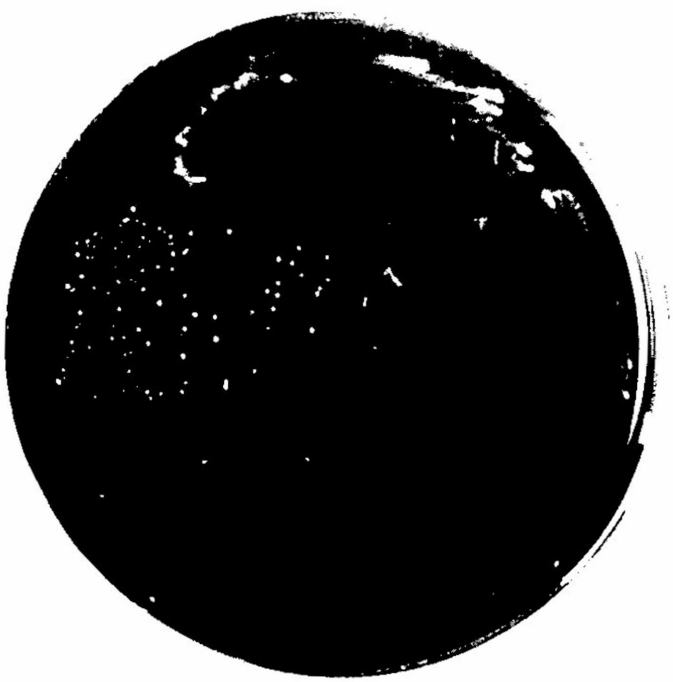
KC416(1) ⇐ ATCC, 17947 (NCTC 8561). Suggested working type culture; smooth form.

Literature

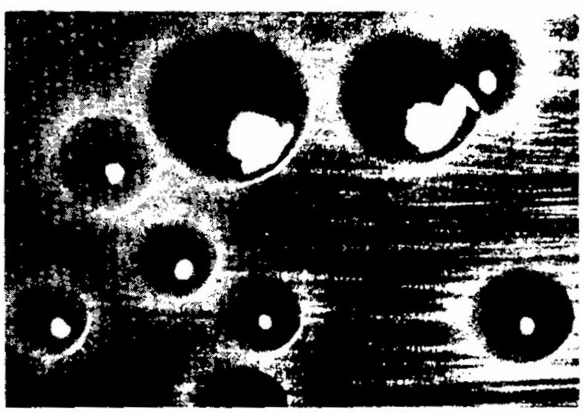
Am J Vet Res 31:462, 1970; Bacteriol Rev 37:522-561, 1973.

Comments

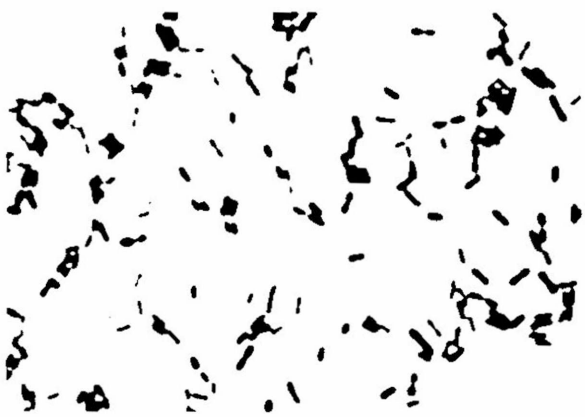
Penicillin-sensitive. Causes "pink-eye" in cattle.



BAP 35°C 24 h ×1.



BAP 35°C 24 h ×6.



Gram stain BAP 35°C 24 h ×1700.

Moraxella bovis

	NUMBER OF STRAINS 7		
TEST PERFORMED	**SIGN**	*% +*	*# + /T*
Morphology	cc,sbr		
Motility; flagella	[nm]		
Action on blood	[β]	100	7/7
Fermentative or oxidative	n-o		
Carbohydrate base	OF		
Acid from:			
Glucose	[ ]	0	0/2
Xylose	–	0	0/2
Mannitol	–	0	0/2
Lactose		0	0/6
Sucrose		0	0/2
Maltose		0	0/3
Catalase	v	14	1w/7
Oxidase	[+]	100	7/7
Growth on:			
MacConkey	[ ]	0	0/7
SS	-	0	0/7
Simmons Citrate		0	0/7
Urea, Christensen's	[ ]	0	0/7
Phenylalanine deaminase		0	0/1
Nitrate reduction	v	14	1/7
Indole	-	0	0/7
TSI slant, acid	-	0	0/7
TSI butt, acid	–	0	0/7
H_2S (TSI butt)	–	0	0/6
H_2S (Pb ac paper)	+	100	4,2w/6
Gelatin hydrolysis[a]	[+]	100	7/7
Litmus milk	pep	100	7/7
Growth at:			
25 C	+	100	7/7
35 C	+	100	7/7
42 C	-	0	0/7
Penicillin sensitivity[b]	+	100	2/2

[a]7-14 days' incubation.
[b]Based on results obtained by streaking a blood agar plate with growth from an 18-36 h culture and then placing a 10-unit penicillin disc on the streaked area. A positive reaction is indicated by the appearance of a zone of inhibition.

Moraxella lacunata

Gram-negative lanceolate coccoid to medium length rod of varying length in pairs or short chains, nonmotile; aerobic; asaccharolytic; strongly oxidase-positive, nitrate-positive; sodium acetate-negative, urea-negative; digests Loeffler blood serum agar (obtained commercially); no growth in nutrient broth.

Sources (25 strains)

Eye (80%), other (20%).

Reference strain

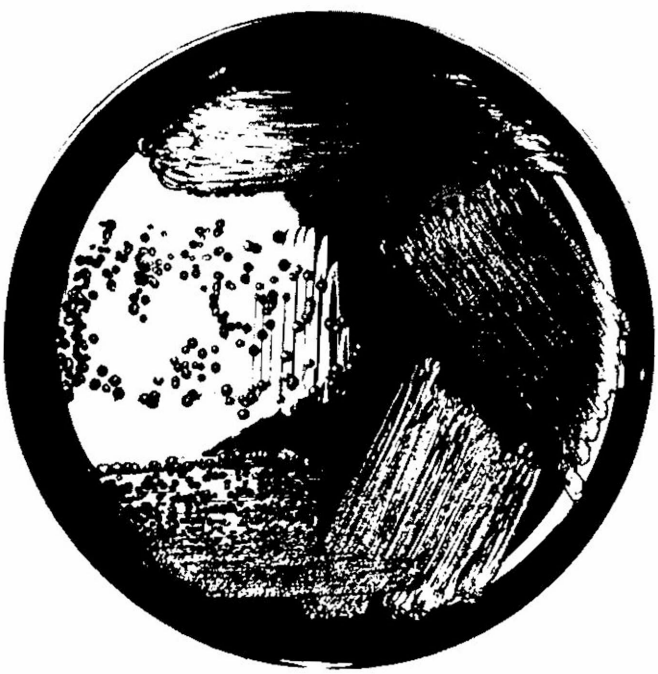
KC1376 ← ATCC, 17967 (neotype strain).

Literature

Ann Inst Pasteur, Paris 62:168-176, 1939; Int J Syst Bacteriol 19:263, 1969, and 21:106, 1971; Bacteriol Rev 37:522-561, 1973.

Comments

Moraxella lacunata is the type species; synonyms are *Moraxella liquefaciens* and *Diplobacillus moraxaxenfeld*. Penicillin-sensitive. Associated with conjunctivitis.



BAP 35°C 24 h ×1 (axial light).



BAP 35°C 24 h ×6 (axial light).



Gram stain BAP 35°C 24 h ×1700.

Moraxella lacunata

NUMBER OF STRAINS 25			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	cc, mr		
Motility: flagella	[nm]		
Action on blood	v	24 al	4/17;2 ly
Fermentative or oxidative	n-o		
Carbohydrate base	OF[a]		
Acid from:			
Glucose	[−]	0	0/12
Xylose	−	0	0/12
Mannitol		0	0/12
Lactose		0	0/12
Sucrose		0	0/12
Maltose		0	0/12
Catalase	+	100	23,1w 24
Oxidase	[+]	100	25/25
Growth on:			
MacConkey	-	4	1w 23
SS	-	0	0/23
Simmons Citrate	-	0	0/23
Sodium acetate	[−]	7	1/15
Urea, Christensen's	[−]	0	0/17
Phenylalanine deaminase	v	31	2,2w/13
Nitrate reduction	[+]	100	24/24
Indole		0	0/20
TSI slant, acid		0	0/11
TSI butt, acid		0	0/12
H_2S (TSI butt)	-	0	0/15
H_2S (Pb ac paper)	-	0	0/15
Gelatin hydrolysis[b]	v	74	17/23
Loeffler slant, digestion	[+]	100	23/23
Litmus milk	v	58 pep	14/24
Pigment	−	4 br sol	1/25
Growth at:			
25°C	v	47	7/15
35°C	v	87	13/15
42°C	-	0	0/15
Esculin hydrolysis	−	0	0/21
Nutrient broth, 0% NaCl	[−]	0	0/20
Nutrient broth, 6% NaCl	−	0	0/20
Penicillin sensitivity[c]	+	100	11/11

[a]Reactions neutral (does not grow or grows poorly) in King's OF medium.
[b]7-14 days' incubation.
[c]Based on results obtained by streaking a blood agar plate with growth from an 18-36 h culture and then placing a 10-unit penicillin disc on the streaked area; a positive reaction is indicated by the appearance of a zone of inhibition.

Moraxella nonliquefaciens

Gram-negative, deeply staining coccoid to filament-length thick rod (may tend to retain crystal violet stain), in pairs and short chains, nonmotile; aerobic; asaccharolytic; strongly oxidase-positive, usually MacConkey-negative; nitrate-positive, unrease-negative, indole- and sodium acetate-negative; no digestion of Loeffler blood serum agar (obtained commercially).

Sources (243 strains)

Nose (21%), throat (12%), eye (11%), nasopharynx (9%), sputum (8%), blood (6%), other (33%).

Reference strains

KC129 ← Henriksen; KC770 ← ATCC, 17953 (NCTC 7784).

Literature

Ann Inst Pasteur, Paris 62:168-176, 1939; Int J Syst Bacteriol 17:127-135, 1967; Bacteriol Rev 37:522-561, 1973.

Comments

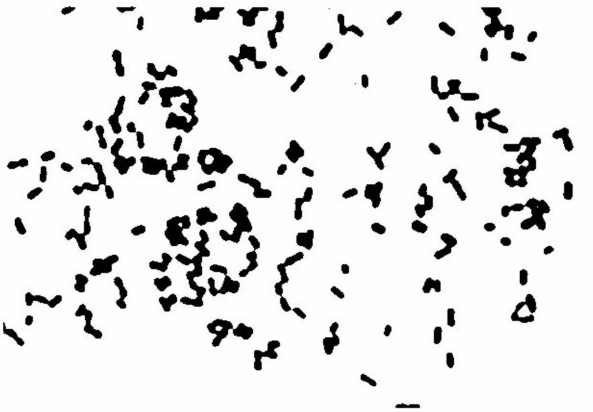
Usually penicillin-sensitive. *Moraxella osloensis* transformation test-negative. Does not grow (or grows poorly) in King's OF medium, on MacConkey agar, and in nutrient broth.



KC129 BAP 35°C 24 h ×1.



KC129 BAP 35°C 24 h ×6.



KC770 Gram stain BAP 35°C 24 h ×1700.

Moraxella nonliquefaciens

	NUMBER OF STRAINS 243		
TEST PERFORMED	**SIGN**	**% +**	**# + /#T**
Morphology	cc,sbr		
Motility: flagella	[nm]		
Action on blood	v	10 ly	21/220,11 al,3 gr,2 LG
Fermentative or oxidative	n-o		
Carbohydrate base	OF[d]		
Acid from:			
Glucose	[ ]	0	0/142
Xylose		0	0/142
Mannitol		0	0/144
Lactose		0	0/144
Sucrose		0	0/143
Maltose		0	0/142
Catalase	+	95	204/214
Oxidase	[+]	100	237/237
Growth on:			
MacConkey	[-]	8(2)	5,15w(4)/239
SS		0	0/239
Simmons Citrate	-	0	0/237
Sodium acetate	[-]	0	0/16
Urea, Christensen's	[-]	0	0/201
Nitrate reduction	[+]	95	225/237
Indole		0	0/237
TSI slant, acid		0	0/227
TSI butt, acid		0	0/229
H_2S (TSI butt)		0	0/230
H_2S (Pb ac paper)	v	83	102,89w/231
Gelatin hydrolysis[b]		0	0/224
Loeffler slant, digestion	[-]	0	0/104
Litmus milk	-	2(1) k	5,1w(3)/233
Pigment	-	3 yel sol	4/117
Growth at:			
25°C	+	93	204/219
35°C	v	88	196/223
42°C	v	15	34/224
Esculin hydrolysis	-	0	0/170
Nutrient broth, 0% NaCl	v	22	39/175
Nutrient broth, 6% NaCl	-	0	0/175
Penicillin sensitivity[c]	+	99	136/137

[a]Reactions neutral (does not grow or grows poorly) in King's OF medium.
[b]7-14 days' incubation.
[c]Based on results obtained by streaking a blood agar plate with growth from an 18-36h culture and then placing a 10-unit penicillin disc on the streaked area. A positive reaction is indicated by the appearance of a zone of inhibition.

Moraxella osloensis

Gram-negative deeply staining coccoid form to thick rod occurring in pairs and short chains, with filaments sometimes observed; may tend to retain crystal violet stain; nonmotile; aerobic; asaccharolytic; strongly oxidase-positive, indole-negative, nitrite-negative, and sodium-acetate-positive; urea-negative; grows in nutrient broth.

Sources (163 strains)

Blood (17%), genital (8%), throat (7%), cerebrospinal fluid (7%), urine (7%), wound (5%), pyogenic lesion (5%), sputum (3%), chest cavity (3%), eye (2%), nasopharyngeal (2%), rectal (2%), other (20%), unknown (12%).

Reference strain

KC1088 = ATCC, 19976 (NCTC 10465) (type strain).

Literature

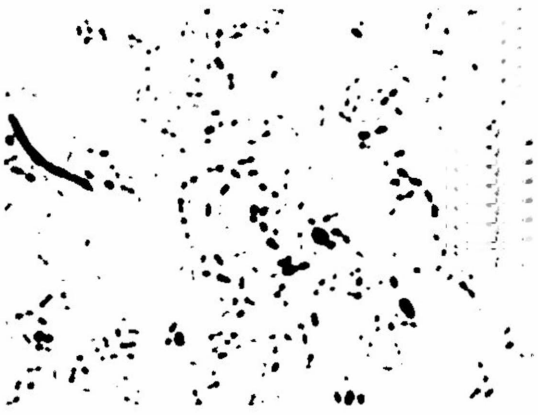
Int J Syst Bacteriol 17:127-135, 1967; J Bacteriol 95: 1520-1541, 1968; Bacteriol Rev 37:522-561, 1973.

Comments

Usually penicillin-sensitive. *Moraxella osloensis* transformation test recommended to confirm identification. An occasional strain may appear "fuzzy" in motility medium, suggesting motility; however, no flagella could be demonstrated. This taxon includes many of the strains formerly called *Mima polymorpha* variety *oxidans*.



BAP 35°C 24 h ×1.



Gram stain BAP 35°C 24 h ×1700.

Moraxella osloensis

	NUMBER OF STRAINS 163		
TEST PERFORMED	**SIGN**	*%* +	# + / T
Morphology	cc,sbr		
Motility; flagella	[nm]		
Action on blood	v	7al	11/147;10 ly, 8 gr, 1 B
Fermentative or oxidative	n-o		
Carbohydrate base	OF		
Acid from:			
Glucose	[−]	0	0/156
Xylose	−	0	0/156
Mannitol	−	0	0/156
Lactose	−	0	0/154
Sucrose		0	0/154
Maltose	−	0	0/154
Catalase	+	95	151/159
Oxidase	[+]	100	158/158
Growth on:			
MacConkey	v	70	111/158
SS	−	0	0/156
Simmons Citrate	−	0	0/156
Sodium acetate	[+]	100	16/16
Urea, Christensen's	[−]	0	0/158
Phenylalanine deaminase	v	14	4/29
Nitrate reduction	v	24	38/159
Nitrite reduction	[−]	0	0/9
Indole	−	0	0/158
TSI slant, acid	−	0	0/159
TSI butt, acid		0	0/159
H_2S (TSI butt)	−	0	0/149
H_2S (Pb ac paper)	v	74	114/153
Gelatin hydrolysis[a]	−	0	0/120
Litmus milk	...	9 k	14/155;2 IR
Pigment	−	6 yel-br sol	10/156
Growth at:			
25 C	+	96	152/158
35 C	+	98	154/157
42 C	v	51	80/157
Esculin hydrolysis	−	0	0/158
Nutrient broth, 0% NaCl	[+]	98	155/158
Nutrient broth, 6% NaCl	v	12	19/158
Penicillin sensitivity[b]	+	92	80/87

[a]7-14 days' incubation.
[b]Based on results obtained by streaking a blood agar plate with growth from an 18-36h culture and then placing a 10-unit penicillin disc on the streaked area. A positive reaction is indicated by the appearance of a zone of inhibition.

Moraxella phenylpyruvica

Gram-negative coccoid to filamentous thick rod (may tend to retain crystal violet stain); nonmotile; fastidious; aerobic; asaccharolytic; strongly oxidase-positive, catalase-positive, urease-positive, deaminates phenylalanine to phenylpyruvic acid; sodium acetate-negative, nitrite-negative.

Sources (52 strains)

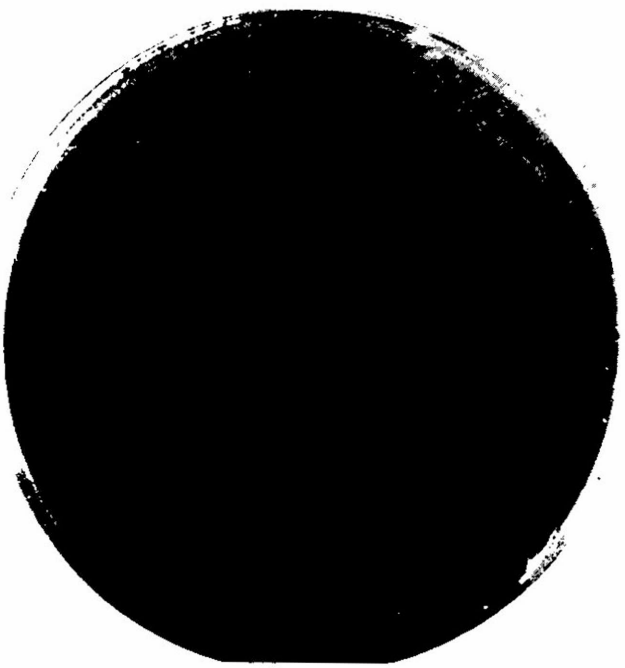
Urine (17%), urogenital tract (15%), blood (13%), cerebrospinal fluid (8%), superficial lesion (6%), peritoneal fluid (6%), fowl (4%), pyogenic lesion (4%), ear (4%), other (19%), unknown (4%).

Reference strain

E.O. King's 2863 (neotype strain), from blood.

Literature

Int J Syst Bacteriol 17:343-360, 1967, and 21:107, 1971; J Clin Pathol 25:959-965, 1972; Bacteriol Rev 37:522-561, 1973.



BAP 35°C 24 h ×1 (axial light).



Gram stain BAP 35°C 24 h ×1700.

Moraxella phenylpyruvica

	NUMBER OF STRAINS 50		
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	[cc,br]		
Motility; flagella	[nm]		
Action on blood	v	16 hy	8,46;6 al,2 gr,1 LG
Fermentative or oxidative	n-o		
Carbohydrate base	OF		
Acid from:			
Glucose	[ ]	0	0/30
Xylose		0	0/30
Mannitol		0	0/30
Lactose		0	0/30
Sucrose		0	0/30
Maltose		0	0/30
Catalase	+	90	30,14w/49
Oxidase	[+]	100	50/50
Growth on:			
MacConkey	v	80(6)	40(3)/50
SS		0	0/50
Simmons Citrate		0	0/50
Sodium acetate	v	43	5,4w/21
Urea, Christensen's	[+]	100	50/50
Phenylalanine deaminase	[+]	97	38/39
Nitrate reduction	v	68	34/50
Nitrite reduction	–	0	0/3
Indole	–	0	0/48
TSI slant, acid	–	0	0/50
TSI butt, acid	–	0	0/50
H_2S (TSI butt)	–	0	0/47
H_2S (Pb ac paper)	v	47	16,7w/49
Gelatin hydrolysis[a]	–	0	0/43
Litmus milk	v	22(20) k	10,2w(9)/46
Pigment	–	8 yel-br sol	4/50
Growth at:			
25 C	v	85	41/48
35 C	+	100	48/48
42 C	v	29	14/48
Esculin hydrolysis		0	0/44
Nutrient broth, 0% NaCl	v	53	21/40
Nutrient broth, 6% NaCl	v	19	8/43
Penicillin sensitivity	v	73	19/26

[a]7-14 days' incubation.

Moraxella urethralis

Gram-negative small coccoid form and rod (may tend to retain crystal violet stain); nonmotile; aerobic; asaccharolytic, strongly oxidase-positive, grows on MacConkey agar; nitrate-negative, nitrite-positive with gas formation; deaminates phenylalanine to phenylpyruvic acid, frequently citrate-positive, urea-negative.

Sources (22 strains)

Urinary tract (68%), ear (14%), other (18%).

Reference strain

KC1290 ← J. L. Mitchell, Brooks AFB ←H. Lautrop, WM20.

Literature

Acta Pathol Microbiol Scand Sect B 78:255-256, 1970; Appl Microbiol 28:355-358, 1974; Bacteriol Rev 37:522-561, 1973.

Comments

Colonies nearly opague. These organisms constitute the bulk of the group formerly called "M-4." Further, this taxon includes some of the strains formerly called *Mima polymorpha* variety *oxidans*.



BAP 35°C 24 h ×1.



Gram stain BAP 35°C 24 h ×1700.

Moraxella urethralis

NUMBER OF STRAINS 22			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	[cc]		
Motility, flagella	[nm]		
Action on blood	v	18 hy	4 22;1 gr
Fermentative or oxidative	n-o		
Carbohydrate base	OF		
Acid from:			
Glucose	[ ]	0	0 22
Xylose	[-]	0	0 22
Mannitol	-	0	0 22
Lactose	-	0	0 22
Sucrose		0	0 22
Maltose		0	0 22
Catalase	+	100	22 22
Oxidase	[+]	100	22 22
Growth on			
MacConkey	[+]	96	21 22
SS		9	2w 22
Simmons Citrate	v	46	10 22
Sodium acetate	v	60	6/10
Urea, Christensen's	[ ]	0	0/22
Phenylalanine deaminase	[+]	100	20/20
Nitrate reduction	[ ]	0	0/22
Nitrite reduction	[+]	100	21/21
Indole		0	0/22
TSI slant, acid		0	0/22
TSI butt, acid		0	0/22
H_2S (TSI butt)		0	0/22
H_2S (Pb ac paper)		9	1,1w/22
Gelatin hydrolysis[a]		0	0/14
Litmus milk	v	36 k	8/22
Pigment		4 amb sol	1 22
Growth at:			
25 C	v	50	11/22
35 C	+	100	22 22
42 C	v	59	13 22
Esculin hydrolysis		0	0 20
Nutrient broth, 0% NaCl	+	96	21 22
Nutrient broth, 6% NaCl	v	59	13 22
Penicillin sensitivity	+	100	13 13

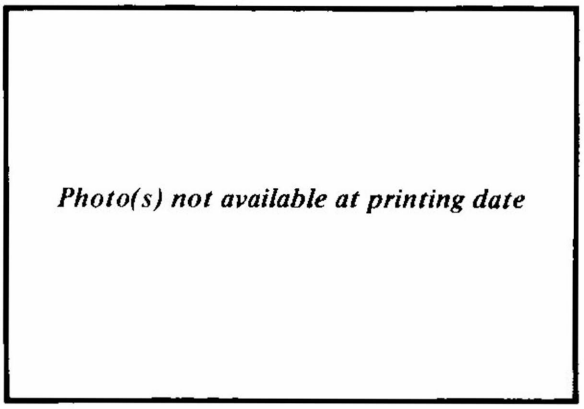
[a]7-14 days' incubation.

Neisseria elongata

Gram-negative rod, often occurring as diplobacilli or in short chains, nonmotile; aerobic; no acid produced from carbohydrates; strongly oxidase-positive; does not grow on MacConkey agar; nitrate- and nitrite-negative; grows on nutrient agar at 35°C and at 25°C; catlase weak or negative; sparse growth on Thayer-Martin media.

Source (one strain)
Nasopharynx.

Literature
J Gen Microbiol 60:67-75, 1970.



Neisseria elongata

	NUMBER OF STRAINS 1
TEST PERFORMED	**SIGN**
Morphology	[cc,srs]
Motility; flagella	[nm]
Fermentative or oxidative	n-o
Carbohydrate base	CTA
Acid from:	
Glucose	[−]
Xylose	−
Mannitol	−
Lactose	
Sucrose	−
Maltose	−
Catalase	[+]
Oxidase	[+]
Growth on:	
MacConkey	[−]
SS	−
Simmons Citrate	−
Sodium acetate	+
Urea, Christensen's	−
Phenylalanine deaminase	−
Nitrate reduction	[−]
Nitrite reduction	
Indole	
TSI slant, acid	
TSI butt, acid	
H_2S (TSI butt)	−
H_2S (Pb ac paper)	+
Gelatin hydrolysis[a]	−
Litmus milk	−
Pigment	−
Growth at:	
25°C	+
35°C	+
42°C	+
Esculin hydrolysis	−
Nutrient broth, 0% NaCl	+
Nutrient broth, 6% NaCl	−
Penicillin sensitivity[b]	+

[a]7-14 days' incubation.
[b]Based on results obtained by streaking a blood agar plate with growth from an 18-36h culture and then placing a 10-unit penicillin disc on the streaked area. A positive reaction is indicated by the appearance of a zone of inhibition.

Neisseria flavescens

Gram-negative coccus occuring singly and in pairs with adjacent sides flattened; nonmotile; grows in air without increased CO_2; produces no acid from carbohydrates; strongly oxidase-positive; nitrate-negative, nitrite-positive; usually grows on nutrient agar at 35° or 25°C; usually produces yellow growth pigment on Loeffler blood serum agar (obtained commercially); produces polysaccharide on medium with 5% sucrose (amylosucrase test); will not grow on Thayer-Martin medium.

Sources (10 strains)

Trachea (1), lymph node (1), throat (1), unknown (7).

Reference strain

KC651 ⇐ ATCC, 13120 (type strain).

Literature

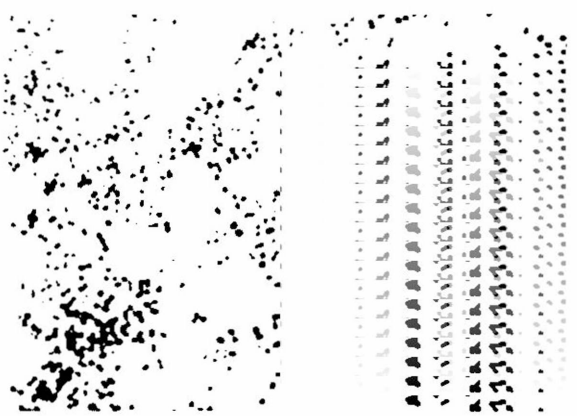
US Public Health Serv Public Health Rep 45:845-849, 1930; J Gen Microbiol 51:387-392, 1968; J Bacteriol 96: 1-5, 1968; J Clin Microbiol 1:294, 1975; Zentralbl Bakteriol Parasitenkd Infektionskr Hyg Abt Orig A230:551-555, 1975.

Comments

Isolations from patients with meningitis and septicemia have been reported. From the number of strains submitted to CDC, this organism appears to be isolated infrequently and apparently is not a problem clinically.



BAP 35°C 24 h ×1 (axial light).



Gram stain BAP 35°C 24 h ×1700.

Neisseria flavescens

	NUMBER OF STRAINS 10		
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	[cd]		
Motility; flagella	[nm]		
Action on blood	v	33 [y	1/3
Fermentative or oxidative	n-o		
Carbohydrate base	CTA		
Acid from:			
Glucose	[ ]	0	0/10
Mannitol	−	0	0/10
Lactose	−	0	0/10
Sucrose	−	0	0/10
Maltose	−	0	0/10
Fructose	[−]	0	0/10
10% Lactose		0	0/3
Catalase	+	100	3/3
Oxidase	[+]	100	10/10
Growth on:			
MacConkey	v	67	1w/3
SS		0	0/3
Simmons Citrate	-	0	0/3
Urea, Christensen's	-	0	0/3
Nitrate reduction	[−]	0	0/10
Nitrite reduction	+	100	1/1
TSI slant, acid	−	0	0/2
TSI butt, acid	−	0	0/2
H_2S (TSI butt)	−	0	0/2
H_2S (Pb ac paper)	+	100	2/2
Gelatin hydrolysis[a]	−	0	0/3
Litmus milk	v	50 k	1w/2
Pigment of Loeffler	[yel]	90	9/10
Growth on nutrient agar:			
25 C	[v]	86	6/7
35 C	+	100	7/7
Amylosucrase	[+]	100	2/2
Growth on Thayer-Martin	[−]	0	0/2

[a]7-14 days' incubation.

Neisseria gonorrhoeae

Gram-negative coccus, occurring singly and in pairs with adjacent sides flattened; nonmotile; grows in air with increased CO_2; usually uses glucose, but not maltose; strongly oxidase-positive; nitrate- and nitrite-negative; does not grow on nutrient agar (without NaCl) either at 35°C or 25°C; most strains (83/86) grow well on Thayer-Martin selective media.

Sources (204 strains)

Cervix (17%), vagina (16%), blood (12%), urethra (11%), joint fluid (7%), eye (6%), urine (4%), other genital sources (5%), throat (2%), cerebrospinal fluid (1%), rectum (1%), other (8%), unknown (10%).

Reference strain

KC1321 ← ATCC, 19424 (suggested working type strain).

Literature

Nomenclator Algarum. Impr du seminaire Padone, 58-59, 1845; J Gen Microbiol 19:555, 1958; Bull WHO 32:449-469, 1965; J Gen Microbiol 51:387-392, 1968.

Comments

Causes gonorrhea. In recent years penicillin-resistant strains have emerged.



Gram stain BAP 35°C 24 h ×1700.

Neisseria gonorrhoeae

	NUMBER OF STRAINS 197		
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	[cd]		
Motility, flagella	[nn]		
Action on blood		0	0/197
Fermentative or oxidative	O		
Carbohydrate base	CTA		
Acid from:			
Glucose	[+][a]	90(3)	127,51w(6)/197
Mannitol		0	0/197
Lactose		0	0/197
Sucrose		0	0/197
Maltose	[ ]	0	0/197
Fructose		0	0/197
ONPG		0	0/19
Oxidase	[+]	100	197/197
Growth on:			
MacConkey	–	0	0/7
Nitrate reduction	[–]	0	0/69
Nitrite reduction	[–]	0	0/10
Pigment on Loeffler	O	10 sl yel	1/10
Phenylalanine		0	0/12
Growth on nutrient agar:			
25°C	[ ]	0	0/149
35°C		0	0/149
Amylosucrase		0	0/14
Growth on Thayer-Martin	[+]	96	83/86

[a]Occasional negative reaction if caps on CTA tubes are not tightened before incubation.

Neisseria lactamica

Gram-negative coccus occurring singly and in pairs with adjacent sides flattened; nonmotile; grows in air with increased CO_2; uses glucose, maltose, and lactose; ONPG-positive; strongly oxidase-positive; nitrate-negative, nitrite-positive; usually grows on nutrient agar (without NaCl) at 35°C, few strains grow at 25°C; grows well on Thayer-Martin selective media.

Sources (287 strains)

Throat (63%), nasopharynx (7%), trachea (2%), lung (1%), sputum (1%), vagina (1%), other (1%), unknown (24%).

Reference strain

A7515, type strain (ATCC 23970), from nasopharynx.

Literature

Appl Microbiol 17:71-77, 1969; J Clin Pathol 26: 399-400, 1973; Amer J Dis Child 130:198-199, 1976.

Comments

Most strains are serologically rough. Rarely isolated from blood or cerebrospinal fluid.



BAP 35°C 24 h ×1.



BAP 35°C 24 h ×6 (axial light).



Gram stain BAP 35°C 24 h ×1700.

Neisseria lactamica

NUMBER OF STRAINS 287			
TEST PERFORMED	**SIGN**	**% +**	**# +/T**
Morphology	[cd]		
Motility; flagella	[nm]		
Action on blood	-	0	0/29
Fermentative or oxidative	F		
Carbohydrate base	CTA		
Acid from:			
Glucose	[+]	96(3)	267,2w(8) 279
Mannitol	-	0	0 269
Lactose	[+]	95(5)	261,5w(13) 279
Sucrose	-	0	0 274
Maltose	[+]	98(2)	270,3w(5) 280
Fructose		0	0 258
10% Lactose	+	92	18,4w 26
ONPG	[+]	100	49,1w 50
Catalase	+	100	29,1w 30
Oxidase	[+]	100	278 278
Growth on:			
MacConkey	-	0	0/33
SS	-	0	0/33
Simmons Citrate	-	0	0/29
Urea, Christensen's	-	0	0/27
Nitrate reduction	[-]	0	0 219
Nitrite reduction	+	100	3/3
TSI slant, acid	v	30	8/27
TSI butt, acid	v	19	4,1w/26
H_2S (TSI butt)	-	0	0/32
H_2S (Pb ac paper)	v	88	11,17w/32
Pigment on Loeffler	v	38 sl yel	15/39
Phenylalanine	-	0	0/4
Growth on nutrient agar:			
25°C	v	22	18,40w(1)/262
35°C	v	74	166/223
Amylosucrase	-	0	0/3
Growth on Thayer-Martin	[+]	100	23/23

Neisseria meningitidis

Gram-negative coccus occurring singly and in pairs with adjacent sides flattened; nonmotile; grows in air with increased CO_2; uses glucose and maltose (some strains do not produce acid from one or both), but not lactose and sucrose; strongly oxidase-positive; nitrate-negative, nitrite-variable; no growth on nutrient agar (without NaCl) at 25°C, but a few carrier-source strains grow at 35°C on this medium; grows well on Thayer-Martin selective media.

Sources (375 strains)

Cerebrospinal fluid (34%), blood (17%), throat (10%), sputum (4%), nasopharynx (3%), eye (1%), trachea (1%), other (3%), unknown (27%).

Reference strain

KC785 ⇐ S. Branham, M1027 (neotype strain) (ATCC 13077).

Literature

Med Res Counc Spec Rep Ser 124:7-142, 1929; Am J Epidemiol 87:643-646, 1968; J Bacteriol 95:1-4, 1968; Int J Syst Bacteriol 20:14, 1970; Br J Vener Dis 50:367-369, 1974.

Comments

Four serogroups are accepted internationally, A, B, C, and D; additional provisional serogroups recognized are Slaterus groups X, Y, Z, and Z' (prime), also group W135. A primary cause of meningitis and septicemia.



Gram stain BAP 35°C 24 h ×1700.

Neisseria meningitidis

	NUMBER OF STRAINS 375		
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	[cd]		
Motility: flagella	[nm]		
Action on blood		2 ly	2/101
Fermentative or oxidative	O		
Carbohydrate base	CTA		
Acid from:			
Glucose	[+][a]	99	247,5w/255
Mannitol	−	0	0/236
Lactose	[−]	0	0/255
Sucrose	[ ]	0	0/238
Maltose	[+]	99	248,4w 254
Fructose		0	0/237
ONPG	−	0	0/44
Oxidase	[+]	100	354/354
Growth on:			
MacConkey	−	0	0/47
Nitrate reduction	−	0	0/160
Nitrite reduction	v	29	2/7
Pigment on Loeffler	−	7 sl yel	6/82
Phenylalanine	−	0	0/26
Growth on nutrient agar:			
25°C	[−]	0	0/207
35°C	−	1	2/209
Amylosucrase	−	0	0/17
Growth on Thayer-Martin	[+]	100	45/45

[a]Occasional negative reaction if caps on CTA tubes are not tightened before incubation.

Neisseria mucosa

Gram-negative coccus occurring singly and in pairs with adjacent sides flattened; nonmotile; grows in air without increased CO_2, also anaerobically; uses glucose (usually), sucrose, maltose, and fructose but not lactose; strongly oxidase-positive; reduces nitrate and nitrite with gas formation; usually grows on nutrient agar (without NaCl) at 35° and 25°C, usually does not grow on Thayer-Martin selective media; produces polysaccharide on medium with 5% sucrose (amylosucrase test).

Sources (30 strains)

Blood (30%), eye (7%), cerebrospinal fluid (7%), wound (7%), urine (7%), sputum (7%), other (13%), unknown (22%).

Reference strain

C2629, from blood.

Literature

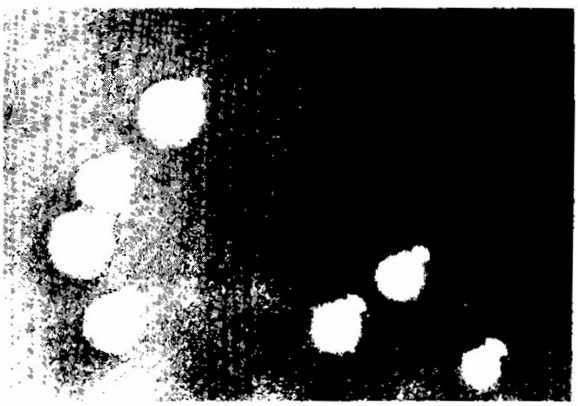
Ann Inst Pasteur, Paris 97:497-510, 1959, and 100:166-179, 1959.

Comments

Occasionally pathogenic for man.



BAP 35°C 24 h ×1.



BAP 35°C 24 h ×6.



Gram stain BAP 35°C 24 h ×1700.

Neisseria mucosa

NUMBER OF STRAINS 30			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	[cd]		
Motility; flagella	[nm]		
Action on blood	v	25 ly	5 20;2 al
Fermentative or oxidative	F		
Carbohydrate base	CTA		
Acid from:			
Glucose	[+ or (+)]	83(10)	25(3) 30
Mannitol	−	0	0 30
Lactose	[−]	0	0 30
Sucrose	[+]	90(10)	27(3) 30
Maltose	+	90(10)	27(3) 30
Fructose	[+]	90(10)	27(3) 30
10% Lactose		0	0 7
Catalase	+	100	8,1w 9
Oxidase	[+]	100	29 29
Growth on:			
MacConkey	v	57(3)	2,2w(2) 7
SS	−	0	0/8
Simmons Citrate	−	0	0/8
Urea, Christensen's	−	0	0/6
Nitrate reduction	[+]	100	29/29
Gas from nitrate	[+]	100	29/29
Nitrite reduction	[+]	100	29/29
TSI slant, acid	+	100	8/8
TSI butt, acid	v	88	7/8
H_2S (TSI butt)	−	0	0/8
H_2S (Pb ac paper)	+	100	7,1w/8
Gelatin hydrolysis[a]	−	0	0 7
Litmus milk	−	0	0 7
Pigment on Loeffler	v	50 sl vel	10 20
Phenylalanine	v	86	6 7
Growth on nutrient agar:			
25 C	[+]	96	24 25
35 C	+	100	16 16
Esculin hydrolysis	−	0	0 5
Amylosucrase	+	100	2 2

[a] 7-14 days' incubation.

Neisseria sicca

Gram-negative coccus occurring singly and in pairs with adjacent sides flattened; nonmotile; grows in air without increased CO_2, also anaerobically; uses glucose (usually), sucrose, maltose, and fructose, but not lactose; strongly oxidase-positive, nitrate-negative, nitrite-positive; usually grows on nutrient agar (without NaCl) at 35° and 25°C; generally does not grow on Thayer-Martin selective media; produces polysaccharide on medium with 5% sucrose (amylosucrase test).

Sources (43 strains)

Throat (19%), sputum (9%), urine (7%), blood (7%), eye (5%), other (12%), unknown (41%).

Reference strain

KC181 ⇐ S. Branham, N5.

Literature

Bergey's Manual, 1st ed. 1923.

Comments

Unable to differentiate with certainty the non-pigmented *N. sicca* and the nonpigmented strains (on Loeffler agar) of the biovar *N. subflava* which was formerly called *N. perflava*.



BAP 35°C 24 h ×1.



BAP 35°C 24 h ×6 (axial light).



Gram stain BAP 35°C 24 h ×1700.

Neisseria sicca

NUMBER OF STRAINS 43			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	[cd]		
Motility: flagella	[nm]		
Action on blood	v	22 ly	4/18;3 β,1 al
Fermentative or oxidative	F		
Carbohydrate base	CTA		
Acid from:			
Glucose	[+ or (+)]	78(14)	24,5w(5) 37
Mannitol	[ ]	0	0 39
Lactose	[ ]	0	0 38
Sucrose	[+]	92(8)	32,4w(3) 39
Maltose	[+]	95(5)	37(2) 39
Fructose	[+]	100	36,2w 38
10% Lactose	-	5	1 21
ONPG	-	0	0 3
Catalase	v	80	15,1w 20
Oxidase	[+]	100	41 41
Growth on:			
MacConkey	v	68	7,8w 22
SS	-	0	0/22
Simmons Citrate	-	0	0/22
Urea, Christensen's	-	0	0/22
Nitrate reduction	[ ]	0	0/37
Nitrite reduction	+	100	2/2
TSI slant, acid	+	100	22 22
TSI butt, acid	+ or (+)	82(9)	16,2w(2) 22
H_2S (TSI butt)	-	0	0/22
H_2S (Pb ac paper)	+	100	21,1w/22
Gelatin hydrolysis[a]	-	0	0 15
Litmus milk	-	0	0 16
Pigment on Loeffler	-	0	0 22
Phenylalanine	v	50	1 2
Growth on nutrient agar:			
25°C	[+]	100	18 18
35°C	+	90(10)	18(2) 20
Esculin hydrolysis	-	0	0 14
Amylosucrase	+	100	3 3
Growth on Thayer-Martin	[-]	0	0 5

[a]7-14 days' incubation.

Neisseria subflava

Gram-negative coccus occurring singly and in pairs with adjacent sides flattened; nonmotile; grows in air without increased CO_2, also anaerobically; uses glucose (usually) and maltose, sometimes sucrose and fructose, lactose negative; strongly oxidase-positive; nitrate-negative, nitrite-positive; usually grows on nutrient agar (without NaCl) at 35°C and 25°C (generally does not grow on Thayer-Martin selective media); usually produces yellow growth pigment on Loeffler blood serum agar; (commercially obtained) may produce polysaccharide on medium with 5% sucrose (amylosucrase test).

Sources (151 strains)

Throat (28%), blood (9%), cerebrospinal fluid (7%), sputum (6%), nasopharynx (4%), urogenital (4%), eye (2%), other (14%), unknown (26%).

Reference strain

KC808 ⇐ ATCC, 14221.

Literature

Generi e le Specie delle Battiericee. Zanaboni e Gabuzzi, Milano, 1889; J Am Med Assoc 195:821-823, 1966.

Comments

Bergey's Manual, 8th ed. combines the former species *N. subflava*, *N. flava*, and *N. perflava* into one species, *N. subflava*.



Gram stain BAP 35°C 24 h ×1700.

