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ENTEROBACTERIACEAE TAXONOMY AND NOMENCLATURE

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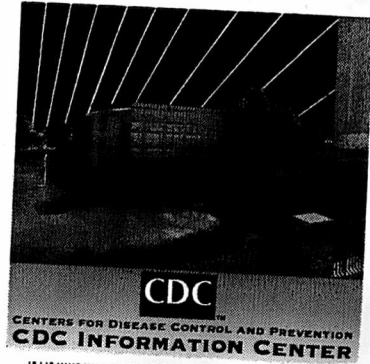
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Enterobacteriaceae

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ENTEROBACTERIACEAE TAXONOMY AND NOMENCLATURE

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Definition (revised) of the family Enterobacteriaceae

The family Enterobacteriaceae consists of gram negative, asporogenous, rod-shaped bacteria that grow well on artificial media. Some species are atrichous, and nonmotile variants of motile species also may occur. Motile forms are peritrichously flagellated. Nitrates are reduced to nitrites, and glucose is fermented with the formation of acid or of acid and gas. The indophenol oxidase test is negative and neither pectate nor alginate is liquefied.

TAXONOMY

At the outset a differentiation should be made between what is meant by Taxonomy and what is meant by Nomenclature. While these two fields or areas are closely related, a clear line of distinction may be drawn between them. One may establish a taxonomic system for a group of related microorganisms and use the letters of an alphabet, Arabic or Roman numerals, the names of places, or practically any other kind of designation one wishes for the different biotypes, serotypes, bacteriophage types, etc. that compose the particular group. A system of classification constructed in one or another of these ways constitutes a taxonomic schema, which may be employed without any reference to formal Nomenclature. Conversely, Nomenclature is concerned primarily with the correct specific names (generic terms and specific epithets) that should be used when referring to microorganisms. As stated in the International Code of Nomenclature of Bacteria and Viruses (14), (Principle 5), "Nomenclature deals with:

(1) The terms which denote the categories of taxa (taxonomic groups or units, such as species, genus, family) and the relative ranks of these categories.

(2) The names which are applied to the individual taxa (taxonomic groups), such as *Bacillus subtilis*, *Streptococcus*, Spirillaceae, Spirochaetales." Thus, it may be seen that Taxonomy may exist without formal Nomenclature, but Nomenclature should be based upon well established taxonomic schemata.

The taxonomic schema for the family Enterobacteriaceae reviewed here is one which evolved from many years of consideration and discussion on the part of the late Dr. P. R. Edwards and the author. It was presented at numerous seminars, workshops, informal discussions, and courses on enteric bacteriology and was well received. The family is composed of several delineated groups of bacteria as listed in the following table:

THE PRINCIPAL DIVISIONS AND GROUPS
OF ENTEROBACTERIACEAE †

Principal Divisions	Groups
Shigella - Escherichia	Shigella Escherichia (<u>E.coli</u> , including Alkalescens-Dispar)
Salmonella - Arizona Citrobacter*	Salmonella Arizona Citrobacter* (including Bethesda-Ballerup)
Klebsiella - Enterobacter** - Serratia	Klebsiella Enterobacter** including Hafnia Serratia
Proteus - Providence	Proteus Providence

† Revised from Ewing and Edwards (1960, 1962).

* Formerly Escherichia freundii.

** Formerly Aerobacter (v. Opinion 28, 1963).

Each group is made up of microorganisms that give similar biochemical reactions. Further, some of these groups are related to each other through similarity of certain biochemical reactions and, in many instances, by means of close antigenic relationships as well. In the outline taxonomic schema given above, groups were placed in the same principal division only if the members of those groups possessed a number of common biochemical characteristics and reliance was not placed on any single characteristic, such as failure to ferment lactose. For example, the Shigella and Escherichia are more closely related to each other, both biochemically and serologically, than either is related to any other group, hence, they are placed in the same principal division. Similarly, Salmonella, Arizona, and Citrobacter are related biochemically and may be said to compose another principal division. Thus, the resulting taxonomic schema consists of only four principal divisions, each of which is composed of two or more groups. It is our belief that this arrangement is more logical than classifications, which place quite different bacteria such as Salmonella and Shigella in the same Tribe simply because members of these groups usually fail to ferment lactose. It will be noted that the Serratia were incorporated into the Klebsiella-Enterobacter-Serratia principal division. This was done because serratiae have many properties in common with Klebsiella and Enterobacter and because studies indicated that there was no justification for a separate tribe Serrateae erected primarily upon pigment production (Ewing, Davis, and Reavis, 1959; Ewing, Davis, and Johnson, 1962).

The principal divisions and groups are erected upon biochemical bases and subgrouping within the various biochemical groups may be accomplished by means of further biochemical tests, on the basis of serology, or by combination of both methods.

In the foregoing discussion of principal divisions nothing has been said about "the paracolon group." This omission is intentional, since in reality, there is no such thing as "a paracolon group." The term "paracolon" has been applied to a wide variety of bacteria, including certain salmonellae, and has lost any meaning it once may have had. Into a so-called "paracolon group" have been placed a large number of diverse microorganisms that have only one common differential characteristic: the inability to ferment lactose promptly. Each group of bacteria that ferments lactose promptly also includes counterparts that do not attack this substrate or do so only after prolonged incubation. Failure to ferment lactose should not exclude a culture from a group of which it is otherwise a typical member. An Escherichia strain and a culture of Enterobacter should not be placed together in a "paracolon group" because each produces acid from lactose after some delay. Instead each should be classified in its appropriate group regardless of its reluctance to utilize lactose, particularly since the rate of lactose fermentation is not a stable characteristic. For example, a "Paracolobactrum coliforme" may be changed to Escherichia coli simply by selection of components that ferment lactose rapidly. Therefore, the writer does not believe that genera should be erected upon the single characteristic of failure to ferment lactose. The author cannot accept the genus Paracolobactrum (Bergey's Manual, 7th ed.) in which totally different microorganisms are placed together simply because of their reluctance to utilize lactose. At the risk of being repetitious, the writer will state again that the group to which a microorganism belongs must be determined by a combination of biochemical reactions, not by a single property. Only in this manner can an orderly arrangement of biochemical groups within the family be achieved.

NOMENCLATURE

The nomenclatural system proposed (Ewing, 1963)* for the family Enterobacteriaceae is based upon the above-mentioned taxonomic system. It is presented below in outline form for consideration, criticism, and comment. Parenthetically, the Subcommittee on Enterobacteriaceae of the American Society for Microbiology approved this nomenclatural system and recommended its adoption by the Society and its inclusion in the next Bergey's Manual (v. Report, 1964).

Family IV ENTEROBACTERIACEAE Rahn

Tribe I ESCHERICHIEAE Bergey, Breed, and Murray

- Genus I Escherichia Castellani and Chalmers
 1. Escherichia coli (Migula) Castellani and Chalmers
- Genus II Shigella Castellani and Chalmers
 1. Shigella dysenteriae (Shiga) Castellani and Chalmers
 2. Shigella flexneri Castellani and Chalmers

* This paper should be consulted for additional references.

3. Shigella boydii Ewing
4. Shigella sonnei (Levine) Weldin

Tribe II SALMONELLEAE Bergey, Breed, and Murray

- Genus I Salmonella Lignières
1. Salmonella choleraesuis (Smith) Weldin
 2. Salmonella typhi (Schroeter) Warren and Scott
 3. Salmonella enteritidis (Gaertner) Castellani and Chalmers

- Genus II Arizona Kauffmann and Edwards
1. Arizona arizonae Kauffmann and Edwards

- Genus III Citrobacter Werkman and Gillen
1. Citrobacter freundii (Braak) Werkman and Gillen

Tribe III KLEBSIELLEAE Trevisan

- Genus I Klebsiella Trevisan
1. Klebsiella pneumoniae (Schroeter) Trevisan
 2. Klebsiella ozaenae (Abel) Bergey, Breed, and Murray
 3. Klebsiella rhinoschleromatis Trevisan

- Genus II Enterobacter Hormaeche and Edwards
1. Enterobacter cloacae (Jordan) Hormaeche and Edwards
 2. Enterobacter aerogenes (Kruse) Hormaeche and Edwards
 3. Enterobacter hafniae (Moeller) Ewing comb. nov.
 4. Enterobacter liquefaciens (Grimes and Hennerty) Ewing comb. nov.