Neisseria subflava

NUMBER OF STRAINS 153			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	[cd]		
Motility: flagella	[nm]		
Action on blood	v	56 ly	28/50;14 β,6 al,1 inc β,1 gr
Fermentative or oxidative	F		
Carbohydrate base	CTA		
Acid from:			
Glucose	v	68(6)	71,25w(8) 141
Mannitol		0	0 143
Lactose	[ ]	0	0 141
Sucrose	[v]	56(5)	63,19w(7) 145
Maltose	[+]	99(1)	132,10w(2) 144
Fructose	[v]	77(5)	87,23w(7) 143
10% Lactose		0	0 33
ONPG	−	0	0 15
Catalase	v	80	47 59
Oxidase	[+]	100	150 150
Growth on:			
MacConkey	v	47(2)	12,13w(1)/53
SS	−	0	0 53
Simmons Citrate		0	0 53
Urea, Christensen's		0	0 51
Nitrate reduction	[ ]	0	0 144
Nitrite reduction	v	80	4 5
TSI slant, acid	v	68	34,3w/54
TSI butt, acid	v	56(4)	23,7w(2)/54
H_2S (TSI butt)	−	0	0 54
H_2S (Pb ac paper)	+	100	45,9w/54
Gelatin hydrolysis[a]	-	0	0 35
Litmus milk	v	14 IR	2 14
Pigment on Loeffler	[yel]	99	146 147
Phenylalanine	v	32	7 22
Growth on nutrient agar:			
25 C	[+]	95	82 86
35 C	+	95	112 118
Amylosucrase	v	73	8 11
Growth on Thayer-Martin	[-]	8	3 39

[a]7-14 days' incubation.

Pasteurella aerogenes

Gram-negative coccobacillus; nonmotile; ferments glucose, xylose, sucrose, and maltose, with gas (some strains received by CDC have fermented lactose weakly after 48 h); grows on MacConkey agar; oxidase- and urease-positive (oxidase often weak); nitrate-positive; indole-negative; usually ornithine decarboxylase-positive.

Sources (16 strains)

Swine (intestine, placenta, lung) (44%), cattle (25%), swine bite (19%), other (12%).

Reference strain

C1485, from pig intestine.

Literature

Am J Vet Res 35:917-922, 1974.

Comment

Usually associated with swine.

Photo(s) not available at printing date

Pasteurella aerogenes

	NUMBER OF STRAINS 16		
TEST PERFORMED	**SIGN**	**% +**	**# +/T**
Morphology	cc,srs		
Motility	[nm]		
Gas from glucose	[+]	100	16/16
Action on blood	v	52 al	9/16:1 ly
Fermentative or oxidative	[F]		
Carbohydrate base	F		
Acid from:			
Glucose	+	100	16/16
Xylose	v	81	13/16
Mannitol	-	6	1/16
Lactose	v	19(38)	3w(6)/16
Sucrose	+	94	15/16
Maltose	+	100	16/16
10% Lactose	v	38	5w/13
Catalase	+	100	15,1w/16
Oxidase	[+]	100	10,6w/16
Growth on:			
MacConkey	[+]	100	15,1w/16
SS	-	6	1w/16
Simmons Citrate	-	0	0/16
Urea, Christensen's	[+]	100	16/16
Nitrate reduction	[+]	100	16/16
Gas from nitrate	-	0	0/16
Indole	[-]	0	0/16
TSI slant, acid	+	94	15/16
TSI butt, acid	+	100	16/16
H_2S (TSI butt)	-	0	0/16
H_2S (Pb ac paper)	+	100	16/16
MR	-	0	0/14
VP	v	29	4w/14
Gelatin hydrolysis[a]	-	0	0/3
Litmus milk	v	56 A	1,8w/16
Growth at:			
25°C	+	94	13,2w/16
35°C	+	100	16/16
42°C	+	94	12,3w/16
Esculin hydrolysis	-	0	0/16
Lysine decarboxylase	-	0	0/16
Arginine dihydrolase	-	0	0/16
Ornithine decarboxylase	v	88	14/16
Nutrient broth, 0% NaCl	+	100	14,2w/16
Nutrient broth, 6% NaCl	-	0	0/16

[a]7-14 days' incubation.

Pasteurella anatipestifer *(species incertae sedis,* Bergey's Manual, 8th ed.)

Gram-negative rod; nonmotile; aerobic; oxidizes glucose and maltose weakly, lactose-negative; does not grow on MacConkey agar; oxidase-positive, nitrate-negative, indole-negative; proteolytic; urea-positive; characteristically produces a brown water-soluble pigment.

Sources (5 strains)

Avian (4), unknown (1).

Reference strain

KC534 <- Cornell University. From white Peking duck.

Literature

Dictionnaire des Bactéries Pathogènes, 28th ed., Paris, 1953.

Comments

Isolated from ducks and ducklings, geese, turkeys, and waterfowl with septicemic disease. This species is called *Moraxella anatipestifer* on the "Approved Lists of Bacterial Names."



BAP 35°C 24 h ×1.



Gram stain BAP 35°C 24 h ×1700.

Pasteurella anatipestifer

	NUMBER OF STRAINS 1
TEST PERFORMED	**SIGN**
Morphology	sts
Motility, flagella	[nm]
Action on blood	ly
Fermentative or oxidative	[O]
Carbohydrate base	OF
Acid from:	
Glucose	[+] [a]
Xylose	-
Mannitol	-
Lactose	[-]
Sucrose	-
Maltose	[+] [a]
Catalase	+
Oxidase	[+]
Growth on:	
MacConkey	[-]
SS	-
Simmons Citrate	-
Urea, Christensen's	+
Nitrate reduction	[-]
Indole	[-]
TSI slant, acid	-
TSI butt, acid	-
H_2S (TSI butt)	-
H_2S (Pb ac paper)	+
Gelatin hydrolysis [b]	+
Litmus milk	-
Pigment	yel-br sol
Growth at:	
25°C	+
35°C	+
42°C	-

[a] Weak reaction.
[b] 7-14 days' incubation.

Pasteurella gallinarum

(name suggested by Hall et al (see reference cited below); not in Bergey's Manual, 8th ed.)

Gram-negative rod and coccoid form; nonmotile; ferments glucose, sucrose, maltose, and sometimes xylose; catalase-and oxidase-positive (often weak); urease- and indole-negative; nitrate-positive.

Sources (10 strains)

Avian (6), lung (1), unknown (3).

Reference strain

KC316 < J. W. Hall, P787.

Literature

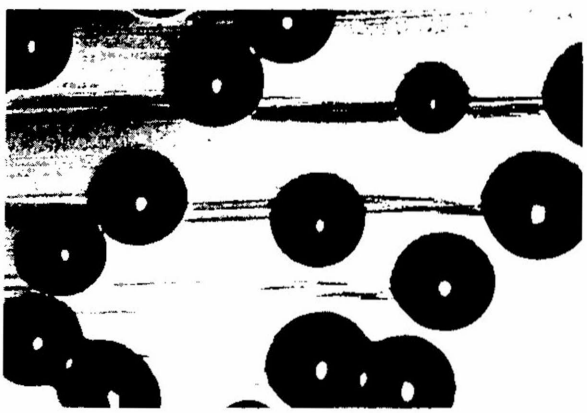
Am J Vet Res 16:598-604, 1955.

Comments

Usually avian-associated.



BAP 35°C 24 h ×1.



BAP 35°C 24 h ×6 (axial light).



Gram stain BAP 35°C 24 h ×1700.

Pasteurella gallinarum

TEST PERFORMED	SIGN	NUMBER OF STRAINS 10	
		% +	# + /T
Morphology	ses		
Motility	[nm]		
Gas from glucose		0	0/10
Action on blood	v	30 al	3/10
Fermentative or oxidative	[F]		
Carbohydrate base	F		
Acid from			
Glucose	+	90/100	9/11/10
Xylose	v	33	3/9
Mannitol		0	0/10
Lactose		0	0/10
Sucrose	+	100	9,1w/10
Maltose	+	100	9,1w/10
10% Lactose	v	17	1/6
Catalase	[+]	100	9,1w/10
Oxidase	[+]	90	4,5w/10
Growth on:			
MacConkey	v	20/100	2/11/10
SS		0	0/10
Simmons Citrate		0	0/10
Urea, Christensen's	[-]	0	0/10
Nitrate reduction	[+]	100	10/10
Gas from nitrate	-	0	0/10
Indole	[ ]	0	0/10
TSI slant, acid	+	100	8,2w/10
TSI butt, acid	+	100	8,2w/10
H_2S (TSI butt)		0	0/10
H_2S (Pb ac. paper)	+	90	6,3w/10
MR	-	0	0/5
VP	v	50	2,3w/8
Gelatin hydrolysis [a]		0	0/10
Litmus milk		10	1w/10
Growth at:			
25 C	v	88	7/8
35 C	+	100	8/8
42 C	v	67	4/6
Esculin hydrolysis		0	0/9
Lysine decarboxylase		0	0/8
Arginine dihydrolase	-	0	0/8
Ornithine decarboxylase	v	25	2/8
Nutrient broth, 0% NaCl	v	25	2w/8
Nutrient broth, 6% NaCl		0	0/9

[a] 7-14 days' incubation.

Pasteurella haemolytica

Gram-negative rod and coccoid forms; nonmotile; ferments glucose, mannitol, and maltose, and other carbohydrates according to biotype; "beta" haemolytic; usually grows on MacConkey agar; catalase-variable, oxidase-positive; urease- and indole-negative; nitrate-positive; lysine decarboxylase-negative, arginine dihydrolase-negative.

Sources (73 strains)

Ewe mastitis (7%), avian sinus (7%), human (4%), other animal sources (bovine, porcine, caprine, rabbit, deer) (82%).

Reference strain

KC228 < L. deS. Smith, from ewe mastitis.

Literature

J Amer Vet Med Assoc 80:711-719, 1932.

Comments

A and T biotypes. Biotype A dies out quickly in culture.



BAP 35°C 24 h × 1.



Gram stain BAP 35°C 24 h ×1700.

Pasteurella haemolytica

	NUMBER OF STRAINS 67					
TEST PERFORMED	**SIGN**	**%**		**#**	
Morphology	STS				
Motility	[nm]				
Gas from glucose		0		0/67	
Action on blood	V	72 β		47/65,3 al,3 gr, 3 ly	
Fermentative or oxidative	[F]				
Carbohydrate base	I				
Number of strains	46 [a]	21 [b]			
Acid from:					
Glucose	+	+	98(2)[a]	90(10)[b]	43/2w(1)/46[a]	15, 4w(2) 2
Xylose	[+]	[–]	96(4)	0	42/2w(2)/46	[illegible]
Mannitol	v	+ or(+)	98(2)	76(24)	40/1/46	[illegible]
Lactose	v	–	11(50)	0	2,3w(23)/46	[illegible]
Sucrose	+	+	98(2)	95(5)	42,3w(1)/46	[illegible]
Maltose	+	+ or(+)	91(6)	72(28)	38,4w(3)/46	[illegible]
Trehalose	–	v	0	80	0/31	[illegible]
Mannose	[–]	[+]	3	95(5)	1/31	13,6w(1) 20
10% Lactose	–	7		1,4w/67		
Catalase	[v]	84(2)		44,7w(1)/61		
Oxidase	[+]	95		54,4w/61		
Growth on:						
MacConkey	[v]	79(6)		40,12w(4)/66		
SS		0		0/67		
Simmons Citrate		0		0/67		
Urea, Christensen's	[–]	0		0/67		
Nitrate reduction	[+]	100		67/67		
Gas from nitrate		0		0/67		
Indole	[–]	0		0/67		
TSI slant, acid	+	100		66,1w/67		
TSI butt, acid	+	100		66,1w/67		
H_2S (TSI butt)		0		0/67		
H_2S (Pb ac paper)	+	100		62,5w/67		
MR		2		1/41		
VP	v	71		22,8w/42		
Gelatin hydrolysis [c]	v	12		7/58		
Litmus milk	v	11(21) A		2,5w(1)/61[3 IR,1 kw		
Growth at:						
25 C	v	73		11,25w/49		
35 C	+	100		46,3w/49		
42 C	v	50		2,22w/49		
Esculin hydrolysis	–[a]	v[b]	0[a]	83[b]	0/46[a]	15/18[b]
Lysine decarboxylase	[–]	3		1/30		
Arginine dihydrolase	[–]	0		0/30		
Ornithine decarboxylase		10		3/30		
Nutrient broth, 0% NaCl	v	65		15,9w/37		
Nutrient broth, 6% NaCl	+	3		1/37		

[a] Biotype A.
[b] Biotype T.
[c] 7-14 days' incubation.

Pasteurella multocida

Gram-negative small coccoid rod; nonmotile; ferments glucose and other carbohydrates without gas; no growth on MacConkey agar; oxidase- and indole-positive (oxidase often weak); urease-negative; nitrate-positive; ornithine decarboxylase-positive.

Sources (306 strains)

Wound (15%), sputum (14%), dog bite (11%), dog or cat gums or tonsils (6%), cat bite (4%), blood (4%), abscess (3%), hand (3%), throat (2%), bronchi (2%), cat scratch (2%), cerebrospinal fluid, ear exudate, pleural fluid, peritoneal fluid, nasopharynx, and foot ulcer (1% each), other (18%), unknown (10%).

Reference strain

D5515, from cerebrospinal fluid.

Literature

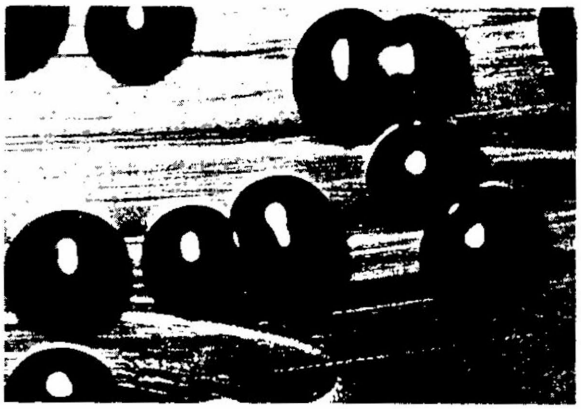
J Bacteriol 37:69-89, 1939; Vet Rev Annot 6:105-128, 1960; Pediatrics 43:295-297, 1969.

Comments

Associated with animals and animal bites. Strains from human respiratory sources usually are mucoid.



BAP 35°C 24 h ×1.



BAP 35°C 24 h ×6 (axial light).



Gram stain BAP 35°C 24 h ×1700.

Pasteurella multocida

		NUMBER OF STRAINS 306					
TEST PERFORMED		**SIGN**			**%**		**# + VE**
Morphology		STS					
Motility		[mm]					
Gas from glucose		[ ]			0		0/297
Action on blood		v			60 al		144/238;14 gr,12 ly,1 LG
Fermentative or oxidative		[F]					
Carbohydrate base		[					
Number of strains	168	66	32	24	7		
Acid from							
Glucose					100		297/297
Xylose					67		199/297
Mannitol					78		231/297
Lactose					8		24/297
Sucrose					100		297/297
Maltose	[ ]	[ ]	[ ]	[ ]	[ ]	2	7/297
10% Lactose					8		21/246
Catalase		+			98		230/49w/283
Oxidase	[ ]	[ ]			97		242/51w/301
Growth on:							
MacConkey		[ ]			2(1)		1/6w(2)/291
SS					0		0/278
Simmons Citrate					0		0/278
Urea, Christensen's		[ ]			0		0/278
Nitrate reduction		[+]			99		299/303
Gas from nitrate					0		0/303
Indole		[+]			99		300/304
TSI slant, acid		+			98(1)		287(2)/293
TSI butt, acid		+			99		290/4w/294
H_2S (TSI butt)		-			0		0/291
H_2S (Pb ac paper)		v			76		220/291
MR		+			1		1/147
VP					0		0/154
Gelatin hydrolysis [1]					0		0/188
Litmus milk		-			3 A		5w,269,2 kw,3 IR
Growth at:							
25 C		+			91		244/267
35 C		+			99		265/268
42 C		v			32		87/268
Esculin hydrolysis					0		0/280
Lysine decarboxylase					0		0/159
Arginine dihydrolase					0		0/159
Ornithine decarboxylase		[+]			94		150/159
Nutrient broth, 0% NaCl		v			68		91,97w/131/280
Nutrient broth, 6% NaCl		-			2		7/275

[1] 7-14 days' incubation.

Pasteurella pneumotropica

Gram-negative rod; nonmotile; ferments (without gas) glucose, xylose, sucrose, maltose, and occasionally lactose, mannitol rarely; may grow on MacConkey agar; oxidase-, urea-, and indole-positive (oxidase often weak); nitrate-positive; ornithine decarboxylase-positive.

Sources (107 strains)

Mice (58%), rats (10%), other small animals (12%), human (cellulitis, throat, rabbit bite, lymph node, pleural fluid) (5%), other (10%), unknown (5%).

Reference strain

KC315 < ATCC, 12555.

Literature

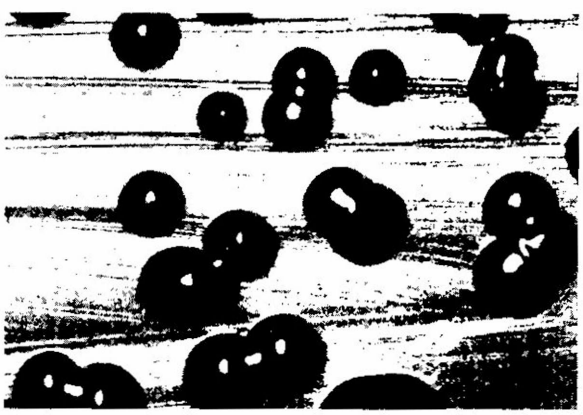
J Infect Dis 86:172-183, 1950; Australian J Exptl Biol Med Sci 31:161, 1953.

Comments

Frequently isolated from mice and rats.



BAP 35°C 24 h ×1.



BAP 35 C 24 h ×6.

Gram stain BAP 35 C 24 h × 1700.

Pasteurella pneumotropica

	NUMBER OF STRAINS 107		
TEST PERFORMED	**SIGN**	**%**	**# +/T**
Morphology	sts		
Motility	[nm]		
Gas from glucose	[ ]	0	0/107
Action on blood	v	32 al	33/104;3 hy,2 gr;1 LG
Fermentative or oxidative	[F]		
Carbohydrate base	F		
Acid from:			
Glucose	+	97(3)	99,5w(3)/107
Xylose	[+ or v]	76(19)	77,4w(20)/107
Mannitol	[-]	2(1)	2(1)/107
Lactose	v	14(39)	9,6w(42)/107
Sucrose	+	97(3)	99,5w(3)/107
Maltose	+	97(3)	99,5w(3)/107
10% Lactose	v	25(3)	5,4w(1)/36
Catalase	+	100	106/106
Oxidase	[+]	99	76,29w/106
Growth on:			
MacConkey	[v]	36(17)	38(18)/107
SS		0	0/107
Simmons Citrate		0	0/107
Urea, Christensen's	[+][a]	95(1)	102(1)/107
Nitrate reduction	[+]	100	107/107
Gas from nitrate	−	0	0/107
Indole	[+]	90	96/107
TSI slant, acid	+	100	102/102
TSI butt, acid	+	97	99/102
H_2S (TSI butt)	−	0	0/103
H_2S (Pb ac paper)	+	100	89,18w/107
MR	−	3	2w/78
VP	v	13	8,3w/82
Gelatin hydrolysis [b]	−	0	0/92
Litmus milk		9 A	5/104;3 IR,2kw
Growth at:			
25°C	v	74	75/101
35°C	+	98	99/101
42°C	−	8	0/64
Esculin hydrolysis	−	0	0/64
Lysine decarboxylase	v	33	9/27
Arginine dihydrolase	−	0	0/27
Ornithine decarboxylase	[+]	100	27/27
Nutrient broth, 0% NaCl	v	84	37/44
Nutrient broth, 6% NaCl	−	2	1/43

[a]May require a drop of serum on slant or a heavy inoculum.

[b]7-14 days' incubation.

***Pasteurella* sp. "n. sp. 1"** (*Pasteurella* "gas") (not cited in Bergey's 8th ed.)

Gram-negative rod; nonmotile; ferments glucose, sucrose, and maltose (sometimes produces slight gas), xylose-and mannitol-negative; no growth on MacConkey agar; oxidase- and indole-positive (oxidase often weak); generally urease-positive; reduces nitrate; ornithine decarboxylase-negative.

Sources (81 strains)

Dog bite (30%), wound (21%), abscess (4%), hand (4%), foot (4%), animal bite (4%), cat mouth (4%), lung, cat bite, throat, blood, dog (2% each), other (18%), unknown (1%).

Reference strain

KC558 < Carpenter, 305-62.

Literature

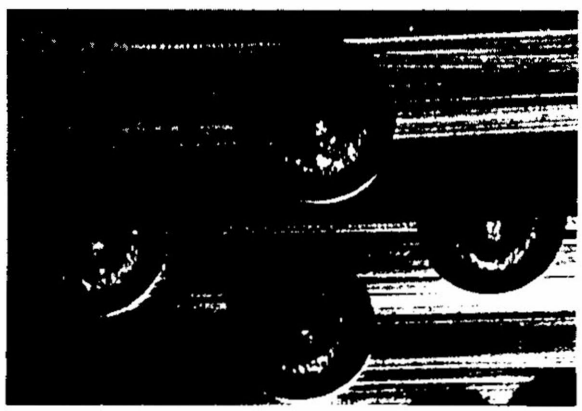
Ann Intern Med 76:275-278, 1972; Winblad S (ed): Contributions to Microbiology and Immunology, vol 2, 1973, pp 170-176.

Comments

Formerly called "*Pasteurella* gas". Thioglycollate broth with glucose and maltose may be useful for detecting gas production, which is often difficult to demonstrate, even with the hot-needle technique (J Infect Dis 3:85-94, 1962). This organism may be a biotype of *P. pneumotropica* (Frederiksen, W., in second reference above).



BAP 35°C 24 h ×1.



BAP 35°C 24 h ×6 (axial light).



Gram stain BAP 35°C 24 h ×1700.

***Pasteurella* sp. "n. sp. 1"** (*Pasteurella* "gas")

NUMBER OF STRAINS 91			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	srs		
Motility	[nm]		
Gas from glucose	v	16 [a]	15/91
Action on blood	v	48 al	34 71;3 hy,2 gr
Fermentative or oxidative	[F]		
Carbohydrate base	F		
Acid from:			
Glucose	+	100	89,2w 91
Xylose	[-]	0	0/91
Mannitol	[-]	0	0/91
Lactose		3	3/91
Sucrose	+	99	89,1w 91
Maltose	[+]	100	84,7w 91
10% Lactose	v	18	7/39
Catalase	+	96	69,18w 91
Oxidase	[+]	98	59,30w 91
Growth on:			
MacConkey	[-]	1	1/91
SS		0	0/91
Simmons Citrate		0	0/91
Urea, Christensen's	[v] [b]	78(3)	68,3w(3)/91
Nitrate reduction	[+]	100	91/91
Gas from nitrate	-	0	0/91
Indole	[+]	100	91/91
TSI slant, acid	+	100	91/91
TSI butt, acid	+	100	91/91
H_2S (TSI butt)		0	0/91
H_2S (Pb ac paper)	+	98	72,17w 91
MR	v	15	3,2w 34
VP	v	28	7,2w 32
Gelatin hydrolysis [c]	v	13	8/62
Litmus milk	v	4 A	4w 91,2 IR,2 dirty,1Dkw
Growth at:			
25 C	v	89	64,17w 91
35 C	+	99	90/91
42 C	v	27	1,24w 91
Esculin hydrolysis		0	0/91
Lysine decarboxylase	+	2	1/46
Arginine dihydrolase	-	0	0/46
Ornithine decarboxylase	[-]	0	0/46
Nutrient broth, 0% NaCl	v	56(1)	5,44w(1)/87
Nutrient broth, 6% NaCl		1	1w/87

[a]37% (15/43) of strains negative for gas in glucose broth were positive for gas in thioglycollate broth when tested with a hot needle.
[b]May require a drop of serum on slant or a heavy inoculum.
[c]7-14 days' incubation.

Pasteurella ureae

Gram-negative pleomorphic rod; nonmotile; ferments glucose, mannitol, sucrose, and maltose, not xylose; no growth on MacConkey agar; oxidase- and urease-positive (oxidase often weak); indole-negative; frequently catalase-negative; reduces nitrate.

Sources (97 strains)

Sputum (67%), bronchi (6%), throat (4%), trachea (4%), nasal (3%), sinus (2%), blood (2%), exudate (2%), cerebrospinal fluid, eye, and otitis media (1% each), unknown (7%).

Reference strain

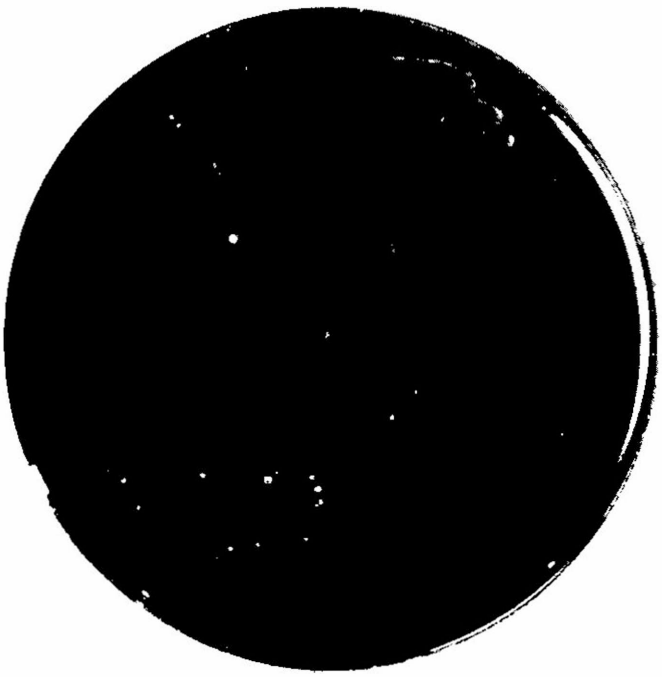
KC518 ← S. D. Henriksen, 218 60.

Literature

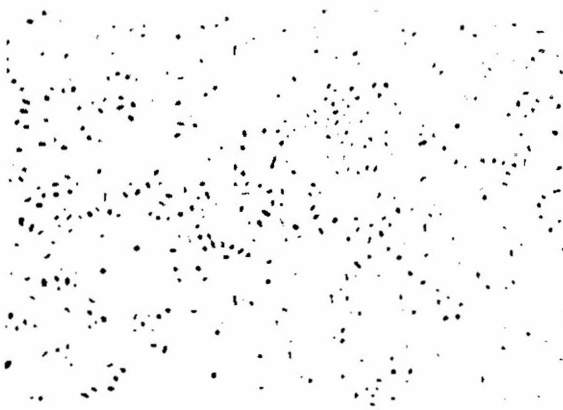
Acta Pathol Microbiol 51:354-367, 1961; J Pathol Bacteriol 83:143-151, 1962; J Clin Pathol 15:247-248, 1962.

Comments

Urea should be inoculated heavily or serum added to slant surface. Associated mainly with respiratory sources (2 blood and 1 CSF isolates); all strains received by CDC have been from human sources.



BAP 35°C 24 h ×1.



Gram stain BAP 35°C 24 h ×1700.

Pasteurella ureae

TEST PERFORMED	SIGN	NUMBER OF STRAINS 97	
		% +	# + / T
Morphology	plr		
Motility	[nm]		
Gas from glucose		0	0/97
Action on blood	v	16 al	14/86;3 hy,2 gr
Fermentative or oxidative	[F]		
Carbohydrate base	F		
Acid from			
Glucose	+	100	87/10w/97
Xylose	[-]	0	0/97
Mannitol	[+]	99(1)	84/12w(1)/97
Lactose	-	0	0/97
Sucrose	+	99(1)	83/13w(1)/97
Maltose	+	91(5)	75/12w(5)/96
10% Lactose	-	0	0/20
Catalase	v	63	34/27w/97
Oxidase	[+]	99	83/10w/94
Growth on			
MacConkey	[-]	0	
SS	-	0	
Simmons Citrate	-	0	
Urea, Christensen's	[+] [a]	100	97/97
Nitrate reduction	[+]	99	93/4w/95
Gas from nitrate	-	0	0/95
Indole	[-]	0	0/93
TSI slant, acid	+	100	95/95
TSI butt, acid	+	99	94/95
H_2S (TSI butt)	-	0	0/97
H_2S (Pb ac paper)	+	91(1)	68/20w(1)/97
MR	-	0	0/97
VP	v	21	0/20w(1)/77
Gelatin hydrolysis [b]	-	0	0/20w(1)/77
Litmus milk	v	90(1)k	0/20w(1)/77
Growth at			77/97
25 C	v	88	77/87
35 C	v	82	69/84
42 C	-	5	4/87
Esculin hydrolysis	-	0	0/94
Lysine decarboxylase	-	0	0/14
Arginine dihydrolase	-	0	0/14
Ornithine decarboxylase	-	0	0/14
Nutrient broth, 0% NaCl	+	0	0/59
Nutrient broth, 6% NaCl	-	0	0/59

[a] May require a drop of serum on slant or a heavy inoculum.

[b] 7-14 days' incubation.

Plesiomonas shigelloides

Gram-negative straight, variable-length rod, motile (tufted polar flagella); ferments glucose, lactose, and maltose (no gas), not mannitol; grows on MacConkey agar; oxidase- and catalase-positive; indole-positive; lysine- and ornithine decarboxylase-positive, arginine dihydrolase-positive; does not require NaCl.

Sources (28 strains)

Stool (25%), spinal fluid (7%), porpoise pus (7%), knee (7%), wound (7%), urine, abscess, blood, lung, paracentesis fluid, calf liver and spleen, porpoise spleen, mouse liver, horse, 1 each (32%), unknown (15%).

Reference strains

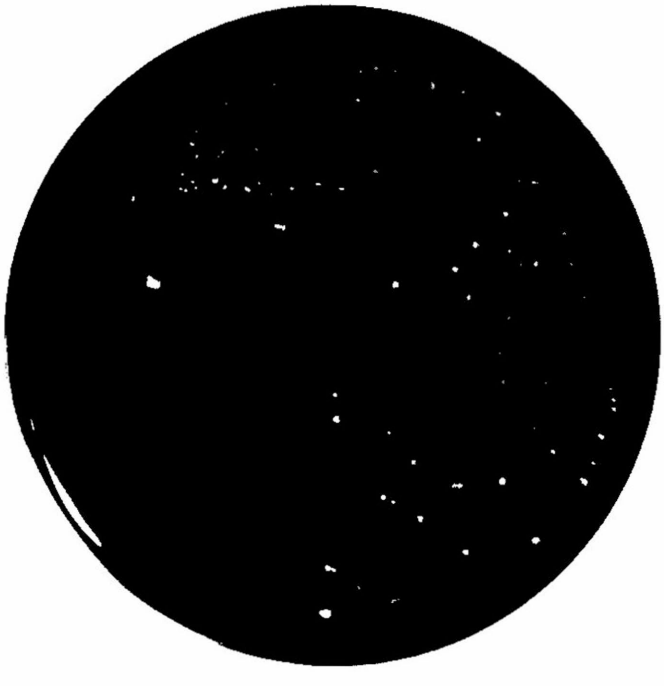
KC1342 < J. L. Mitchell, Brooks AFB (ATCC 14029) (type strain); B3196, from spleen.

Literature

Center for Disease Control: Studies on the *Aeromonas* Group. Atlanta, CDC, 1961; Zentralbl Bakteriol Parasitenkd Infektionskr Hyg Abt Orig 186:316-327, 1962.

Comments

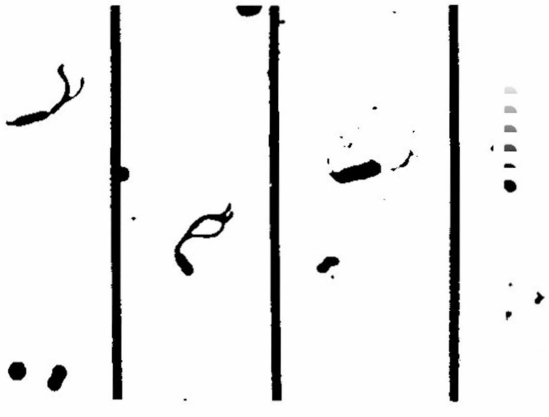
Formerly called *Aeromonas shigelloides*. Sometimes found with shigellae in patients with dysentery.



KC1342 BAP 35°C 24 h ×1.



KC1342 Gram stain BAP 35°C 24 h ×1700.



B3196 (plates 1-2), KC1342 (plates 3-4) Flagell stains HIA 35°C 24 h ×1700.

Plesiomonas shigelloides

NUMBER OF STRAINS 28			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	mrs		
Motility: flagella	[m].p.1-4		
Gas from glucose		0	0/28
Action on blood	v	41 ly	11/27,10β,3 al
Fermentative or oxidative	[F]		
Carbohydrate base	F		
Acid from			
Glucose	+	96(4)	27(1)/28
Xylose		0	0/28
Mannitol	[ ]	0	0/28
Lactose	+	92(4)	23,2w(1)/28
Sucrose		0	0/28
Maltose	+	96(4)	27(1)/28
10% Lactose	v	38(8)	8,2w(2)/26
Catalase	+	93	26/28
Oxidase	[+]	96	27/28
Growth on:			
MacConkey	[+]	100	27,1w/28
SS	+	96	27/28
Simmons Citrate		0	0/28
Urea, Christensen's		0	0/28
Nitrate reduction	+	100	28/28
Indole	+	96	27/28
TSI slant, acid	v	11	3/28
TSI butt, acid	+	100	26,2w/28
H-S (TSI butt)	+	0	0/28
H-S (Pb ac paper)	v	86	10,14w/28
MR	v	82	20,3w/28
VP		4	1/28
Gelatin hydrolysis [a]		0	0/23
Litmus milk	v	63 A	3,14w/28,1 IR
Growth in nutrient broth:			
0% NaCl	[+]	100	13/13
6% NaCl	v	30(8)	2,2w(1)/13
Growth at:			
25 C	+	100	28/28
35 C	+	100	28/28
42 C	v	89	25/28
Esculin hydrolysis	+	0	0/28
Lysine decarboxylase	[+]	100	12/12
Arginine dihydrolase	[+]	100	12/12
Ornithine decarboxylase	[+]	100	12/12

[a] 7-14 days' incubation.

Pseudomonas acidovorans

Gram-negative medium to large rod, motile (polar tuft of three or more flagella); aerobic; grows on MacConkey agar and usually on SS agar; oxidase positive; produces a weakly acid or neutral reaction in mannitol; alkaline in glucose, xylose, lactose, sucrose, and maltose; uses acetamide; reduces nitrate without gas formation; urease-negative; does not produce H_2S on TSI agar.

Sources (67 strains)

Urine (19%), blood (16%), intravenous tubing (9%), water (6%), sputum (6%), other (34%), unknown (10%).

Reference strain

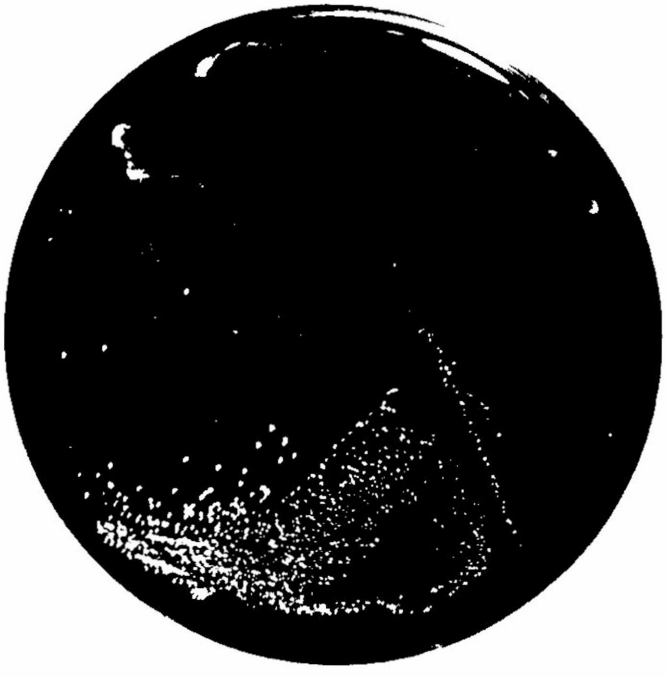
KC1069 < R. Y. Stanier, RYS14 (ATCC 15668, type strain).

Literature

den Dooren de Jong LE: Thesis, Delft, Technische Hoogeschool, 1926; J Gen Microbiol 43:159-271, 1966; Manual of Clinical Microbiology, 2nd ed. p 262.

Comments

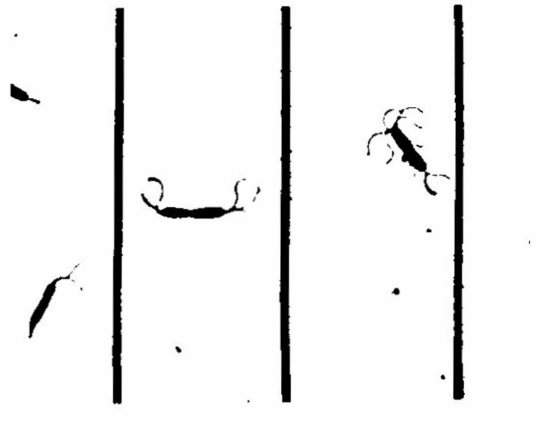
P. acidovorans and *P. testosteroni* are included by Hugh in the species *Comamonas terrigena*. Cells are predominantly straight, occasionally curved; indole is not produced, but some strains cause a vivid yellow reaction in the test medium with xylene extraction and the addition of Ehrlich's reagent. Hugh and Welch reported (Bacteriol Proc p 148, 1969) that some strains produce anthranilic acid and kynurenine in tryptone broth and that these and other tryptophan derivatives cause Kovacs' reagent to become orange. Stanier et al, 1966, reported that DL-arginine, betaine, and testosterone are not used.



BAP 35°C 24 h ×1.



Gram stain BAP 35°C 24 h ×1700.



Flagella stain BAP 35°C 24 h ×1700.

Pseudomonas acidovorans

NUMBER OF STRAINS 64			
TEST PERFORMED	**SIGN**	**%+**	**#+/T**
Morphology	rods		
Motility, flagella	[m,p,-2]		
Action on blood	v	38 [v	23/60,13 gr,7 br,5 EG,
Fermentative or oxidative	F/O		
Carbohydrate base	OF		
Acid from			
Glucose	[ ]	0	0/64
Xylose	[ ]	0	0/64
Mannitol	[+]	100	64w/64
Lactose		0	0/64
Sucrose		0	0/64
Maltose		0	0/64
Fructose	+	100	11w/11
Catalase	+	100	64/64
Oxidase	[+]	100	54,9w/63
Growth on:			
MacConkey	[+]	100	64/64
SS	v	67(19)	43(12)/64
Cetrimide		5	3/63
Simmons Citrate	+	94(5)	60(3)/64
Urea, Christensen's	[-]	0(3)	(2)/67
Nitrate reduction	+	98	63/64
Gas from nitrate	[-]	0	0/64
Indole	-	0	0/64
TSI slant, acid	-	0	0/64
TSI butt, acid		0	0/64
H_2S (TSI butt)	[ ]	0	0/64
H_2S (Pb ac paper)	v	56	36/64
Gelatin hydrolysis [a]	v	12	8/64
Litmus milk	k	97	62/64
Pigment			
Fluorescent	v	23	14/64
Soluble	v	47 yel-ta	18/38
Growth at:			
25 C	+	100	64/64
35 C	+	100	64/64
42 C	v	31(11)	20/64
Esculin hydrolysis		0	0/64
Lysine decarboxylase		0	0/64
Arginine dihydrolase		0	0/64
Ornithine decarboxylase		0	0/64
Nutrient broth, 0% NaCl	+	100	45/45
Nutrient broth, 6% NaCl	-	7	4/57

[a] 7-14 days' incubation.

Pseudomonas aeruginosa

Gram-negative rod; motile (single, occasionally two, polar flagella); aerobic; uses glucose, xylose, and usually mannitol, not sucrose or maltose; grows on MacConkey, SS, and cetrimide agars; oxidase- and arginine dihydrolase-positive, lysine decarboxylase-negative; reduces nitrate to gas; indole-negative; grows at 42 C; acetamide and 2-ketogluconate-positive; proteolytic; may produce pyocyanin (blue), pyoverdin (yellow-green) (fluoresces with a Woods lamp), pyorubrin (red), and pyomelanin (brown) water-soluble pigments.

Sources (201 strains)

Urine (29%), sputum (18%), environmental (8%), ear (5%), other (28%), unknown (12%).

Reference strains

KC1072 < R. Y. Stanier, 45; KC1073 < R. Y. Stanier, 52.

Literature

Migula W: Syst der Bakterien 2. G. Fischer, Jena, 1900; J Gen Microbiol 43:159-271, 1966; Int J Syst Bacteriol 20:15, 1970.

Comments

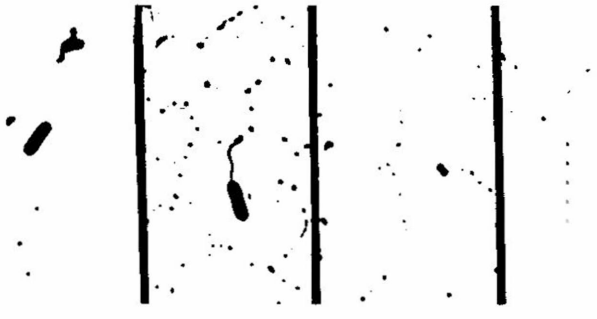
Growth frequently exhibits a metallic sheen; mucoid strains occur and frequently may be atypical biochemically. To our knowledge *P. aeruginosa* is the only species of *Pseudomonas* that produces pyocyanin.



KC1073 BAP 35°C 24 h ×1.



KC1072 Gram stain BAP 35°C 24 h ×1700.



KC1072 Flagella stain BAP 35°C 24 h ×1700.

Pseudomonas aeruginosa

		NUMBER OF STRAINS 201	
TEST PERFORMED	**SIGN**	**% +**	**# +/T**
Morphology	mrs		
Motility, flagella	[m,p,1-2]		
Action on blood	v	42 LG	70/168,33 β,32 ly,17 gr,9 br
Fermentative or oxidative	[0]		
Carbohydrate base	OF		
Acid from			
Glucose	[+]	97(1)	195(2)/201
Xylose	+	90(1)	182(2)/201
Mannitol	v	70(3)	141(6)/201
Lactose		1	1(1)/201
Sucrose	[ ]	0	0/201
Maltose	[ ]	0(-1)	(1)/201
Catalase	+	100	167/167
Oxidase	[+]	99	163/165
Growth on			
MacConkey	[+]	100	166/166
SS	+	96	158/165
Cetrimide	[+]	94(2)	156(4)/165
Simmons Citrate	+	95(1)	158(1)/166
Urea, Christensen's	v	48(9)	75,3w(15)/161
Nitrate reduction	+	98	163/167
Gas from nitrate	[+]	93	156/167
Indole	[ ]	0	0/168
TSI slant, acid		0	0/164
TSI butt, acid		0	0/164
H-S (TSI, butt)		0	0/168
H-S (Pb ac paper)		4	2,2w/168
Gelatin hydrolysis [a]	[v]	82	111/135
Litmus milk	[v]	89 pep	153/172;2 IR,2 k,2 c
Pigment			
Pyoverdin	[v]	65	131/201
Pyocyanin	[v]	46	92/201
Pyorubrin	v	25	22/85
Other	v	23 pyomelanin	47/201
Growth at:			
25 C	+	100	201/201
35 C	+	100	201/201
42 C	[+]	100	201/201
Esculin hydrolysis		0	0/156
Lysine decarboxylase	[ ]	0	0/15
Arginine dihydrolase	[+]	100	15/15
Ornithine decarboxylase	+	0	0/15
Nutrient broth, 0% NaCl	+	100	148/148
Nutrient broth, 6% NaCl	v	65	96/147
Acetamide	[+]	100	70,1w/71
2 Ketogluconate	+	96	27/28

[a] 7-14 days' incubation.

Pseudomonas alcaligenes

Gram-negative rod or filament; motile (1-2 polar flagella); aerobic; grows on MacConkey agar; does not use carbohydrates; oxidase-positive, urease-negative, usually does not grow at 42 C; sometimes reduces nitrate, without gas.

Sources (26 strains)

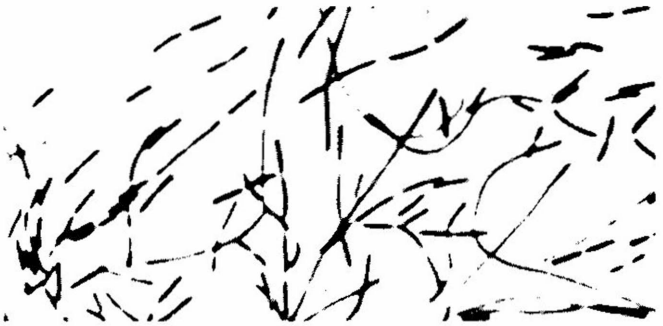
Urine (15%), blood (12%), sputum (12%), ear (8%), tissue culture (8%), other (41%), unknown (4%).

Reference strain

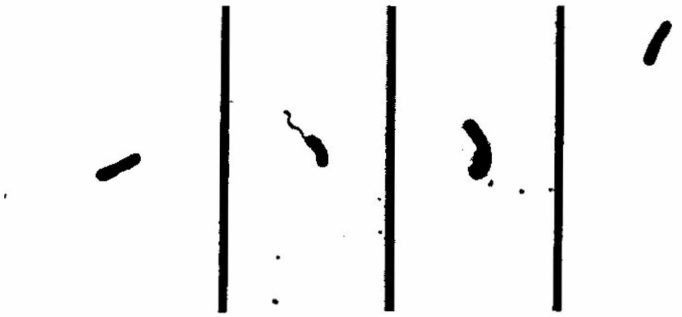
KC1075 < ATCC, 14909 (proposed neotype).

Literature

J Infect Dis 43:330-334, 1928; Int Bull Bacteriol Nomenel Taxon 14:103-107, 1964; J Gen Microbiol 43:243-247, 1966; Int J Syst Bacteriol 26:421-426, 1976; Manual of Clinical Microbiology, 3rd ed. pp 304-306.



Gram stain BAP 35°C 24 h ×1700.



Flagella stain BAP 35°C 24 h ×1700.

Pseudomonas alcaligenes

NUMBER OF STRAINS 26			
TEST PERFORMED	**SIGN**	**%**	**#+/#T**
Morphology	mrs		
Motility; flagella	[m;p,1-2]		
Action on blood	v	31 ly	8 26;3 I G,2 gr,1 al,1 β
Fermentative or oxidative	[n-o]		
Carbohydrate base	OF		
Acid from:			
Glucose	[-]	0	0 26
Xylose	[-]	0	0 26
Mannitol		0	0 26
Lactose		0	0 26
Sucrose		0	0 26
Maltose	[-]	0	0 26
Fructose	[-]	0	0 12
Catalase	+	92	24 26
Oxidase	[+]	96	25 26
Growth on:			
MacConkey	[+]	96	25 26
SS	v	38(8)	10(2) 26
Cetrimide	v	15(4)	4(1) 26
Simmons Citrate	v	57(8)	15(2) 26
Urea, Christensen's	[-]	0	0 25
Nitrate reduction	v	54	14 26
Gas from nitrate	[-]	0	0 26
Indole		0	0 26
TSI slant, acid		0	0 26
TSI butt, acid		0	0 26
H_2S (TSI butt)		0	0 26
H_2S (Pb ac paper)	v	65	17 26
Gelatin hydrolysis [a]		0	0 18
Litmus milk	v	46 k	12 26;4 IR
Pigment:			
Soluble	v	32 fa	12 19;1 sl yel
Growth at:			
25 C	+	100	26 26
35 C	+	100	26 26
42 C	-	0	0 26
Esculin hydrolysis		0	0 26
Lysine decarboxylase		0	0 8
Arginine dihydrolase	v	12	1 8
Ornithine decarboxylase	-	0	0 8
Nutrient broth, 0% NaCl	+	95	18 19
Nutrient broth, 6% NaCl	v	41	9 22

[a] 7-14 days' incubation.

Pseudomonas cepacia

Gram-negative, straight or slightly curved rod; motile (polar tuft of three or more flagella); oxidizes glucose, xylose, mannitol, lactose, and maltose; usually lysine decarboxylase-positive, arginine dihydrolase-negative; grows on MacConkey agar; sometimes reduces nitrate, without gas.

Sources (159 strains)

Urine (31%), blood (16%), cerebrospinal fluid (8%), bronchial washing (7%), Detergicide (6%), sputum (6%), abscess, exudate, and wound (6%), other (9%), unknown (11%).

Reference strain

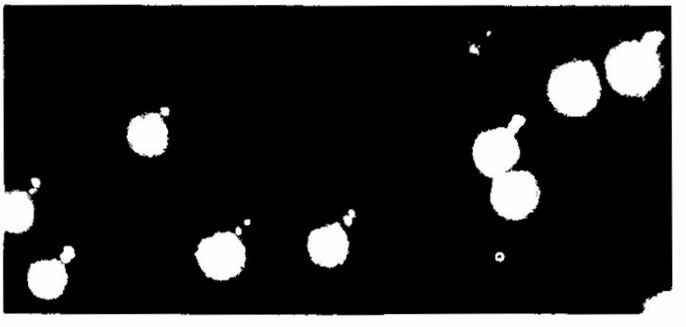
KC1371 < ATCC 25416.

Literature

Phytopathology 40:115-117, 1950; J Lab Clin Med 38:374-383, 1951; J Gen Microbiol 43:159-271, 1966; N Eng J Med 279:361-362, 1968; Int J Syst Bacteriol 20:255-257,1970; J Gen Microbiol 60:199-214, 1970; Lancet 1:1188-1191, 1970; Can J Microbiol 16:351-362, 1970; Lancet 1:375-377, 1971; Int J Syst Bacteriol 22:127-138, 1972; J Infect Dis 125:613-618, 1972; Int J Syst Bacteriol 25:187-201, 1975.



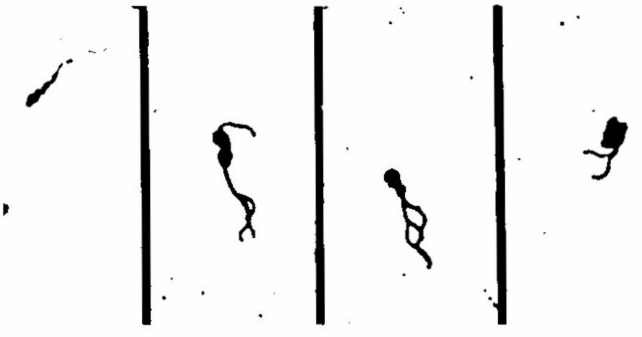
BAP 35°C 24 h ×1.



BAP 35°C 24 h ×6.



Gram stain BAP 35°C 24 h ×1700.



Flagella stain FB 25°C 24 h ×1700.

Comments

Synonyms *P. multivorans*, *P. kingii*, EO-1 (Eugonic Oxidizer). Surface growth dies out quickly; survives "disinfection" with quaternary ammonium compounds. Some strains produce a yellow growth pigment on TSI agar and Kligler's iron agar, but not on media which do not contain iron; others produce a chartreuse growth pigment on common laboratory media; most strains exhibit no growth pigment.

Pseudomonas cepacia

NUMBER OF STRAINS 159			
TEST PERFORMED	**SIGN**	**% +**	**# + T**
Morphology	mrs		
Motility; flagella	[m,p,-2]		
Action on blood	v	47 ly	65 139,30 LG,16 gr,17 lay,9 al
Fermentative or oxidative	[0]		
Carbohydrate base	OF		
Acid from:			
Glucose	[+]	100	159 159
Xylose	+	100	159 159
Mannitol	[+]	100	159 159
Lactose	[+]	99(1)	156,2w(1) 159
Sucrose	v	86(1)	137(1) 159
Maltose	[+]	99(1)	157(2) 159
Catalase	+	99	109,44w 155
Oxidase	v	86	53,80w 154
Growth on:			
MacConkey	[+]	100	155 155
SS	v	6(18)	9(27) 154
Cetrimide	v	44(22)	69(34) 155
Simmons Citrate	+	94(5)	146(8) 156
Urea, Christensen's	v	60(18)	93(28) 155
Nitrate reduction	v	57	87 152
Gas from nitrate	[ ]	0	0 152
Indole		0	0 152
TSI slant, acid		1	2 157
TSI butt, acid		0	0 157
H_2S (TSI butt)		0	0 140
H_2S (Pb ac paper)		0	0 141
Gelatin hydrolysis [a]	v	20	24 118
Litmus milk	pep	94	132 140,6 IR,1 k
Pigment:			
Insoluble	v	60 yel(TSI) [b]	95 159
Growth at:			
25 C	+	98	142 145
35 C	+	100	145 145
42 C	v	83	120 145
Esculin hydrolysis	v	63(6)	85(8) 135
Lysine decarboxylase	[v]	80	32 40
Arginine dihydrolase	[ ]	0	0 40
Ornithine decarboxylase	v	48	19 40
Nutrient broth, 0% NaCl	+	100	132 132
Nutrient broth, 6% NaCl	+	7	9 135

[a] 7-14 days' incubation.

[b] 60% of the *P. cepacia* strains produced a yellow pigment which occurs only on iron-containing media, e.g. TSI; this usually is not evident before 42-48h. Sometimes a chartreuse pigment occurs on ordinary media.

Pseudomonas diminuta

Gram-negative short to long rods, motile (single polar flagellum with distinctive, very short wavelength); aerobic; grows on MacConkey agar; usually does not use carbohydrates, although some strains oxidize glucose; produces oxidase; rarely reduces nitrate to nitrite; indole negative.

Sources (68 strains)

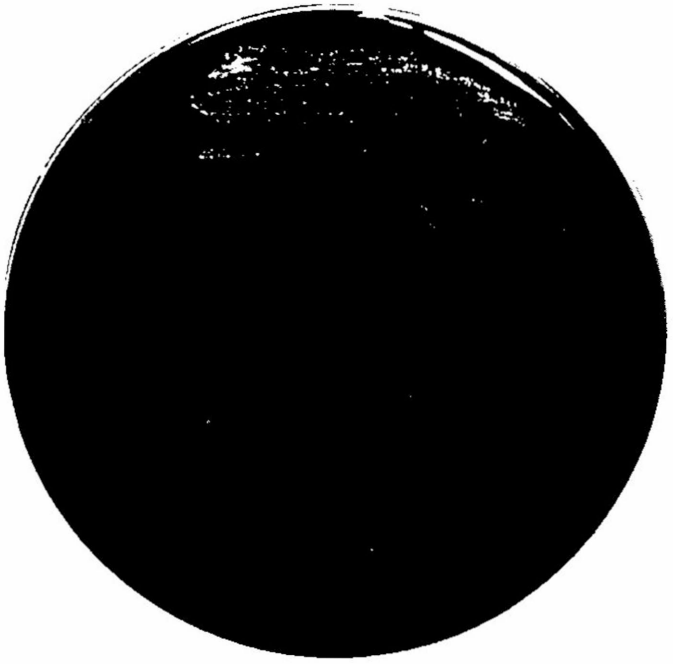
Blood (29%), cerebrospinal fluid (10%), urine (10%), wound (7%), other (32%), unknown (12%).

Reference strains

KC679 < ATCC 11568 (type strain); D7054 (1), from toe wound.

Literature

J Gen Microbiol 10:68-70, 1954, and 25:400,1961, and 53:349-361, 1968; J Clin Microbiol 1:339, 1975.



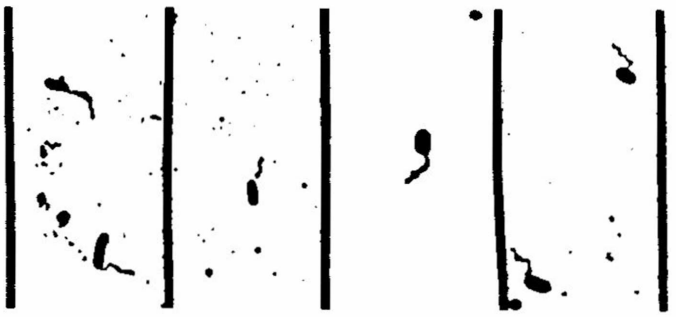
KC679 BAP 35°C 24 h ×1.



KC679 BAP 35°C 24 h ×6.



KC679 Gram stain BAP 35°C 24 h ×1700.



KC679 (plates 1 and 2) and D7054 (plates 3 and 4) flagella stain BAP 35°C 24 h ×1700.

Comment

This organism distinctively produces a brown, water-soluble pigment on heart infusion agar with tyrosine.

Pseudomonas diminuta

NUMBER OF STRAINS 68			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	rs		
Motility, flagella	[m;p,1-2] [a]		
Action on blood	v	69 LG or gr	38,55;6 br,6 ly,5 lav
Fermentative or oxidative	n-o or O		
Carbohydrate base	OF		
Acid from:			
Glucose	[v]	21(9)	13(6) 68
Xylose	[ ]	0	0/68
Mannitol		0	0/68
Lactose		0	0/68
Sucrose		0	0/68
Maltose	[ ]	0	0/68
Catalase	+	98	67/68
Oxidase	[+]	100	68/68
Growth on:			
MacConkey	[+]	97(3)	66(2)/68
SS		1(1)	1(1)/68
Cetrimide	-	0(1)	(1)/68
Simmons Citrate		1	1w/68
Urea, Christensen's	v	0(13)	(9)/68
Nitrate reduction	[ ]	3	2/68
Gas from nitrate	[ ]	0	0/68
Indole	[-]	0	0/68
TSI slant, acid		0	0/68
TSI butt, acid		0	0/68
H_2S (TSI butt)	-	0	0/68
H_2S (Pb ac paper)	v	34	23/68
Gelatin hydrolysis [b]	v	68	46/68
Litmus milk	v	35 k	17,48,15 IR,9 pep
Pigment:			
Soluble	[br-ta]	96	64,67,2 yel,1 or
Growth at:			
25 C	+	100	68/68
35 C	+	100	68/68
42 C	v	38	26/68
Esculin hydrolysis	-	5(1)	3(1)/68
Lysine decarboxylase	-	0	0/6
Arginine dihydrolase	-	0	0/6
Ornithine decarboxylase	-	0	0/6
Nutrient broth, 0% NaCl	+	100	58/58
Nutrient broth, 6% NaCl	v	21(2)	12(1)/56

[a] Flagella have a short wavelength with low amplitude.

[b] 7-14 days' incubation.

Pseudomonas fluorescens

Gram-negative short to long rods; motile (polar tuft of three or more flagella); aerobic; uses glucose, xylose, often sucrose, and sometimes other carbohydrates; aerobic; grows on MacConkey and SS agars; oxidase-positive; arginine dihydrolase-positive, lysine decarboxylase-negative; liquefies gelatin at 25 C; usually does not reduce nitrate; indole negative; usually prefers to grow at 25 C, grows poorly at 35 C, does not grow at 42 C; produces a fluorescent yellow-green pigment (pyoverdin), does not produce pyocyanin; acetamide-negative.

Sources (155 strains)

Sputum (30%), blood (9%), urine (8%), cerebrospinal fluid (6%), other (40%), unknown (7%).

Reference strain

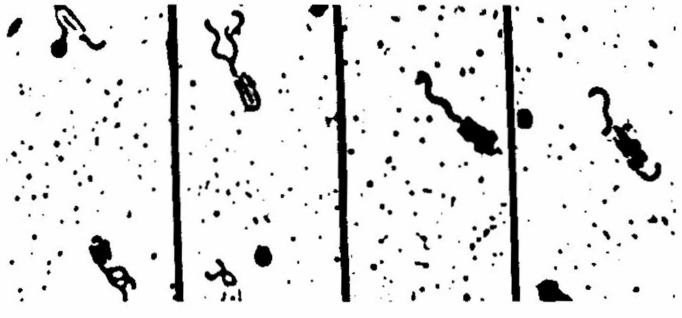
KC678 < ATCC 13525 (neotype strain).

Literature

Migula, W: Bakteriaceae, in Engler und Prantl, Pflanzenfamilien, W. Engelmann, Leipzig, Teil I., 1895; J Gen Microbiol 21:221-263, 1959; Int Bull Bacterial Nomencl Taxon 14:145-155, 1964; Int J Syst Bacteriol 20:18, 1970; yale J Biol Med 44:265-273, 1971; Appl Microbiol 25:107-110, 1973.



Gram stain BAP 35°C 24 h ×1700.



Flagella stain FB 25°C 24 h ×1700.

Pseudomonas fluorescens

NUMBER OF STRAINS 155			
TEST PERFORMED	**SIGN**	**% +**	**# +/T**
Morphology	mrs		
Motility; flagella	[m,p,+2]		
Action on blood	v	50 ly	60 119;16 gr,6 LG,3 lav
Fermentative or oxidative	[O]		
Carbohydrate base	OF		
Acid from:			
Glucose	[+]	100	155 155
Xylose	+	100	155 155
Mannitol	v	53(2)	80(3) 151
Lactose	v	24(3)	38(5) 155
Sucrose	[v]	48	75 155
Maltose	[ ]	2	3 155
Catalase	+	99(1)	149(1) 150
Oxidase	[+]	97	148,2w 154
Growth on:			
MacConkey	[+]	100	152 152
SS	[+ ort +]	86(7)	130(1) 152
Cetrimide	+	89	135 151
Simmons Citrate	+	93(3)	142(5) 152
Urea, Christensen's	v	21(31)	32(47) 151
Nitrate reduction	v	19	29 151
Gas from nitrate	[ ]	3	5 151
Indole	[ ]	0	0 155
TSI slant, acid		0	0 145
TSI butt, acid	-	0	0 145
H_2S (TSI, butt)	-	0	0 155
H_2S (Pb ac paper)	v	12	5,14w 152
Gelatin hydrolysis [a]	[+]	100	75 75
Litmus milk	[pep]	95	146 154;7 k,1 A
Pigment:			
Pyoverdin	[+]	96	141 147
Pyocyanin	[−]	0	0 147
Pyorubrin	−	0	0 147
Other	−	4 br sol	6w 147;3 yel sol
Growth at			
5 C	+	95(1)	136(1) 143
25 C	+	100	153 153
35 C	+	90	138 153
42 C	[−]	0	0 153
Esculin hydrolysis	−	0	0 138
Lysine decarboxylase	[−]	0	0 37
Arginine dihydrolase	[+]	97	36 37
Ornithine decarboxylase	−	0	0 37
Nutrient broth, 0% NaCl	+	99	147 149
Nutrient broth, 6% NaCl	v	43	64 149
Acetamide	v	6(12)	1(2) 17

[a] 7-14 days' incubation, 25°C.

Pseudomonas mallei

Gram-negative rod; nonmotile; aerobic; oxidizes glucose and sometimes (delayed) xylose, mannitol, lactose, and maltose, but not sucrose; growth on MacConkey agar is variable; usually oxidase-negative, reduces nitrate to nitrite without gas; produces arginine dihydrolase, not lysine decarboxylase; does not use citrate (Simmons), digest gelatin, or grow at 42 C.

Sources (8 strains)

Equine abscess (1), unknown (7).

Reference strain

See "Comments" below.

Literature

Ann Inst Pasteur Paris 106:479-483, 1964; J Gen Microbiol 43:293-313, 1966; J Bacteriol 101:827-835, 1970.

Comments

Etiologic agent of glanders, primarily a natural equine disease. Considered to be too hazardous for routine laboratory study.

Photo(s) not available at printing date

Pseudomonas mallei

NUMBER OF STRAINS 8			
TEST PERFORMED	**SIGN**	**% +**	**# + [a]T**
Morphology	ss		
Motility, flagella	[nm]		
Action on blood	ly	100	8/8
Fermentative or oxidative	[0]		
Carbohydrate base	OF		
Acid from:			
Glucose	[+]	100	7/1w/8
Xylose	v	12(50) [b]	1w/4/8
Mannitol	v	62(14) [b]	5w/1/8
Lactose	v	12(62) [a]	1w/5/8
Sucrose	[+]	0	0/8
Maltose	v	0(75) [b]	(6)/8
Catalase	+	100	8/8
Oxidase	v	25	2/8
Growth on			
MacConkey	v	88	7w/8
SS		0	0/8
Cetrimide		0	0/8
Simmons Citrate	[+]	0	0/8
Urea, Christensen's	v	12	1/8
Nitrate reduction	[+]	100	8/8
Gas from nitrate	[+]	0	0/8
Indole		0	0/8
TSI slant, acid		0	0/8
TSI butt, acid		0	0/8
H S (TSI butt)		0	0/8
H S (Pb ac paper)	+	100	8/8
Gelatin hydrolysis [b]		0	0/8
Litmus milk		0	0/8
Pigment			
Soluble		0	0/8
Insoluble		0	0/8
Growth at			
25 C	+	100	8/8
35 C	+	100	8/8
42 C	[+]	0	0/8
Esculin hydrolysis		0	0/8
Lysine decarboxylase	[+]	0	0/8
Arginine dihydrolase	[+]	100	8/8
Ornithine decarboxylase		0	0/8
Nutrient broth, 0% NaCl	+	100	8/8
Nutrient broth, 6% NaCl		0	0/8

[a]Some delayed carbohydrate reactions required 7-14 days' incubation.

[b]7-14 days' incubation

Pseudomonas maltophilia

Gram-negative short to medium rod; motile (polar tuft of three or more flagella); aerobic; oxidizes glucose (frequently weak or delayed) and maltose, but not mannitol; grows on MacConkey agar; catalase-positive, oxidase-negative; lysine decarboxylase-positive; hydrolyzes gelatin; sometimes reduces nitrate, but without gas; does not produce H_2S in TSI agar.

Sources (228 strains)

Blood (14%), sputum (14%), urine (10%), wound (10%), throat (5%), other (37%), unknown (10%).

Reference strains

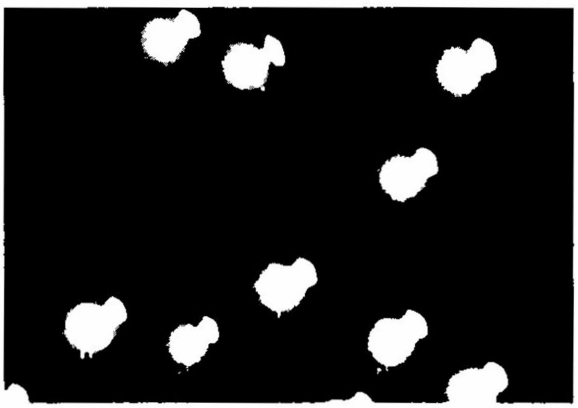
KC1078 ← ATCC 13637 (type strain); C3507, from stool of mice.

Literature

J Gen Microbiol 26:123-132, 1961; Med Welt 3:177-178, 1965; J Gen Microbiol 43:159-271, 1966; Amer J Clin Pathol 51:58-61, 1969.



KC1078 BAP 35°C 24 h ×1.



KC1078 BAP 35°C 24 h ×6.



KC1078 Gram stain BAP 35°C 24 h ×1700.



C3507 Flagella stain FB 25°C 24 h ×1700.

Comments

Produces a lavender-green color on rabbit blood agar at 18-24 h, and a distinctive brown water-soluble pigment on heart infusion agar with tyrosine.

Pseudomonas maltophilia

NUMBER OF STRAINS 228			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	mrs		
Motility: flagella	[m].p.-2		
Action on blood	v	81 LG	158/194,27 lav,15 ly,6 gr,3 β
Fermentative or oxidative	[0]		
Carbohydrate base	OF		
Acid from			
Glucose	[+ or (+)]	85(5)	6,56w(4)/73
Xylose	v	35(1)	2,24w(1)/73
Mannitol	[ ]	0	0/73
Lactose	v	60(1)	44w(1)/73
Sucrose	v	63(1)	46w(1)/73
Maltose	[+] [a]	100	67,6w/73
Catalase	[+]	100	226/226
Oxidase	[b]	0	0/73
Growth on:			
MacConkey	[+]	100	228/228
SS	v	22(21)	51(49)/228
Cetrimide		2(7)	5(17)/227
Simmons Citrate	v	34(12)	77(28)/226
Urea, Christensen's	v	3(12)	6(27)/227
Nitrate reduction	v	39	88/226
Gas from nitrate	[ ]	0	0/226
Indole		0	0/226
TSI slant, acid		0	0/226
TSI butt, acid	-	0	0/226
H-S (TSI butt)	[-]	0	0/228
H-S (Pb ac paper)	+	95	217/228
Gelatin hydrolysis [c]	[+]	93	178/191
Litmus milk	pep	96	214/224
Pigment:			
Soluble	br-ta	98	223/228
Growth at			
25 C	+	99	224/225
35 C	+	99	224/225
42 C	v	48	108/225
Esculin hydrolysis	v	39	87/224
Lysine decarboxylase	[+]	93	52/56
Arginine dihydrolase	-	0	0/56
Ornithine decarboxylase	-	0	0/56
Nutrient broth, 0% NaCl	+	100	225/225
Nutrient broth, 6% NaCl	v	22	49/225

[a]Acid reaction in maltose usually stronger than acid reactions in the other carbohydrates listed.

[b]14 of 73 strains which were negative when the reagent was applied directly to growth on a blood agar plate were positive when tested by Kovacs' method. 10 of 14 were recorded as only weakly positive.

[c]7-14 days' incubation.

Pseudomonas marginata

Gram-negative rod; motile (1-2 polar flagella); aerobic; oxidizes glucose, xylose, and mannitol, but not maltose; grows on MacConkey agar; oxidase-negative; nitrate-negative; peptonizes litmus milk; urea-positive; does not produce lysine decarboxylase or arginine dihydrolase. (These reactions are those of *P. marginata* ATCC 10248 on the test media of CDC's Special Bacteriology Laboratory.)

Source

Gladiolus.

Reference strain

ATCC 10248.

Literature

J Gen Microbiol 60:211, 1970.

Comments

The reference strain oxidized lactose weakly in 8-21 days when removed from the 35 C incubator after 7 days and held at ambient temperature (25°-28°C). In Bergey's Manual, 8th Edition, it is stated that *P. marginata* is motile, with polar multitrichous flagellation, that the oxidase reaction is positive but very weak in some strains, that it is isolated from decayed onions, *Gladiolus* spp. and *Iris* spp., and that it is pathogenic for them. We have several similar cultures from water, blood, cerebrospinal fluid, sputum, and other sources.

Photo(s) not available at printing date

Pseudomonas marginata

NUMBER OF STRAINS 1 (Type Strain, ATCC 10248)	
TEST PERFORMED	**SIGN**
Morphology	rods
Motility; flagella	[m];p.1-2 [a]
Fermentative or oxidative	[O]
Carbohydrate base	OF
Acid from:	
Glucose	[+]
Xylose	+
Mannitol	[+]
Lactose	[b]
Sucrose	
Maltose	[ ]
Catalase	+
Oxidase	[ ] [c]
Growth on:	
MacConkey	[+]
SS	
Cetrimide	(+)
Simmons Citrate	+
Urea, Christensen's	+
Nitrate reduction	-
Gas from nitrate	
Indole	-
TSI slant, acid	-
TSI butt, acid	-
H_2S (TSI butt)	-
H_2S (pb ac paper)	
Gelatin hydrolysis [d]	-
Litmus milk	(pep)
Pigment	
Soluble	-
Insoluble	-
Growth at:	
25 C	+
35 C	+
42 C	
Esculin hydrolysis	
Lysine decarboxylase	[ ]
Arginine dihydrolase	[ ]
Ornithine decarboxylase	
Nutrient broth, 0% NaCl	+
Nutrient broth, 6% NaCl	-

[a]Polar multitrichous flagellation according to Bergey's Manual (8th ed.).

[b]OF lactose held at room temperature after the 7th day became acid between 7th and 21st day.

[c]Oxidase is positive but very weak in some strains according to Bergey's Manual (8th ed.).

[d]7-14 days' incubation.

Pseudomonas mendocina

Gram-negative short to medium length slender rod; motile (single polar flagellum); aerobic; oxidizes glucose and xylose only; does not hydrolyze starch; grows on MacConkey and SS agars; oxidase-positive reduces nitrate to gas; produces arginine dihydrolase, but not lysine decarboxylase; 2-ketogluconate-negative; acetamide-negative-, produces no H_2S on TSI agar; non proteolytic.

Sources (3 strains)

Water (2), soil (1).

Reference strains

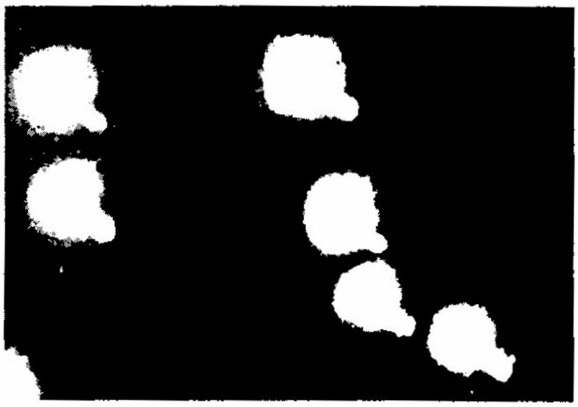
KC1140 ← N. J. Palleroni, CH20 (ATCC 25412); KC1218 ← ATCC, 25411 (Palleroni CH50) (type strain); KC1220 ← ATCC, 25413 (Palleroni CH120).

Literature

J Gen Microbiol 43:159-271, 1966, and 60:215-23, 1970.



KC1140 BAP 35°C 24 h ×1.



KC1140 BAP 35°C 24 h ×6.



KC1140 Gram stain BAP 35°C 24 h ×1700.



KC1140 Flagella stain BAP 35°C 24 h ×1700.

Comments

P. mendocina is similar to nonpigmented strains of *P. aeruginosa*, except that the latter is acetamide and 2-ketogluconate positive.

A small group of similar organisms which oxidize mannitol were included in previously published "Round-Table" charts as Vb-2. Such strains were isolated from a variety of sources (two from inline transducer and one each from urine, vagina, wound, claviele node, serous otitis, and exudate). These strains are not included in this manual. Further studies are needed to establish their taxonomy.

Pseudomonas mendocina

NUMBER OF STRAINS 3			
TEST PERFORMED	**SIGN**	**%**	**# + /T**
Morphology	mrs		
Motility; flagella	[m];p,1-2		
Action on blood	ly	100	1/1
Fermentative or oxidative	[O]		
Carbohydrate base	OF		
Acid from:			
Glucose	[+]	100	3/3
Xylose	+	100	3/3
Mannitol		0	0/3
Lactose	[−]	0	0/3
Sucrose	−	0	0/3
Maltose	[−]	0	0/3
Starch	−	0	0/3
Catalase	+	100	1,2w/3
Oxidase	[+]	100	3/3
Growth on:			
MacConkey	[+]	100	3/3
SS	[+]	100	3/3
Cetrimide	+	100	3/3
Simmons Citrate	+	100	3/3
Urea, Christensen's	v	33	1/3
Nitrate reduction	+	100	3/3
Gas from nitrate	[+]	100	3/3
Indole	−	0	0/3
TSI slant, acid	−	0	0/3
TSI butt, acid	−	0	0/3
H_2S (TSI, butt)	[−]	0	0/3
H_2S (Pb ac paper)	+	100	3/3
Gelatin hydrolysis [a]	[−]	0	0/3
Litmus milk	(k)	0(100)	(3)/3
Pigment	sl yel ins	100	3/3
Growth at:			
25°C	+	100	3/3
35°C	+	100	3/3
42°C	+	100	3/3
Esculin hydrolysis	−	0	0/3
Lysine decarboxylase	[−]	0	0/3
Arginine dihydrolase	[+]	100	3/3
Ornithine decarboxylase	−	0	0/3
Nutrient broth, 0% NaCl	+	100	3/3
Nutrient broth, 6% NaCl	+	100	1/1
Acetamide	[−]	0	0/3
2-Ketogluconate	[−]	0	0/3

[a] 7-14 days' incubation.

Pseudomonas mesophilica

Gram-negative pleomorphic vacuolated rod with large intracellular fat bodies; motile (single polar flagellum); aerobic; oxidizes xylose, methanol, and sometimes glucose (weakly), but not lactose; oxidase-positive; indole-negative; pink to coral growth pigment.

Sources (90 strains)

Blood (24%), bronchus (9%), sputum (6%), nebulizer (6%), cerebrospinal fluid (3%), eye (3%), water (3%), urine (2%), bone (2%), chest fluid (2%), exudate (2%), other (25%), unknown (13%).

Reference strains

8143, from blood, bone marrow, and liver biopsy of the same patient; B4139, from sputum; D1912, source unknown.

Literature

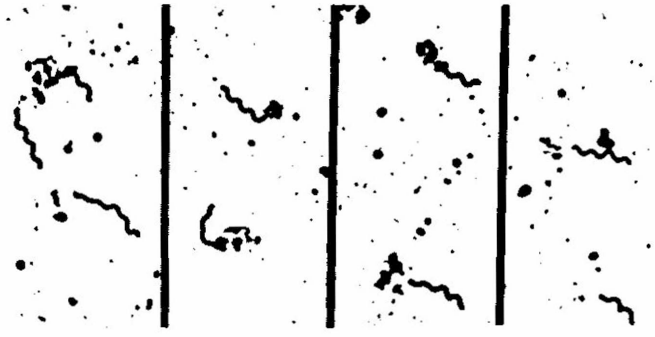
Am Rev Respir Dis 90:437-447, 1965; J Bacteriol 88:1065-1070, 1964; Int J Syst Bacteriol 29:373-378, 1979.

Comments

Formerly called *Vibrio extorquens*. Pale-staining Gram-positive members may be observed. May prefer media without blood. May be a cause of systemic illness as a secondary invader. Has been found as a contaminant of laboratory media and is called the "Red Phantom."



D1912 Gram stain HIA 35°C 24 h ×1700.



B4139 Flagella stain FB 25°C 24 h ×1700.

Pseudomonas mesophilica

NUMBER OF STRAINS 90			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	[vps-mr]		
Motility; flagella	[m].p.1		
Action on blood		8 ly	4 47;1 al
Fermentative or oxidative	[0]		
Carbohydrate base	OF		
Acid from			
Glucose	v	40	4,32w 90
Xylose	[+]	94	51,34w 90
Mannitol		2	2w 90
Lactose	[ ]	0	0 90
Sucrose		0	0 90
Maltose		2	2w 90
Catalase	+	100	82,6w 88
Oxidase	[+]	96	72,3w 78
Growth on:			
MacConkey	v	15	7,6w(1) 89
SS		0	0 89
Simmons Citrate		2(3)	2(3) 89
Urea, Christensen's	v	29(26)	23(20) 78
Nitrate reduction	v	25	19,3w 88
Gas from nitrate	–	0	0 88
Indole	[–]	0	0 85
TSI slant, acid	–	0	0/83
TSI butt, acid	–	0	0/75
H_2S (TSI butt)	–	0	0/84
H_2S (Pb ac paper)	v	47	9,31w/85
Gelatin hydrolysis [a]	–	0	0/57
Litmus milk	v	49 k	43/87
Pigment	[pk-co]	99	89/90
Growth at:			
25°C	+	98	81/83
35 C	+	93	76/82
42 C	v	12	9/73
Esculin hydrolysis	–	0	0/81
Nutrient broth, 0% NaCl	+	93	83/89
Nutrient broth, 6% NaCl	–	0	0 79

[a] 7-14 days' incubation.

Pseudomonas paucimobilis

Gram-negative medium, sometimes long rods; motility difficult to demonstrate, especially at 35°C (single, occasionally two polar flagella); produces a yellow insoluble growth pigment; aerobic; does not usually grow on MacConkey agar; uses glucose, xylose, lactose, sucrose, and maltose, not mannitol; oxidase-variable; usually does not produce urease nor reduce nitrate; lysine decarboxylase-negative, arginine dihydrolase-negative.

Sources (134 strains)

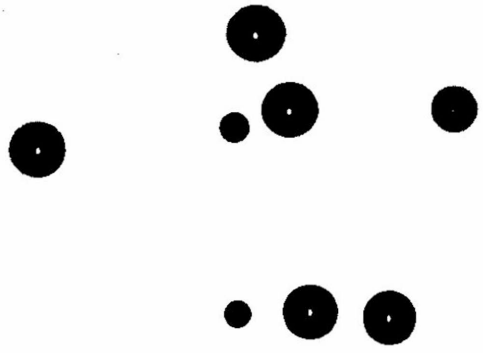
Blood (24%), environmental contaminant (9%), humidifier (8%), water (7%), urine (7%), wound (7%), cerebrospinal fluid (6%), ear, eye, throat, abdomen, vagina, dialysis fluid (2% each), other (16%), unknown (4%).

Reference strain

B2966, from dialysis fluid.



BAP 35°C 24 h ×1.



BAP 35°C 24 h ×6 (axial light).



Gram stain BAP 35°C 24 h ×1700.



Flagella stain FB 25°C 24 h ×1700.

Literature

Int J Syst Bacteriol 27:133-146, 1977.

Comment

Formerly called IIk-1.

Pseudomonas paucimobilis

NUMBER OF STRAINS 134			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	mrs		
Motility, flagella	[m];p,1-2 [a]		
Action on blood	v	32 gr	13 40
Fermentative or oxidative	[O]		
Carbohydrate base	OF		
Acid from:			
Glucose	[+]	93(7)	103,22w(9) 134
Xylose	+	96(4)	129(5) 134
Mannitol	[ ]	0	0 134
Lactose	[+]	93(7)	116,8w(10) 134
Sucrose	+	93(7)	116,9w(9) 134
Maltose	[+]	97(3)	125,5w(4) 134
Catalase	+	95	127 134
Oxidase	v	75	72,28w 134
Growth on:			
MacConkey	v	10(13)	14(17) 134
SS		0	0 134
Cetrimide		0	0 134
Simmons Citrate		6(1)	8(1) 134
Urea, Christensen's	[ ]	6(3)	8(4) 133
Nitrate reduction		3	3,1w 134
Gas from nitrate		0	0 134
Indole		0	0 134
TSI slant, acid	v	20(3)	14(2) 70
TSI butt, acid	v	6(31)	8(41) 134
H_2S (TSI butt)		0	0 132
H_2S (Pb ac paper)	v	76	100 132
Gelatin hydrolysis [b]	-	2	3 118
Litmus milk	v	60 IR	24 40,6 k,5 A, 4 pep
Pigment:			
Insoluble	[yel]	95	121 128,3 or
Growth at:			
25 C	+	100	129 129
35 C	+	100	129 129
42 C	v	24	31 129
Esculin hydrolysis	+	91	108 119
Lysine decarboxylase	[-]	0	0 26
Arginine dihydrolase	[-]	8	2 26
Ornithine decarboxylase	-	0	0 26
Nutrient broth, 0% NaCl	+	93	124 133
Nutrient broth, 6% NaCl		4	5 133

[a] Usually appears nonmotile in motility medium.

[b] 7-14 days' incubation.

Pseudomonas pickettii

(not cited in Bergey's Manual, 8th ed.)

Gram-negative short to medium length rod, motile (single polar flagellum); aerobic; oxidizes glucose and xylose; grows on MacConkey agar; oxidase-positive; citrate-positive; urease-positive; reduces nitrate with gas formation (may be difficult to detect); lysine decarboxylase- and arginine dihydrolase-negative; grows in nutrient broth.

Sources (54 strains)

Urine (22%), nasopharynx (20%), abscess and wound (18%), blood (13%), other (12%), unknown (15%).

Reference strain

KC1295 < ATCC, 27511 (type strain).

Literature

Int J Syst Bacteriol 23:15-19, 1973; J Clin Microbiol 1:61-64, 1975.

Comments

Motility weak or delayed, not readily detectable. Gas production during denitrification also may not be readily detected unless Stanier's semi-aerobic nitrate medium is used.

See ∇a-1 (biovar of *P. pickettii*) and *P. thomasii*.



BAP 35°C 24 h ×1.



Gram stain BAP 35°C 24 h ×1700.



Flagella stain FB 25°C 24 h ×1700.

Pseudomonas pickettii

NUMBER OF STRAINS 54			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	mrs		
Motility; flagella	[m],p,1-2 [a]		
Action on blood	v	21 ly	6 29,5 al,4 LG,2 gr
Fermentative or oxidative	[0]		
Carbohydrate base	OF		
Acid from:			
Glucose	[+]	100	54/54
Xylose	[+]	100	54/54
Mannitol		0	0/54
Lactose	[ ]	0	0/54
Sucrose		0	0/54
Maltose	[ ]	0	0/54
Catalase	+	100	54/54
Oxidase	[+]	100	54/54
Growth on:			
MacConkey	[+]	100	54/54
SS		0	0/54
Cetrimide		0	0/54
Simmons Citrate	+	100	54/54
Urea, Christensen's	[+]	100	54/54
Nitrate reduction	+	100	54/54
Gas from nitrate	[+] [b]	100	54/54
Indole		0	0/54
TSI slant, acid		0	0/54
TSI butt, acid		0	0/54
H_2S (TSI butt)		0	0/54
H_2S (Pb ac paper)	v	38	11/29
Gelatin hydrolysis [c]	v	33	12/36
Litmus milk	k	90	26/29
Growth at:			
25 C	+	100	54/54
35 C	+	100	54/54
42 C	+	94	51/54
Esculin hydrolysis		0	0/54
Lysine decarboxylase	[ ]	0	0/54
Arginine dihydrolase	[+]	0	0/54
Ornithine decarboxylase	+	0	0/54
Nutrient broth, 0% NaCl	[+]	100	29/29
Nutrient broth, 6% NaCl	+	3	1/29

[a]Weakly motile, may be difficult to demonstrate.

[b]May be negative if not tested in semi-aerobic nitrate medium.

[c]7-14 days' incubation.

Pseudomonas pseudoalcaligenes

Gram-negative medium length rod; motile (1-2 polar flagella); aerobic; grows on MacConkey agar; usually oxidizes fructose, not glucose or maltose; oxidase-positive; grows at 42°C; urease-negative; usually reduces nitrate without gas; does not produce H_2S on TSI agar.

Sources (19 strains)

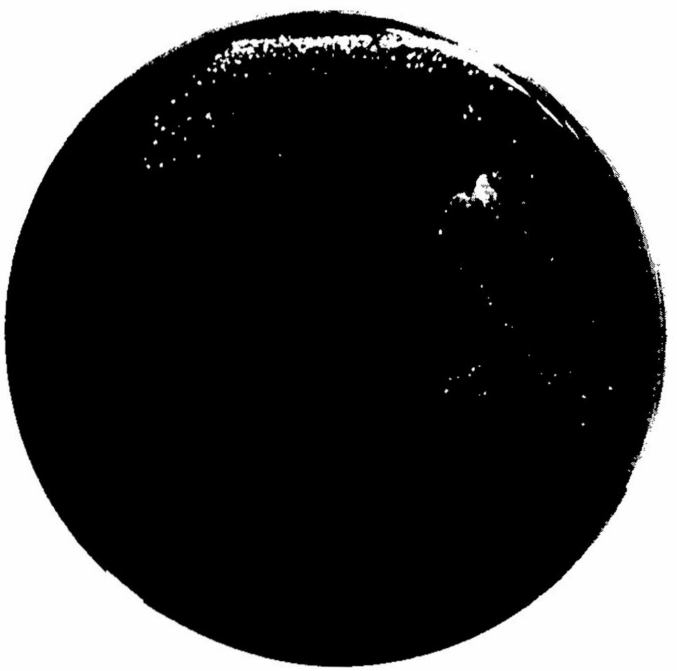
Urine (16%), cerebrospinal fluid (16%), environmental (16%), other (42%), unknown (10%).

Reference strain

KC946 ⇐ Pickett, K401.

Literature

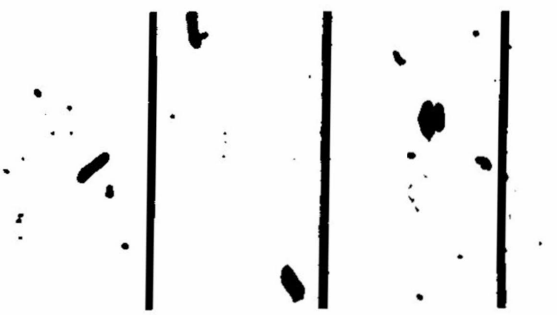
J Gen Microbiol 43:159-271, 1966; Manual of Clinical Microbiology, 3rd ed., pp 304-306.



BAP 35°C 24 h ×1.



Gram stain BAP 35°C 24 h ×1700.



Flagella stain BAP 35°C 24 h ×1700.