- Genus III Serratia Bizio
1. Serratia marcescens Bizio (Serratia marcescens subspecies marcescens)
 - 1a. Serratia marcescens subspecies kiliensis (Lehmann and Neumann) Ewing, et al.

Tribe IV PROTEAE Castellani and Chalmers

- Genus I Proteus Hauser
1. Proteus vulgaris Hauser
 2. Proteus mirabilis Hauser
 3. Proteus morganii (Winslow et al.) Rauss
 4. Proteus rettgeri (Hadley et al.) Rustigian and Stuart

- Genus II Providencia Kauffmann and Edwards
1. Providencia alcalifaciens (DeSalles Gomes) Ewing comb. nov.
 2. Providencia stuartii (Buttiaux et al.) Ewing comb. nov.

N.B. The first species listed in each genus is the type species.

It is expected that additional genera will be added in future; for example, the genus Edwardsiella (Ewing et al., 1964, 1965), the type species of which is Edwardsiella tarda. Also it is probable that a new tribe will be established, i.e., EDWARDSIELLEAE.

The nomenclature given in the foregoing outline is employed in the series of tables that follow. These tables are self-explanatory and contain the essential information required in the differentiation of the genera of Enterobacteriaceae. The reactions listed are those given by the majority of cultures but notes are included where necessary to explain certain important exceptions. The methods used in the determination of the biochemical reactivities of the microorganisms may be termed "recommended" or "standard" methods. A compilation of these methods is available (Ewing, 1960).

TABLE 1

Differentiation of the Tribes of ENTEROBACTERIACEAE by
Biochemical Methods

Substrate or Test	Tribes			
	ESCHERICHIEAE	SALMONELLEAE	KLEBSIELLEAE	PROTEAE
Indol	+ or -	-	-	+ or -
Methyl red	+	+	-	+
Voges-Proskauer	-	-	+	-
Simmons' citrate	-	+	+	d
Hydrogen sulfide (TSI or PI agar)	-	+	-	d
Urease	-	-	- or (+)	+ or -
KCN	-	- or +	+	+
Phenylalanine deami- nase	-	-	-	+

N.B. Salmonella typhi, Salmonella enteritidis bioserotype Paratyphi A and some rare biotypes fail to utilize citrate. Cultures of S. enteritidis bioser. Paratyphi A and some rare biotypes may fail to produce hydrogen sulfide. Some cultures of P. mirabilis may yield a positive Voges-Proskauer test.

- + , 90% or more positive within 1 or 2 days
- , 90% or more, no reaction
- (+), delayed positive 3 or more days
- d, different biochemical reactions, +, (+), -.
- + or -, majority of strains +, some cultures negative.
- or +, majority of cultures negative, some strains positive.

Table 2

Differentiation within the Tribe ESCHERICHIEAE¹

Substrate or test	<u>Escherichia</u>			<u>Shigella</u>		
	Sign	%+	(%+)*	Sign	%+	(%+)*
Gas from glucose	+	90.7		-	2.1 ²	
Lactose	+	90.8	(5.1)	-	0.3	(11.4) ³
Sucrose	d	48.9	(5.6)	-	0.9	(31.1) ³
Salicin	d	40	(14)	-	0	
Motility	+ or -	69.1		-	0	
Indol	+	99.2		- or +	39.8	
Lysine decarboxylase	d	87.9	(1.2)	-	0	
Arginine dihydrolase	d	17.2	(44.8)	d	9.5	(17.3)
Ornithine decarboxylase	d	63.4	(7.1)	-	20 ³	
Esculin	d	30.9	(19.7)	-	0	
Sodium acetate	+or(+)	83.9	(9.7)	-	0	
Christensen's citrate	d	24.4	(21.2)	-	0	
Mucate	+	96.3		-	0 ³	

¹ Based on the results obtained with 1425 cultures of Escherichia (Ewing, Davis, and Martin, in press) and 5166 strains of Shigella (Ewing, Martin, Wathen, and Jaugstetter, in press).

* Figures in parentheses indicate percentages of delayed reactions (3 days or more)

² Certain biotypes of S. flexneri 6 form gas

³ S. sonnei strains usually ferment lactose and sucrose slowly and cultures of this species decarboxylate ornithine (v. table 3). Some strains of S. sonnei utilize mucate (v. table 4).