Pseudomonas pseudoalcaligenes

NUMBER OF STRAINS 19			
TEST PERFORMED	**SIGN**	**% +**	**# + / T**
Morphology	mrs		
Motility, flagella	[m,p,l-2]		
Action on blood	v	26 ly	5 19;4 LG;3 gr,1 al
Fermentative or oxidative	[n-o]		
Carbohydrate base	OF		
Acid from:			
Glucose	[ ]	0	0 19
Xylose	v	32	6w 19
Mannitol		0	0 19
Lactose		0	0 19
Sucrose		0	0 19
Maltose	[ ]	0	0 19
Fructose	[v]	82	14w 17
Catalase	+	95	14,4w 19
Oxidase	[+]	100	19 19
Growth on:			
MacConkey	[+]	100	19 19
SS	v	84	15,1w 19
Cetrimide	v	37(10)	7,2w 19
Simmons Citrate	v	43(10)	8(2) 19
Urea, Christensen's	[ ]	5	1w 19
Nitrate reduction	+	90	17 19
Gas from nitrate	[-]	0	0 19
Indole	-	0	0 19
TSI slant, acid		0	0 19
TSI butt, acid	-	0	0 19
H_2S (TSI butt)	[-]	0	0 19
H_2S (Pb ac paper)	+	95	16,2w 19
Gelatin hydrolysis [a]	-	0	0 9
Litmus milk	v	37 k	7 19;2 IR
Pigment:			
Soluble	v	26 yel br	5 19
Growth at:			
25 C	+	100	19 19
35 C	+	100	19 19
42 C	[+]	100	19 19
Esculin hydrolysis	-	0	0 19
Lysine decarboxylase	-	0	0 3
Arginine dihydrolase	+	100	3 3
Ornithine decarboxylase	-	0	0 3
Nutrient broth, 0% NaCl	+	100	19 19
Nutrient broth, 6% NaCl	v	68	13 19

[a] 7-14 days' incubation.

Pseudomonas pseudomallei

Gram-negative rod, motile (polar tufts of three or more flagella); aerobic; oxidizes glucose, xylose, mannitol, lactose, maltose, and sometimes sucrose; grows on MacConkey agar; oxidase-positive; hydrolyzes arginine, but does not decarboxylate lysine; proteolytic; reduces nitrate, usually with formation of gas. Usually shows characteristic white opaque growth with a sheen by 48 h; at first colonies are smooth and convex, but slowly they become unbonate with an uneven, wrinkled surface. In a young culture at 35°C most cells will show bipolar staining; after 48 h most cells are oval to round, and only the periphery stains; such cells may be mistaken for endospores.

Sources (70 strains)

Sputum (21%), wound (14%), animal isolate (13%), blood (10%), abscess or drainage (6%), lung (4%), skin (3%), other (including one prostatic fluid) (15%), unknown (14%).

Reference strains

KC871(1) ← WRAMIR, 295 MP-G; KC872 ← WRAMIR, 3MP-h.

Literature

Bergey's Manual, 7th ed., pp. 89-152; J Gen Microbiol 25:400, 1961; J Bacteriol 101:827-835, 1970; Am J Clin Pathol 55:596-603, 1971.

Comments

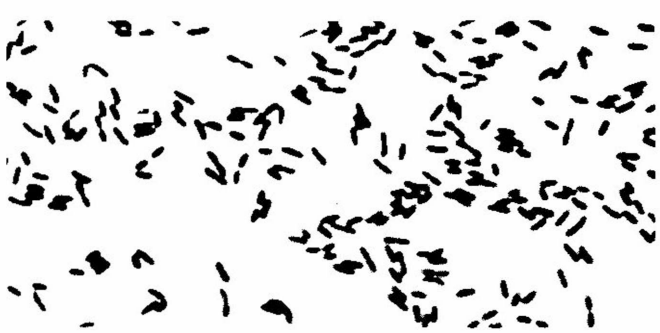
Causes melioidosis, an endemic glanders-like disease. Most infections are acquired in the Far East. Melioidosis was reported in American military personnel during World War II and during the Viet Nam



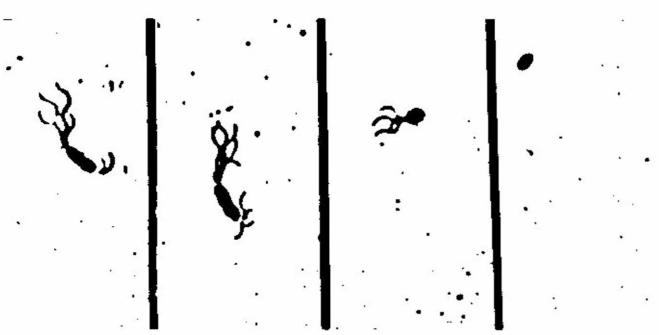
KC872 BAP 35°C 24 h ×1.



KC871(1) BAP 35°C 24 h ×6.



KC871(1) Gram stain BAP 35°C 24 h ×1700.



KC871(1) Flagella stain BAP 35°C 24 h ×1700.

conflict. Laboratory infections have been reported. Frequently this organism has a characteristic musty, earthy odor; it is resistant to colistin. The amount of gas formed in the reduction of nitrate may be small or may be demonstrated only at 25°C. *P. stutzeri* may be confused with *P. pseudomallei;* however, *P. stutzeri* usually develops a slightly yellow growth pigment, does not oxidize lactose, is rarely proteolytic, and is motile by a single polar flagellum. Also, *P. cepacia* may be confused with *P. pseudomallei* when gas is not formed readily from nitrate; however, *P. cepacia* is lysine-positive and arginine-negative.

Pseudomonas pseudomallei

	NUMBER OF STRAINS 70		
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	mrs		
Motility, flagella	[m],p,>2		
Action on blood	v	40 gr	23/57;17 hy,11 LG,6 lav
Fermentative or oxidative	[O]		
Carbohydrate base	OF		
Acid from			
Glucose	[+]	100	70/70
Xylose	+ or (+)	86(14)	58,2w(10)/70
Mannitol	+	94(6)	66(4)/70
Lactose	[+]	99(1)	69(1)/70
Sucrose	v	66(4)	42,4w(3)/70
Maltose	[+]	99(1)	69(1)/70
Catalase	+	100	58,1w/59
Oxidase	[+]	100	55,6w/61
Growth on:			
MacConkey	[+]	100	62/62
SS	v	8(31)	5(19)/61
Cetrimide		7(3)	5(2)/68
Simmons Citrate	v	77(4)	53(3)/69
Urea, Christensen's	v	13(8)	9(6)/69
Nitrate reduction	[+]	100	70/70
Gas from nitrate	[+] [a]	100	70/70
Indole	−	0	0/60
TSI slant, acid	v	72	49/68
TSI butt, acid	−	0	0/68
H_2S (TSI butt)	−	0	0/70
H_2S (Pb ac paper)	v	26	18w/69
Gelatin hydrolysis [b]	v	79	46/58
Litmus milk	pep	96	67/70,2 IR
Pigment:			
Insoluble	v	51 white	28/54;21 yel
Growth at:			
25°C	+	100	55/55
35°C	+	100	55/55
42°C	+	100	55/55
Esculin hydrolysis	v	59	34/58
Lysine decarboxylase	[−]	0	0/8
Arginine dihydrolase	[+]	100	8/8
Ornithine decarboxylase	−	0	0/8
Nutrient broth, 0% NaCl	+	100	61/61
Nutrient broth, 6% NaCl	v	12	7/61

[a]The volume of gas may be small. Gas may not be detected unless incubated at 25°C.

[b]7-14 days' incubation.

Pseudomonas putida

Gram-negative medium to slightly long rod, motile (polar tuft of three or more flagella); aerobic; oxidizes glucose and xylose, but not sucrose; grows on MacConkey agar; oxidase-positive; usually produces pyoverdin, a fluorescent yellow-green pigment; does not grow at 42°C; does not reduce nitrate; does not liquefy gelatin at 25°C; lysine decarboxylase-negative, arginine dihydrolase-positive; acetamide-negative.

Sources (99 strains)

Urine (18%), blood (7%), cosmetics (7%), sputum (7%), lung (6%), cerebrospinal fluid (5%), throat (5%), other (33%), unknown (12%).

Reference strain

KC1074 ≤ R. Y. Stainer, 90.

Literature

J Gen Microbiol 43:299, 1966; Appl Microbiol 25:107-110, 1973.

Comments

Similar to *P. fluorescens*, except for lack of gelatinase and certain other proteolytic enzymes.



BAP 35°C 24 h ×1.



BAP 35°C 24 h ×6.



Gram stain BAP 35°C 24 h ×1700.



Flagella stain BAP 35°C 24 h ×1700.

Pseudomonas putida

NUMBER OF STRAINS 16			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	rnrs		
Motility; flagella	[m;p,c2]		
Action on blood	v	38 gr	6/16;4 gr & ly,4 ly
Fermentative or oxidative	[O]		
Carbohydrate base	OF		
Acid from:			
Glucose	[+]	100	16/16
Xylose	+	100	16/16
Mannitol	v	25	3,1w/16
Lactose	v	25(13)	4(2)/16
Sucrose	[−]	0	0/16
Maltose	[v]	31	3,2w/16
Catalase	+	100	16/16
Oxidase	[+]	100	16/16
Growth on:			
MacConkey	[+]	100	16/16
SS	+	100	16/16
Cetrimide	v	81(6)	13(1)/16
Simmons Citrate	+	94(6)	15(1)/16
Urea, Christensen's	v	13(44)	2(7)/16
Nitrate reduction	[−]	0	0/16
Gas from nitrate	−	0	0/16
Indole		0	0/16
TSI slant, acid	-	0	0/16
TSI butt, acid		0	0/16
H_2S (TSI, butt)	-	0	0/16
H_2S (Pb ac paper)	-	6	1w/16
Gelatin hydrolysis [a]	[-]	0	0/16
Litmus milk	v	62 k	10/16
Pigment:			
Pyoverdin	[+]	93	15/16
Pyocyanin		0	0/16
Pyorubrin	−	0	0/16
Growth at:			
25°C	+	100	16/16
35°C	+	100	16/16
42°C	[−]	0	0/16
Esculin hydrolysis	-	0	0/16
Lysine decarboxylase	[-]	0	0/16
Arginine dihydrolase	[+]	100	16/16
Ornithine decarboxylase	−	0	0/16
Nutrient broth, 0% NaCl	+	100	16/16
Nutrient broth, 6% NaCl	v	44	3,4w/16
Acetamide	[-]	0	0/13

[a] 7-14 days' incubation at 25°C.

Pseudomonas putrefaciens

(Bergey's Manual, 8th ed.: *species incertae sedis)*

Gram-negative short to long rod or filament; motile (1-2 polar flagella); aerobic; grows on MacConkey agar; produces oxidase and ornithine decarboxylase; produces hydrogen sulfide in TSI agar; reduces indicator in litmus milk overnight; also reduces nitrate to nitrite without gas.

Two biotypes are recognized: biotype 1 oxidizes glucose, sucrose, and maltose, and grows heavily in nutrient broth without sodium chloride after overnight incubation; biotype 2 does not oxidize glucose and grows poorly or not at all in nutrient broth without sodium chloride (prefers or requires NaCl), and may not grow in OF basal medium (King) (there is no NaCl in this medium), although it grows well in Difco OF basal medium, in which it produces an alkaline reaction.

Sources Biotype 1 (24 strains)

Stool (17%), throat (12%), hand (8%), water (8%), other (38%), unknown (17%).

Sources Biotype 2 (26 strains)

Wound (19%), blood, ear, stool, sea water, sputum, water (8% each), other (19%), unknown (14%).

Reference strains

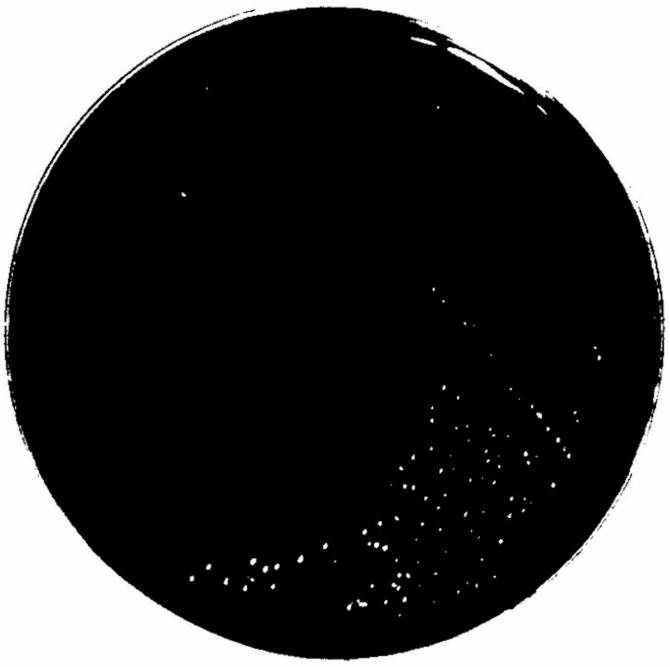
KC988 ← ATCC, 8073; D3845, from foot.

Literature

Appl Microbiol 19:176, 1970; J Conf Public Health Lab Dir 28:168-187, 1970; Appl Microbiol 24:798-800, 1972.

Comment

Formerly called Ib.



KC988 BAP 35°C 24 h ×1.



KC988 BAP 35°C 24 h ×6.



KC988 Gram stain BAP 35°C 24 h ×1700.



D3845 Flagella stain FB 25°C 24 h ×1700.

Pseudomonas putrefaciens (biotype 1)

NUMBER OF STRAINS 24			
TEST PERFORMED	**SIGN**	**% +**	**# +/T**
Morphology	mrs		
Motility, flagella	[m][p,1-2		
Action on blood	v	75 I,G	18 24;2 ly,2 al,1 br, 1 β
Fermentative or oxidative	O or n-o		
Carbohydrate base	OF		
Acid from:			
Glucose	[v]	17(33)	4(8) 24
Xylose	[ ]	0	0 24
Mannitol		0	0 24
Lactose		0	0 24
Sucrose	[-]	96(4)	23(1) 24
Maltose	+	92(8)	22(2) 24
Catalase	+	100	24 24
Oxidase	[+]	100	24 24
Growth on:			
MacConkey	[+]	100	24 24
SS		0(8)	(2) 24
Cetrimide		4	1 24
Simmons Citrate		4(4)	1(1) 24
Urea, Christensen's		4(8)	1(2) 24
Nitrate reduction	[+]	100	24 24
Gas from nitrate	[-]	0	0 24
Indole	-	0	0 24
TSI slant, acid		0	0 24
TSI butt, acid	-	0	0 24
H_2S (TSI butt)	[+]	96	23 24
H_2S (Pb ac paper)	+	100	24 24
Gelatin hydrolysis [a]	v	65	13 20
Litmus milk	v	61 pep	14 23;9 IR
Pigment:			
Soluble	v	71 br-ta	17 24
Growth at:			
25 C	+	100	24 24
35 C	+	96	23 24
42 C	v	38	9 24
Esculin hydrolysis		0	0 24
Lysine decarboxylase		0	0 24
Arginine dihydrolase	-	0	0 24
Ornithine decarboxylase	[+]	100	24 24
Nutrient broth, 0% NaCl	[+]	100	23 23
Nutrient broth, 6% NaCl	v	43	10 23

[a] 7-14 days' incubation.

Pseudomonas putrefaciens (biotype 2)

	NUMBER OF STRAINS 26		
TEST PERFORMED	**SIGN**	% +	# + /T
Morphology	mrs		
Motility; flagella	[m];p,1-2		
Action on blood	LG	92	24/26
Fermentative or oxidative	[n-o]		
Carbohydrate base	OF		
Acid from:			
Glucose	[ ]	0	0/26
Xylose	[ ]	0	0/26
Mannitol		0	0/26
Lactose		0	0/26
Sucrose		0	0/26
Maltose		0	0/26
Catalase	+	100	26/26
Oxidase	[+]	100	26/26
Growth on:			
MacConkey	[+]	100	26/26
SS	+	92(4)	24(1)/26
Cetrimide	–	0	0/26
Simmons Citrate		8	2/26
Urea, Christensen's	v	42	11/26
Nitrate reduction	[+]	100	26/26
Gas from nitrate	[–]	0	0/26
Indole	–	0	0/26
TSI slant, acid	–	0	0/26
TSI butt, acid	–	0	0/26
H_2S (TSI butt)	[+]	100	26/26
H_2S (Pb ac paper)	+	100	26/26
Gelatin hydrolysis [a]	+	100	23/23
Litmus milk	v	50 IR & pep	13/26,4 IR,3 pep
Pigment:			
Soluble	br-yel	100	26/26
Growth at:			
25 C	+	100	26/26
35°C	+	100	26/26
42°C	v	23	6/26
Esculin hydrolysis	–	0	0/25
Lysine decarboxylase	–	0	0/3
Arginine dihydrolase	–	0	0/3
Ornithine decarboxylase	[+]	100	3/3
Nutrient broth, 0% NaCl	[+]	0	0/26
Nutrient broth, 6% NaCl	[+]	100	26/26

[a] 7-14 days' incubation.

***Pseudomonas* sp.** CDC Group 1

Gram-negative straight to slightly curved rod, motile (1-2 polar flagella); aerobic; usually grows on MacConkey agar; does not utilize carbohydrates; oxidase-positive; urease-negative, reduces both nitrate and nitrite to gas; produces no H_2S in TSI agar.

Sources (31 strains)

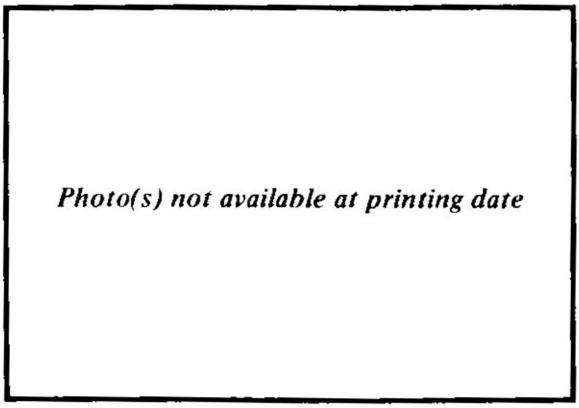
Urine (26%), cerebrospinal fluid (13%), blood (10%), throat (10%), environment (6%), 1 strain each from sputum, lung, ear, toe-nail exudate, knee fluid, cul-de-sac pericardium, leg lesion, and horse uterus (29%), unknown (6%).

Literature

None.

Comment

This is a group of pseudomonads that was referred to as "*Pseudomonas denitrificans*" in our laboratory and was included in the 1972 preliminary revision of The Identification of Unusual Pathogenic Gram Negative Bacteria (E .O. King); however, at that time we did not have a reference strain. In 1978 we received *P. denitrificans* 13867 from the American Type Culture Collection. After studying strain 13867 we learned that the group we had called "*P. denitrificans*" was unlike the ATCC strain. *P. denitrificans*, as exemplified by ATCC 13867, was a glucose oxidizer that grew on MacConkey agar, and was oxidase-positive. It did not produce soluble pigments; indole was not produced; H_2S in TSI was negative; lysine decarboxylase was negative and arginine dihydrolase was positive; nitrate was reduced with gas formation; urease was negative; an acid reaction was produced in OF glucose, lactose, maltose, and xylose (the xylose reaction was delayed); and an alkaline reaction was produced in mannitol and sucrose. Our laboratory has received a number of similar cultures (17) from various sources (blood, peritoneal fluid, urine, water, sputum, pleural fluid, and intravenous amphotericin solution). In Bergey's Manual, 8th ed., *Pseudomonas denitrificans* is included in *species incertae sedis*. Recently the species name *Pseudomonas denitrificans* (Christensen) Bergey et al. 1923 was rejected by the Judicial Commission of the International Committee on Systematic Bacteriology (Int J Syst Bacteriol 32; 466, 1982).



Pseudomonas sp. CDC Group 1 [a]

NUMBER OF STRAINS 31			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	mrc		
Motility: flagella	[m,p,1-2]		
Action on blood	v	33 ly or gr	10/30;2 al,1 LG
Fermentative or oxidative	[n-o]		
Carbohydrate base	OF		
Acid from:			
Glucose	[ ]	0	0/31
Xylose	[ ]	0	0/31
Mannitol	–	0	0/31
Lactose	–	0	0/31
Sucrose	–	0	0/31
Maltose	[–]	0	0/31
Catalase	v	81	19,6w/31
Oxidase	[+]	100	30,1w/31
Growth on:			
MacConkey	[+]	97(3)	30(1)/31
SS	v	30(6)	9(2)/31
Cetrimide	v	13(6)	4(2)/31
Simmons Citrate	v	42(6)	13(2)/31
Urea, Christensen's	[–]	3(7)	1(2)/30
Nitrate reduction	+	100	31/31
Gas from nitrate	[+]	100	31/31
Indole	–	0	0/31
TSI slant, acid	–	0	0/31
TSI butt, acid	–	0	0/31
H_2S (TSI butt)	[ ]	0	0/31
H_2S (Pb ac paper)	v	68	10,11w/31
Gelatin hydrolysis [b]		4	1/26
Litmus milk	v	39 k	12/31
Pigment:			
Soluble	v	60 yel-br	12/20
Growth at:			
25°C	+	97	30/31
35°C	+	97	30/31
42°C	v	48	15/31
Esculin hydrolysis	–	0	0/28
Lysine decarboxylase	–	0	0/3
Arginine dihydrolase	v	33	1/3
Ornithine decarboxylase	–	0	0/3
Nutrient broth, 0% NaCl	+	93	26/28
Nutrient broth, 6% NaCl	v	14	4/28

[a] Referred to as *P. denitrificans* in 1972 "Round Table".

[b] 7-14 days' incubation.

Pseudomonas stutzeri

Gram-negative medium to long rod; motile (single polar flagellum); aerobic; oxidizes glucose, xylose, maltose, and (usually) mannitol, not lactose; hydrolyzes starch; grows on MacConkey agar; oxidase-positive; reduces nitrate to gas; indole-negative; does not grow on cetrimide agar; no H_2S on TSI agar; nonproteolytic; arginine dihydrolase- and lysine decarboxylase-negative.

Sources (28 strains)

Wound (14%), urogenital (14%), blood (7%), fecal material (7%), nasopharynx (7%), cerebrospinal fluid, heel fluid, tooth socket, bronchial wash, (1 each) (14%), other (nonhuman) (12%), unknown (25%).

Reference strain

KC1077 ← ATCC, 17587 (Stanier's 220).

Literature

Am J Clin Pathol 43:357-360, 1965; J Gen Microbiol 43:159-271, 1966, and 60:215-231, 1970.

Comments

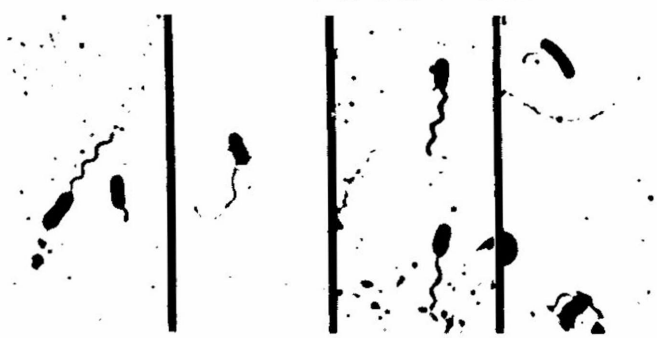
Formerly called Vb-1. A nonfluorescent pseudomonad; produces smooth and rough, slightly yellow, wrinkled colonies.



BAP 35°C 24 h ×6.



Gram stain BAP 35°C 24 h ×1700.



Flagella stain BAP 35°C 24 h ×1700.

Pseudomonas stutzeri

NUMBER OF STRAINS 28			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	mrs		
Motility; flagella	[m;p,1-2]		
Action on blood	v	26 ly	7,27;6 gr,3 LG,3 br
Fermentative or oxidative	[0]		
Carbohydrate base	OF		
Acid from:			
Glucose	[+]	96(4)	25,2w(1) 28
Xylose	+	93(7)	25,1w(2) 28
Mannitol	+ or(+)	89(4)	10,15w(1) 28
Lactose	[-]	0	0 28
Sucrose		0	0 28
Maltose	[+]	100	13,15w 28
Starch	+	100	5 5
Catalase	+	100	25,3w 28
Oxidase	[+]	100	28 28
Growth on:			
MacConkey	[+]	100	28 28
SS	v	54	13,2w 28
Cetrimide		4	1w 28
Simmons Citrate	+ or(+)	82(14)	23(4) 28
Urea, Christensen's	v	33(22)	9(6) 27
Nitrate reduction	+	100	28 28
Gas from nitrate	[+]	100	28 28
Indole	[-]	0	0 28
TSI slant, acid	-	0	0/28
TSI butt, acid	-	0	0/28
H_2S (TSI, butt)	[-]	0	0/28
H_2S (Pb ac paper)	v	36	4,6w/28
Gelatin hydrolysis [a]	[-]	0	0/22
Litmus milk	v	57 k	16/28;4 IR,1 pep
Pigment	v	86 sl yel ins	24 28
Growth at:			
25°C	+	100	26/26
35 C	+	100	26/26
42 C	v	69	16,2w/26
Esculin hydrolysis	-	0	0 27
Lysine decarboxylase	[-]	0	0 28
Arginine dihydrolase	[-]	0	0/28
Ornithine decarboxylase	-	0	0 28
Nutrient broth, 0% NaCl	+	96	24 25
Nutrient broth, 6% NaCl	+ or(+)	80(16)	19,1w(4) 25
Acetamide	-	0	0 3
2-Ketogluconate	-	0	0 5

[a] 7-14 days' incubation.

Pseudomonas testosteroni

Gram-negative medium to long rod; motile (polar tuft of three or more flagella); aerobic; grows on MacConkey agar, most strains do not grow on SS agar; does not use carbohydrates; oxidase-positive, usually reduces nitrate (without gas); most strains do not use acetamide; does not produce H_2S on TSI agar.

Sources (28 strains)

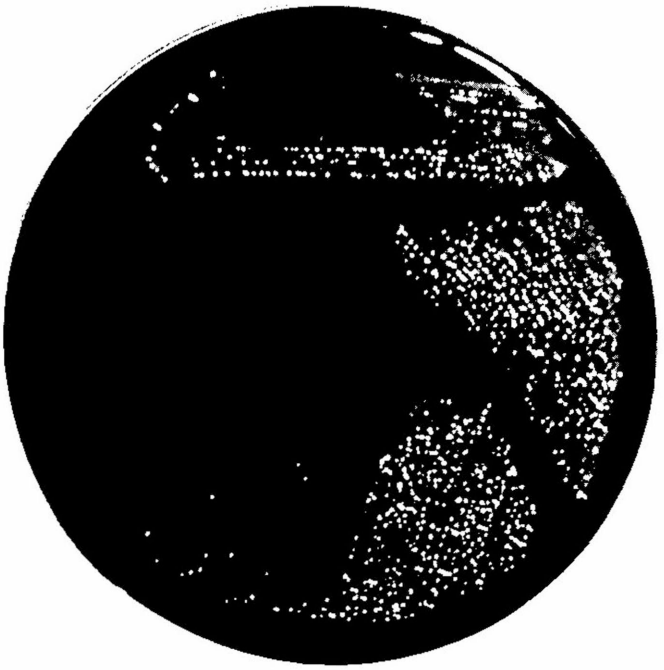
Sputum (18%), peritoneal fluid (18%), urine (11%), urinary catheter (7%), appendix (7%), stool (7%), other (14%), unknown (18%).

Reference strain

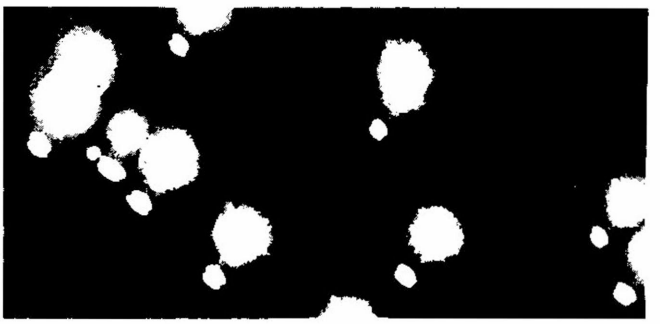
KC1071 < ATCC, 11996 (type strain).

Literature:

Int Bull Bacteriol Nomencl Taxon 15:125-132, 1965; J Gen Microbiol 43:237-242, 1966; Manual of Clinical Microbiology, 2nd ed., p 262.



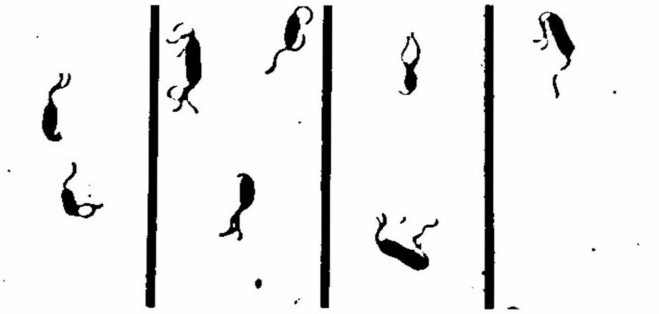
BAP 35°C 24 h ×1.



BAP 35°C 24 h ×6.



Gram stain BAP 35°C 24 h ×1700.



Flagella stain FB 25°C 24 h ×1700.

Comments:

Indole is not produced, and only an occasional strain (3 of 28 tested) gives a yellow reaction when tested for indole (see "Comments" under *P. acidovorans*); it is reported that this organism uses testosterone (hence the name), but that other pseudomonads and some fungi may also do so.

Pseudomonas testosteroni

	NUMBER OF STRAINS 28		
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	pr r		
Motility, flagella	[m;p,>2]		
Action on blood	v	32 ly	9 28;4 LG,2 gr,1 br
Fermentative or oxidative	[n-o]		
Carbohydrate base	OF		
Acid from:			
Glucose	[ ]	0	0 28
Xylose	[ ]	0	0 28
Mannitol	[ ]	0	0 28
Lactose	-	0	0 28
Sucrose		0	0 28
Maltose	–	0	0 28
Fructose	[–]	6 [a]	1w 16
Catalase	+	96	21,6w 28
Oxidase	[+]	100	26,2w 28
Growth on:			
MacConkey	[+]	96(4)	27(1)/28
SS	v	21(11)	6(3)/28
Cetrimide	-	0(4)	(1)/28
Simmons Citrate	v	47(7)	13(2)/28
Urea, Christensen's	v	7(14)	2(4) 28
Nitrate reduction	+	96	27 28
Gas from nitrate	[–]	0	0/28
Indole	–	0	0/28
TSI slant, acid	–	0	0/28
TSI butt, acid		0	0/28
H_2S (TSI butt)	[ ]	0	0/28
H_2S (Pb ac paper)	v	61	14,3w 28
Gelatin hydrolysis [b]	-	0	0 18
Litmus milk	v	86 k	24 28
Pigment:			
Soluble	v	27 yel-br	6 22
Growth at:			
25°C	+	100	25 25
35°C	+	100	25 25
42°C	v	68	17 25
Esculin hydrolysis	–	0	0 28
Lysine decarboxylase	–	0	0 17
Arginine dihydrolase	-	0	0 17
Ornithine decarboxylase	-	0	0 17
Nutrient broth, 0% NaCl	+	100	28 28
Nutrient broth, 6% NaCl	v	14	4 28

[a]Fructose reaction weak.

[b]7-14 days' incubation.

Pseudomonas thomasii

(not cited in Bergey's Manual, 8th ed.)

Gram-negative short to medium length rod; motile (single polar flagellum); aerobic; oxidizes glucose, xylose, mannitol, lactose, and maltose, but not sucrose; grows on MacConkey agar; oxidase-positive; does not usually reduce nitrate; arginine dihydrolase-negative and lysine and ornithine decarboxylases-negative.

Sources (31 strains)

Blood (26%), sputum (13%), abscess (10%), urine (6%), other (39%), unknown (6%).

Reference strains

C3408 ← I. Phillips, 73-9288; C1700, from blood.

Literature

Lancet 1:1258-1260, 1972, and 2:284-285, 1973; J Med Microbiol 9:493-495, 1976.

Comments

This group of cultures is similar to a culture received from Ian Phillips, London, England, designated "*Pseudomonas thomasii*". Numerical taxonomy studies indicate *P. thomasii* may be a biovar of *P. pickettii* (J Gen Microbiol 114:137-147, 1979).



C3408 BAP 35°C 24 h ×1.



C1700 Gram stain BAP 35°C 24 h ×1700.



C1700 Flagella stain BAP 35°C 24 h ×1700.

Pseudomonas thomasii

NUMBER OF STRAINS 31			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	rnfs		
Motility, flagella	[m,p,1-2]		
Action on blood	v	24 gr	7,29;6 ly,2 LG,1 inc β
Fermentative or oxidative	[0]		
Carbohydrate base	OF		
Acid from:			
Glucose	[+]	100	31/31
Xylose	+	100	31/31
Mannitol	[+]	100	3,28w/31
Lactose	[+]	100	31/31
Sucrose	[ ]	0	0/31
Maltose	[+]	100	31/31
Catalase	v	87	5,22w/31
Oxidase	[+]	100	21,10w/31
Growth on:			
MacConkey	[+]	100	31/31
SS		0	0/31
Cetrimide		0(3)	0[w]/31
Simmons Citrate	+	100	31/31
Urea, Christensen's	+ or (+)	81(39)	25(6)/31
Nitrate reduction	v	13	4/31
Gas from nitrate	[-]	0	0/31
Indole	-	0	0/31
TSI slant, acid		0	0/31
TSI butt, acid		0	0/31
H_2S (TSI butt)	-	0	0/31
H_2S (Pb ac paper)	v	23	1,8w/31
Gelatin hydrolysis [a]	v	30	8/27
Litmus milk	v	52 k	15/29;5 IR,2 pep
Pigment:			
Soluble	v	33 vel-ta	10/31
Growth at:			
25 C	+	97	17,13w/31
35 C	+	100	31/31
42 C	v	84	21,5w/31
Esculin hydrolysis	-	0	0/31
Lysine decarboxylase	[-]	0	0/31
Arginine dihydrolase	[-]	3	1/31
Ornithine decarboxylase	-	0	0/31
Nutrient broth, 0% NaCl	+	100	31/31
Nutrient broth, 6% NaCl	-	0	0/31

[a] 7-14 days' incubation.

Pseudomonas vesicularis

Gram-negative medium to long, slender rod, motile (single polar flagellum with distinctively short wavelength); aerobic; usually grows on MacConkey agar; oxidizes glucose, maltose, and sometimes xylose; produces oxidase; does not usually reduce nitrate to nitrite.

Sources (47 strains)

Cerebrospinal fluid (15%), blood (15%), urine (11%), vagina (8%), eye (4%), wound (4%), iceballs (4%), other (37%), unknown (2%).

Reference strain

D3834, from urine.

Literature

Int Bull Bacteriol Nomencl Taxon 14:165-168, 1964; J Gen Microbiol 53:349-361, 1968; J Clin Microbiol 1:339, 1975.

Comments

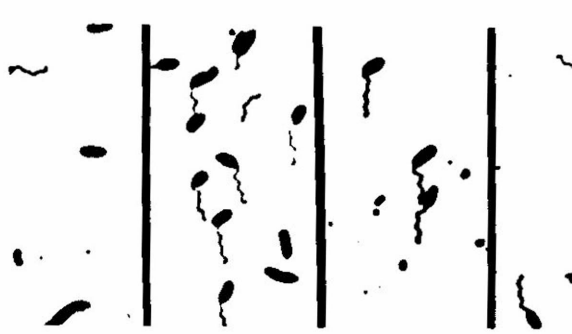
Formerly called "*Pseudomonas vesiculare*". Sixty-six percent of strains received at CDC produced a tan-brown, water-soluble pigment on heart infusion agar with tyrosine, and 15% of the strains produced a yellow growth pigment.



BAP 35°C 24 h ×1.



Gram stain BAP 35°C 24 h ×1700.



Flagella stain FB 25°C 24 h ×1700.

Pseudomonas vesicularis

NUMBER OF STRAINS 47			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	rnrs		
Motility: flagella	[m,p,1-2] [a]		
Action on blood	v	46 LG	12,26,6 ly,3 gr,3 lav
Fermentative or oxidative	O		
Carbohydrate base	OF		
Acid from:			
Glucose	[+ or (+)]	87(9)	27,13w(4) 46
Xylose	[v]	21(2)	10(1) 47
Mannitol		0	0/47
Lactose		0	0/47
Sucrose		0	0/47
Maltose	[+]	98(2)	46(1) 47
Catalase	v	74(11)	35(5) 47
Oxidase	[+]	94	44/47
Growth on:			
MacConkey	[v]	81(8)	38(4) 47
SS		6(2)	3(1) 47
Cetrimide		0	0/47
Simmons Citrate		2(2)	1(1) 47
Urea, Christensen's		0(8)	(4) 47
Nitrate reduction	[-]	6	3/47
Gas from nitrate	[-]	0	0/47
Indole		0	0/47
TSI slant, acid		0	0/47
TSI butt, acid		0	0/47
H_2S (TSI butt)		0	0/47
H_2S (Pb ac paper)	v	68	32/47
Gelatin hydrolysis [b]	v	40	17/42
Litmus milk	v	33 IR	7 21;7 k,4 pep
Pigment:			
Insoluble	v	15 yel	7/47;3 or
Soluble	v	66 br-ta	31/47;9 yel,4 amb,4 or
Growth at:			
25 C	+	96	45/47
35 C	+	98	46/47
42 C	v	38	18/47
Esculin hydrolysis	v	79(5)	33(2) 42
Lysine decarboxylase		0	0/11
Arginine dihydrolase		0	0/11
Ornithine decarboxylase	–	0	0/11
Nutrient broth, 0% NaCl	+	98	39/40
Nutrient broth, 6% NaCl	v	17	7/40

[a]Flagella have a short wavelength with low amplitude.

[b]7-14 days' incubation.

Streptobacillus moniliformis

Gram-negative pleomorphic rod, frequently in chains and tangled filaments with bulbous or "*Monilia*"-like swellings; nonmotile; usually requires 20% serum or ascitic fluid or 10% whole blood; facultatively anaerobic; ferments glucose and maltose weakly, but not mannitol or lactose; no growth on MacConkey agar; catalase-and oxidase-negative; does not reduce nitrate.

Sources (15 strains)

Blood (13%), rat bite (1%), peacock lung (1%).

Reference strains

KC199 ← R. Wickelhausen, strain Vancouver; D7626, from blood.

Literature

C R Acad Sci, Paris 180:1188-1190, 1925; *Streptobacillus moniliformis* Infections, in Harris, A., Coleman, M. (eds): Diagnostic Procedures and Reagents, 4th ed. New York, American Public Health Assn., 1963, pp 642-651; Vet Rec 79:95-100,1966.

Comment

Grows in broth producing discrete "fluff-ball-like" colonies. A causative agent of rat-bite fever in humans.



D7626 Gram stain Thio 35°C 24 h ×1700.

Streptobacillus moniliformis

NUMBER OF STRAINS 16			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	[plr,ch]		
Motility	nm		
Action on blood		0	0/5
Fermentative or oxidative			
Carbohydrate base	F [a]		
Acid from:			
Glucose	[+ or (+)]	67(27)	4,6w(4) 15
Xylose	–	0(7)	(1) 15
Mannitol	[–]	0	0 15
Lactose	[–]	0	0 15
Sucrose	–	0	0 15
Maltose	[+ or (+)]	67(33)	4,6w(5) 15
Catalase	[–]	0	0 9
Oxidase	[–]	0	0 7
Growth on:			
MacConkey	[–]	0	0 11
SS	–	0	0 10
Simmons Citrate	–	0	0 10
Urea, Christensen's		0	0/7
Nitrate reduction	[–]	0	0/13
Indole		0	0/12
TSI slant, acid	v	71	5w/7
TSI butt, acid	v	57	4w/7
H_2S (TSI butt)	–	0	0/7
H_2S (Pb ac paper)	v	71	5/7
Gelatin hydrolysis [b]		0	0/3
Growth at:			
25°C	+	100	2,3w/5
35°C	+	100	5,5
42°C	v	40	2w/5
Nutrient broth, 0% NaCl	–	0	0 8
Nutrient broth, 6% NaCl	–	0	0/8

[a] Usually requires 20% serum or ascitic fluid.

[b] 7-14 days' incubation.

Tatumella ptyseos

Gram-negative short to medium straight rod with occasional filaments, motile at 25°C (may be delayed) (polar and lateral flagella); ferments glucose (no gas), xylose (variable), and sucrose, not maltose; grows on MacConkey but not SS agars; oxidase-, urease-, and indole-negative, V-P usually positive; gelatin not liquefied; lysine- and ornithine decarboxylase-negative.

Sources (41 strains)
Sputum (63%), throat (15%), blood (10%), other (12%).

Reference strain
D7425, from sputum.

Literature
J Clin Micro 14:79-88, 1981

Comment
Formerly called EF-9.



Gram stain HIA 35°C 24 h ×1700.

Tatumella ptyseos

NUMBER OF STRAINS 41			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	cc,srs		
Motility; flagella	[m];p,L [a]		
Gas from glucose	[−]	0	0/41
Action on blood	v	18 al	7/39,6 ly,1 br
Fermentative or oxidative	[F]		
Carbohydrate base	F		
Acid from:			
Glucose	[+]	100	41/41
Xylose	v	24(51)	8,1w(21)/41
Mannitol	-	0	0/41
Lactose	-	0	0/41
Sucrose	[+]	100	40,1w/41
Maltose	[−]	0	0/41
10% Lactose	v	0(44)	(4)/9
Catalase	+	100	41/41
Oxidase	[-]	7	2,1w/41
Growth on:			
MacConkey	[+]	93	35,3w/41
SS	[−]	2	1w/39
Simmons Citrate	v	10(7)	4(3)/41
Urea, Christensen's	[−]	0	0/41
Nitrate reduction	+	90	37/41
Indole	[−]	0	0/41
TSI slant, acid	v	39	16/41
TSI butt, acid	+ or(+)	88(2)	35,1w(1)/41
H_2S (TSI butt)	−	0	0/41
H_2S (Pb ac paper)	+	93	34,4w/41
MR	v	28	1,9w/37
V-P	[v]	81	29,1w/37
Gelatin hydrolysis [b]	[-]	0	0/17
Litmus milk	v	11(6) A	4w(2w)/35
Growth in nutrient broth:			
0% NaCl	+	98	26,14w/41
6% NaCl	v	32	5,8w/41
Growth at:			
25°C	+	95	39/41
35°C	+	98	27,13w/41
42°C	−	7	3w/41
Esculin hydrolysis	v	88	35/40
Lysine decarboxylase	[−]	0	0/40
Arginine dihydrolase	v	12	3,2w/40
Ornithine decarboxylase	[−]	0	0/40

[a]Motile at 25°C, nonmotile at 35°C.

[b]7-14 days' incubation.

Vibrio alginolyticus

Gram-negative, usually curved rod, motile (single polar flagellum); requires NaCl for growth; ferments glucose, mannitol, sucrose, and maltose without gas, not lactose; grows on MacConkey and TCBS agars; oxidase- and indole-positive; lysine and sometimes ornithine decarboxylase-positive, but arginine dihydrolase-negative; V-P-positive; NaCl tolerance $\geq 10\%$.

Sources (57 strains)

Ear (26%), wound and abscess (25%), water (9%), sputum (7%), eye (5%), crabmeat (5%), other (23%).

Reference strains

KC1404 ← ATCC, 17749 (type strain); D2276, from ear wound.

Literature

Jpn J Microbiol 5:447-486, 1961; Jpn J Med Sci Biol 21:359-362, 1968.

Comment

Cited in Bergey's Manual, 8th ed. as *V. parahaemolyticus* biotype 2. Associated with local lesions, not implicated in gastroenteritis.



BAP 35°C 24 h ×1.



Gram stain BAP 35°C 24 h ×1700.



Flagella stain BAP 35°C 24 h ×1700.