N.B. Obviously there is no difficulty in the differentiation of typical E. coli cultures and shigellae. However, the anaerogenic nonmotile, varieties of E. coli, some of which are often referred to as Alkalescens-Dispar types, may require closer examination before they can be definitely classified as E. coli. In attempting to classify a particular strain as E. coli or as a member of the genus Shigella, the biochemical reactivities of the culture should be considered as a whole. Shigellae are much less reactive than E. coli strains and a culture that produces acid promptly (i.e., within 24 hrs.) from all, or most of a wide variety of carbohydrates, such as maltose, rhamnose, xylose, sorbitol, and dulcitol, undoubtedly is not a member of the genus Shigella.

+, 90% or more positive within 1 or 2 days. -, 90% or more, no reaction. (+) positive reaction 3 or more days. d, different biochemical reactions, +, (+), -. +or-, majority of strains +, some cultures negative. -or+, majority of cultures negative, some strains positive.

Table 3

The decarboxylase reactions of shigellae and *E. coli* including nonmotile anaerogenic biotypes such as "Alkalescens-Dispar"

Genera and species	No. tested	Lysine			Arginine			Ornithine		
		Sign	%+ *	(%+) **	Sign	%+ *	(%+) **	Sign	%+ *	(%+) **
<i>S. dysenteriae</i>	352	-	0	0	d	5.4	(42.3)	-	0	0
<i>S. flexneri</i>	1817	-	0	0	d	8.3	(2)	-	0	0
<i>S. boydii</i>	363	-	0	0	d	21	(35.7)	-	2.5	0
<i>S. sonnei</i>	633	-	0	0	d	8.8	(41.9)	+	99.8 ^b	0
<i>E. coli</i>	505	d	87.9	(1.2)	d	17.2	(44.8)	d	63.4	(7.1)
"Alkalescens-Dispar" biotypes	94	d	73.4	(3.2)	d	38.3	(57.4)	d	17	0

* %+, percentage of positive reactions within 1 or 2 days

** (%+), percentage of positive reactions after 3 or 4 days

^a these few cultures all were *S. boydii* ser. 13, a very rare serotype

^b decarboxylation of ornithine is a characteristic of *S. sonnei*
Note that the *Shigella* cultures tested did not decarboxylate lysine whereas the majority of strains of *E. coli* did and that only a small percentage of *S. flexneri* possessed an arginine dihydrolase system (10.3%+ in 1 to 4 days).

Table 4

The reactions of shigellae and *E. coli* in acetate, Christensen's citrate, and mucate media

Genera and species	Sodium acetate ¹			Christensen's citrate			Sodium mucate		
	No. tested	Sign	%+ (%+)*	No. tested	Sign	%+ (%+)*	No. tested	Sign	%+ (%+)*
<i>S. dysenteriae</i>	50	-	0 0	294	-	0 0	63	-	0 0
<i>S. flexneri</i>	100	-	0 0	1375	-	0 0	423	-	0 0
<i>S. boydii</i>	50	-	0 0	442	-	0 0	123	-	0 0
<i>S. sonnei</i>	100	-	0 0	209	-	0 0	165	d	6.4 ^w (30.3 ^w)
<i>E. coli</i>	156	+	or(+) 84 (9.6)	1229	d	7.4 (19.7)	134	+	96.3 0
"Alkalescens-Dispar" bio-types	173	+	or(+) 89.6 (4.7)	200	d	75 (12.5)	61	d	29.5 (27.9)

¹ Adapted from Trabulsi and Ewing (1962)

* Figures in parentheses indicate percentages of delayed reactions (3 to 7 days)

^w Weakly positive reaction

Table 5
Differentiation within the Tribe SALMONELLEAE

Test or substrate	<u>Salmonella</u> ¹			<u>Arizona</u> ²			<u>Citrobacter</u> ³		
	Sign	%+	(%+)*	Sign	%+	(%+)*	Sign	%+	(%+)*
Urease	-	0		-	0		d	69.4	(6.9)
KCN	-	0.3	(0.3)	-	8.7		+	96.2	(0.9)
Gelatin (22 C)	-		(1.3)	(+)		(92)	-		(0.9)
Lysine decarboxylase	+	97.7		+	100		-	0	
Ornithine decarboxylase	+	100		+	100		d	17.2	(0.2)
Lactose	-	1		d	61.3	(16.7)	(+)or+	39.3	(50.9)
Sucrose	-	0.7		-	4.7		d	15.3	(9.4)
Dulcitol	+	98.3		-	0		d	59.4	(0.7)
Inositol	d	42.8	(1)	-	0		-	3.3	(1.9)
Cellobiose	(+)or+	5.4	(88.1)	d	1	(72)	+or(+)	60.8	(38)
Malonate	-	0.7		+	92.6	(0.7)	d	21.8	(0.7)
Jordan's tartrate	+	92.5	(1.1)	-	5.3	(19.3)	+	100	
Beta galactosidase	-	1.5		+	100		+or-	74.4	
Organic acids **									
citrate	+	96	(4)	+or(+)	78.7	(19.3)	(+)or+	49.2	(49.5)
D-tartrate	+	91	(5.3)	(+)or-		(83.3)	(+)		(90.9)

* Figures in parentheses indicate percentages of delayed reactions (3 or more days)

**Organic acid media of Kauffmann and Petersen, 1956 (v. table 6)

+, positive within one or two days' incubation (90% or more)

(+), positive reaction after 3 or more days

-, no reaction (90% or more)

+or-, majority of strains positive, some cultures negative

-or+, majority of cultures negative, occasional strains positive

(+) or +, majority of reactions delayed, some occur within 1 or 2 days

d, different reactions: +, (+), -

1, summary of 315 cultures (Ewing and Ball, 1966)

2, summary of 150 strains (Ewing and Fife, 1965)

3, summary of 582 cultures (Davis and Ewing, 1965)

N.B. The majority of salmonellae ferment dulcitol promptly, but S. typhi, S. enteritidis biosero. Paratyphi A and Pullorum, and S. cholerae-suis, and a few others do not. Members of the genus Arizona are uniformly negative on this substrate. Bioser. Paratyphi A is lysine negative. S. typhi is ornithine negative.

Table 6

Reactions of members of the tribe SALMONELLEAE in
organic acid media of Kauffmann and Petersen (1956)*

Genus	No.	Citrate					D-tartrate				
		1**	2	5	14	-	1**	2	5	14	-
<u>Salmonella</u>	299	245(81.9)	42(14.1)	8(2.7)	4(1.3)	0	272(91)	4(1.3)	3(1)	9(3)	11(3.7)
<u>Arizona</u>	150	7(4.7)	111(74)	23(15.3)	6(4)	3(2)	0	0	68(45.3)	57(38)	25(16.7)
<u>Citrobacter</u>	268	4(1.5)	143(53.3)	117(43.7)	0	4(1.5)	0	0	119(44.4)	122(45.5)	27(10.1)

* From Ewing, 1965, and Ewing, Fife, and Davis (1965)

** Days of incubation

N.B. Numerals in parentheses indicate percentages

Table 7
Differentiation within the tribe KLEBSIELLEAE

Test or substrate	<u>Klebsiella pneumonia</u>		<u>Enterobacter</u>								<u>Serratia marcescens</u> subsp. <u>marcescens</u>	
			<u>.cloacae</u>		<u>aerogenes</u>		<u>hafniae</u>		<u>liquefaciens</u>			
	Sign	%+	Sign	%+	Sign	%+	Sign	%+	Sign	%+	Sign	%+
Gas from:												
glucose	+	96.5	+	100	+	100	+	100	+	94.1	+ or -	61.3
adonitol	+ or -	83.7	- or +	28.4	+	98.7	-	0	-	1.5	-	0
inositol	+	91.9	-	4.5	+	100	-	0	d	23.5	-	0
glycerol	+	92.5	-	5.5	+	100	+	100	d	82.3	-	0
cellobiose	+	95.7	+	100	+	98.7	d	88	d	25	-	0
Sorbitol	+	99.4	+	94.5	+	100	-	0	+	97	+	98.3
Raffinose	+	99.7	+	97	+	96	-	0	d	89.7	-	0.9
Rhamnose	+	99.3	+	92	+	98.7	+	93	-	0	-	0
Arabinose	+	99.9	+	99.5	+	100	+	96	+	92.6	-	0.2
Methyl red:												
37 C	- or +	13.3	-	0.3	-	0	+ or -	54	+ or -	75	- or +	14
22 C							-	1	- or +	25	-	8.8
Voges-Proskauer:												
37 C	+	91.1	+	99.5	+	100	+ or -	65	- or +	30.9	+	100
22 C							+	99	+ or -	79.4	+	100
Lysine decarboxylase	+	97.2	-	0.5	+	98.7	+	100	+ or -	82.4	+	99.8
Arginine dihydrolase	-	0.9	+	96.5	-	0	-	9	-	4.4	-	3
Ornithine decarboxylase	-	0	+	96	+	98.7	+	100	+	98.5	+	99.8