Vibrio alginolyticus

NUMBER OF STRAINS 58			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	mrc		
Motility: flagella	(m)p.1-2		
Gas from glucose	[ ]	0	0/58
Action on blood	v	46 LG	26/57;12 β,9 gr,6 al,1 ly
Fermentative or oxidative	[F]		
Carbohydrate base	F		
Acid from:			
Glucose	+	100	58/58
Xylose		0	0/58
Mannitol	+	100	56,2w/58
Lactose	[ ]	0	0/58
Sucrose	[+]	100	58/58
Maltose	+	100	58/58
10% Lactose		0	0/27
Catalase	+	97	44,12w/58
Oxidase	[+]	100	58/58
Growth on:			
MacConkey	[+]	100	58/58
SS	v	77(4)	43(2)/56
Simmons Citrate	v	66	23/35
Urea, Christensen's	−	4	2/56
Nitrate reduction	+	100	58/58
Indole	+ [a]	90	50/55
TSI slant, acid	+	100	58/58
TSI butt, acid	+	100	58/58
H_2S (TSI butt)	−	0	0/58
H_2S (Pb ac paper)	v	66	19,19w/58
MR	v [a]	19	1,9w/52
V-P	[+] [a]	93	43,8w/55
Gelatin hydrolysis [b]	+	94	43/46
Litmus milk	v	72 pep	42/58;3 k,1 A,1 IR
Growth in nutrient broth:			
0% NaCl	[−]	0	0/58
6% NaCl	[+]	100	30/30
8% NaCl	+	100	24/24
10% NaCl	[+]	100	24/24
Growth at:			
25°C	+	94 [c]	51/54
35°C	v	87 [c]	48/55
42°C	v	27 [c]	15/55
Esculin hydrolysis	v	34	10,10w/58
Lysine decarboxylase	[+] [a]	100	50/50
Arginine dihydrolase	[−] [a]	0	0/50
Ornithine decarboxylase	v [a]	57	28/49
ONPG	−	0	0/6

[a]Add 0.15 ml of 20% NaCl per ml.

[b]7-14 days' incubation.

[c]Higher % may grow if HIA is used.

Vibrio anguillarum

Gram-negative, usually curved rod; motile (single polar flagellum); requires NaCl for growth; ferments glucose, mannitol, sucrose, and maltose; grows (delayed) on MacConkey agar; oxidase- and indole-positive; arginine dihydrolase-positive, lysine decarboxylase-negative; NaCl tolerance 5%.

Sources

Usually associated with diseases of fish.

Reference strain

KC1293 ← J. L. Mitchell, Brooks AFB, Texas ← ATCC, 19264.

Literature

Int J Syst Bacteriol 21:64-68, 1971; Int J Syst Bacteriol 16:124-125, 1966; J Gen Microbiol 24:247-252, 1961.



BAP 25°C 24 h ×1
(incident plus transmitted light).

Vibrio anguillarum

(Suggested "working type" strain, ATCC 19264)

TEST PERFORMED	NUMBER OF STRAINS 1 SIGN
Morphology	mrc
Motility: flagella	[m]:p,1-2
Gas from glucose	
Action on blood	β
Fermentative or oxidative	[F]
Carbohydrate base	F
Acid from:	
Glucose	+
Xylose	−
Mannitol	−
Lactose	
Sucrose	+
Maltose	+
10% Lactose	
Catalase	+
Oxidase	[+]
Growth on:	
MacConkey	[(+)]
SS	
Simmons Citrate	
Urea, Christensen's	
Nitrate reduction	+
Indole	+, [a]
TSI slant, acid	+
TSI butt, acid	+
H_2S (TSI butt)	−
H_2S (Pb ac paper)	+
MR	+, [a]
V-P	−, [a]
Gelatin hydrolysis [b]	
Litmus milk	+
Growth in nutrient broth:	
0% NaCl	[+]
6% NaCl	[ ] [c]
Growth at:	
25 C	+
35 C	+
42 C	
Esculin hydrolysis	
Lysine decarboxylase	[ ] [a]
Arginine dihydrolase	[+] [a]
Ornithine decarboxylase	[a]

[a] Add 0.15 ml of 20% NaCl per ml.

[b] 7-14 days' incubation.

[c] Grows in 0.5 - 5.0% NaCl.

Vibrio cholerae (O:1 Group)

Gram-negative curved or straight rod, motile (single polar flagellum); ferments glucose, mannitol, and other carbohydrates; grows on MacConkey and TCBS agars; oxidase- and indole-positive; lysine- and ornithine decarboxylase-positive, arginine dihydrolase-negative; "string test"-positive; does not require NaCl.

Sources (8 strains)

Storm drain (3), stool (1), abdominal abscess (1), septic tank (1), intestine (1), water (1).

Reference strain

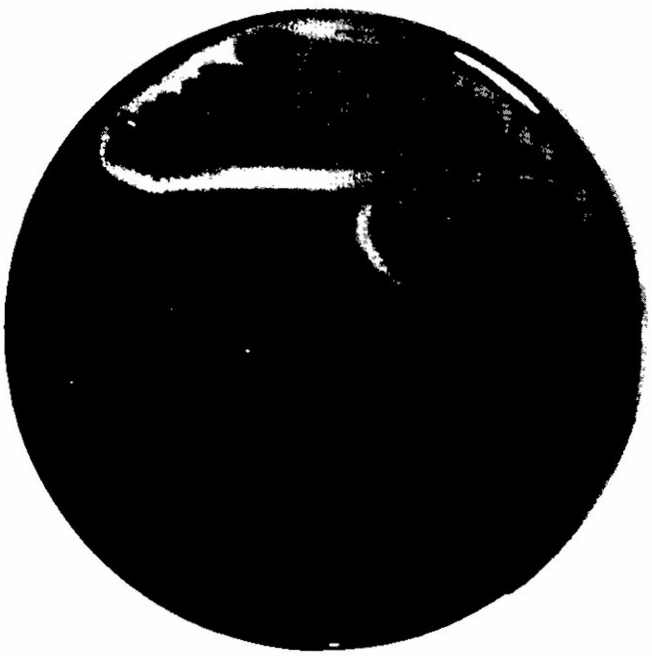
KC655 ← W. B. Cherry, G5977.

Literature

Indian J Med Res 54:839-847, 1966; Int Bull Bacteriol Nomencl Taxon 15:185-186, 1965; J Gen Microbiol 19:544, 1958.

Comments

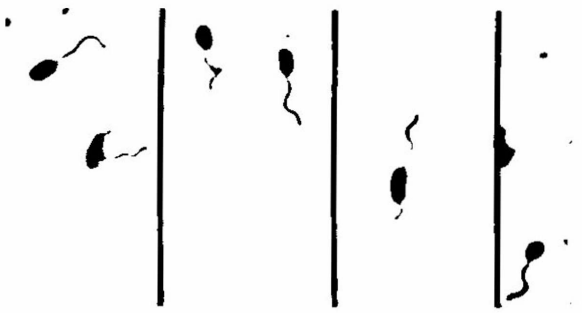
Causes Asiatic cholera; agglutinates in cholera O:1 antiserum.



BAP 35°C 24 h ×1 (incident plus transmitted light).



Gram stain BAP 35°C 24 h ×1700.



Flagella stain BAP 35°C 24 h ×1700.

Vibrio cholerae
(O:1 Group)

NUMBER OF STRAINS 9			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	rnic		
Motility: flagella	[m].p.1-2		
Gas from glucose		0	0/9
Action on blood	v	80 β&gr	7/9;1 I.G.1 gr
Fermentative or oxidative	[F]		
Carbohydrate base	F		
Acid from:			
Glucose	+	100	9/9
Xylose		0	0/9
Mannitol	[+]	100	9/9
Lactose	v	11(33)	1(3)/9
Sucrose	+	100	8,1w/9
Maltose	+	100	9/9
10% Lactose	v	12	1/9
Catalase	+	90	8/9
Oxidase	[+]	100	9/9
Growth on:			
MacConkey	[+]	100	9/9
SS	+	100	9/9
Simmons Citrate	+	100	9/9
Urea, Christensen's		0	0/9
Nitrate reduction	+	90	8/9
Indole	+	100	9/9
TSI slant, acid	+	90	8/9
TSI butt, acid	+	100	9/9
H_2S (TSI butt)	-	0	0/9
H_2S (Pb ac paper)	+	100	6,3w/9
MR	-	10	1/9
V-P	+	90	8/9
Gelatin hydrolysis [a]	+	100	8/8
Litmus milk	pep	90	8/9
Growth in nutrient broth:			
0% NaCl	[+]	100	8/8
6% NaCl	+	90	7/8
Growth at:			
25°C	+	100	8/8
35°C	+	100	8/8
42°C	+	90	7/8
Esculin hydrolysis	v	20	1,1w/9
Lysine decarboxylase	[+]	100	9/9
Arginine dihydrolase	[-]	0	0/9
Ornithine decarboxylase	[+]	90	8/9

[a] 7-14 days' incubation.

Vibrio cholerae (other than 0:1 Group)

Gram-negative curved rod, motile (single polar flagellum); ferments glucose, mannitol, and other carbohydrates; grows on MacConkey and TCBS agars; oxidase- and indole-positive; lysine- and ornithine decarboxylase-positive, arginine dihydrolase-negative; does not require NaCl; "string test"-positive.

Sources (73 strains)

Stool (38%), blood (15%), ear (5%), sewage (5%), other (32%), unknown (5%).

Reference strains

None photographed; colonial and cell morphologies same as those of *Vibrio cholerae* (0:1 Group).

Literature

Bull WHO 32:627-732, 1965; Amer J Trop Med Hyg 14:412-418, 1965; Jpn J Med Sci 20:265-280, 1967; Ann Int Med 88:602-606, 1978.

Comments

Also known as *Vibrio* "noncholera" or nonagglutinable (NAG) vibrio; biochemically indistinguishable from *V. cholerae*, except that a small percent of strains examined did not ferment sucrose (*Vibrio mimicus*, J Clin Microbiol *14*:631-639 (1981)); does not agglutinate in *Vibrio* Group 0:1 antisera; serologically heterogeneous. Included within *V. cholerae* in Bergey's Manual (8th ed.).

Photo(s) not available at printing date

Vibrio cholerae
(Other than 0:1 Group)[a]

NUMBER OF STRAINS 73			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	mrc		
Motility; flagella	[m];p,1-2		
Gas from glucose		0	0/73
Action on blood	-	96 β	64(20)β,29β & gr,15β & LG) 67,3gr
Fermentative or oxidative	[F]		
Carbohydrate base	F		
Acid from:			
Glucose	+	100	73/73
Xylose		0	0/71
Mannitol	[+]	96(3)	70(2)/73
Lactose	v	11(27)	2,6w(19)/71
Sucrose	v	89	64/72
Maltose	+	97(3)	71(2)/73
10% Lactose	v	9(24)	4(18)/46
Catalase	+	98	52,2w/55
Oxidase	[+]	100	71/71
Growth on:			
MacConkey	[+]	100	71/71
SS	v	84	57/68
Simmons Citrate	+	96(1)	67(1)/70
Urea, Christensen's		4(3)	1,2w(2)/69
Nitrate reduction	+	100	72/72
Indole	+	100	68,2w/70
TSI slant, acid	v	36	25/70
TSI butt, acid	+	99	69/70
H_2S (TSI butt)		0	0/64
H_2S (Pb ac paper)	v	64	21,20w/64
MR	v	30	3,18w/70
V-P	v	84	49,5w/64
Gelatin hydrolysis [b]	+	100	38/38
Litmus milk	v	75 pep	52/69;8 A,7 IR
Growth in nutrient broth:			
0% NaCl	[+]	100	70/70
6% NaCl	v	84	54/64
Growth at:			
25°C	+	100	68/68
35°C	+	100	68/68
42°C	v	88	60/68
Esculin hydrolysis		0	0/61
Lysine decarboxylase	[+]	100	70/70
Arginine dihydrolase	[-]	0	0/70
Ornithine decarboxylase	[+]	100	70/70

[a]Commonly called "non-cholera" vibrios.
[b]7-14 days' incubation.

Vibrio damsela

Gram-negative curved rod; motile (single polar or subpolar flagellum); requires NaCl for growth; ferments glucose and maltose; grows on MacConkey agar; oxidase-, urease-, and arginine dihydrolase-positive, lysine decarboxylase-variable, ornithine decarboxylase-negative; indole-negative; NaCl tolerance < 8%.

Sources (23 strains)

Fish or other marine animals (52%), wound (12%), water tank (8%), seafood (8%), other (leg ulcer, urine, shorebird) (16%), unknown (4%).

Reference strain

A3864, from a porpoise heart.

Literature

Science 214:1139-1140, 1981.

Comment

Marine-associated.

Photo(s) not available at printing date

Vibrio damsela

TEST PERFORMED	SIGN	% +	# +/T
	NUMBER OF STRAINS 23		
Morphology	mrs,mrc		
Motility, flagella	[m],p,1-2		
Gas from glucose	v	9(4)	2(1) 23
Action on blood	v	87 β	20/23;2 al,1 ly
Fermentative or oxidative	[F]		
Carbohydrate base	F		
Acid from:			
Glucose	+	100	23 23
Xylose	-	0	0 23
Mannitol	[-]	0	0 23
Lactose	[-]	0	0 23
Sucrose	-	0(9)	(2) 23
Maltose	[+]	100	23 23
10% Lactose	-	0	0 22
Catalase	v	71	11,4w 21
Oxidase	[+]	91	12,9w/23
Growth on:			
MacConkey	[+]	100	15,8w/23
SS	v	39(4)	4,5w(1)/23
Simmons Citrate	-	0	0/23
Urea, Christensen's	[+]	100	23 23
Nitrate reduction	+	100	23/23
Indole	[-] [a]	0	0/23
TSI slant, acid	v	26(4)	6(1) 23
TSI butt, acid	+	91	21 23
H_2S (TSI butt)	-	0	0/23
H_2S (Pb ac paper)	v	83	12,7w/23
MR	v [a]	50	5/10
V-P	v [a]	85	11 13
Gelatin hydrolysis [b]	-	0	0 8
Litmus milk	v	22 A	5w/23
Growth in nutrient broth:			
0% NaCl	[-]	0	0 23
6% NaCl	[+]	100	23 23
8% NaCl	-	10	2/19
Growth at:			
25 C	+	95 [c]	20 21
35°C	+	95 [c]	21 22
42 C	v	23 [c]	1,4w 23
Esculin hydrolysis	v	14	3 22
Lysine decarboxylase	[v] [a]	27(4)	6(1) 22
Arginine dihydrolase	[+] [a]	100	21 21
Ornithine decarboxylase	[-] [a]	0	0 21

[a]Add 0.15 ml of 20% NaCl per ml.

[b]7-14 days' incubation.

[c]A higher percent may grow if HIA is used.

Vibrio fluvialis

Gram-negative rod; motile (single polar flagellum); requires NaCl for growth (tolerance ≤ 8%); ferments glucose (possibly with slight gas), mannitol, sucrose, and maltose. Grows on MacConkey agar; oxidase-positive, indole-variable; arginine dihydrolase-positive.

Sources (25 strains)

Rectal swab (passengers from the Orient upon arrival in the U.S.) (52%), sea water (Hawaiian sources, mainly, but one from Alabama) (16%), oyster (12%), stool (12%), bronchoscopy (4%), bovine spleen (Hawaiian source) (4%).

Reference strain

D2326, from stool.

Literature

Int J Syst Bacteriol 28:99-111, 1978; J Appl Bacteriol 50:73-94, 1981.

Comments

Formerly called EF-6. This organism is associated with marine environments and food poisoning outbreaks; pathogenicity is not fully established. The organism is referred to as "Group F" in the first reference cited above.

Photo(s) not available at printing date

Vibrio fluvialis

NUMBER OF STRAINS 25			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	mrs,mrc		
Motility; flagella	[m],p,1-2		
Gas from glucose	v	48	12/25
Action on blood	v	44 β	7/16;6 LG,2 β gr,1 gr
Fermentative or oxidative	[F]		
Carbohydrate base	F		
Acid from:			
Glucose	+	100	25/25
Xylose		0	0/25
Mannitol	[+]	100	25/25
Lactose	−	0(8)	(2)/25
Sucrose	[+]	100	25/25
Maltose	+	100	25/25
10% Lactose	v	0(14)	(2)/14
Catalase	v	64	5,11w/25
Oxidase	[+]	100	25/25
Growth on:			
MacConkey	[+]	92(8)	23(2)/25
SS	v	64	4,12w/25
Simmons Citrate	v	84	21/25
Urea, Christensen's	−	0	0/25
Nitrate reduction	+	92	23/25
Indole	v [a]	24	3,3w/25
TSI slant, acid	+	100	25/25
TSI butt, acid	+	100	25/25
H_2S (TSI butt)	−	0	0/25
H_2S (Pb ac paper)	v	84	13,8w/25
MR	v [a]	44	3,8w/25
V-P	v [a]	16	4/25
Gelatin hydrolysis [b]	+	100	8/8
Litmus milk	pep	92	23/25;1 A
Growth in nutrient broth:			
0% NaCl	[−]	0	0/25
6% NaCl	[+]	100	25/25
8% NaCl	v	65	15/25
10% NaCl	−	0	0/25
Growth at:			
25°C	+	100	25/25
35°C	+	100	25/25
42°C	v	40 [c]	10/25
Esculin hydrolysis	v	16	3,1w/25
Lysine decarboxylase	[−] [a]	0	0/25
Arginine dihydrolase	[+] [a]	96	24/25
Ornithine decarboxylase	− [a]	0	0/25

[a] Add 0.15 ml of 20% NaCl.

[b] 7-14 days' incubation.

[c] Higher % may grow if HIA is used.

Vibrio hollisae

Gram-negative rod, with some cells slightly curved, motile with one to two polar flagella; requires NaCl for growth; ferments glucose; growth on MacConkey agar-variable, did not grow on TCBS; oxidase- and indole-positive; urease-negative; lysine- and ornithine decarboxylase-negative; arginine dihydrolase usually negative; V-P-negative; NaCl tolerance $\leq 7\%$.

Sources (11 strains)

Stool (9), blood (1), wound (1).

Literature

J Clin Microbiol 15: 395-401, 1982.

Comment

The majority of stool isolates were found in pure culture in patients with diarrhea.

Photo(s) not available at printing date

Vibrio hollisae

NUMBER OF STRAINS 12			
TEST PERFORMED	**SIGN**	**% +**	**# +/T**
Morphology	mrc		
Motility; flagella	m;p,1-2		
Gas from glucose		0	0/12
Action on blood	v	50 β	6/12;2/lv,1/lv/LG
Fermentative or oxidative	[F]		
Carbohydrate base	F		
Acid from:			
Glucose	+	100	12/12
Xylose	−	0	0/12
Mannitol	−	0	0/12
Lactose		0	0/12
Sucrose	−	0	0/12
Maltose	[−]	0	0/12
10% Lactose	−	0	0/4
Catalase	+	100	8/8w/11
Oxidase	+	100	12/12
Growth on:			
MacConkey	v	64(18)	6/8w(2)/11
SS	−	0	0/11
Simmons Citrate	−	9	1/11
Urea, Christensen's	[−]	0	0/11
Nitrate reduction	+	100	12/12
Indole	[+] [a]	100	11/lw/12
TSI slant, acid	v	18	2/11
TSI butt, acid	+	100	11/11
H_2S (TSI butt)	−	0	0/11
H_2S (Pb ac paper)	+	91	6/8w/11
MR	.. [a]	0	0/11
V-P	− [a]	0	0/11
Gelatin hydrolysis [b]	(v)	0(20)	(1)/5
Litmus milk	v	25 Aw	2/8
Growth in nutrient broth:			
0% NaCl	[−]	0	0/12
6% NaCl	[+]	100	12/12
8% NaCl	−	0	0/12
Growth at:			
25 C	+	100	10/10
35 C	+	100	12/12
42 C	+	100	9/9
Esculin hydrolysis	−	0	0/10
Lysine decarboxylase	[−] [b]	0	0/12
Arginine dihydrolase	[−] [b]	8	1/12
Ornithine decarboxylase	[−] [b]	0	0/12

[a]Add 0.15 ml of 20% NaCl per ml.

[b]7-14 days' incubation.

Vibrio parahaemolyticus

Gram-negative usually curved rod; motile (single polar flagellum); requires NaCl for growth, ferments glucose, mannitol, and maltose, not lactose and usually not sucrose; grows on MacConkey and TCBS agars; oxidase- and indole-positive; lysine- and ornithine decarboxylase-positive; V-P negative; NaCL tolerance ≥ 8%, <10%.

Sources (158 strains)

Stool (36%), seafood (5%), wound (5%), other (including 2 from blood) (12%), unknown (42%).

Reference strain

KC1405 ← ATCC, 17802 (type strain); D3942, from clam.

Literature

Jpn J Med Sci Biol 16:161-188, 1963; J Conf Public Health Dir 28:82-92, 1970; Appl Microbiol 23:26-33, 1972; Int J Syst Bacteriol 24:449, 1974.

Comments

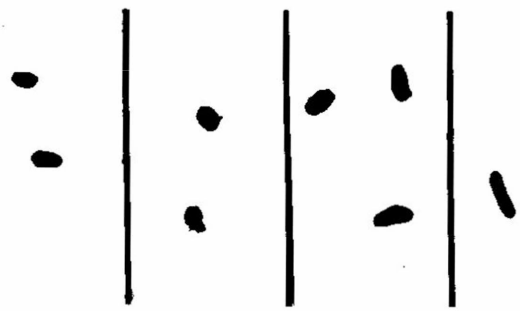
Cited in Bergey's Manual, 8th ed., as *V. parahaemolyticus*, biotype 1. Cause of gastroenteritis. Mixed polar and peritrichous flagella on some agar cultures grown for 16 h at 20°C.



KC1405 BAP 35°C 24 h ×1.



KC1405 Gram stain BAP 35°C 24 h ×1700.



D3942 Flagella stain BAP 35°C 24 h ×1700.

Vibrio parahaemolyticus

NUMBER OF STRAINS 160			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	mrc		
Motility, flagella	[m]:p,1-2		
Gas from glucose	–	0	0/160
Action on blood	v	32 LG	39/120;31 β,22 gr,18 ly
Fermentative or oxidative	[F]		
Carbohydrate base	F		
Acid from:			
Glucose	+	100	160/160
Xylose	–	0	0/155
Mannitol	[+]	98(1)	149,8w(1)/159
Lactose	[–]	0	0/160
Sucrose	[–]	2	3/157
Maltose	+	96	152,2w/160
10% Lactose	-	0	0/61
Catalase	+	93	76,19w/102
Oxidase	[+]	100	155/155
Growth on:			
MacConkey	[+]	100	93/93
SS	+	94	84/89
Simmons Citrate	v	76(11)	74(11)/97
Urea, Christensen's	–	8	8/101
Nitrate reduction	+	100	160/160
Indole	+ [a]	100	160/160
TSI slant, acid	–	3	3/104
TSI butt, acid	+	97	101/104
H_2S (TSI butt)	–	0	0/104
H_2S (Pb ac paper)	v	35(1)	11,26w(1)/104
MR	v [a]	57	67/118
V-P	[–] [a]	1	1w/136
Gelatin hydrolysis [b]	+	98	113/115
Litmus milk	v	86 pep	131/152;9 A,3 k,1 IR,1 c
Growth in nutrient broth:			
0% NaCl	[–]	0	0/141
6% NaCl	[+]	100	115/115
8% NaCl	+	98	42/43
10% NaCl	[–]	3	2/65
Growth at:			
25°C	+	98 [c]	56/57
35°C	+	91 [c]	85/93
42°C	v	43 [c]	34/79
Esculin hydrolysis	v	10(3)	7,1w(2)/79
Lysine decarboxylase	[+] [b]	100	94/94
Arginine dihydrolase	[–] [b]	0	0/94
Ornithine decarboxylase	[+] [b]	98(1)	92(1)/94
ONPG	–	0	0/17

[a]Add 0.15 ml of 20% NaCl per ml.

[b]7-14 days' incubation.

[c]Higher % may grow if HIA is used.

Vibrio vulnificus

Gram-negative, usually curved pleomorphic rod; motile (single polar flagellum); requires NaCl for growth; ferments glucose, lactose (sometimes weak or delayed), and certain other carbohydrates without gas, but not sucrose and xylose; grows on MacConkey and TCBS agars; oxidase- and indole-positive; lysine- and ornithine decarboxylase-positive; V-P-negative; NaCl tolerance less than 8%.

Sources (47 strains)

Blood (49%), wound (17%), abscess, leg, leg fluid, corneal ulcer, and thumb (each 4%), other (11%), unknown (2%).

Reference strain

A8694, from blood.

Literature

J Clin Microbiol 3:425-431, 1976; Arch Mikrobiol 110:101-120, 1976; Int J Syst Bacteriol 27:194-199, 1977; Lancet ii (27 October):903, 1979.

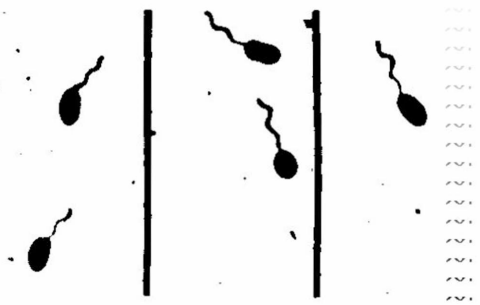
Comments

Previously referred to as "lactose-positive *Vibrio*"; also called *Beneckea vulnifica*. Associated with septicemias and local lesions.



BAP 35°C 24 h ×1.

Gram stain BAP 35°C 24 h ×1700.



Flagella stain FB 25°C 24 h ×17[illegible]

Vibrio vulnificus

	NUMBER OF STRAINS 47		
TEST PERFORMED	**SIGN**	**% +**	**# +/T**
Morphology	mtc		
Motility; flagella	[m],p,1-2		
Gas from glucose	[ ]	0	0/47
Action on blood	v	77 β	27,35;4 LG,3 gr,1 hy
Fermentative or oxidative	[F]		
Carbohydrate base	F		
Acid from:			
Glucose	+	100	47/47
Xylose	−	0	0/47
Mannitol	v	62	29/47
Lactose	[+ or(+)]	87(11)	16,25w(5)/47
Sucrose	[ ]	2	1/47
Maltose	+	100	47/47
10% Lactose	v	16(35)	6(13)/37
Catalase	+	100	25,12w/37
Oxidase	[+]	100	47/47
Growth on:			
MacConkey	[+]	100	47/47
SS	v	42(6)	16,4w(3)/47
Simmons Citrate	v	74(11)	35(5)/47
Urea, Christensen's	−	0	0/47
Nitrate reduction	+	100	47/47
Indole	+ [a]	100	47/47
TSI slant, acid	v	21	10/47
TSI butt, acid	+	100	47/47
H_2S (TSI butt)		0	0/47
H_2S (Pb ac paper)	+	91	36,7w/47
MR	v [a]	25(2)	9,2w(1)/45
V-P	[−] [a]	0	0/46
Gelatin hydrolysis [b]	+	97	38/39
Litmus milk	v	32(11) A	15(5)/47;6 pep
Growth in nutrient broth:			
0% NaCl	[−]	0	0/47
6% NaCl	[+]	95	36/38
8% NaCl	[−]	5	2/38
10% NaCl	−	0	0/38
Growth at:			
25°C	+	100	45/45
35°C	+	100	45/45
42°C	v	36 [c]	16/44
Esculin hydrolysis	v	9(2)	3,1w(1)/45
Lysine decarboxylase	[+] [a]	100	39/39
Arginine dihydrolase	[−] [a]	0	0/39
Ornithine decarboxylase	+ or(+) [a]	89(8)	34(3)/38
ONPG	+	100	20/20

[a]Add 0.15 ml of 20% NaCl per ml.

[b]7-14 days' incubation.

[c]Higher % may grow if HIA is used.

Yersinia enterocolitica

Gram-negative short to medium length rod; motile below 30°C (peritrichous flagella); ferments glucose (slight gas may be produced), xylose, maltose, mannitol, usually sucrose, and sometimes lactose (late); grows on MacConkey agar; oxidase- and citrate-negative; urease- and ornithine decarboxylase-positive; V-P-positive (25°C).

Sources (83 strains)

Blood (17%), water (12%), stool (10%), abscess (8%), animal (8%), food (6%), eye (5%), sputum (5%), other (29%).

Reference strain

KC1052 ← R. Hugh, 2845 ← N. Y. State Health Dept. "*Bacterium enterocoliticum*" 33114.

Literature

Int J Syst Bacteriol 25:336, 1975, and 26:180-194, 1976; Curr Microbiol 4:201-206, 1980.

Comments

Ubiquitous; European strains usually indole-negative, U. S. strains usually indole-positive; both O and H antigens have been shown. Some srains previously included in *Y. enterocolitica* have been assigned to three new species: *Y. kristensenii*, *Y. frederiksenii*, and *Y. intermedia*.



BAP 35°C 24 h ×1.



Gram stain 35°C 24 h ×1700.



Flagella stain FB 25°C 24 h ×1700.

Yersinia enterocolitica [a]

NUMBER OR STRAINS 83			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	mrs		
Motility; flagella	[m];pe [b]		
Gas from glucose	v	36	30/83
Action on blood	v	24 ly	20/83;14 gr,12 al,2 LG,1 lav
Fermentative or oxidative	[F]		
Carbohydrate base	F		
Acid from:			
Glucose	[+]	100	83/83
Xylose	+	97(2)	80(2)/83
Mannitol	+	100	83/83
Lactose	v	18(18)	15(15)/83
Sucrose	[+]	98	81/83
Maltose	+	92(7)	76(6)/83
10% Lactose	v	30(22)	7(5)/23
Catalase	+	100	80/80
Oxidase	[−]	0	0/80
Growth on:			
MacConkey	[+]	100	83/83
SS	+	90(6)	75(5)/83
Simmons Citrate	−	0	0/81
Urea, Christensen's	[+ or(+)]	70(30)	58(25)/83
Nitrate reduction	+	100	83/83
Indole	v	79(1)	65(1)/83
TSI slant, acid	+	100	81/81
TSI butt, acid	+	100	81/81
H_2S (TSI butt)	−	0	0/81
H_2S (Pb ac paper)	+	99	80/81
MR	v	63(10)	50(8)/79
V-P	[+] [c]	98	40/41
Gelatin hydrolysis [d]	−	0	0/37
Litmus milk	A	96	46/48
Growth in nutrient broth:			
0% NaCl	+	100	78/78
6% NaCl	v	35	28/79
Growth at:			
25°C	+	100	79/79
35°C	+	100	79/79
42°C	v	81	64/79
Esculin hydrolysis	v	28	22/78
Lysine decarboxylase	[−]	0	0/82
Arginine dihydrolase	[−]	0	0/82
Ornithine decarboxylase	[+]	100	82/82

[a] Some of the strains identified as *Y. enterocolitica* now would be identified as *Y. kristensenii* (sucrose − , D-trehalose + , L-rhamnose − , D-melibiose −), *Y. frederiksenii* (sucrose + , D-trehalose + , L-rhamnose + , D-melibiose −), or *Y. intermedia* (sucrose + , D-trehalose + , L-rhamnose + , D-melibiose +).

[b] Motile at 25°C, nonmotile at 35°C.

[c] Incubated at 25°C.

[d] 7-14 days' incubation.

Yersinia pestis

Gram-negative; coccoid to rod-shaped; nonmotile; ferments glucose, xylose, mannitol, and usually maltose, lactose and sucrose negative; grows on MacConkey agar; oxidase-, indole-, and urease-negative; reduces nitrate; lysine- and ornithine decarboxylase-negative, arginine dihydrolase-negative.

Sources (37 strains)

Blood (22%), animal (11%), cerebrospinal fluid (5%), other (11%), unknown (51%).

Reference strain

KC171 ← M. Moody.

Literature

Antonie van Leeuwenhoek J Microbiol Serol 10:15-16, 1944; J Gen Microbiol 19:554, 1958; Int J Syst Bacteriol 16:88-89, 1966; Health Lab Sci 5: 38-45, 1968.

Comments

Causative agent of plague. Sensitive to *Y. pestis* phage at 35° and 25°C. Often "fried egg-like" colonies on blood agar. May show bipolar staining ("safety-pin" appearance) with Wayson's stain.



BAP 35°C 24 h ×1.



BAP 35°C 24 h ×6.



Gram stain BAP 35°C 24 h ×1700.

Yersinia pestis

NUMBER OF STRAINS 37			
TEST PERFORMED	**SIGN**	**% +**	**# · /T**
Morphology	srs		
Motility; flagella	[nm]		
Gas from glucose	[−]	0	0/37
Action on blood	v	19 ly	7/39;2 al
Fermentative or oxidative	[F]		
Carbohydrate base	F		
Acid from:			
Glucose	[+]	100	37/37
Xylose	+	100	37/37
Mannitol	+	100	37/37
Lactose	[−]	0	0/37
Sucrose	[−]	0	0/37
Maltose	v	89	33/37
10% Lactose	−	0	0/26
Catalase	+	100	27/27
Oxidase	[−]	0	0/31
Growth on:			
MacConkey	[+]	100	31/31
SS	−	3(3)	1(1)/31
Simmons Citrate	−	0	0/29
Urea, Christensen's	[−]	0	0/31
Nitrate reduction	[+]	100	31/31
Indole	[−]	0	0/31
TSI slant, acid	v	50	18/36
TSI butt, acid	+	97	35/36
H_2S (TSI butt)	−	0	0/29
H_2S (Pb ac paper)	+	100	29/29
MR	+ or(+)	86(11)	24(3)/28
V-P	−	0	0/28
Gelatin hydrolysis [a]	−	0	0/13
Litmus milk	v	11 A	3w/27
Growth in nutrient broth:			
0% NaCl	+	100	27/27
6% NaCl	−	4	1/27
Growth at:			
25°C	+	100	28/28
35°C	+	96	27/28
42°C	−	0	0/28
Esculin hydrolysis	+	100	28/28
Lysine decarboxylase	[−]	0	0/19
Arginine dihydrolase	[−]	0	0/19
Ornithine decarboxylase	[−]	0	0/19

[a] 7-14 days' incubation.

Yersinia pseudotuberculosis

Gram-negative; coccoid to rod-shaped; motile (peritrichous) below 30°C; ferments glucose, xylose, mannitol, and maltose, not lactose and sucrose; grows on MacConkey agar; oxidase- and indole-negative; reduces nitrate; urease-positive; lysine- and ornithine decarboxylase-negative, arginine dihydrolase-negative; V-P-negative.

Sources (34 strains)

Human (blood and other) (26%), monkey (various organs and blood) (50%), miscellaneous animal isolations (24%).

Reference strain

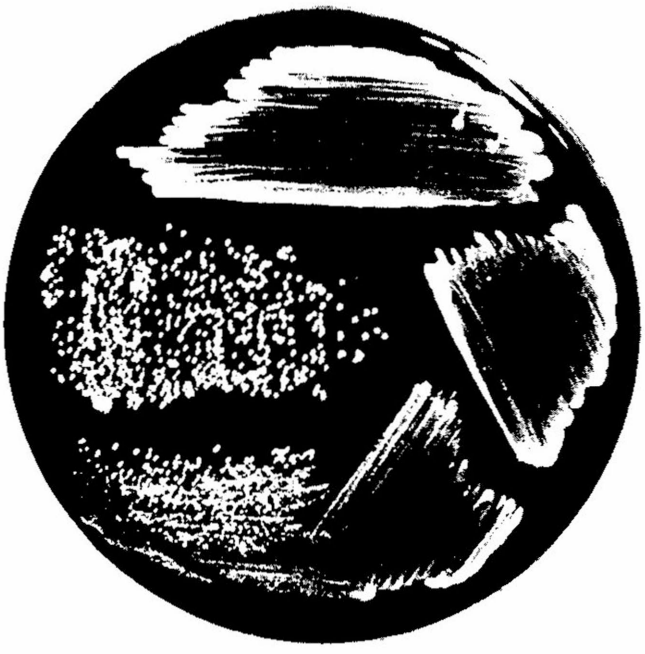
KC172 ← M. Moody.

Literature

Acta Pathol Microbiol Scand 64:213-223, 1965; Int J Syst Bacteriol 26:180-194, 1976.

Comments

Usually sensitive to *Yersinia pestis* phage at 35°-37°C, but not at 25°C; associated with mesenteric lymphadenitis suggesting appendicitis.



BAP 35°C 24 h ×1.



BAP 35°C 24 h ×6.



Gram stain BAP 35°C 24 h ×1700.

Yersinia pseudotuberculosis

	NUMBER OF STRAINS 34		
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	srs		
Motility: flagella	[m]:pe [a]		
Gas from glucose	-	0	0/34
Action on blood	v	47 ly	16/34,4 gr,2 al
Fermentative or oxidative	[F]		
Carbohydrate base	F		
Acid from:			
Glucose	[+]	100	34/34
Xylose	+	100	34/34
Mannitol	+	100	34/34
Lactose	[-]	0	0/34
Sucrose	[-]	0	0/34
Maltose	+	97(3)	33(1)/34
10% Lactose	-	0	0/14
Catalase	+	100	29/29
Oxidase	[-]	3	1w/29
Growth on:			
MacConkey	[+]	100	29/29
SS	v	17(41)	5(12)/29
Simmons Citrate	-	0	0/26
Urea, Christensen's	[+]	100	34/34
Nitrate reduction	[+]	100	34/34
Indole	[-]	0	0/34
TSI slant, acid	-	7	2/27
TSI butt, acid	+	93	25/27
H_2S (TSI butt)	-	0	0/26
H_2S (Pb ac paper)	v	81	21/26
MR	+	100	23/23
V-P	[-]	0	0/21
Gelatin hydrolysis [b]	-	0	0/20
Litmus milk	v	25 k	6/24
Growth in nutrient broth:			
0% NaCl	+	100	9/9
6% NaCl	-	0	0/9
Growth at:			
25°C	+	100	26/26
35°C	+	100	26/26
42°C	v	15(31)	4(8)/26
Esculin hydrolysis	+	100	17/17
Lysine decarboxylase	[-]	0	0/16
Arginine dihydrolase	[-]	0	0/16
Ornithine decarboxylase	[-]	0	0/16

[a]Usually motile at 25°C, nonmotile at 35°C.

[b]7-14 days' incubation.

IIe

Gram-negative slightly pleomorphic rod (cells sometimes appear thin to very thin in the central region, with thicker ends, the so-called "II forms"); motility not demonstrated; grows well aerobically; uses glucose and maltose, but not mannitol and lactose; no growth on MacConkey agar; weakly indole-positive; nitrate not reduced and esculin not hydrolyzed; produces no insoluble pigment; does not hydrolyze gelatin.

Sources (18 strains)

Blood (22%), urine (17%), genital site (17%), other (28%), unknown (16%).

Reference strains

B5408, from blood; KC198 ← ATCC, 10811 ← J. M. Coffey, New York Dept Publ Hlth, 33444.

Literature

None known.

Comment

Groups IIe and IIh differ mainly in the esculin reaction.



B5408 BAP 35°C 24 h ×1.



KC198 Gram stain BAP 35°C 24 h ×1700

IIe

NUMBER OF STRAINS 18			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	srs, II		
Motility; flagella	[nm]		
Action on blood	v	13 LG	2/15;2 br,1 gr,1 al
Fermentative or oxidative	[0]		
Carbohydrate base	OF		
Acid from:			
Glucose	[+]	100	18/18
Xylose		0	0/18
Mannitol	[ ]	0	0/18
Lactose	[ ]	0	0/18
Sucrose	-	0	0/18
Maltose	[+]	100	18/18
Catalase	v	88	15/17
Oxidase	v	88	15/17
Growth on:			
MacConkey	[-]	7	1/15
SS		0	0/16
Simmons Citrate	-	0	0/16
Urea, Christensen's	-	0	0/16
Nitrate reduction	[-]	0	0/16
Indole	[+]	100	16/16
TSI slant, acid	-	0	0/16
TSI butt, acid	-	0	0/16
H_2S (TSI butt)	-	0	0/16
H_2S (Pb ac paper)	v	78	14/18
Gelatin hydrolysis [a]	[-]	0	0/16
Litmus milk	v	12 IR	2/16;1 pep,1 k
Pigment:			
Insoluble	-	0	0/17
Soluble	br-yel	82	14/17
Growth at:			
25°C	+	100	16/16
35°C	+	100	16/16
42°C	-	0	0/16
Esculin hydrolysis	[-]	0	0/17
Nutrient broth, 0% NaCl	v	88	15/17
Nutrient broth, 6% NaCl	0	0	0/17

[a] 7-14 days' incubation.

IIf

Gram-negative slightly pleomorphic rod (cells sometimes appear thin to very thin in the central region, with thickened ends, the so-called "H forms"), motility not demonstrated; grows well aerobically; does not use carbohydrates; MacConkey-negative, oxidase-positive; produces indole weakly; does not break down urea or nitrate; proteolytic.

Sources (87 strains)

Urine (45%), vagina (15%), cervix (15%), other (17%), unknown (8%).

Reference strain

B4353, from vagina.

Literature

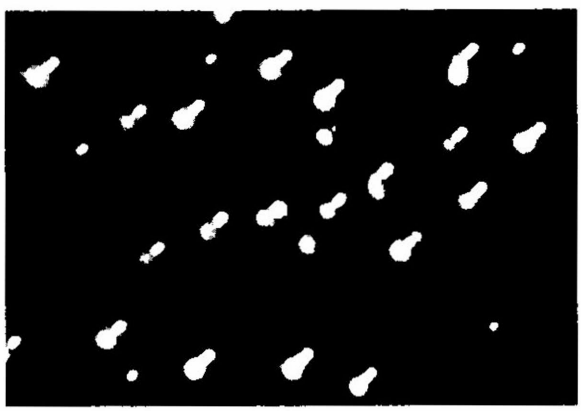
Antonie van Leeuwenhoek J Microbiol Serol 39:473-480, 1973.

Comments

Isolated from genitourinary tract, primarily female. Grows on Thayer-Martin selective media. Produces very heavy confluent growth on heart infusion agar in 24 h; cells are encapsulated.



BAP 35°C 24 h ×1.



BAP 35°C 24 h ×6.



Gram stain BAP 35°C 24 h ×1700.

IIf

NUMBER OF STRAINS 87			
TEST PERFORMED	**SIGN**	*% +*	*# + /T*
Morphology	srs, II		
Motility; flagella	[nm]		
Action on blood	v	76 LG	62 81;7 lav, 3 gr, 2 br
Fermentative or oxidative	[n-o]		
Carbohydrate base	OF		
Acid from:			
Glucose	[-]	0	0 87
Xylose	[-]	0	0 87
Mannitol	–	0	0 87
Lactose		0	0 87
Sucrose		0	0 87
Maltose		0	0 87
Catalase	+	98	74,11w 87
Oxidase	[+]	100	86,1w 87
Growth on:			
MacConkey	[ ]	0(10)	(9) 87
SS		0	0 87
Simmons Citrate	[-]	0	0 87
Urea, Christensen's	[-] [a]	0	0 86;(27 pk)
Nitrate reduction		0	0 87
Indole	[+]	100	51,36w 87
TSI slant, acid	–	0	0/87
TSI butt, acid	–	0	0/87
H_2S (TSI butt)	–	0	0/87
H_2S (Pb ac paper)	+	95	83/87
Gelatin hydrolysis [b]	[+]	100	79/79
Litmus milk	v	39 pep	34/87;15 IR,3 k
Pigment:			
Insoluble	-	0	0 87
Soluble	br-ta	98	85/87
Growth at:			
25°C	v	58	45/77
35°C	+	100	77/77
42°C	v	70	54 77
Esculin hydrolysis	–	0	0 87
Lysine decarboxylase	-	0	0 2
Arginine dihydrolase	–	0	0 2
Ornithine decarboxylase	–	0	0 2
Nutrient broth, 0% NaCl	+	99	85 86
Nutrient broth, 6% NaCl	–	7	6 86

[a] In 3-7 days 27 of 86 strains produced pink reactions which might be interpreted as weakly positive.
[b] 7-14 days' incubation.

IIh

Small gram-negative slightly pleomorphic rod (cells sometimes appear thin to very thin in the central region, with thicker ends, the so-called "II forms"); motility not demonstrated; grows well aerobically; uses glucose and maltose, but not mannitol or lactose; MacConkey-negative, oxidase-positive; produces indole; hydrolyzes esculin; does not break down urea or nitrate; produces no growth pigment; nonproteolytic.

Sources (21 strains)

Animal (38%), cerebrospinal fluid (10%), ear (10%), other human (42%).

Reference strains

781, source unknown; D3016, from guinea pig kidney.

Literature

None known.

Comment

Groups IIh and IIe differ mainly in the esculin reaction.



781 Gram stain BAP 35°C 24 h ×1700.

IIh

NUMBER OF STRAINS 21			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	srs, II		
Motility; flagella	[nm]		
Action on blood	v	32 LG	6/19;4 gr,3 al (stab)
Fermentative or oxidative	[0]		
Carbohydrate base	OF		
Acid from:			
Glucose	[+ or(+)]	85(15)	17(3) 20
Xylose	−	5	1w/20
Mannitol	[−]	0	0 20
Lactose	[−]	0	0 20
Sucrose	−	0	0 20
Maltose	[+]	95	19 20
Catalase	+	100	21 21
Oxidase	[+]	100	21 21
Growth on:			
MacConkey	[−]	0	0 21
SS	−	0	0 21
Simmons Citrate	−	0	0 21
Urea, Christensen's	−	0	0 21
Nitrate reduction	[−]	0	0 21
Indole	[+]	100	18,3w/21
TSI slant, acid	−	0	0 21
TSI butt, acid	−	5	1w 21
H_2S (TSI butt)	−	0	0/21
H_2S (Pb ac paper)	+	100	21/21
Gelatin hydrolysis [a]	[−]	7	1/15
Litmus milk	−	7 IR	1/21
Pigment:			
Insoluble	−	0	0/21
Soluble	br	100	19/19
Growth at:			
25°C	+	100	21 21
35°C	+	100	21 21
42°C	−	5	1/21
Esculin hydrolysis	[+]	100	21/21
Nutrient broth, 0% NaCl	v	86	18/21
Nutrient broth, 6% NaCl	−	5	1/21

[a] 7-14 days' incubation.

IIi

Gram-negative slightly pleomorphic rod (cells sometimes appear thin to very thin in the central region, with thicker ends, the so-called "II forms"), motility not demonstrated; grows well aerobically; uses glucose, xylose, lactose, sucrose, and maltose, but not mannitol; reaction on TSI agar is alkaline slant, no change in the butt; no growth on MacConkey agar; oxidase-, catalase-, and indole-positive; nonproteolytic.

Sources (23 strains)

Blood (35%), urine (26%), wound (13%), other (13%), unknown (13%).

Reference strain

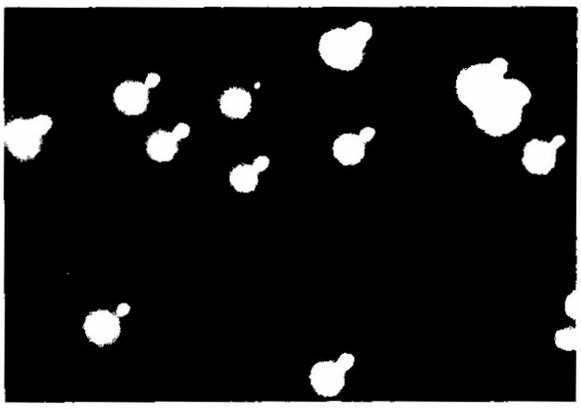
C9138, from leg wound.

Literature

None known.



BAP 35°C 24 h ×1.



BAP 35°C 24 h ×6.



Gram stain BAP 35°C 24 h ×1700.

IIi

NUMBER OF STRAINS 23			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	srs, II		
Motility; flagella	[nm]		
Action on blood	v	39 LG	9/23;5 ly,1 lav,1 gr,1 al
Fermentative or oxidative	[0]		
Carbohydrate base	OF		
Acid from:			
Glucose	[+]	91(9)	21(2) 23
Xylose	+ or(+)	87(13)	16,4w(3) 23
Mannitol	[−]	0	0/23
Lactose	+	91(9)	21(2) 23
Sucrose	[+]	91(9)	21(2) 23
Maltose	+	91(9)	21(2) 23
Catalase	[+]	100	23/23
Oxidase	[+]	100	21,2w/23
Growth on:			
MacConkey	[ ]	0	0/22
SS	−	0	0/22
Simmons Citrate	−	0	0/22
Urea, Christensen's	v	14(18)	3(4)/22
Nitrate reduction	−	0	0/23
Indole	[+]	100	10,13w/23
TSI slant, acid		0	0/23
TSI butt, acid	[ ]	0	0/23
H_2S (TSI butt)	−	0	0/23
H_2S (Pb ac paper)	v	70	16/23
Gelatin hydrolysis [a]	[−]	0	0/16
Litmus milk	−	4 A	1/22
Pigment:			
Insoluble	v	22 yel	3,2w/23
Soluble	v	48 yel-ta	11/23
Growth at:			
25°C	+	100	22/22
35°C	+	100	22/22
42°C	v	36	8/23
Esculin hydrolysis	+	96	22/23
Lysine decarboxylase	−	0	0/3
Arginine dihydrolase	−	0	0/3
Ornithine decarboxylase	−	0	0/3
Nutrient broth, 0% NaCl	+	100	23/23
Nutrient broth, 6% NaCl	−	9	2/23

[a] 7-14 days' incubation.

IIj

Gram-negative medium to long rod, may exhibit "II"-forms; motility not demonstrated; does not use carbohydrates; usually does not grow or change indicator in OF carbohydrate media; oxidase-positive; no growth on MacConkey agar; indole- and urease-positive; nitrate not reduced.

Sources (41 strains)

Dogbite (34%), cat bite and scratch (15%), abscess and wound (10%), canine isolation (10%), sputum (5%), cerebrospinal fluid (5%), other (16%), unknown (5%).

Reference strains

B3969, from eye tissue; B2704, from cerebrospinal fluid.

Literature

Manual of Clinical Microbiology, 2nd ed., pp 284-285; J Clin Microbiol 7:223-231, 1978.

Comments

Growth on rabbit blood agar is usually sticky. Associated with animal bite wounds.



B2704 Gram stain BAP 35°C 24 h ×1700.

IIj

NUMBER OF STRAINS 41			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	s-lr, II		
Motility: flagella	[nm]		
Action on blood	v	36 LG	15/41;4 al,3 gr,2 lav,1 β
Fermentative or oxidative	[n-o]		
Carbohydrate base	OF		
Acid from:			
Glucose	[−]	0	0/41
Xylose	[−]	0	0/41
Mannitol	−	0	0/41
Lactose	−	0	0/41
Sucrose	−	0	0/41
Maltose	−	0	0/41
Catalase	+	100	39,2w/41
Oxidase	[+]	100	39,1w/40
Growth on:			
MacConkey	[−]	2	12/41
SS	−	0	0/41
Simmons Citrate	−	0	0/41
Urea, Christensen's	[+]	100	40/40
Nitrate reduction	[−]	0	0/40
Indole	[+]	98	31,9w/41
TSI slant, acid	−	0	0/41
TSI butt, acid	−	0	0/40
H_2S (TSI butt)	−	0	0/41
H_2S (Pb ac paper)	v	59	21,2w/39
Gelatin hydrolysis [a]	+	98	39/40
Litmus milk	v	18 pep	7/40;3 k,1 IR
Pigment:			
Insoluble	−	0	0/39
Soluble	ta-yel	100	39/39
Growth at:			
25°C	v	30	12/40
35°C	+	95	38/40
42°C	−	10	4/40
Esculin hydrolysis	−	0	0/15
Lysine decarboxylase	−	0	0/2
Arginine dihydrolase	+	100	2/2
Ornithine decarboxylase	−	0	0/2
Nutrient broth, 0% NaCl	v	15	6/40
Nutrient broth, 6% NaCl	−	0	0/40

[a] 7-14 days' incubation.

IVc-2

Gram-negative short to medium rod, may stain irregularly, motile (peritrichous flagella); aerobic; grows on MacConkey agar, not on SS medium; does not use carbohydrates; oxidase-positive; urease test partially positive in 24 h; usually nitrate-negative (no gas if positive); no H_2S formed on TSI agar.

Sources (37 strains)

Water (19%), blood (14%), eye (8%), sputum (8%), urine (5%), throat (5%), tissue culture contaminant (5%), peritoneal fluid (5%), other (15%), unknown (16%).

Referene strains

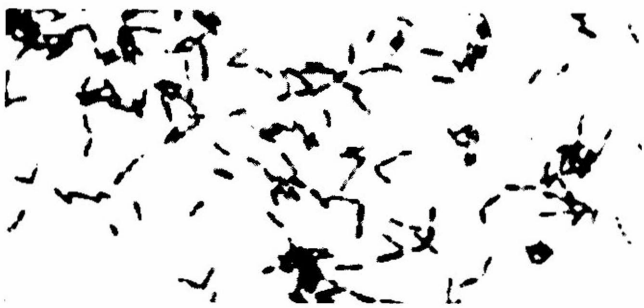
A1036, from blood; D3518, from peritoneal fluid.

Literature

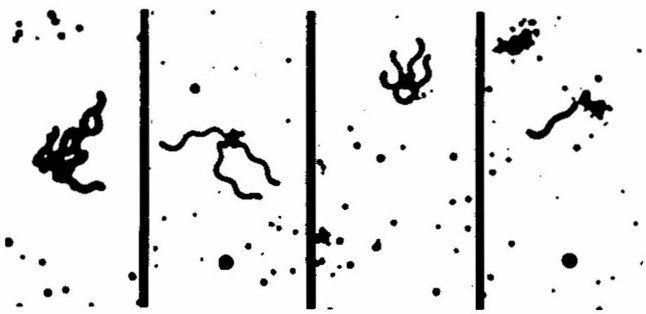
J Clin Microbiol 14:39-47, 1981.

Comment

Closely resembles *Bordetella bronchiseptica* except for nitrate and SS reactions.



A1036 Gram stain BAP 35°C 24 h ×1700.



D3518 Flagella stain FB 25°C 24 h ×1700.

IVc-2

NUMBER OF STRAINS 36			
TEST PERFORMED	**SIGN**	**% +**	**# +/T**
Morphology	srs		
Motility: flagella	[m;pe]		
Action on blood	v	53 hy	8/15;4 gr,2 br,1 al
Fermentative or oxidative	[n-o]		
Carbohydrate base	OF		
Acid from:			
Glucose	[ ]	0	0/36
Xylose	[-]	0	0/36
Mannitol		0	0/36
Lactose	–	0	0/36
Sucrose	–	0	0/36
Maltose	–	0	0/36
Catalase	+	100	36/36
Oxidase	[+]	100	37/37
Growth on:			
MacConkey	[+]	94(6)	34(2)/36
SS	[-]	3(6)	1(2)/36
Cetrimide	-	0(3)	(1)/36
Simmons Citrate	+	100	36/36
Urea, Christensen's	[+]	100	36/36
Nitrate reduction	[v]	11	4/36
Gas from nitrate	[–]	0	0/36
Indole	–	0	0/36
TSI slant, acid	–	0	0/36
TSI butt, acid	–	0	0/36
H_2S (TSI butt)	[–]	0	0/35
H_2S (Pb ac paper)	v	51	18/35
Gelatin hydrolysis [a]	–	0	0/30
Litmus milk	k	97	35/36
Pigment	v	22 yel-ta sol	8/36
Growth at:			
25°C	+	94	34/36
35°C	+	100	36/36
42°C	v	86	31/36
Esculin hydrolysis	–	0	0/30
Nutrient broth, 0% NaCl	+	100	36/36
Nutrient broth, 6% NaCl	v	11	4/36

[a] 7-14 days' incubation.

IVe

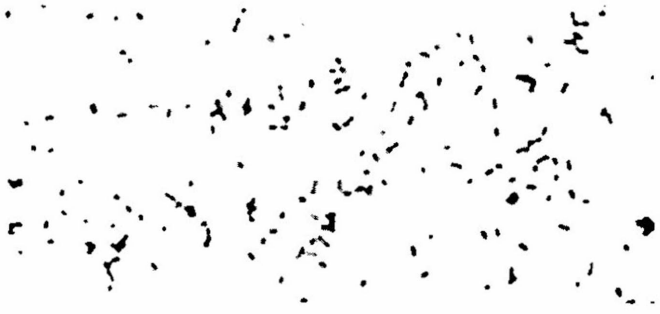
Small coccoid gram-negative rod; motile (peritrichous, often with one long polar and only a few lateral flagella); aerobic; does not use carbohydrates; oxidase-positive; frequently grows on MacConkey agar, but not on SS agar; strongly urease-positive; nitrate and nitrite reduced, usually with some gas production; no H_2S formed on TSI agar.

Sources (37 strains)
Urine (92%), blood (3%), unknown (5%).

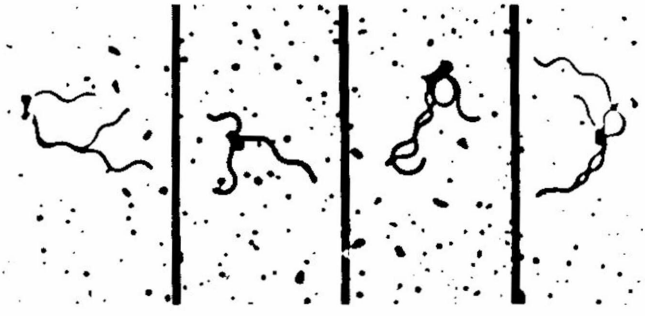
Reference strains
D456 and D3926, from urine.

Literature
Manual of Clinical Microbiology, 2nd ed., pp 277-279.

Comments
Most frequent source has been urine, particularly from male. Motility may be delayed, or difficult to demonstrate.



D3926 Gram stain BAP 35°C 24 h ×1700.



D3926 Flagella stain FB 25°C 24 h ×1700.

IVe

TEST PERFORMED	SIGN	% +	# + /T
	NUMBER OF STRAINS 37		
Morphology	srs		
Motility; flagella	[m;pe] [a]		
Action on blood	v	71 [v	5 7;1 gr;1 br
Fermentative or oxidative	[n-o]		
Carbohydrate base	OF		
Acid from:			
Glucose	[ ]	0	0 37
Xylose	[ ]	0	0 37
Mannitol		0	0 37
Lactose		0	0 37
Sucrose	–	0	0 37
Maltose	–	0	0 37
Catalase	+	100	37 37
Oxidase	[+]	100	37 37
Growth on:			
MacConkey	[v]	62(27)	23(10) 37
SS	[–]	5	2 37
Cetrimide	–	0(3)	0(1) 37
Simmons Citrate	v	14(16)	5(6) 37
Urea, Christensen's	[+]	97	36 37
Nitrate reduction	[+]	100	37 37
Gas from nitrate	[v]	60	22 37
Indole	–	0	0 37
TSI slant, acid	–	0	0 36
TSI butt, acid	–	0	0 36
H_2S (TSI butt)	[–]	0	0 36
H_2S (Pb ac paper)	v	38	14 37
Gelatin hydrolysis [b]	-	0	0 33
Litmus milk	v	65 k	24 37
Pigment	–	3 yel sol	1 37
Growth at:			
25°C	v	67	22 33
35°C	v	88	29 33
42°C	v	18	6 33
Esculin hydrolysis	–	0	0 32
Nutrient broth, 0% NaCl	v	19(3)	7(1) 37
Nutrient broth, 6% NaCl	v	14(5)	5(2) 37

[a]Motility may be delayed or difficult to demonstrate.

[b]7-14 days' incubation.

Ya-1

(biovar of *Pseudomonas pickettii*)

Gram-negative medium length rod; motility difficult to demonstrate (1-2 polar flagella); aerobic; uses glucose, xylose, lactose, and maltose; oxidase-positive; grows on MacConkey agar; reduces nitrate, some strains with gas formation; urease produced; lysine decarboxylase and arginine dihydrolase not produced.

Sources (70 strains)

Blood (11%), water (10%), sputum (10%), urine (10%), cerebrospinal fluid (7%), eye (6%), exudate, dialysis, lymph node, cervix, and trachea (3% each), other (25%), unknown (6%).

Reference strains

C6712, from ear exudate; D3927, from conjunctiva.

Literature

Ann Intern Med 84:51-52, 1975; J Gen Microbiol 120: 439-446, 1980.

Comment

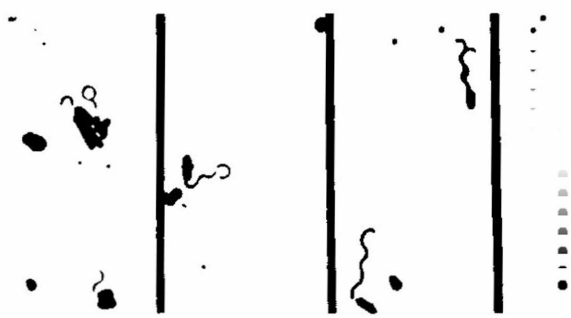
Genetic studies have shown that Ya-1, which differs from the type strain of *P. pickettii* in the oxidation of lactose and maltose, is a biovar of *P. pickettii*.



C6712 BAP 35°C 24 h ×1.



D3927 Gram stain BAP 35°C 24 h ×1700.



D3927 Flagella stain FB 25°C 24 h ×1700.

Ya-1

NUMBER OF STRAINS 70			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	rnrs		
Motility: flagella	[m;p,1-2] [a]		
Action on blood	v	56 ly	14/25;5 LG,4 al,2 gr
Fermentative or oxidative	[0]		
Carbohydrate base	OF		
Acid from:			
Glucose	[+]	100	70/70
Xylose	[+]	100	70/70
Mannitol	−	0	0/70
Lactose	[+]	100	70/70
Sucrose		0	0/70
Maltose	[+]	100	69/69
Catalase	+	100	70w/70
Oxidase	[+]	100	70/70
Growth on:			
MacConkey	[+]	99(1)	69(1)/70
SS	−	0	0/70
Cetrimide	−	1	1/70
Simmons Citrate	+	99	69/70
Urea, Christensen's	[+]	100	70/70
Nitrate reduction	+	100	70/70
Gas from nitrate	[v]	86	60/70
Indole	−	0	0/70
TSI slant, acid		0	0/70
TSI butt, acid	−	0	0/70
H_2S (TSI butt)	-	0	0/70
H_2S (Pb ac paper)	v	67	47/70
Gelatin hydrolysis [b]	v	12	7/59
Litmus milk	v	83 k	40/48;2 pep
Pigment:			
Soluble	v	36 yel-ta	25/70
Growth at:			
25°C	+	100	69/69
35°C	+	100	69/69
42°C	v	83	57/69
Esculin hydrolysis	−	0	0/70
Lysine decarboxylase	[−]	0	0/53
Arginine dihydrolase	[−]	6	3/53
Ornithine decarboxylase	−	0	0/53
Nutrient broth, 0% NaCl	+	100	70/70
Nutrient broth, 6% NaCl	−	4	3/70

[a]Weakly motile, may be difficult to demonstrate.
[b]7-14 days' incubation.

Vb-3

Gram-negative medium rods; motile (single, occasionally two polar flagella); aerobic; uses glucose, xylose, maltose, and (some strains) mannitol, but not lactose and sucrose; hydrolyzes starch; oxidase-positive; grows on MacConkey agar; reduces nitrate to gas; no H_2S produced on TSI agar; usually nonproteolytic; produces arginine dihydrolase, but not lysine decarboxylase.

Sources (65 strains)

Wound (12%), ear (11%), leg (8%), blood (8%), sputum (6%), eye (5%), cerebrospinal fluid (5%), urine (5%), knee abscess (3%), dialysis supply tank (3%), other (31%), unknown (3%).

Reference strain

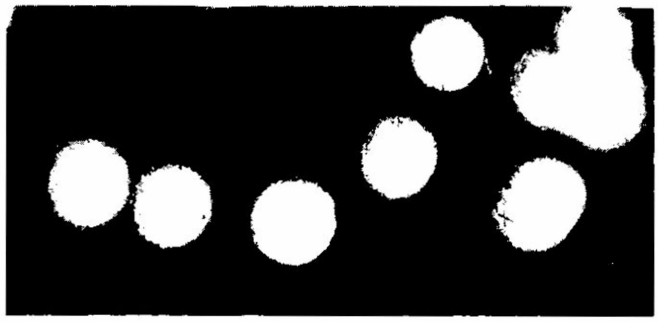
D2986, from leg wound.

Literature

None known.



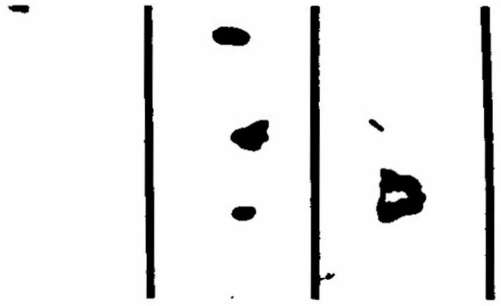
BAP 35°C 24 h ×1.



BAP 35°C 24 h ×6.



Gram stain BAP 35°C 24 h ×1700.



Flagella stain FB 25°C 24 h ×1700.

Comments

Very similar to *Pseudomonas stutzeri*, possibly a biotype (arginine dihydrolase production is positive with ∇b-3 and negative with *P. stutzeri*); genetic studies are needed to determine the species status of this group.

∇b-3

NUMBER OF STRAINS 65			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	mrs		
Motility; flagella	[m;p,1-2]		
Action on blood	v	32 gr	20,63;17 ly,11 br&ly,7 LG
Fermentative or oxidative	[0]		
Carbohydrate base	OF		
Acid from:			
Glucose	[+]	100	61,4w/65
Xylose	+	97(3)	61,2w(2)/65
Mannitol	v	65(9)	19,23w(6)/65
Lactose	[−]	0	0/65
Sucrose	−	0	0/65
Maltose	[+]	95(5)	42,20w(3)/65
Starch	+	100	9,1w/10
Catalase	+	98	61,3w/65
Oxidase	[+]	100	62,3w/65
Growth on:			
MacConkey	[+]	100	65/65
SS	+	100	64,1w/65
Cetrimide	v	18(2)	4,8w(1)/65
Simmons Citrate	+	92(6)	60(4)/65
Urea, Christensen's	v	12(45)	7,1w(29)/65
Nitrate reduction	+	100	65/65
Gas from nitrate	[+]	100	65/65
Indole	−	0	0/65
TSI slant, acid	−	0	0/65
TSI butt, acid	−	0	0/65
H_2S (TSI, butt)	[−]	0	0/65
H_2S (Pb ac paper)	v	5(14)	3(9)/65
Gelatin hydrolysis [a]	[−]	4	2/45
Litmus milk	v	69 k	44/64;14 IR,2 pep
Pigment	yel ins	95	21,41w/65;7 yel-br sol
Growth at:			
25°C	+	98	64/65
35°C	+	100	65/65
42°C	+	91	56,3w/65
Esculin hydrolysis	−	0	0/65
Lysine decarboxylase	[−]	0	0/65
Arginine dihydrolase	[+]	100	65/65
Ornithine decarboxylase	−	0	0/65
Nutrient broth, 0% NaCl	+	100	61/61
Nutrient broth, 6% NaCl	v	85(3)	45,7w(2)/61
Acetamide	−	0	0/8
2-Ketogluconate	−	10	1w/10

[a] 7-14 days' incubation.

∇d *(Achromobacter sp.)*

Gram-negative rod; motile (peritrichous; frequently individual cells have only a single long flagellum either polar, subpolar, or lateral with a fairly short wavelength); oxidizes glucose, xylose, mannitol, sucrose, and maltose (these reactions may be delayed), but not lactose; grows on MacConkey and SS agars; oxidase- and urease-positive; reduces nitrate with gas formation; indole-negative.

Sources (52 strains)

Blood (23%), urine (12%), sputum (10%), wound (8%), throat (6%), stool and rectum (4%), other (35%), unknown (1%).

Reference strains

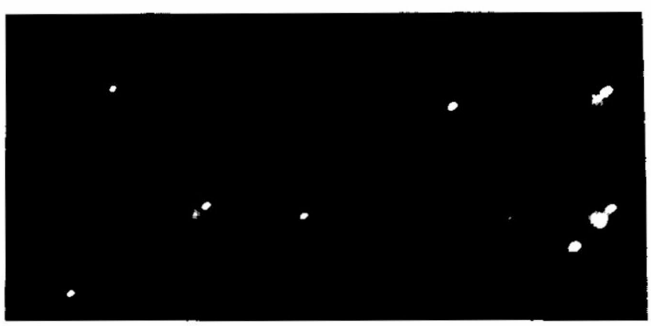
D1857, from wound; D2701, from blood.

Literature

Manual of Clinical Microbiology, 2nd ed., p 280; J Clin Microbiol 5:172-177, 1977.



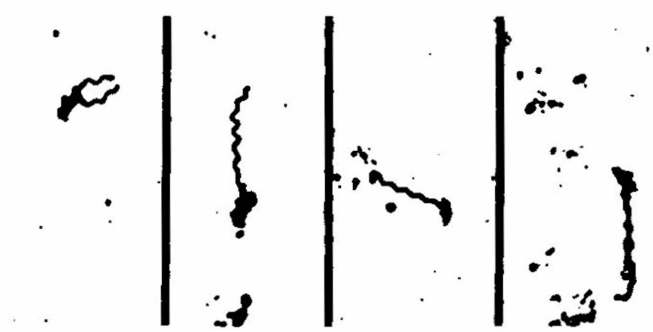
D2701 BAP 35°C 24 h ×1.



D2701 BAP 35°C 24 h ×6.



D1857 BAP 35°C 24 h ×1700.



D2701 FB 25°C 24 h ×1700.

Comments

This organism, unlike *Agrobacterium radiobacter*, does not oxidize lactose. The group Vd is composed of organisms formerly designated as groups Vd-1 and Vd-2 and *Achromobacter* sp. biotypes 1 and 2. Most of the strains which had been designated group Vd-3 have been identified as strains of *A. radiobacter*.

Vd (*Achromobacter* sp.)

	NUMBER OF STRAINS 71		
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	mrs		
Motility; flagella	[m;pe] [a]		
Action on blood	v	23 LG	16/71;13 ly,10 gr,5 lav,5 br,2 β
Fermentative or oxidative	[0]		
Carbohydrate base	OF		
Acid from:			
Glucose	[+ or (+)]	86(13)	61(9)/71
Xylose	+	96(4)	68(3)/71
Mannitol	v	46(34)	33(24)/71
Lactose	[-]	0	0/71
Sucrose	v	28(25)	20(18)/71
Maltose	v	32(25)	23(18)/71
Catalase	+	100	71/71
Oxidase	[+]	100	71/71
Growth on:			
MacConkey	[+]	100	71/71
SS	[+]	96(1)	68(1)/71
Cetrimide	−	0(3)	(2)/71
Simmons Citrate	v	54(13)	38(9)/71
Urea, Christensen's	[+]	92(8)	65(6)/71
Nitrate reduction	+	100	71/71
Gas from nitrate	[+]	99	70/71
Indole	[−]	0	0/71
TSI slant, acid	−	0	0/71
TSI butt, acid	−	0	0/71
H_2S (TSI butt)	v	49	27,8w/71
H_2S (Pb ac paper)	+	100	71/71
Gelatin hydrolysis [b]	−	0	0/71
Litmus milk	v	85 k	60/71;2 IR
Pigment	v	11 br-yel sol	8/71
Growth at:			
25°C	+	100	71/71
35°C	+	100	71/71
42°C	v	56	40/71
Esculin hydrolysis	v	38(11)	27(8)/71
Lysine decarboxylase	−	0	0/22
Arginine dihydrolase	v	68	15/22
Ornithine decarboxylase	−	0	0/22
Nutrient broth, 0% NaCl	+	100	58/58
Nutrient broth, 6% NaCl	v	45	26/58
3-Ketolactonate	−	0	0/5

[a]Frequently individual cells have only a single flagellum, either polar, subpolar, or lateral.

[b]7-14 days' incubation.

Ve-1

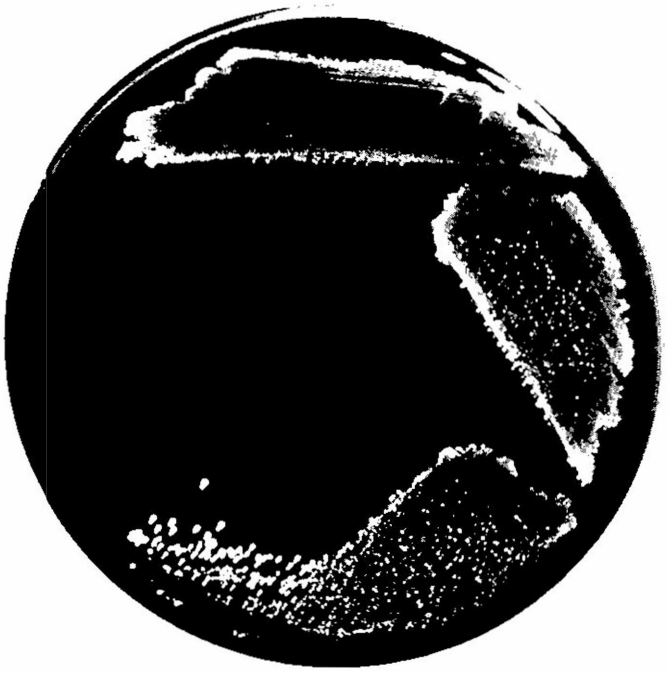
Gram-negative short to medium length, slightly thick rod, sometimes paired; motile (polar tuft of three or more flagella); cells often appear spindle-shaped and vacuolated; smooth and rough, wrinkled, adherent colonies may occur; produces a yellow growth pigment. Aerobic; grows on MacConkey agar,oxidase-negative; uses glucose, xylose, maltose, usually mannitol (often weakly), sometimes lactose and occasionally sucrose; produces arginine dihydrolase but not lysine decarboxylase; hydrolyzes esculin; usually reduces nitrate to nitrite.

Sources (18 strains)

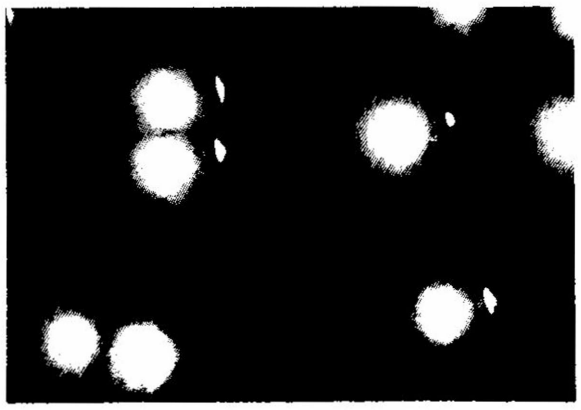
Blood (3), urine (2), lung, cyst, skin, wound, throat, pleural fluid, eye, knee (1 each), unknown (5).

Reference strain

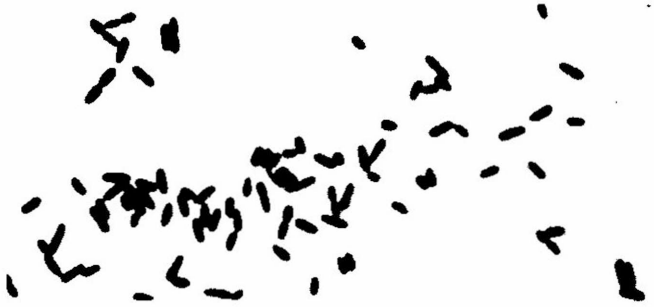
D4029(1), from human skin.



BAP 35°C 24 h ×1.



BAP 35°C 24 h ×6.



Gram stain BAP 35°C 24 h ×1700.



Flagella stain HIA 35°C 24 h ×1700.

Literature

Can J Microbiol 16:351-362, 1970; J Clin Microbiol 1:384-389, 1975.

Comment

Cellular and colonial morphologies similar to those of Ve-2.

Ve-1

NUMBER OF STRAINS 18			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	mrs		
Motility, flagella	[m;p,-2]		
Action on blood	v	29 ly	5 17;4 gr,2 LG
Fermentative or oxidative	[0]		
Carbohydrate base	OF		
Acid from:			
Glucose	[+]	100	18 18
Xylose	+	100	18 18
Mannitol	[v]	82(6)	6,8w(1) 17
Lactose	v	6(28)	1w(5) 18
Sucrose	–	6	1 18
Maltose	+	100	17,1w 18
Catalase	+	100	15,3w 18
Oxidase	[–]	0	0 18
Growth on:			
MacConkey	[+]	100	18/18
SS	v	67	12/18
Cetrimide	–	0	0/18
Simmons Citrate	+	100	18 18
Urea, Christensen's	v	39(44)	7(8)/18
Nitrate reduction	v	67	12/18
Gas from nitrate		0	0/18
Indole		0	0/18
TSI slant, acid	–	0	0/18
TSI butt, acid	–	0	0 18
H_2S (TSI butt)	–	0	0 18
H_2S (Pb ac paper)	–	6	1w 18
Gelatin hydrolysis [a]	v	53	8 15
Litmus milk	v	61 k	11 18;3 pep,2 IR
Pigment:			
Insoluble	[yel]	94	17 18
Growth at:			
25°C	+	100	17 17
35°C	+	100	17 17
42°C	+	94	16 17
Esculin hydrolysis	[+]	100	18 18
Lysine decarboxylase	[–]	0	0 10
Arginine dihydrolase	[+]	100	10 10
Ornithine decarboxylase	–	0	0 10
Nutrient broth, 0% NaCl	+	100	18 18
Nutrient broth, 6% NaCl	v	83	15 18

[a] 7-14 days' incubation.

Ve-2

Gram-negative rod; motile (1-2 polar flagella); produces a yellow growth pigment; aerobic; oxidase-negative; grows on MacConkey agar; uses glucose, xylose, mannitol, maltose, and sometimes lactose and sucrose; does not hydrolyze esculin; arginine dihydrolase-variable, lysine decarboxylase usually negative; does not usually reduce nitrate.

Sources (37 strains)

Urine (14%), eye (13%), wound (11%), blood (11%), skin (8%), sputum (5%), other (24%), unknown (14%).

Reference strain

D7021, source unknown.

Literature

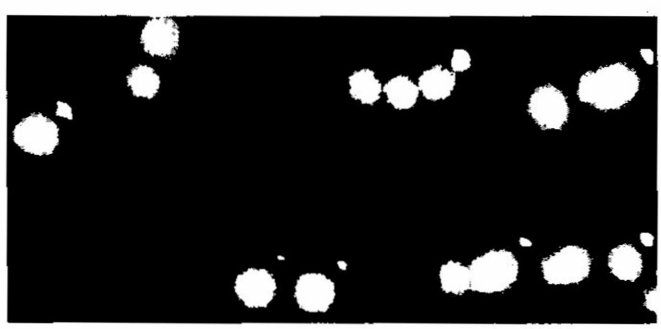
Can J Microbiol 16:351-362, 1970; J Clin Microbiol 1: 384-389, 1975.

Comment

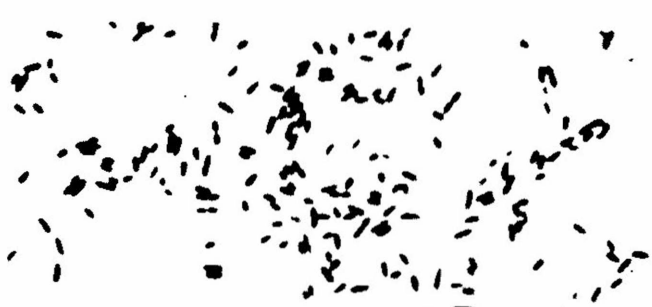
Cellular and colonial morphologies similar to those of Ve-1.



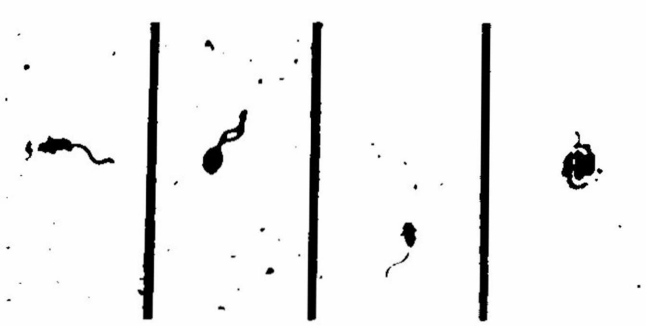
BAP 35°C 24 h ×1.



BAP 35°C 24 h ×6.



Gram stain BAP 35°C 24 h ×1700.



Flagella stain BAP 35°C 24 h ×1700.

Ye-2

NUMBER OF STRAINS 36			
TEST PERFORMED	**SIGN**	**% +**	**# + /T**
Morphology	srs		
Motility, flagella	[m;p,1-2]		
Action on blood	v	43 [γ	12,28;3 LG,2 β
Fermentative or oxidative	[O]		
Carbohydrate base	OF		
Acid from:			
Glucose	[+]	100	36/36
Xylose	+	100	35,1w/36
Mannitol	[+]	100	29,7w/36
Lactose	v	14(22)	2,3w(8)/36
Sucrose	v	25	9/36
Maltose	+	97(3)	30,5w(1)/36
Catalase	+	94	34/36
Oxidase	[−]	0 [a]	0/36
Growth on:			
MacConkey	[+]	100	36/36
SS	v	22	7/28
Cetrimide	v	25(28)	9w(10)/36
Simmons Citrate	+	97	35/36
Urea, Christensen's	v	77	27/35
Nitrate reduction	[−]	6	2/36
Gas from nitrate	−	0	0/36
Indole	−	0	0/35
TSI slant, acid	−	0	0/36
TSI butt, acid	−	0	0/36
H_2S (TSI butt)	−	0	0/36
H_2S (Pb ac paper)	+	97	35w/36
Gelatin hydrolysis [b]	v	17	4/23
Litmus milk	v	57 k	20/35
Pigment:			
Insoluble	[yel]	100	35/35
Growth at:			
25 C	+	100	33/33
35 C	+	100	33/33
42 C	v	33	11/33
Esculin hydrolysis	[−]	0	0/34
Lysine decarboxylase	[−]	7	2/30
Arginine dihydrolase	v	14	4/30
Ornithine decarboxylase	−	3	1/30
Nutrient broth, 0% NaCl	+	100	32/32
Nutrient broth, 6% NaCl	v	62	20/32

[a] A few strains were weakly positive by the Kovacs' method.
[b] 7-14 days' incubation.

APPENDIX

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GRAM-NEGATIVE ORGANISMS: AN APPROACH TO IDENTIFICATION (GUIDE TO PRESUMPTIVE IDENTIFICATION)

Gram-Negative Organisms: An Approach to Identification (Guide to Presumptive Identification) was developed in response to the question, "How can we identify these organisms in the clinical laboratory without doing the total number of tests that are done in a reference laboratory?" The Guide is based on results obtained with conventional procedures (described in the manual *Identification of Unusual Pathogenic Gram-Negative Aerobic and Facultatively Anaerobic Bacteria*).

The Guide consists of three parts: 1) a key for the identification of gram-negative aerobes, 2) 12 tables, and 3) a code book. The key contains 12 categories based on the method by which an organism produces acid from glucose, its ability to grow on MacConkey agar, and the oxidase reaction. There is a corresponding table for each category.

In the key and the tables, a species or unnamed group is considered positive for a "key" characteristic if 90% or more of the strains have been positive for that characteristic. If 11% through 89% of the strains have been positive for that characteristic, the organism is considered to be variable for the characteristic. If only 10% or less of the strains have been positive, the organism is considered to be negative for the characteristic. If a species or unnamed group is variable for one or more of the three "key" characteristics, the species or group is listed in more than one category of the key. For example, if a species ferments glucose 95% of the time, grows on MacConkey agar 65% of the time, and is oxidase-positive 2% of the time, it is listed, because of the variable MacConkey reaction, in two categories of the key (and on the two corresponding tables): 1) glucose fermenters, MacConkey-positive, oxidase-negative and 2) glucose fermenters, MacConkey-negative, oxidase-negative.

The first step in arriving at a presumptive identification is to determine the three key characteristics of the culture being studied. After this has been done, the organism is tested for the additional reactions or characteristics included in the table listing the various species and unnamed groups of bacteria that have the same three key characteristics. For example, if an unknown culture ferments glucose, grows on MacConkey agar, and has a positive oxidase reaction, it should be tested for the additional reactions listed in Table 2: lysine and ornithine decarboxylase, arginine dihydrolase, indole, urease, and fermentation of mannitol, lactose, and sucrose. A presumptive identification is reached by comparing the results obtained in these tests with the reactions listed for the various organisms in the table.

An alternative method for reaching the presumptive identification is to translate the results into a number (the number of digits in the number varies for each table). As shown at the top of each table, each reaction has been assigned a numerical value of 1, 2, or 4. The reactions to be used for calculating the value of each digit are separated by the bold lines. For Table 2 the first digit is determined by the lysine, arginine, and ornithine reactions, the second digit by the indole, urea, and mannitol reactions, and the third digit by the lactose and sucrose reactions. The calculation of the three-digit number for an organism which produces positive reactions for ornithine decarboxylase, indole, urease, and fermentation of sucrose is illustrated below. The number is 432.

To find which organism(s) may produce these reactions, the number 432 is found in the Code Book on the pages titled glucose fermenters, MacConkey-positive, oxidase-positive. The presumptive identification for the organism in the illustration is *Pasteurella pneumotropica*.

	Lysine	Arginine	Ornithine	Indole	Urea	Mannitol	Lactose	Sucrose
Numerical value of positive result	1	2	4	1	2	4	1	2
Test result	—	—	+	+	+	—	—	+
Score of result	0	0	4	1	2	0	0	2
Sum of scores (code number)	4			3			2	

Some of the code numbers may be generated from the reactions produced by more than one species. In these instances the organisms which would produce the number are listed along with additional characteristics which can be used to differentiate those organisms. For example, the reactions of both *Vibrio parahaemolyticus* and *V. vulnificus* may generate the code number 550. The Code Book indicates that the ONPG and cellobiose reactions can be used to differentiate these two species of bacteria.

For each of the tables in the Guide, there is a corresponding set of code numbers. In the Key for Identification of Gram-negative Aerobes, the table number and the pages of the Code Book which correspond to each category of the key are indicated.

The media which can be used to determine the key reactions are:

Triple sugar iron agar
OF glucose
OF base, control
} (mode of glucose utilization)
MacConkey agar (growth)
Blood agar (oxidase test)

The media used for the presumptive identification of gram-negative aerobic bacteria are listed below for each of the 12 tables.

Table 1. Glucose fermenters, MacConkey-positive/oxidase-negative

Urea agar	Lactose broth
Tryptone broth (indole)	Sucrose broth
Lysine, Moeller base	Motility agar (incubate at 35°C)
Arginine, Moeller base	Motility agar (incubate at 25°C)
Ornithine, Moeller base	SS agar slant
Moeller base, control	

Table 2. Glucose fermenters, MacConkey-positive/oxidase-positive

Lysine, Moeller base	Mannitol broth
Arginine, Moeller base	Lactose broth
Ornithine, Moeller base	Sucrose broth
Moeller base, control	20% NaCl solution, sterile
Tryptone broth (indole)	MR broth (optional test)
Urea agar	MR broth with 3% NaCl (optional test)

One drop of the 20% NaCl solution is added to each 1 ml of the Moeller base media and tryptone broth to enhance the growth of sodium-requiring organisms. The use of MR broth (which is essentially void of NaCl) and MR broth with 3% NaCl is to aid in the identification of the sodium-requiring organisms.

Table 3. Glucose fermenters, MacConkey-negative/oxidase-negative

Heart infusion agar slant (catalase)	Maltose broth
Nitrate broth, with insert tube	Xylose broth
Tryptone broth (indole)	Esculin agar slant
Lactose broth	

Table 4. Glucose fermenters, MacConkey-negative/oxidase-positive

Heart infusion agar slant (catalase)	Moeller base, control
Tryptone broth (indole)	Xylose broth
Urea agar	Mannitol broth
Nitrate broth, with insert tube	Sucrose broth
Ornithine, Moeller base	Maltose broth
	Blood agar (hemolysis, if not previously inoculated)

Table 5. Glucose oxidizers, MacConkey-positive/oxidase-negative

OF mannitol	Lysine, Moeller base
OF lactose	Arginine, Moeller base
OF sucrose	Moeller base, control
OF base, control	Esculin agar slant
Nitrate broth, with insert tube	Motility agar

Table 6. Glucose oxidizers, MacConkey-positive/oxidase-positive

Flo agar slant	Urea agar
Tech agar slant	OF xylose
Tryptone broth (indole)	OF mannitol
TSI slant (if not previously inoculated)	OF lactose
Lysine, Moeller base	OF sucrose
Arginine, Moeller base	OF maltose
Moeller base, control	OF base, control
Nitrate broth, with insert tube	

To aid in the identification of apyocyanogenic strains of *Pseudomonas aeruginosa*, it is recommended that cultures also be tested for the ability to grow at 42°C. *P. aeruginosa* grows at 42° while *P. fluorescens* and *P. putida* do not.

Table 7. Glucose oxidizers, MacConkey-negative/oxidase-negative

Urea agar	OF maltose
Tryptone broth (indole)	OF base, control
Nitrate broth, with insert tube	Arginine, Moeller base
OF xylose	Moeller base, control
OF sucrose	

Table 8. Glucose oxidizers, MacConkey-negative/oxidase-positive

Tryptone broth (indole)	OF lactose
Urea agar	OF sucrose
OF xylose	OF maltose
OF mannitol	OF base, control

Table 9. Nonoxidizers, MacConkey-positive/oxidase-negative

OF maltose	Motility agar
OF xylose	Urea agar
OF base, control	Nitrate broth, with insert tube
	Blood agar (hemolysis, if not previously inoculated)

Table 10. Nonoxidizers, MacConkey-positive/oxidase-positive

OF mannitol	SS agar slant
OF xylose	Motility agar
OF base, control	Heart infusion agar slant (incubate at 25°C for flagella stain)
TSI slant (if not previously inoculated)	Nitrite broth, with insert tube (optional test)
Urea agar	
Nitrate broth, with insert tube	

Table 11. Nonoxidizers, MacConkey-negative/oxidase-negative

Urea agar
Nitrate broth, with insert tube
Motility agar

Table 12. Nonoxidizers, MacConkey-negative/oxidase-positive

Tryptone broth (indole)	Nitrate broth, with insert tube
Urea agar	Motility agar
OF xylose	Heart infusion agar slant (catalase)
OF base, control	

NOTES

All strains were not tested in every test.

Symbols:

− = 0−10% +
v = 11−89% +
\+ = 90−100% + within 48 h
+ or(+) = <90% + in 48 h, >90% + in 3-7 days

Test results are recorded as above; numbers are percent-positive reactions.

Some *Bacillus* species may give a negative Gram stain reaction. The possibility that an organism, particularly if it does not grow on MacConkey agar, may be a *Bacillus* species should be considered if it can not be identified as a species of gram-negative bacteria.

Microscopic morphology is important for the recognition of some genera such as *Neisseria* and *Campylobacter*.

GUIDE TO PRESUMPTIVE IDENTIFICATION: KEY

GLUCOSE FERMENTERS

Table No.		Code Book Page(s)
	MacConkey-positive	
1	Oxidase-negative . . . . . *Chromobacterium violaceum, Enterobacteriaceae, Haemophilus aphrophilus,* HB-5, *Tatumella ptyseos, Yersinia enterocolitica, Y. pestis, Y. pseudotuberculosis.*	361
2	Oxidase-positive . . . . . *Actinobacillus equuli, A. lignieresii, A. suis, Aeromonas hydrophila, Chromobacterium violaceum,* EF-4a, *Haemophilus aphrophilus,* HB-5, *Kingella kingae, Neisseria mucosa, N. sicca, N. subflava, Pasteurella aerogenes, P. gallinarum, P. haemolytica, P. pneumotropica, Plesiomonas shigelloides, Vibrio alginolyticus, V. anguillarum, V. cholerae* (0:1 Group and other 0 Groups), *V. damsela, V. fluvialis, V. hollisae, V. parahaemolyticus, V. vulnificus.*	361-362
	MacConkey-negative	
3	Oxidase-negative . . . . . *Actinobacillus actinomycetemcomitans, Capnocytophaga* spp. (DF-1), DF-3, *Gardnerella vaginalis, Haemophilus aphrophilus,* HB-5, *Pasteurella multocida*, P. pneumotropica*, P. ureae*, Pasteurella* sp. "n. sp. 1."*	363
4	Oxidase-positive . . . . . *Actinobacillus actinomycetemcomitans, A. equuli, A. lignieresii, A. suis, Cardiobacterium hominis,* DF-2, EF-4a, *Haemophilus aphrophilus,* HB-5, *Kingella denitrificans, K. indologenes, K. kingae, Neisseria lactamica, N. mucosa, N. sicca, N. subflava, Pasteurella gallinarum, P. haemolytica, P. multocida, P. pneumotropica, P. ureae, Pasteurella* sp. "n. sp. 1", *Vibrio anguillarum, V. hollisae.*	364-366

GLUCOSE OXIDIZERS

	MacConkey-positive	
5	Oxidase-negative . . . . . *Acinetobacter calcoaceticus (A. anitratus), Brucella canis, Pseudomonas cepacia, P. mallei, P. maltophilia, P. marginata, P. paucimobilis,* Ⅴe-1, Ⅴe-2.	367-368
6	Oxidase-positive . . . . . *Achromobacter xylosoxidans, Agrobacterium radiobacter, Brucella abortus, B. canis, B. melitensis, B. suis,* EF-4b, EO-2, *Flavobacterium breve, F. meningosepticum, F. multivorum, F. spiritivorum, Flavobacterium* sp. (Ⅱb), *Pseudomonas aeruginosa, P. cepacia, P. diminuta, P. fluorescens, P. mallei, P. mendocina, P. mesophilica, P. paucimobilis, P. pickettii, P. pseudomallei, P. putida, P. putrefaciens* biotype 1, *P. stutzeri, P. thomasii, P. vesicularis,* Ⅴa-1, Ⅴb-3, Ⅴd (*Achromobacter* sp.).	368-373

*Included in this section because oxidase reaction may vary with the reagent used.

Table No.		Code Book Page(s)
	MacConkey-negative	
7	Oxidase-negative . . . . . *Brucella canis, Francisella tularensis, Pseudomonas mallei, P. paucimobilis,* IIe.	373
8	Oxidase-positive . . . . . *Brucella abortus, B. canis, B. melitensis, B. suis,* EF-4b, EO-2, *Flavobacterium meningosepticum, F. spiritivorum, Flavobacterium* species (IIb), *Neisseria gonorrhoeae, N. lactamica, N. meningitidis, Pasteurella anatipestifer, Pseudomonas mallei, P. mesophilica, P. paucimobilis, P. vesicularis,* IIe, IIh, IIi.	373-374
	NONOXIDIZERS	
	MacConkey-positive	
9	Oxidase-negative . . . . . *Acinetobacter calcoaceticus (A. lwoffi), Bordetella parapertussis, Brucella canis, Pseudomonas maltophilia.*	375
10	Oxidase-positive . . . . . *Achromobacter xylosoxidans, Alcaligenes denitrificans, A. faecalis, A. odorans, Bordetella bronchiseptica, Brucella abortus, B. canis, B. melitensis, Campylobacter fetus* subsp. *fetus, C. fetus* subsp. *jejuni, Flavobacterium odoratum,* M-5, M-6, *Moraxella atlantae, M. osloensis, M. phenylpyruvica, M. urethralis, Neisseria flavescens, N. mucosa, N. sicca, N. subflava, Pseudomonas acidovorans, P. alcaligenes, P. diminuta, P. mesophilica, P. pseudoalcaligenes, P. putrefaciens* biotype 1, *P. putrefaciens* biotype 2, *P. testosteroni, P. vesicularis, Pseudomonas* sp. CDC Group 1, IVc-2 IVe, Vd (*Achromobacter* sp.).	375-377
	MacConkey-negative	
11	Oxidase-negative . . . . . *Brucella canis, Francisella tularensis.*	377
12	Oxidase-positive . . . . . *Bordetella pertussis, Branhamella catarrhalis, Brucella abortus, B. canis, B. melitensis, Campylobacter fetus* subsp. *fetus, C. fetus* subsp. *jejuni, C. fetus* subsp. *venerealis, Eikenella corrodens,* M-5, M-6, *Moraxella atlantae, M. bovis, M. lacunata, M. nonliquefaciens, M. osloensis, M. phenylpyruvica, Neisseria elongata, N. flavescens, N. mucosa, N. sicca, N. subflava, Pseudomonas mesophilica, P. vesicularis,* IIf, IIj, IVe.	377-378

Table 1: Glucose Fermenters, MacConkey-positive/oxidase-negative

Tests	Number of Strains	Glucose	Growth on MacConkey	Oxidase	Urea	Indole	Lysine	Arginine	Ornithine	Lactose	Sucrose	Motility 35°C	Motility 25°C	Growth on SS
Numerical Value of Positive Result					1	2	4	1	2	4	1	2	4	1
Chromobacterium violaceum	37	+ 100	+ 100	V 67	V 5(14)	V 21	– 0	+ 100	– 0	– 0	V 20(6)	+ *	+	+ or (+) 71(23)
Enterobacteriaceae		+	+	–										
Haemophilus aphrophilus	203	+ 98(2)	V 19	V 28	– 0	– 0	– 0	– 0	– 0	+ 96(4)	+ 99(1)	–	–	– 0
HB-5	44	+ 100	V 34(23)	V 54	– 0	+ 100	– 0	– 0	– 0	– 0	– 0	–	–	– 0
Tatumella ptyseos	41	+ 100	+ 93	– 7	– 0	– 0	– 0	V 12	– 0	– 0	+ 100	–	V	– 2
Yersinia enterocolitica	83	+ 100	+ 100	– 0	+ or (+) 70(30)	V 79(1)	– 0	– 0	+ 100	V 18(18)	+ 98	–	+	+ 90(6)
Yersinia pestis	37	+ 100	+ 100	– 0	– 0	– 0	– 0	– 0	– 0	– 0	– 0	–	–	– 3(3)
Yersinia pseudotuberculosis	34	+ 100	+ 100	– 3	+ 100	– 0	– 0	– 0	– 0	– 0	– 0	–	V	V 17(41)

*Where blank spaces occur either the percentages were not calculated or they were not available.

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Table 2: Glucose Fermenters, MacConkey-positive/oxidase-positive

Tests	Number of Strains	Glucose	Growth on MacConkey	Oxidase	Lysine	Arginine	Ornithine	Indole	Urea	Mannitol	Lactose	Sucrose
Numerical Value of Positive Result					1	2	4	1	2	4	1	2
Actinobacillus equuli	19	+ 100	V 84(5)	+ 100	– 0	– 0	– 0	– 0	+ 100	+ 100	+ 95	+ 100
Actinobacillus lignieresii	30	+ 96(4)	V 67(8)	+ 100	– 0	– 0	– 0	– 0	+ 100	+ 91(9)	V 17(61)	+ 96(4)
Actinobacillus suis	33	+ 94(6)	+ or(+) 82(12)	+ 100	– 0	– 0	– 0	– 0	+ 97(3)	V 54	+ or(+) 79(18)	+ 94(6)
Aeromonas hydrophila	115	+ 97(3)	+ 100	+ 99	V 17	+ 99	– 0	+ 92	– 2	+ 100	V 20(9)	V 87(1)
Chromobacterium violaceum	37	+ 100	+ 100	V 67	– 0	+ 100	– 0	V 21	V 5(14)	– 0	– 0	V 20(6)
EF-4a	97	+ 100	V 42(8)	+ 100	– 0	V 77(2)	– 0	– 0	– 0	– 0	0	– 0
Haemophilus aphrophilus	203	+ 98(2)	V 19	V 28	– 0	– 0	– 0	– 0	– 0	– 0	+ 96(4)	+ 99(1)
HB-5	44	+ 100	V 34(23)	V 54	– 0	– 0	– 0	+ 100	– 0	– 0	– 0	– 0
Kingella kingae	33	+ or(+) 42(48)	V 33(3)	+ 100	–	–	–	– 0	– 0	– 0	– 0	– 0
Neisseria mucosa	30	+ or(+) 83(10)	V 57(3)	+ 100	–	–	–	–	– 0	– 0	– 0	+ 90(10)
Neisseria sicca	43	+ or(+) 78(14)	V 68	+ 100	–	–	–	–	– 0	– 0	– 0	+ 92(8)
Neisseria subflava	153	V 68(6)	V 47(2)	+ 100	–	–	–	–	– 0	– 0	– 0	V 56(5)
Pasteurella aerogenes	16	+ 100	+ 100	+ 100	– 0	– 0	V 88	– 0	+ 100	+ 6	V 19(38)	+ 94
Pasteurella gallinarum	10	+ 90(10)	V 20(10)	+ 90	– 0	– 0	V 25	– 0	– 0	– 0	– 0	+ 100
Pasteurella haemolytica	67	+ 96(4)	V 79(6)	+ 95	– 3	– 0	– 10	– 0	– 0	V 30(9)	V 7(34)	+ 97(3)
Pasteurella pneumotropica	107	+ 97(3)	V 36(17)	+ 99	V 33	– 0	+ 100	+ 90	+ 95(1)	– 2(1)	V 14(39)	+ 97(3)
Plesiomonas shigelloides	28	+ 96(4)	+ 100	+ 96	+ 100	+ 100	+ 100	+ 96	– 0	– 0	+ 92(4)	– 0
Vibrio alginolyticus	58	+ 100	+ 100	+ 100	+ 100	– 0	V 57	+ 90	– 4	+ 100	– 0	+ 100
Vibrio anguillarum	1	+	(+)	+	–	+	–	+	–	+	–	+
Vibrio cholerae (01 Group and other O Groups)	82	+ 100	+ 100	+ 100	+ 100	– 0	+ 99	+ 100	– 4(3)	+ 96(2)	V 11(28)	+ 90
Vibrio damsela	23	+ 100	+ 100	+ 91	V 27(4)	+ 100	– 0	– 0	+ 100	– 0	– 0	– (9)
Vibrio fluvialis	25	+ 100	+ 92(8)	+ 100	– 0	+ 96	– 0	V 24	– 0	+ 100	– (8)	+ 100
Vibrio hollisae	12	+ 100	V 64(18)	+ 100	– 0	– 8	– 0	+ 100	– 0	– 0	– 0	– 0
Vibrio parahaemolyticus	160	+ 100	+ 100	+ 100	+ 100	+ 0	+ 98(1)	+ 100	– 8	+ 98(1)	– 0	– 2
Vibrio vulnificus	47	+ 100	+ 100	+ 100	+ 100	0	+ or(+) 89(8)	+ 100	– 0	V 62	+ or(+) 87(11)	– 2

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Table 3: Glucose Fermenters, MacConkey-negative/oxidase-negative

Tests	Number of Strains	Glucose	Growth on MacConkey	Oxidase	Catalase	Nitrate Reduction	Indole	Lactose	Maltose	Xylose	Esculin Hydrolysis
Numerical Value of Positive Result					1	2	4	1	2	4	1
Actinobacillus actinomycetemcomitans	120	+ or(+) 83(16)	– 4(1)	V 19	+ 99	+ 100	– 0	– 0	+ or(+) 80(15)	V 33(9)	– 0
Capnocytophaga spp. (DF-1)	155	+ 90(10)	– 0	– 7	– 7	V 63	– 0	V 75(11)	+ or(+) 86(14)	– 0	V 81(2)
DF-3	21	+ or(+) 86(14)	– 0	– 0	– 0	– 0	V 71(14)	+ or(+) 52(43)	+ or(+) 81(19)	+ or(+) 86(14)	+ 100
Gardnerella vaginalis	126	+ or(+) 81(13)	– 5	– 6	– 2	– 0	– 0	– (1)	+ or(+) 87(13)	V 36(9)	– 0
Haemophilus aphrophilus	203	+ 98(2)	V 19	V 28	– 5	+ 100	– 0	+ 96(4)	+ 99(1)	– 0	– 0
HB-5	44	+ 100	V 34(23)	V 54	– 2	+ 100	+ w 100	– 0	– 0	– 0	– 0
Pasteurella multocida	306	+ 100	– 2(1)	+ 97	+ 98	+ 99	+ 99	– 8	– 2	V 67	– 0
Pasteurella pneumotropica	107	+ 97(3)	V 36(17)	+ 99	+ 100	+ 100	+ 90	V 14(39)	+ 97(3)	+ or(+) 76(19)	– 0
Pasteurella ureae	97	+ 100	– 0	+ 99	V 63	+ 99	– 0	– 0	+ 91(5)	– 0	– 0
Pasteurella sp., "n. sp. 1"	91	+ 100	– 1	+ 98	+ 96	+ 100	+ 100	– 3	+ 100	– 0	– 0

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Table 4: Glucose Fermenters, MacConkey-negative/oxidase-positive

Tests	Number of Strains	Glucose	Growth on MacConkey	Oxidase	Catalase	Indole	Urea	Nitrate reduction	Nitrate to gas	Ornithine Decarboxylase	Xylose	Mannitol	Sucrose	Maltose	Beta like Hemolysis
Numerical Value of Positive Result					1	2	4	1	2	4	1	2	4	1	2
Actinobacillus actinomycetemcomitans	120	+ or (+) 83(16)	– 4(1)	V 19	+ 99	– 0	– 0	+ 100	– 0	–	V 33(9)	V 66(16)	– 0	+ or (+) 80(15)	– 0
Actinobacillus equuli	19	+ 100	V 83(5)	+ 100	V 68(5)	– 0	+ 100	+ 100	– 0	– 0	+ 100	+ 100	+ 100	+ 95	– 0
Actinobacillus lignieresii	30	+ 96(4)	V 67(8)	+ 100	V 89	– 0	+ 100	+ 100	– 0	– 0	+ or (+) 87(13)	+ 91(9)	+ 96(4)	+ or (+) 83(17)	– 4
Actinobacillus suis	33	+ 94(6)	+ or (+) 82(12)	+ 100	V 85	– 0	+ 97(3)	+ 100	– 0	– 0	+ 94(6)	V 54	+ 94(6)	+ 94(6)	V 76
Cardiobacterium hominis	65	+ or (+) 80(20)	– 0	+ 100	1	+ 100	– 0	– 0	–	–	– 0	+ or (+) 52(43)	+ or (+) 63(35)	+ or (+) 69(31)	– 0
DF-2	27	V 67(18)	– 0	+ 96	+ 100	– 0	– 0	– 0	– 0	– 0	– 0	– 0	– 0	+ or (+) 81(19)	– 4
EF-4a	97	+ 100	V 42(8)	+ 100	+ 100	– 0	– 0	+ 97	V 62	– 0	– 0	– 0	– 0	– 0	– 1
Haemophilus aphrophilus	203	+ 98(2)	V 19	V 28	– 5	– 0	– 0	+ 100	– 0	– 0	– 0	– 0	+ 99(1)	+ 99(1)	– 0
HB-5	44	+ 100	V 34(23)	V 54	– 2	+ 100	– 0	+ 100	– 0	– 0	– 0	– 0	– 0	– 0	– 0
Kingella denitrificans	60	+ or (+) 30(62)	– 0	+ 100	– 10	– 0	– 0	+ 93	V 88	–	– 0	– 0	– 0	– 0	– 0
Kingella indologenes	1	+	–	+	–	+	–	–	–	–	–	–	+	(+)	–
Kingella kingae	33	+ or (+) 42(48)	V 33(3)	+ 100	– 0	– 0	– 0	– 3(3)	– 0	-	– 0	- 0	– 0	+ or (+) 76(24)	V 84
Neisseria lactamica	287	+ 96(3)	– 0	+ 100	+ 100	–	– 0	– 0	–	–	–	– 0	– 0	+ 98(2)	– 0
Neisseria mucosa	30	+ or (+) 83(10)	V 57(3)	+ 100	+ 100	–	– 0	+ 100	+ 100	-	–	– 0	+ 90(10)	+ 90(10)	– 0
Neisseria sicca	43	+ or (+) 78(14)	V 68	+ 100	V 80	–	– 0	– 0	–	-		- 0	+ 92(8)	+ 95(5)	– 7
Neisseria subflava	153	V 68(6)	V 47(2)	+ 100	V 80	–	- 0	– 0			-	– 0	V 56(5)	+ 99(1)	– 9
Pasteurella gallinarum	10	+ 90(10)	V 20(10)	+ 90	+ 100	– 0	– 0	+ 100	– 0	V 25	V 33	– 0	+ 100	+ 100	– 0
Pasteurella haemolytica	67	+ 98(2)	V 79(6)	+ 95	V 84(2)	– 0	– 0	+ 100	– 0	– 10	V 66(3)	V 30(9)	+ 97(3)	+ or (+) 85(13)	V 72
Pasteurella multocida	306	+ 100	– 2(1)	+ 97	+ 98	+ 99	– 0	+ 99	- 0	+ 94	V 67	V 78	+ 100	– 2	– 0
Pasteurella pneumotropica	107	+ 97(3)	V 36(17)	+ 99	+ 100	+ 90	+ 95(1)	+ 100	– 0	+ 100	+ or (+) 76(19)	– 2(1)	+ 97(3)	+ 97(3)	– 0
Pasteurella ureae	97	+ 100	– 0	+ 99	V 63	– 0	+ 100	+ 99	– 0	- 0	- 0	+ 99(1)	+ 99(1)	+ 91(5)	– 0
Pasteurella sp., "n. sp. 1"	91	+ 100	– 1	+ 98	+ 96	+ 100	V 78(3)	+ 100	- 0	– 0	– 0	- 0	+ 99	+ 100	– 0
Vibrio anguillarum	1	+	(+)	+	+	+	-	+	-		-	+	+	+	+
Vibrio hollisae	12	+ 100	V 64(8)	+ 100	+ 100	+ 100	– 0	+ 100	0	– 0	0	– 0	– 0	– 0	V 50

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Table 5: Glucose Oxidizers, MacConkey-positive/oxidase-negative

Tests	Number of Strains	Glucose	Growth on MacConkey	Oxidase	OF Mannitol	OF Lactose	OF Sucrose	Nitrate Reduction	Lysine	Arginine	Esculin Hydrolysis	Motility
Numerical Value of Positive Result					1	2	4	1	2	4	1	2
Acinetobacter calcoaceticus (*A. anitratus*)	501	+ 100	+ 99(1)	− 0	2	+ 97(2)	0	1			0	0
Brucella canis	28	+ or (+) 81(14)	V 12(29)	V 72	0	− 0	0	+ 100	−		0	0
Pseudomonas cepacia	159	+ 100	+ 100	V 86	+ 100	+ 99(1)	V 86(1)	V 52	V 80	0	V 63(6)	+
Pseudomonas mallei	8	+ 100	V 88	V 25	V 62(14)	V 12(62)	0	+ 100	0	+ 100	0	− 0
Pseudomonas maltophilia	228	+ or (+) 85(5)	+ 100	0	− 0	V 60(1)	V 63(1)	V 39	+ 93	0	V 39	+
Pseudomonas marginata	1	+	+	−	+	−		−				+
Pseudomonas paucimobilis	134	+ 93(7)	V 10(13)	V 75	− 0	+ 93(7)	+ 93(7)	3	0	8	+ 91	V
Ye-1	18	+ 100	+ 100	0	V 82(6)	V 6(28)	6	V 67	− 0	+ 100	+ 100	+
Ye-2	36	+ 100	+ 100	0	+ 100	V 14(22)	V 25	6	− 7	V 14	0	+

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Table 6: Glucose Oxidizers, MacConkey-positive/oxidase-positive

Tests	Number of Strains	Glucose	Growth on MacConkey	Oxidase	Pyocyanin	Pyoverdin (Fluorescin)	Indole	H_2S, TSI	Lysine Decarboxylase	Arginine Dihydrolase	Nitrate Reduction	Nitrate to Gas	Urea	OF Xylose	OF Mannitol	OF Lactose	OF Sucrose	OF Maltose
Numerical Value of Positive Result					1	2	4	1	2	4	1	2	4	1	2	4	1	2
Achromobacter xylosoxidans	135	V 78	+ 100	+ 100	– 0	– 0	– 0	– 0	– 0	V 13	+ 100	V 60	– 0	+ 99	– 0	– 0	– 0	– 0
Agrobacterium radiobacter	38	+ 100	+ 100	+ 100	– 0	– 0	– 0	V 8(5)	– 0	– 3	V 87	– 5	+or(+) 89(11)	+ 100	+ 100	+ 100	+ 100	+ 100
Brucella species	347	+or(+) 80(10)	V 23(27)	+ 92	– 0	– 0	– 0	– 0	–	–	+ 100	V 44	+ 99	+ 90(10)	– 0	– 0	– 0	– 0
EF-4b	34	+or(+) 70(26)	V 65(6)	+ 100	– 0	– 0	– 0	– 0	– 0	– 0	+ 97	– 0	– 0	– 0	– 0	– 0	– 0	– 0
EO-2	93	+ 100	V 63(11)	+ 100	– 0	– 0	– 0	– 0	– 0	– 0	V 85	– 2	V 74(3)	+ 99(1)	V 10(10)	+ 91(6)	– 0	V 20(1)
Flavobacterium breve	3	+ 100	+ 100	+ 100	– 0	– 0	+ 100	– 0	– 0	– 0	– 0	– 0	– 0	– 0	– 0	– 0	– 0	+ 100
Flavobacterium meningosepticum	148	+ 95(4)	V 89(3)	+ 99	– 0	– 0	+ 100	– 0	–	–	– 0	–	– 3(5)	– 2(1)	+ 91(8)	V 42(15)	– 0	+ 93(7)
Flavobacterium multivorum	22	+ 100	+ 100	+ 100	– 0	– 0	– 0	– 0	– 0	– 0	– 0	– 0	+ 95	+ 100	– 0	+ 100	+ 100	+ 100
Flavobacterium spiritivorum	11	+ 100	V (55)	+ 100	– 0	– 0	– 0	– 0	– 0	V 25	– 0	– 0	+or(+) 55(36)	+ 91(9)	+ 100	+ 100	+ 100	+ 100
Flavobacterium sp. (IIb)	155	+ 92(6)	V 54(9)	+ 96	– 0	– 0	+ 98	– 1	–	–	V 22	– 0	V 14(28)	V 30(1)	– 10	– 0	V 13(1)	+ 92(6)
Pseudomonas aeruginosa	201	+ 97(1)	+ 100	+ 99	V 46	V 65	– 0	– 0	– 0	+ 100	+ 98	+ 93	V 48(9)	+ 90(1)	V 70(3)	– 1	– 0	– (1)
Pseudomonas cepacia	159	+ 100	+ 100	V 86	– 0	– 0	– 0	– 0	V 80	– 0	V 57	– 0	V 60(18)	+ 100	+ 100	+ 99(1)	V 86(1)	+ 99(1)
Pseudomonas diminuta	68	V 21(9)	+ 97(3)	+ 100	– 0	– 0	– 0	– 0	– 0	– 0	– 3	– 0	V (13)	– 0	– 0	– 0	– 0	– 0
Pseudomonas fluorescens	155	+ 100	+ 100	+ 97	– 0	+ 96	– 0	– 0	– 0	+ 97	V 19	– 3	V 21(31)	+ 100	V 53(2)	V 24(3)	V 48	– 2
Pseudomonas mallei	8	+ 100	V 88	V 25	– 0	– 0	– 0	– 0	– 0	+ 100	+ 100	– 0	V 12	V 12(50)	V 62(14)	V 12(62)	– 0	V (75)
Pseudomonas mendocina	3	+ 100	+ 100	+ 100	– 0	– 0	– 0	– 0	– 0	+ 100	+ 100	+ 100	V 33	+ 100	– 0	– 0	– 0	– 0
Pseudomonas mesophilica	90	V 40	V 15	+ 96	– 0	– 0	– 0	– 0	–	–	V 25	– 0	V 29(26)	+ 94	– 2	– 0	– 0	– 2
Pseudomonas paucimobilis	134	+ 93(7)	V 10(13)	V 75	– 0	– 0	– 0	– 0	– 0	– 8	– 3	– 0	– 6(3)	+ 96(4)	– 0	+ 93(7)	+ 93(7)	+ 97(3)
Pseudomonas pickettii	54	+ 100	+ 100	+ 100	– 0	– 0	– 0	– 0	– 0	– 0	+ 100	+ 100*	+ 100	+ 100	– 0	– 0	– 0	– 0
Pseudomonas pseudomallei	70	+ 100	+ 100	+ 100	– 0	– 0	– 0	– 0	– 0	+ 100	+ 100	+ 100*	V 13(8)	+or(+) 86(14)	+ 94(6)	+ 99(1)	V 66(4)	+ 99(1)
Pseudomonas putida	16	+ 100	+ 100	+ 100	– 0	+ 93	– 0	– 0	– 0	+ 100	– 0	– 0	V 13(44)	+ 100	V 25	V 25(13)	– 0	V 31
Pseudomonas putrefaciens, biotype 1	24	V 17(33)	+ 100	+ 100	– 0	– 0	– 0	+ 96	– 0	– 0	+ 100	– 0	V 4(8)	– 0	– 0	– 0	+ 96(4)	+ 92(8)
Pseudomonas stutzeri	28	+ 96(4)	+ 100	+ 100	– 0	– 0	– 0	– 0	– 0	– 0	+ 100	+ 100	V 33(22)	+ 93(7)	+or(+) 89(4)	– 0	– 0	+ 100
Pseudomonas thomasii [a]	31	+ 100	+ 100	+ 100	– 0	– 0	– 0	– 0	– 0	– 3	V 13	– 0	+or(+) 81(19)	+ 100	+ 100	+ 100	– 0	+ 100
Pseudomonas vesicularis	47	+or(+) 87(9)	V 81(8)	+ 94	– 0	– 0	– 0	– 0	– 0	– 0	– 6	– 0	– (8)	V 21(2)	– 0	– 0	– 0	+ 98(2)
∇a-1[b]	70	+ 100	+ 99(1)	+ 100	– 0	– 0	– 0	– 0	– 0	– 6	+ 100	V 86	+ 100	+ 100	– 0	+ 100	– 0	+ 100
∇b-3	65	+ 100	+ 100	+ 100	– 0	– 0	– 0	– 0	– 0	+ 100	+ 100	+ 100	V 12(45)	+ 97(3)	V 65(9)	– 0	– 0	+ 95(5)
∇d (*Achromobacter* sp.)	71	+or(+) 86(13)	+ 100	+ 100	– 0	– 0	– 0	V 49	– 0	V 68	+ 100	+ 99	+ 92(8)	+ 96(4)	V 46(34)	– 0	V 28(25)	V 32(25)

*The volume of gas may be small. Gas may not be detected unless incubated at 25°C or in semianaerobic nitrate broth; therefore, in arriving at code numbers, the possibility that this reaction may appear negative was allowed for.

[a] Possibly a biovar of *P. pickettii*.

[b] Shown by DNA hybridization to be a biovar of *P. pickettii*.

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Table 7: Glucose Oxidizers, MacConkey-negative/oxidase-negative

Tests	Number of Strains	Glucose	Growth on MacConkey	Oxidase	Urea	Indole	Nitrate Reduction	OF Xylose	OF Sucrose	OF Maltose	Arginine Dihydrolase
Numerical Value of Positive Result					1	2	4	1	2	4	1
Brucella canis	28	+ or(+) 81(14)	V 12(29)	V 72	+ 100	– 0	+ 100	+ or(+) 81(19)	– 0	– 0	–
*Francisella tularensis**	53	+ or(+) 86(14)	– 0	– 2	– 0	– (3)	– 4	-	-	–	–
Pseudomonas mallei	8	+ 100	V 88	V 25	V 12	– 0	+ 100	V 12(50)	– 0	V (75)	+ 100
Pseudomonas paucimobilis	134	+ 93(7)	V 10(13)	V 75	– 6(3)	– 0	– 3	+ 96(4)	+ 93(7)	+ 97(3)	8
IIe	18	+ 100	– 7	V 88	– 0	+ 100	– 0	– 0	– 0	+ 100	–

*Carbohydrate medium containing cystine or cysteine must be used to demonstrate acid production from carbohydrates. Identification on basis of enhancement of growth by or requirement for cystine or cysteine in media and by serology.

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Table 8: Glucose Oxidizers, MacConkey-negative/oxidase-positive

Tests	Number of Strains	Glucose	Growth on MacConkey	Oxidase	Indole	Urea	OF Xylose	OF Mannitol	OF Lactose	OF Sucrose	OF Maltose
Numerical Value of Positive Result					1	2	4	1	2	4	1
Brucella species	347	+ or (+) 80(10)	V 23(27)	+ 92	– 0	+ 99	+ 98(10)	0	– 0	– 0	0
EF 4b	34	+ or (+) 70(26)	V 65(6)	+ 100	0	– 0	0	0	+ 0	0	0
EO-2	93	+ 100	V 63(11)	+ 100	– 0	V 74(3)	+ 99(1)	V 10(10)	+ 91(6)	0	V 20(1)
Flavobacterium meningosepticum	148	+ 95(4)	+ or (+) 89(3)	+ 99	+ 100	3(5)	2(1)	+ 91(8)	V 42(15)	– 0	+ 93(7)
Flavobacterium spiritivorum	11	+ 100	V (55)	+ 100	– 0	+ or (+) 55(36)	+ 91(9)	+ 100	+ 100	+ 100	+ 100
Flavobacterium species (IIb)	155	+ 92(6)	V 54(9)	+ 96	+ 98	V 14(28)	V 30(1)	10	0	V 13(1)	+ 92(6)
Neisseria gonorrhoeae[1]	197	+ 90(3)	– 0	+ 100				0	– 0	– 0	0
Neisseria lactamica[1]	287	+ 96(3)	– 0	+ 100	–	0		0	+ 95(5)	– 0	+ 98(2)
Neisseria meningitidis[1]	375	+ 99	0	+ 100	–	–	–	– 0	0	0	+ 99
Pasteurella anatipestifer	1	+		+		+	–				+
Pseudomonas mallei	8	+ 100	V 88	V 25	– 0	V 12	V 12(50)	V 62(14)	V 12(62)	0	V (75)
Pseudomonas mesophilica	90	V 40	V 15	+ 96	– 0	V 29(26)	+ 94	2	0	0	– 2
Pseudomonas paucimobilis	134	+ 93(7)	V 10(13)	V 75	– 0	– 6(3)	+ 96(4)	– 0	+ 93(7)	+ 93(7)	+ 97(3)
Pseudomonas vesicularis	47	+ or (+) 87(9)	V 81(8)	+ 94	0	(8)	V 21(2)	0	0	– 0	+ 98(2)
IIe	18	+ 100	7	V 88	+ 100	0	– 0	0	– 0	0	+ 100
IIh	21	+ or (+) 85(15)	– 0	+ 100	+ 100	0	– 5	– 0	– 0	– 0	+ 95
IIi	23	+ 91(9)	– 0	+ 100	+ 100	V 14(18)	+ or (+) 87(13)	0	+ 91(9)	+ 91(9)	+ 91(9)

*Carbohydrate reactions obtained in CTA medium. *Neisseria* species also recognized by morphology.

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Table 9: Nonoxidizers, MacConkey-positive/oxidase-negative

Tests	Number of Strains	Glucose	Growth on MacConkey	Oxidase	OF Maltose	Motility	Urea	Nitrate Reduction	"Beta" Hemolysis	OF Xylose
Numerical Value of Positive Result					1	2	4	1	2	4
Acinetobacter calcoaceticus (A. lwoffi)	253	– 0	+ 90(7)	– 0	– 0	– 0	– 5(4)	– 3	V 29	– 0
Bordetella parapertussis	7	– 0	+ 100	– 0	– 0	– 0	+ 100	– 0	+ 100	– 0
Brucella canis	28	+ ort +) 81(14)	V 12(29)	V 72	– 0	– 0	+ 100	+ 100	– 0	+ ort +) 81(19)
Pseudomonas maltophilia	228	+ ort +) 85(5)	+ 100	– 0	+ 100	+	V 3(12)	V 39	– 2	V 35(1)

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Table 10: Nonoxidizers, MacConkey-positive/oxidase-positive

Tests	Number of Strains	Glucose	Growth on MacConkey	Oxidase	OF Mannitol	OF Xylose	Nitrate to Gas	Nitrate Reduction	H_2S TSI	Urea	Growth on SS	Motility	1-2 Polar Flagella	> 2 Polar Flagella	Peritrichous (lateral & polar)
Numerical Value of Positive Result					1	2	4	1	2	4	1	2	4	1	2
Achromobacter xylosoxidans	135	V 78	+ 100	+ 100	– 0	+ 99	V 60	+ 100	– 0	– 0	+ 98	+	–	–	+
Alcaligenes denitrificans	34	– 0	+ 100	+ 100	– 0	– 0	+ 100	+ 100	– 0	V 12(3)	V 65	+	–	–	+
Alcaligenes faecalis	69	– 0	+ 100	+ 100	– 0	– 0	– 0	V 45	– 0	– (1)	V 78(2)	+	–	–	+
Alcaligenes odorans	49	– 0	+ 100	+ 100	– 0	– 0	– 0	– 0	– 0	– 2	+ 100	+	–	–	+
Bordetella bronchiseptica	85	– 0	+ 100	+ 100	– 1	– 7	– 0	+ 92	– 0	+ 99	+ 99	+	–	–	+
Brucella species	347	+or(+) 80(10)	V 23(27)	+ 92	– 0	+ 90(10)	V 44	+ 100	– 0	+ 99	– 0	–	–	–	–
Campylobacter fetus subsp. *fetus*	113	– 0	+or(+) 83(12)	+ 100	– 0	– 0	– 0	+ 100	– 0	– 0	– 0	+	+	–	–
Camplylobacter fetus subsp. *jejuni*	41	– 0	V 44(12)	+ 97	– 0	– 0	– 0	+ 100	– 0	– 0	– 0	+	+	–	–
Flavobacterium odoratum	74	– 0	+ 91(5)	+ 99	– 0	– 0	–	– 0	– 0	+ 100	V 30(11)	–	–	–	–
M-5	59	– 0	V 42(20)	+ 100	– 0	– 0	–	– 0	– 0	– 0	– 0	–	–	–	–
M-6	40	– 0	V 22(28)	+ 100	– 0	– 0	–	+ 100	– 0	– 0	– 0	–	–	–	–
Moraxella atlantae	23	– 0	+or(+) 87(13)	+ 100	– 0	– 0	–	– 5	– 0	– 0	– 0	–	–	–	–
Moraxella osloensis	163	– 0	V 70	+ 100	– 0	– 0	–	V 24	– 0	– 0	– 0	–	–	–	–
Moraxella phenylpyruvica	50	– 0	V 80(6)	+ 100	– 0	– 0	–	V 68	– 0	+ 100	– 0	–	–	–	–
Moraxella urethralis	22	– 0	+ 96	+ 100	– 0	– 0	–	– 0	– 0	– 0	– 9	–	–	–	–
Neisseria flavescens	10	– 0	V 67	+ 100	– 0	–	–	– 0	– 0	– 0	– 0	–	–	–	–
Neisseria mucosa	30	+or(+) 83(10)	V 57(3)	+ 100	– 0	–	+ 100	+ 100	– 0	– 0	– 0	–	–	–	–
Neisseria sicca	43	+or(+) 78(14)	V 68	+ 100	– 0	–	–	– 0	– 0	– 0	– 0	–	–	–	–
Neisseria subflava	153	V 68(6)	V 47(2)	+ 100	– 0	–	–	– 0	– 0	– 0	– 0	–	–	–	–
Pseudomonas acidovorans	64	– 0	+ 100	+ 100	+ 100	– 0	– 0	+ 98	– 0	– (3)	V 67(19)	+	–	+	–
Pseudomonas alcaligenes	26	– 0	+ 96	+ 96	– 0	– 0	– 0	V 54	– 0	– 0	V 38(8)	+	+	–	–
Pseudomonas diminuta	68	V 21(9)	+ 97(3)	+ 100	– 0	– 0	– 0	– 3	– 0	V (13)	– 1(1)	+	+	–	–
Pseudomonas mesophilica	90	V 40	V 15	+ 96	– 2	+ 94	– 0	V 25	– 0	V 29(26)	– 0	+	+	–	–
Pseudomonas pseudoalcaligenes	19	– 0	+ 100	+ 100	– 0	V 32	– 0	+ 90	– 0	– 5	V 84	+	+	–	–
Pseudomonas putrefaciens, biotype 1	24	V 17(33)	+ 100	+ 100	– 0	– 0	– 0	+ 100	+ 96	V 4(8)	– (8)	+	+	–	–
Pseudomonas putrefaciens, biotype 2	26	– 0	+ 100	+ 100	– 0	– 0	– 0	+ 100	+ 100	V 42	+ 92(4)	+	+	–	–
Pseudomonas testosteroni	28	– 0	+ 96(4)	+ 100	– 0	– 0	– 0	+ 96	– 0	V 7(14)	V 21(11)	+	–	+	–
Pseudomonas vesicularis	47	+or(+) 87(9)	V 81(8)	+ 94	– 0	V 21(2)	– 0	– 6	– 0	– (8)	– 6(2)	+	+	–	–
Pseudomonas sp. CDC Group 1	31	– 0	+ 97(3)	+ 100	– 0	– 0	+ 100	+ 100	– 0	V 3(7)	V 30(6)	+	+	–	–
IVc-2	36	– 0	+ 94(6)	+ 100	– 0	– 0	– 0	V 11	– 0	+ 100	– 3(6)	+	–	–	+
IVe	37	– 0	V 62(27)	+ 100	– 0	– 0	V 60	+ 100	– 0	+ 97	– 5	V	–	–	V*
Vd (*Achromobacter* sp.)	71	+or(+) 86(13)	+ 100	+ 100	V 46(34)	+ 96(4)	+ 99	+ 100	V 49	+ 92(8)	+ 96(1)	+	–	–	+

*Motility may be variable, but flagella, if present, are always peritrichous (usually 1-2 polar and/or lateral).

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Table 11: Nonoxidizers, MacConkey-negative/oxidase-negative

Tests	Number of Strains	Glucose	Growth on MacConkey	Oxidase	Urea	Nitrate Reduction	Motility
Numerical Value of Positive Result					1	2	4
Brucella canis	28	+ or (+) 81(14)	V 12(29)	V 72	+ 100	+ 100	–
Francisella tularensis	53	+ or (+)* 86(14)	– 0	– 2	– 0	– 4	–

*Glucose reaction determined in cysteine agar with phenol red indicator

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Table 12: Nonoxidizers, MacConkey-negative/oxidase-positive

Tests	Number of Strains	Glucose	Growth on MacConkey	Oxidase	Indole	Urea	OF Xylose	Nitrate Reduction	Motility	Catalase
Numerical Value of Positive Result					1	2	4	1	2	4
Bordetella pertussis	51		−	+ 94	−	− 0	−	−		+ 100
Branhamella catarrhalis	74	0	5	+ 100	0	− 0	− 0	+ 92	−	+ 100
Brucella species	347	+ or (+) 80(10)	V 23(27)	+ 92	− 0	+ 99	+ 90(10)	+ 100		+ 100
Campylobacter fetus subsp. *fetus*	113	0	+ or (+) 83(12)	+ 100	− 0	− 0	− 0	+ 100	+	+ 99(1)
Campylobacter fetus subsp. *jejuni*	41	0	V 44(12)	+ 97	− 0	− 0	− 0	+ 100	+	+ 100
Campylobacter fetus subsp. *venerealis*	2	− 0	0	+ 100	− 0	0	0	+ 100	+	+ 100
Eikenella corrodens	506	0	0	+ 100	− 0	0	0	+ 99		− 8
M-5	59	0	V 42(20)	+ 100	− 0	− 0	− 0	− 0	−	+ 100
M-6	40	− 0	V 22(28)	+ 100	− 0	0	0	+ 100		8
Moraxella atlantae	23	− 0	+ or (+) 87(13)	+ 100	− 0	− 0	− 0	− 5	−	+ 91
Moraxella bovis	7	− 0	0	+ 100	− 0	0	− 0	V 14	− 0	V 14
Moraxella lacunata	25	− 0	4	+ 100	− 0	0	− 0	+ 100		+ 100
Moraxella nonliquefaciens	243	− 0	− 8(2)	+ 100	− 0	− 0	− 0	+ 95	−	+ 95
Moraxella osloensis	163	− 0	V 70	+ 100	− 0	− 0	− 0	V 24	−	+ 95
Moraxella phenylpyruvica	50	− 0	V 80(6)	+ 100	− 0	+ 100	0	V 68	−	+ 90
Neisseria elongata	1		−	+	−		−		−	−
Neisseria flavescens	10	0	V 67	+ 100	−	− 0	−	− 0		+ 100
Neisseria mucosa	30	+ or (+) 83(10)	V 57(3)	+ 100	−	− 0	−	+ 100	−	+ 100
Neisseria sicca	43	+ or (+) 78(14)	V 68	+ 100		0		− 0		V 80
Neisseria subflava	153	V 68(6)	V 47(2)	+ 100	−	− 0		0		V 80
Pseudomonas mesophilica	90	V 40	V 15	+ 96	0	V 29(26)	+ 94	V 25	V	+ 100
Pseudomonas vesicularis	47	+ or (+) 87(9)	V 81(8)	+ 94	0	− (8)	V 21(2)	− 6	+	V 74(11)
IIi	87	− 0	− (10)	+ 100	+ 100	− 0	− 0	− 0	−	+ 98
IIj	41	− 0	− 2	+ 100	+ 98	+ 100	0	− 0	−	+ 100
IVc	37	− 0	V 62(27)	+ 100	− 0	+ 97	− 0	+ 100	V	+ 100

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GUIDE TO PRESUMPTIVE IDENTIFICATION: CODE BOOK

GLUCOSE FERMENTERS: MacConkey-positive/oxidase negative

These organisms must be differentiated from the species of *Enterobacteriaceae*, which have not been included on the chart.

Numerical Code		Numerical Code	
0000	*Yersinia pestis*	1170	*Chromobacterium violaceum*
0010	*Tatumella ptyseos*	1171	*Chromobacterium violaceum*
0050	*Tatumella ptyseos*	1251	*Yersinia enterocolitica*
0110	*Tatumella ptyseos*	1651	*Yersinia enterocolitica*
0150	*Tatumella ptyseos*	2000	HB-5
0160	*Chromobacterium violaceum*	2160	*Chromobacterium violaceum*
0161	*Chromobacterium violaceum*	2161	*Chromobacterium violaceum*
0170	*Chromobacterium violaceum*	2170	*Chromobacterium violaceum*
0171	*Chromobacterium violaceum*	2171	*Chromobacterium violaceum*
0251	*Yersinia enterocolitica*	2251	*Yersinia enterocolitica*
0410	*Haemophilus aphrophilus*	2651	*Yersinia enterocolitica*
0651	*Yersinia enterocolitica*	3160	*Chromobacterium violaceum*
1000	*Yersinia pseudotuberculosis*	3161	*Chromobacterium violaceum*
1001	*Yersinia pseudotuberculosis*	3170	*Chromobacterium violaceum*
1040	*Yersinia pseudotuberculosis*	3171	*Chromobacterium violaceum*
1041	*Yersinia pseudotuberculosis*	3251	*Yersinia enterocolitica*
1160	*Chromobacterium violaceum*	3651	*Yersinia enterocolitica*
1161	*Chromobacterium violaceum*		

GLUCOSE FERMENTERS: MacConkey-positive/oxidase-positive

Numerical Code	
000	EF-4a: catalase +, 100%; rod; maltose neg., 0%. *Kingella kingae:* catalase neg., 0%; rod; maltose + or (+), 76(24)%. *Neisseria subflava:* catalase v, 80%; coccus; determine reactions for differentiation of *Neisseria* species; maltose +, 99(1)%.
002	*Pasteurella haemolytica:* rarely both mannitol and lactose neg.; beta-like hemolysis v, 72%; rod. *Pasteurella gallinarum:* mannitol and lactose neg., 0%; beta-like hemolysis neg., 0%; rod. *Neisseria subflava*, *Neisseria sicca*, *Neisseria mucosa*: cocci; determine reactions for differentiation of *Neisseria* species.
003	*Pasteurella haemolytica:* acid from xylose v, 66(3)%; catalase v, 84(2)%; beta-like hemolysis v, 72%; usually from animal source. *Haemophilus aphrophilus:* acid from xylose neg., 0%; catalase neg., 5%; beta-like hemolysis neg., 0%; usually from human source.
010	HB-5: catalase neg. 0%; motility neg., 0%. *Vibrio hollisae:* catalase +, 100%; motility v, 50(25)%.
022	*Pasteurella aerogenes:* gas from glucose +, 100%; esculin hydrolysis neg., 0%. *Actinobacillus suis:* gas from glucose neg., 0%; esculin hydrolysis +, 100%.
023	*Pasteurella aerogenes*, *Actinobacillus suis*: see 022

Glucose Fermenters: MacConkey-positive/oxidase-positive (continued)

Numerical Code	
042	*Pasteurella haemolytica*
043	*Pasteurella haemolytica*
062	*Actinobacillus lignieresii:* esculin hydrolysis neg., 0%. *Actinobacillus suis:* esculin hydrolysis +, 100%.
063	*Actinobacillus equuli:* esculin hydrolysis neg., 0%; acid from trehalose +, 100%. *Actinobacillus lignieresii:* esculin hydrolysis neg., 0%; acid from trehalose neg., 0%. *Actinobacillus suis:* esculin hydrolysis +, 100%.
110	*Vibrio vulnificus*
111	*Vibrio vulnificus*
150	*Vibrio vulnificus*
151	*Vibrio vulnificus*
152	*Vibrio alginolyticus*
200	EF-4a: growth on SS neg., 1%; citrate neg., 3(1)%. *Chromobacterium violaceum:* growth on SS + or (+), 71(23)%; citrate v, 68(9)%; usually purple pigmented.
202	*Chromobacterium violaceum*
210	*Chromobacterium violaceum*
212	*Chromobacterium violaceum*
220	*Vibrio damsela:* NaCl required +, 100%; maltose +, 100%; non-pigmented *Chromobacterium violaceum:* NaCl required neg., 0%; maltose neg., 3%; usually purple pigmented.
222	*Chromobacterium violaceum*
230	*Chromobacterium violaceum*
232	*Chromobacterium violaceum*
242	*Vibrio fluvialis*
250	*Aeromonas hydrophila*
251	*Aeromonas hydrophila*
252	*Aeromonas hydrophila:* NaCl required neg., 0%; gelatin hydrolysis +, 96%. *Vibrio fluvialis:* NaCl required +, 100%; growth in 6% NaCl +, 100%; gelatin hydrolysis +, 100%. *Vibrio anguillarum:* NaCl required +; growth in 6% NaCl neg.; gelatin hydrolysis neg. (1 strain).
253	*Aeromonas hydrophilia*
320	*Vibrio damsela*
350	*Aeromonas hydrophila*
351	*Aeromonas hydrophila*
352	*Aeromonas hydrophila*
353	*Aeromonas hydrophila*
402	*Pasteurella gallinarum*
422	*Pasteurella aerogenes*
423	*Pasteurella aerogenes*
432	*Pasteurella pneumotropica*
433	*Pasteurella pneumotropica*
510	*Vibrio vulnificus*
511	*Vibrio vulnificus*
532	*Pasteurella pneumotropica*
533	*Pasteurella pneumotropica*
550	*Vibrio parahaemolyticus:* ONPG neg., 0%; cellobiose neg., 0%. *Vibrio vulnificus:* ONPG +, 100%; cellobiose +, 100%.
551	*Vibrio vulnificus*
552	*Vibrio alginolyticus:* NaCl required +, 100%. *Vibrio cholerae:* NaCl required neg., 0%.
553	*Vibrio cholerae*
711	*Plesiomonas shigelloides*

GLUCOSE FERMENTERS: MacConkey-negative/oxidase-negative

Numerical Code	
000	*Capnocytophaga:* long to filamentous rods; lactose v, 86%. *Gardnerella vaginalis:* short to medium length rods; delayed, incomplete hemolysis on rabbit and human blood agar; lactose neg., 1%.
001	*Capnocytophaga:* continue incubation of xylose for 7 days, neg., 0%; long to filamentous rods. DF-3: continue incubation of xylose for 7 days, + or (+), 86(14)%; predominantly coccoid and short rods.
010	*Capnocytophaga*
011	*Capnocytophaga* DF-3 } see 001
020	*Capnocytophaga* *Gardnerella vaginalis* } see 000
021	*Capnocytophaga* DF-3 } see 001
030	*Capnocytophaga*
031	*Capnocytophaga* DF-3 } see 001
040	*Gardnerella vaginalis*
041	DF-3
046	*Gardnerella vaginalis*
051	DF-3
061	DF-3
071	DF-3
200	*Capnocytophaga*
201	*Capnocytophaga*
210	*Capnocytophaga*
211	*Capnocytophaga*
220	*Capnocytophaga:* urea neg., 0%; mannitol neg., 0%. *Pasteurella ureae:* urea +, 100%; mannitol +, 99(1)%.
221	*Capnocytophaga*
230	*Capnocytophaga:* long to filamentous rods; esculin hydrolysis v, 83%. *Haemophilus aphrophilus:* coccoid to short rods; esculin hydrolysis neg., 0%.
231	*Capnocytophaga*
300	*Actinobacillus actinomycetemcomitans*
320	*Actinobacillus actinomycetemcomitans:* urea neg., 0%. *Pasteurella ureae:* urea +, 100%.
340	*Actinobacillus actinomycetemcomitans*
360	*Actinobacillus actinomycetemcomitans*
401	DF-3
411	DF-3
421	DF-3
431	DF-3
441	DF-3
451	DF-3
461	DF-3
471	DF-3
600	HB-5
700	*Pasteurella multocida*
720	*Pasteurella* sp. "new species 1": ornithine neg., 0%; xylose neg., 0%. *Pasteurella pneumotropica:* ornithine +, 100%; xylose + or (+), 76(19)%.
740	*Pasteurella multocida*
760	*Pasteurella pneumotropica*
770	*Pasteurella pneumotropica*

GLUCOSE FERMENTERS: MacConkey-negative/oxidase-positive

Numerical Code	
0000	*Kingella kingae*
0001	*Kingella kingae:* rod *Neisseria subflava:* coccus
0002	*Kingella kingae*
0003	*Kingella kingae* *Neisseria subflava* } see 0001
0041	*Neisseria subflava:* yellow pigmentation on Loeffler slant +, 99%. *Neisseria sicca:* not pigmented on Leoffler slant.
0043	*Neisseria subflava*
0100	*Kingella denitrificans*
0140	*Pasteurella haemolytica*
0141	*Pasteurella haemolytica:* gas from glucose neg., 0%; catalase v, 84(2)%. *Haemophilus aphrophilus:* gas from glucose +, 98%; catalase neg., 5%.
0142	*Pasteurella haemolytica*
0143	*Pasteurella haemolytica*
0150	*Pasteurella haemolytica*
0151	*Pasteurella haemolytica*
0152	*Pasteurella haemolytica*
0153	*Pasteurella haemolytica*
0160	*Pasteurella haemolytica*
0161	*Pasteurella haemolytica*
0162	*Pasteurella haemolytica*
0163	*Pasteurella haemolytica*
0170	*Pasteurella haemolytica*
0171	*Pasteurella haemolytica*
0172	*Pasteurella haemolytica*
0173	*Pasteurella haemolytica*
0300	*Kingella denitrificans*
1000	DF-2 (if maltose reaction is delayed)
1001	DF-2: long thin rod *Neisseria lactamica:* coccus; ONPG +, 100%. *Neisseria subflava:* coccus; ONPG neg., 0%.
1003	*Neisseria subflava*
1041	*Neisseria subflava* *Neisseria sicca* } see 0041
1043	*Neisseria subflava*
1100	EF-4a: TSI slant acid, neg., 3%. *Actinobacillus actinomycetemcomitans:* no strains have been encountered which were negative in xylose, mannitol, sucrose, and maltose; TSI slant acid, +, 100%.
1101	*Actinobacillus actinomycetemcomitans*
1110	*Actinobacillus actinomycetemcomitans*
1111	*Actinobacillus actinomycetemcomitans*
1120	*Actinobacillus actinomycetemcomitans*
1121	*Actinobacillus actinomycetemcomitans*
1130	*Actinobacillus actinomycetemcomitans*
1131	*Actinobacillus actinomycetemcomitans*
1140	*Pasteurella haemolytica*
1141	*Pasteurella haemolytica:* generally not from an avian source. *Pasteurella gallinarum:* predominantly avian sources.
1142	*Pasteurella haemolytica*
1143	*Pasteurella haemolytica*
1150	*Pasteurella haemolytica*

Glucose Fermenters: MacConkey-negative/oxidase-positive (continued)

Numerical Code	
1151	*Pasteurella haemolytica* *Pasteurella gallinarum* } see 1141
1152	*Pasteurella haemolytica*
1153	*Pasteurella haemolytica*
1160	*Pasteurella haemolytica*
1161	*Pasteurella haemolytica*
1162	*Pasteurella haemolytica*
1163	*Pasteurella haemolytica*
1170	*Pasteurella haemolytica*
1171	*Pasteurella haemolytica*
1172	*Pasteurella haemolytica*
1173	*Pasteurella haemolytica*
1300	EF-4a
1341	*Neisseria mucosa*
1541	*Pasteurella gallinarum*
1551	*Pasteurella gallinarum*
2000	*Cardiobacterium hominis*
2001	*Cardiobacterium hominis*
2020	*Cardiobacterium hominis*
2021	*Cardiobacterium hominis*
2040	*Cardiobacterium hominis:* continue incubation of mannitol, +or(+), 52(43)%; alkaline phosphatase neg.; casein neg.; Tween 20 and 40 hydrolysis neg. *Kingella indologenes:* mannitol neg. at 7 days; alkaline phosphatase +; casein +; Tween 20 and 40 hydrolysis +.
2041	*Cardiobacterium hominis* *Kingella indologenes* } see 2040
2060	*Cardiobacterium hominis*
2061	*Cardiobacterium hominis*
2100	HB-5
3100	*Vibrio hollisae*
3102	*Vibrio hollisae*
3141	*Pasteurella* sp., "new species 1"
3163	*Vibrio anguillarum*
3540	*Pasteurella multocida*
3550	*Pasteurella multocida*
3560	*Pasteurella multocida*
3570	*Pasteurella multocida*
4151	*Actinobacillus suis*
4153	*Actinobacillus suis*
4160	*Actinobacillus lignieresii*
4161	*Actinobacillus lignieresii:* continue incubation of xylose for 7 days, + or(+), 87(13)%; usually animal source or history of animal contact; galactose +, 90(5)%. *Pasteurella ureae:* human sources, mainly respiratory; xylose negative at 7 days; galactose neg., 0%.
4170	*Actinobacillus lignieresii*
4171	*Actinobacillus lignieresii:* esculin neg., 0%; trehalose neg., 0%. *Actinobacillus suis:* esculin +, 100%; trehalose +, 100%. *Actinobacillus equuli:* esculin neg., 0%; trehalose +, 100%.
4173	*Actinobacillus suis*
5151	*Actinobacillus suis*
5153	*Actinobacillus suis*
5160	*Actinobacillus lignieresii*

Glucose Fermenters: MacConkey-negative/oxidase-positive (continued)

Numerical Code		
5161	*Actinobacillus lignieresii* *Pasteurella ureae*	see 4161
5170	*Actinobacillus lignieresii*	
5171	*Actinobacillus lignieresii* *Actinobacillus suis* *Actinobacillus equuli*	see 4171
5173	*Actinobacillus suis*	
7141	*Pasteurella* sp. "new species 1"	
7541	*Pasteurella pneumotropica*	
7551	*Pasteurella pneumotropica*	

GLUCOSE OXIDIZERS: MacConkey-positive/oxidase-negative

Numerical Code	
010	*Brucella* species, probably *B. canis:* confirm by serology and dye inhibition tests.
022	*Pseudomonas maltophilia*
023	*Pseudomonas maltophilia*
032	*Pseudomonas maltophilia*
033	*Pseudomonas maltophilia*
043	Ve-1
050	*Pseudomonas mallei*
053	Ve-1
102	*Pseudomonas marginata* (one strain): OF maltose neg.; non-pigmented. Ve-2: OF maltose +, 97(3)%; yellow insoluble pigment, 100%.
142	Ve-2
143	Ve-1
150	*Pseudomonas mallei*
153	Ve-1
200	*Acinetobacter calcoaceticus (A. anitratus)*
222	*Pseudomonas maltophilia*
223	*Pseudomonas maltophilia*
232	*Pseudomonas maltophilia*
233	*Pseudomonas maltophilia*
243	Ve-1
250	*Pseudomonas mallei*
253	Ve-1
302	Ve-2: Yellow insoluble pigment +, 100%; H_2S on lead acetate paper over TSI +, 97%; 1-2 polar flagella. *Pseudomonas cepacia:* variable pigmentation; H_2S on lead acetate paper over TSI neg., 0%; > 2 polar flagella.
303	*Pseudomonas cepacia*
312	*Pseudomonas cepacia*
313	*Pseudomonas cepacia*
322	*Pseudomonas cepacia*
323	*Pseudomonas cepacia*
332	*Pseudomonas cepacia*
333	*Pseudomonas cepacia*
342	Ve-2
343	Ve-1
350	*Pseudomonas mallei*
353	Ve-1
422	*Pseudomonas maltophilia*
423	*Pseudomonas maltophilia*
432	*Pseudomonas maltophilia*
433	*Pseudomonas maltophilia*
502	Ve-2
542	Ve-2
601	*Pseudomonas paucimobilis*
603	*Pseudomonas paucimobilis*
622	*Pseudomonas maltophilia*
623	*Pseudomonas maltophilia*
632	*Pseudomonas maltophilia*
633	*Pseudomonas maltophilia*

Glucose Oxidizers: MacConkey-positive/oxidase-negative (continued)

Numerical Code		
702	∇e-2 *Pseudomonas cepacia*	see 302
703	*Pseudomonas cepacia*	
712	*Pseudomonas cepacia*	
713	*Pseudomonas cepacia*	
722	*Pseudomonas cepacia*	
723	*Pseudomonas cepacia*	
732	*Pseudomonas cepacia*	
733	*Pseudomonas cepacia*	
742	∇e-2	

GLUCOSE OXIDIZERS: MacConkey-positive/oxidase-positive

Numerical Code		
00002	*Pseudomonas vesicularis*	
00010	*Pseudomonas mesophilica*	
00012	*Pseudomonas vesicularis*	
00050	EO-2	
00052	EO-2	
00053	*Pseudomonas paucimobilis*	
00070	EO-2	
00072	EO-2: nonmotile *Pseudomonas thomasii:* motile, <3 polar flagella *Pseudomonas cepacia:* motile, >2 polar flagella	
00073	*Agrobacterium radiobacter:* polar & lateral flagella; H_2S (lead acetate)+, 100%. 3-ketolactonate +, 100%. *Pseudomonas cepacia:* polar flagella; H_2S (lead acetate) neg., 0%; 3-ketolactonate neg., 0%. *Flavobacterium spiritivorum:* non-motile; H_2S (lead acetate) v, 56%; 3-ketolactonate neg., 0%.	
00100	EF-4b	
00110	*Achromobacter xylosoxidans:* citrate +, 95%; peritrichous flagella; growth on SS +, 98%; no pigment. *Pseudomonas mesophilica:* citrate neg., 2(3)%; polar flagella; growth on SS neg., 0%; pink to coral insoluble pigment, +, 99%.	
00150	EO-2	
00152	EO-2	
00170	EO-2	
00172	EO-2 *Pseudomonas thomasii* *Pseudomonas cepacia*	see 00072
00173	*Agrobacterium radiobacter* *Pseudomonas cepacia*	see 00073
00310	*Achromobacter xylosoxidans*	
00312	*Pseudomonas stutzeri*	
00332	*Pseudomonas stutzeri*	
00400	*Pseudomonas diminuta*	
00410	*Pseudomonas mesophilica*	
00450	EO-2	
00452	EO-2	

Glucose Oxidizers: MacConkey-positive/oxidase-positive (continued)

Numerical Code

00453 *Flavobacterium multivorum*

00470 EO-2

00472 EO-2

Pseudomonas thomasii, *Pseudomonas cepacia* } see 00072

00473 *Agrobacterium radiobacter*, *Pseudomonas cepacia*, *Flavobacterium spiritivorum* } see 00073

00510 *Pseudomonas mesophilica:* motile; pink to coral insoluble pigment +, 99%; pleomorphic rods, vacuolated; Simmons citrate neg., 2(3)%.
Brucella: nonmotile; nonpigmented; tiny, coccoid; Simmons citrate neg., 0%.
Pseudomonas pickettii: (if gas from nitrate is not detected) weakly motile; nonpigmented; rods (not pleomorphic); Simmons citrate +, 100%.

00550 EO-2

00552 Va-1: motile; rod.
EO-2: nonmotile; coccoid.

00570 EO-2

00572 EO-2
Pseudomonas thomasii, *Pseudomonas cepacia* } see 00072

00573 *Agrobacterium radiobacter*, *Pseudomonas cepacia* } see 00073

00710 *Pseudomonas pickettii:* motile, polar flagella; growth on SS neg., 0%; rod.
Vd: motile, peritrichous flagella, predominantly polar & lateral; growth on SS +, 96(1)%.
Brucella: nonmotile; growth on SS neg., 0%; tiny, coccoid.

00711 Vd

00712 *Pseudomonas stutzeri:* motile, polar flagella; H_2S on lead acetate paper v, 36%.
Vd: motile, polar and lateral flagella; H_2S on lead acetate paper +, 100%.

00713 Vd

00730 Vd

00731 Vd

00732 *Pseudomonas stutzeri*, Vd } see 00712

00733 Vd

00752 Va-1

01073 *Agrobacterium radiobacter*

01103 *Pseudomonas putrefaciens*, biotype 1

01173 *Agrobacterium radiobacter*

01473 *Agrobacterium radiobacter*

01503 *Pseudomonas putrefaciens*, biotype 1

01573 *Agrobacterium radiobacter*

01710 Vd

01711 Vd

01713 Vd

01730 Vd

01731 Vd

01732 Vd

01733 Vd

Glucose Oxidizers: MacConkey-positive/oxidase-positive (continued)

Numerical Code	
02072	*Pseudomonas cepacia*
02073	*Pseudomonas cepacia*
02172	*Pseudomonas cepacia*
02173	*Pseudomonas cepacia*
02472	*Pseudomonas cepacia*
02473	*Pseudomonas cepacia*
02572	*Pseudomonas cepacia*
02573	*Pseudomonas cepacia*
04073	*Flavobacterium spiritivorum*
04100	*Pseudomonas mallei*
04102	*Pseudomonas mallei*
04110	*Pseudomonas mallei:* nonmotile; growth on SS neg., 0%; 42°C neg., 0%. *Achromobacter xylosoxidans:* motile; growth on SS +, 98%; 42°C v, 84%.
04112	*Pseudomonas mallei*
04120	*Pseudomonas mallei*
04122	*Pseudomonas mallei*
04130	*Pseudomonas mallei*
04132	*Pseudomonas mallei*
04140	*Pseudomonas mallei*
04142	*Pseudomonas mallei*
04150	*Pseudomonas mallei*
04152	*Pseudomonas mallei*
04160	*Pseudomonas mallei*
04162	*Pseudomonas mallei:* nonmotile. *Pseudomonas pseudomallei:* motile.
04163	*Pseudomonas pseudomallei*
04170	*Pseudomonas mallei*
04172	*Pseudomonas mallei:* nonmotile. *Pseudomonas pseudomallei:* motile.
04173	*Pseudomonas pseudomallei*
04310	*Pseudomonas aeruginosa:* glucose reaction as strong or stronger than xylose reaction; polar flagella; acetamide +, 100%. *Achromobacter xylosoxidans:* xylose reaction stronger than glucose reaction; peritrichous flagella. *Pseudomonas mendocina:* glucose reaction as strong or stronger than xylose reaction, polar flagella; acetamide neg., 0%.
04312	∇b-3
04330	*Pseudomonas aeruginosa*
04332	∇b-3
04362	*Pseudomonas pseudomallei*
04363	*Pseudomonas pseudomallei*
04372	*Pseudomonas pseudomallei*
04373	*Pseudomonas pseudomallei*
04473	*Flavobacterium spiritivorum*
04500	*Pseudomonas mallei*
04502	*Pseudomonas mallei*
04510	*Pseudomonas mallei*
04512	*Pseudomonas mallei*
04520	*Pseudomonas mallei*
04522	*Pseudomonas mallei*
04530	*Pseudomonas mallei*
04532	*Pseudomonas mallei*
04540	*Pseudomonas mallei*

Glucose Oxidizers: MacConkey-positive/oxidase-positive (continued)

Numerical Code	
04542	*Pseudomonas mallei*
04550	*Pseudomonas mallei*
04552	*Pseudomonas mallei*
04560	*Pseudomonas mallei*
04562	*Pseudomonas mallei*
04570	*Pseudomonas mallei*
04572	*Pseudomonas mallei*
04710	*Pseudomonas aeruginosa:* acetamide +, 100%; polar flagella; H_2S(PbAc)neg., 4%. *Pseudomonas mendocina:* acetamide neg., 0%; polar flagella; H_2S(PbAc)+, 100%. ∇d: acetamide neg., 4%; peritrichous flagella, predominantly polar & lateral.
04711	∇d
04712	∇b-3: polar flagella ∇d: polar and lateral flagella
04713	∇d
04730	*Pseudomonas aeruginosa:* acetamide +, 100%; polar flagella. ∇d: acetamide neg., 4%; peritrichous flagella.
04731	∇d
04732	∇b-3, ∇d } see 04712
04733	∇d
04762	*Pseudomonas pseudomallei*
04763	*Pseudomonas pseudomallei*
04772	*Pseudomonas pseudomallei*
04773	*Pseudomonas pseudomallei*
05710	∇d
05711	∇d
05712	∇d
05713	∇d
05730	∇d
05731	∇d
05732	∇d
05733	∇d
14310	*Pseudomonas aeruginosa*
14330	*Pseudomonas aeruginosa*
14710	*Pseudomonas aeruginosa*
14730	*Pseudomonas aeruginosa*
24010	*Pseudomonas fluorescens:* gelatin + at 25°C. *Pseudomonas putida:* gelatin neg. at 25°C.
24011	*Pseudomonas fluorescens*
24012	*Pseudomonas putida*
24030	*Pseudomonas fluorescens*, *Pseudomonas putida* } see 24010
24031	*Pseudomonas fluorescens*
24032	*Pseudomonas putida*
24050	*Pseudomonas fluorescens*, *Pseudomonas putida* } see 24010
24051	*Pseudomonas fluorescens*
24052	*Pseudomonas putida*
24070	*Pseudomonas fluorescens*, *Pseudomonas putida* } see 24010
24071	*Pseudomonas fluorescens*
24072	*Pseudomonas putida*
24110	*Pseudomonas fluorescens*

Glucose Oxidizers: MacConkey-positive/oxidase-positive (continued)

Numerical Code		
24111	*Pseudomonas fluorescens*	
24130	*Pseudomonas fluorescens*	
24131	*Pseudomonas fluorescens*	
24150	*Pseudomonas fluorescens*	
24151	*Pseudomonas fluorescens*	
24170	*Pseudomonas fluorescens*	
24171	*Pseudomonas fluorescens*	
24310	*Pseudomonas aeruginosa*	
24330	*Pseudomonas aeruginosa*	
24410	*Pseudomonas fluorescens* *Pseudomonas putida*	see 24010
24411	*Pseudomonas fluorescens*	
24412	*Pseudomonas putida*	
24430	*Pseudomonas fluorescens* *Pseudomonas putida*	see 24010
24431	*Pseudomonas fluorescens*	
24432	*Pseudomonas putida*	
24450	*Pseudomonas fluorescens* *Pseudomonas putida*	see 24010
24451	*Pseudomonas fluorescens*	
24452	*Pseudomonas putida*	
24470	*Pseudomonas fluorescens* *Pseudomonas putida*	see 24010
24471	*Pseudomonas fluorescens*	
24472	*Pseudomonas putida*	
24510	*Pseudomonas fluorescens*	
24511	*Pseudomonas fluorescens*	
24530	*Pseudomonas fluorescens*	
24531	*Pseudomonas fluorescens*	
24550	*Pseudomonas fluorescens*	
24551	*Pseudomonas fluorescens*	
24570	*Pseudomonas fluorescens*	
24571	*Pseudomonas fluorescens*	
24710	*Pseudomonas aeruginosa*	
24730	*Pseudomonas aeruginosa*	
34310	*Pseudomonas aeruginosa*	
34330	*Pseudomonas aeruginosa*	
34710	*Pseudomonas aeruginosa*	
34730	*Pseudomonas aeruginosa*	
40002	*Flavobacterium* sp. (IIb): OF trehalose +, 100% (14 strains); ONPG v, 57% (14 strains); *frank* yellow insoluble pigment +, 99% (155 strains); esculin hydrolysis v, 70% (149 strains). *Flavobacterium breve:* OF trehalose neg., 0% (2 strains); ONPG neg., 0% (3 strains); esculin hydrolysis neg., 0% (3 strains); *slight* yellow insoluble pigment +, 100% (3 strains).	
40003	*Flavobacterium* sp. (IIb)	
40012	*Flavobacterium* sp. (IIb)	
40013	*Flavobacterium* sp. (IIb)	
40022	*Flavobacterium meningosepticum*	
40062	*Flavobacterium meningosepticum*	
40102	*Flavobacterium* sp. (IIb)	
40103	*Flavobacterium* sp. (IIb)	
40112	*Flavobacterium* sp. (IIb)	

Glucose Oxidizers: MacConkey-positive/oxidase-positive (continued)

Numerical Code		Numerical Code	
40113	*Flavobacterium* sp. (IIb)	40502	*Flavobacterium* sp. (IIb)
40402	*Flavobacterium* sp. (IIb)	40503	*Flavobacterium* sp. (IIb)
40403	*Flavobacterium* sp. (IIb)	40512	*Flavobacterium* sp. (IIb)
40412	*Flavobacterium* sp. (IIb)	40513	*Flavobacterium* sp. (IIb)
40413	*Flavobacterium* sp. (IIb)		

GLUCOSE OXIDIZERS: MacConkey-negative/oxidase-negative

Numerical Code		Numerical Code	
070	*Pseudomonas paucimobilis*	500	*Brucella* sp., probably *B. canis*
240	IIe	501	*Pseudomonas mallei*
401	*Pseudomonas mallei*	510	*Brucella* sp., probably *B. canis*
411	*Pseudomonas mallei*	511	*Pseudomonas mallei*
441	*Pseudomonas mallei*	541	*Pseudomonas mallei*
451	*Pseudomonas mallei*	551	*Pseudomonas mallei*

GLUCOSE OXIDIZERS: MacConkey-negative/oxidase-positive

(Recognize *Neisseria* by morphology as well as by biochemical reactions)

Numerical Code	
000	*Pseudomonas mallei:* arginine dihydrolase +, 100%; rod. EF-4b: arginine dihydrolase neg., 0%; rod. *Neisseria gonorrhoeae:* coccus.
001	*Pseudomonas mallei:* nonmotile; nitrate reduction +, 100%; arginine dihydrolase +, 100%; rod. *Pseudomonas vesicularis:* motile; nitrate reduction neg., 3%; arginine dihydrolase neg., 0%; rod. *Neisseria meningitidis:* coccus.
010	*Pseudomonas mallei*
011	*Pseudomonas mallei*
020	*Pseudomonas mallei*
021	*Pseudomonas mallei:* rod. *Neisseria lactamica:* coccus.
030	*Pseudomonas mallei*
031	*Pseudomonas mallei*
101	*Flavobacterium* sp. (IIb): esculin hydrolysis v. 70%; insoluble yellow pigment +, 99%. IIe: esculin hydrolysis neg., 0%; insoluble yellow pigment neg., 0%. IIh: esculin hydrolysis +, 100%; insoluble yellow pigment neg., 0%.
111	*Flavobacterium meningosepticum*
131	*Flavobacterium meningosepticum*
141	*Flavobacterium* sp. (IIb)
161	IIi
200	*Pseudomonas mallei*
201	*Pseudomonas mallei:* nitrate reduction +, 100%. *Pasteurella anatipestifer:* nitrate reduction neg. (one strain tested).
210	*Pseudomonas mallei*
211	*Pseudomonas mallei*
220	*Pseudomonas mallei*
221	*Pseudomonas mallei*

Glucose Oxidizers: MacConkey-negative/oxidase-positive (continued)

Numerical Code	
230	*Pseudomonas mallei*
231	*Pseudomonas mallei*
301	*Flavobacterium* sp. (IIb)
341	*Flavobacterium* sp. (IIb)
361	IIi
400	*Pseudomonas mallei:* nonmotile; non-pigmented. *Pseudomonas mesophilica:* motile: pink to coral insoluble pigment +, 99%.
401	*Pseudomonas mallei* *Pseudomonas vesicularis* } see 001
410	*Pseudomonas mallei*
411	*Pseudomonas mallei*
420	*Pseudomonas mallei:* arginine dihydrolase +, 100%. EO-2: arginine dihydrolase neg., 0%.
421	*Pseudomonas mallei* EO-2 } see 420
430	*Pseudomonas mallei* EO-2 } see 420
431	*Pseudomonas mallei* EO-2 } see 420
461	*Pseudomonas paucimobilis*
471	*Flavobacterium spiritivorum*
501	*Flavobacterium* sp. (IIb)
541	*Flavobacterium* sp. (IIb)
561	IIi
600	*Pseudomonas mesophilica:* motile; pink to coral insoluble pigment +, 99%; pleomorphic vacuolated rods. *Brucella:* nonmotile; nonpigmented; tiny, coccoid. *Pseudomonas mallei:* nonmotile; nonpigmented; rod.
601	*Pseudomonas mallei*
610	*Pseudomonas mallei*
611	*Pseudomonas mallei*
620	*Pseudomonas mallei* EO-2 } see 420
621	*Pseudomonas mallei* EO-2 } see 420
630	*Pseudomonas mallei* EO-2 } see 420
631	*Pseudomonas mallei* EO-2 } see 420
671	*Flavobacterium spiritivorum*
701	*Flavobacterium* sp. (IIb)
741	*Flavobacterium* sp. (IIb)
761	IIi

NON-OXIDIZERS: MacConkey-positive/oxidase-negative

Numerical Code	
00	*Acinetobacter calcoaceticus (A. lwoffi, Mima polymorpha)*
02	*Acinetobacter calcoaceticus (A. lwoffi, Mima polymorpha)*
30	*Pseudomonas maltophilia*
31	*Pseudomonas maltophilia*
34	*Pseudomonas maltophilia*
35	*Pseudomonas maltophilia*
41	*Brucella* sp., possibly *B. canis*
42	*Bordetella parapertussis*
45	*Brucella* sp., possibly *B. canis*
70	*Pseudomonas maltophilia*
71	*Pseudomonas maltophilia*
74	*Pseudomonas maltophilia*
75	*Pseudomonas maltophilia*

NON-OXIDIZERS: MacConkey-positive/oxidase-positive

Numerical Code	
0000	*Neisseria flavescens:* coccus; CTA maltose neg., 0%. *Neisseria subflava:* coccus; CTA maltose +, 99(1)%; yellow pigment on Loeffler slant +, 99%. *Neisseria sicca:* coccus; CTA maltose +, 95(5)%; no pigment on Loeffler slant, 0%. *Moraxella osloensis:* short rod; phenylalanine v, 14%; nitrite reduction neg., 0%; *M. osloensis* transformation +. *Moraxella atlantae:* short rod; phenylalanine neg., 0%; nitrite reduction v, 20%; *M. osloensis* transformation neg.; appears fastidious but grows on MacConkey agar. *Moraxella urethralis:* predominantly small coccoid forms; phenylalanine +, 100%; nitrite reduction +, 100%; *M. osloensis* transformation neg. M-5: long rod forms; phenylalanine v, 73%; nitrite reduction v, 84%; *M. osloensis* transformation neg.; associated with dog-bite wounds.
0022	*Alcaligenes faecalis*
0032	*Alcaligenes faecalis:* nitrite reduction neg., 0%; no fruity odor. *Alcaligenes odorans:* nitrite reduction +, 100%; usually has a fruity odor.
0060	*Pseudomonas diminuta:* OF maltose neg., 0%; flagella with short wavelength and low amplitude. *Pseudomonas vesicularis:* OF maltose +, 98(2)%; flagella similar to those of *P. diminuta* but with slightly longer wavelength. *Pseudomonas alcaligenes:* OF maltose neg., 0%; flagella with "normal" wavelength and amplitude.
0070	*Pseudomonas alcaligenes*
0100	*Moraxella osloensis:* catalase +, 95%; nitrite reduction neg., 0%. M-6: catalase neg., 8%; nitrite reduction +, 100% (may require incubation for 48 hr., gas not produced).
0121	*Pseudomonas testosteroni*
0122	*Alcaligenes faecalis*
0131	*Pseudomonas testosteroni*
0132	*Alcaligenes faecalis*

Non-Oxidizers: MacConkey-positive/oxidase-positive (continued)

Numerical Code	
0160	*Campylobacter fetus* subspecies *jejuni:* thin, curved, and wavy rods; growth at 42°C; nalidixic acid-sensitive; microaerophilic. *Campylobacter fetus* subspecies *fetus:* thin, curved, and wavy rods; usually no growth at 42°C nalidixic acid-resistant; microaerophilic. *Pseudomonas alcaligenes:* straight or slightly curved rods; OF fructose neg., 0%; growth at 42°C neg., 0%; aerobic. *Pseudomonas pseudoalcaligenes:* straight or slightly curved rods; OF fructose v, 82%; growth at 42°C +, 100%; aerobic.
0170	*Pseudomonas alcaligenes* *Pseudomonas pseudoalcaligenes* } see 0160
0321	*Pseudomonas putrefaciens,* biotype 1
0331	*Pseudomonas putrefaciens,* biotype 2
0400	*Moraxella phenylpyruvica:* nitrite reduction neg., 0%; gelatin hydrolysis neg., 0%; yellow insoluble pigment neg., 0%. *Flavobacterium odoratum:* Nitrite reduction v, 83%; gelatin hydrolysis +, 96%; yellow insoluble pigment v, 85%.
0410	*Flavobacterium odoratum*
0422	IVc-2
0460	*Pseudomonas diminuta*
0500	*Moraxella phenylpyruvica:* coccoid to filamentous thick rods. IVe: small coccoid to short rods, some filaments; motility may be delayed or difficult to detect.
0502	IVe
0520	IVe
0521	*Pseudomonas testosteroni*
0522	IVe: citrate v, 14(16)%; small coccoid to short rods, may have filamentous forms; urine is major source, 92%; predominantly from male patients. IVc-2: citrate +, 100%; medium rods; rarely from urine, 5%.
0531	*Pseudomonas testosteroni*
0532	*Bordetella bronchiseptica*
0760	*Pseudomonas putrefaciens,* biotype 1
0770	*Pseudomonas putrefaciens,* biotype 2
1121	*Pseudomonas acidovorans*
1131	*Pseudomonas acidovorans*
2060	*Pseudomonas mesophilica:* OF maltose neg., 2%; pink to coral insoluble pigment +, 99%; pleomorphic vacuolated rods. *Pseudomonas vesicularis:* OF maltose +, 98(2)%; pink to coral insoluble pigment neg., 0%.
2160	*Pseudomonas pseudoalcaligenes:* pink to coral insoluble pigment neg., 0%; growth at 42°C+, 100%. *Pseudomonas mesophilica:* pink to coral insoluble pigment +, 99%; growth at 42°C neg., 0%; pleomorphic vacuolated rods.
2132	*Achromobacter xylosoxidans*
2170	*Pseudomonas pseudoalcaligenes*
2460	*Pseudomonas mesophilica*
2500	*Brucella*
2560	*Pseudomonas mesophilica*
4100	*Neisseria mucosa*
4122	*Alcaligenes denitrificans*
4132	*Alcaligenes denitrificans*
4160	*Pseudomonas denitrificans*
4170	*Pseudomonas denitrificans*
4500	IVe
4502	IVe
4520	IVe

Non-Oxidizers: MacConkey-positive/oxidase-positive (continued)

Numerical Code	
4522	*Alcaligenes denitrificans:* urea not rapid, "average" length flagella. IVe: urea rapid, flagella relatively long.
4532	*Alcaligenes denitrificans*
4560	*Pseudomonas denitrificans*
4570	*Pseudomonas denitrificans*
6132	*Achromobacter xylosoxidans*
6500	*Brucella*
6532	Vd
6732	Vd
7532	Vd
7732	Vd

NON-OXIDIZERS: MacConkey-negative/oxidase-negative

Numerical Code	
0	*Francisella tularensis*
3	*Brucella* sp., possibly *B. canis*

NON-OXIDIZERS: MacConkey-negative/oxidase-positive

Numerical Code	
00	*Neisseria elongata:* rod; gelatin not hydrolyzed. *Moraxella bovis:* rod; gelatin hydrolysis +, 100%; probably an animal source. *Neisseria subflava:* coccus; yellow pigment on Loeffler slant +, 99%. *Neisseria sicca:* coccus; yellow pigment on Loeffler slant, neg., 0%.
01	*Moraxella bovis:* gelatin hydrolysis +, 100%; beta-like hemolysis of blood agar. M-6: gelatin hydrolysis neg., 0%; nitrite reduction + without gas, 100%. *Eikenella corrodens:* gelatin hydrolysis neg., 0%; nitrite reduction neg., 0%; lysine v, 82%; ornithine +, 98%. *Kingella denitrificans:* gelatin hydrolysis neg., 0%; nitrite reduction v with gas, 88%.
02	*Pseudomonas vesicularis*
04	*Moraxella osloensis:* rod; gelatin hydrolysis neg., 0%; sodium acetate +, 100%. *Moraxella bovis:* rod; gelatin hydrolysis +, 100%. *Moraxella atlantae:* rod; gelatin hydrolysis neg., 0%. M-5: longer rod than generally formed by *Moraxella* species; gelatin hydrolysis neg., 0%; associated with dog-bite wounds. *Neisseria flavescens:* coccus; CTA maltose neg., 0%. *Neisseria subflava:* coccus; CTA maltose +, 99(1)%; yellow pigment on Loeffler slant +, 99%. *Neisseria sicca:* coccus; CTA maltose +, 95(5)%; yellow pigment on Loeffler slant neg., 0%. *Bordetella pertussis:* special media required for growth or enhancement of growth; confirmation by agglutination or direct fluorescent antibody reactions; hemolytic on Bordet-Gengou medium.
05	*Moraxella lacunata:* rod; sodium acetate neg., 7%; digestion of Loeffler slant +, 100%; not hemolytic. *Moraxella nonliquefaciens:* rod; sodium acetate neg., 0%; digestion of Loeffler slant neg., 0%; not hemolytic. *Moraxella osloensis:* rod; sodium acetate +, 100%; digestion of Loeffler slant neg., 0%; not hemolytic. *Moraxella bovis:* rod; beta-like hemolysis +, 100%. *Branhamella catarrhalis:* coccus; gas from nitrate neg., 0%. *Neisseria mucosa:* coccus; gas from nitrate +, 100%.

Non-Oxidizers: MacConkey-negative/oxidase-positive (continued)

Numerical Code	
06	*Pseudomonas vesicularis*
07	*Campylobacter fetus* (all subspecies)
14	IIf
24	*Moraxella phenylpyruvica*
25	*Moraxella phenylpyruvica:* acid from OF xylose, neg. at 7 days, 0%; gas from nitrate neg., 0%. IVe: OF xylose neg. at 7 days; gas from nitrate v, 60%; recheck for motility and/or flagella.
27	IVe
34	IIj
42	*Pseudomonas vesicularis*
44	*Pseudomonas mesophilica*
45	*Pseudomonas mesophilica*
46	*Pseudomonas mesophilica:* acid from OF maltose neg., 2%. *Pseudomonas vesicularis:* acid from OF maltose +, 98(2)%; if OF glucose held for 7 days, will be acid, 96%.
47	*Pseudomonas vesicularis*
64	*Pseudomonas mesophilica*
65	*Pseudomonas mesophilica:* pleomorphic rod; recheck motility (+); pink to coral insoluble pigment. *Brucella:* tiny coccoid forms; recheck motility ( ); no insoluble pigment.
66	*Pseudomonas mesophilica*
67	*Pseudomonas mesophilica*

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