Table 7 (cont'd)
Differentiation within the tribe KLEBSIELLEAE

Test or substrate	<u>Klebsiella pneumonia</u>		<u>Enterobacter</u>								<u>Serratia marcescens</u> subsp. <u>marcescens</u>	
			<u>cloacae</u>		<u>aerogenes</u>		<u>hafniae</u>		<u>liquefaciens</u>		Sign	%+
	Sign	%+	Sign	%+	Sign	%+	Sign	%+	Sign	%+		
Malonate	+	92.5	+or-	80.6	+or-	74.7	+or-	74	-	7.4	-	1.9
Mucate	+	92.8	+or-	75.6	+	94.7	-	0	-	0	-	0
Urease	+	94.5	+or-	64.7	-	2.7	-	3	d	23.5	d	52.4
Gelatin 22 C	-	3.3	(+)	(96)	(+)or-	(77.3)	-	0	+	100	+	97.2
Motility	-	0	+	94.5	+	97.3	+	93	+	97.1	+	99.1
Growth on synthetic alginate medium	+or(+)	88.5	-	0	-	0	-	0	-or(+)	(20.3)	-	0
DNase	-	0	-	0	-	0	-	0	+	96.8	+	100

¹ Adapted from Fife, Ewing and Davis (1965) and Martin and Ewing (in press)

+, positive within 1 or 2 days' incubation (90 percent or more)

(+), positive reaction after 3 or more days

-, no reaction (90 percent or more)

+ or -, majority of strains positive, occasional cultures negative

- or +, majority of cultures negative, occasional strains positive

(+) or +, majority of reactions delayed, some occur within 1 or 2 days

d, different reactions: +, (+), -. In this table (only) the percentages given with the symbol "d" are based upon the positive reactions obtained within 7 days of incubation.

N.B. When gas is formed from glucose by *Serratia*, the volumes are small (10% or less)

Numerals in parentheses indicate percentage of delayed reactions (3 or more days)

Table 8
Differentiation within the genus Klebsiella
(Tests of particular usefulness)

Test or substrate	<u>K. pneumoniae</u>		<u>K. ozaenae</u>		<u>K. rhino- schleromatis</u>	
	Sign	%+ (%+)*	Sign	%+ (%+)*	Sign	%+ (%+)*
Urease	+	94.5	d	9.5 (10.3)	-	0
Methyl red	-or+	13.3	+	99.1	+	100
Voges-Proskauer	+	91.1	-	0	-	0
Citrate (Simmons')	+	97.7	d	31.9 (31)	-	0
Organic acids **						
citrate	+or-	64.4	-or+	18	-	0
D-tartrate	+or-	67.1	-or+	36	-	0
Malonate	+	92.5	-	4	+	95.5
Mucate	+	92.8	-or+	24	-	0
Lysine decarboxylase	+	97.2	-or+	48	-	0
Gas from glucose	+	96.5	d	64 (2)	-	0
Lactose	+	98.2 (1.4)	(+) or +	24.1 (70.7)	(+) or -	(72.8)
Dulcitol	-or+	31.5	-	0	-	0

¹ Adapted from Fife, Ewing, and Davis (1965)

+, positive within 1 or 2 days' incubation (90 percent or more)

(+), positive reaction after 3 or more days

-, no reaction (90 percent or more)

+ or -, majority of strains positive, occasional cultures negative

- or +, majority of cultures negative, occasional strains positive

(+) or +, majority of reactions delayed, some occur within 1 or 2 days

d, different reactions: +, (+), -

*

Numerals in parentheses indicate percentage of delayed reactions (3 or more days)

** Method of Kauffmann and Petersen (1956)

Table 9

Differentiation of Enterobacter aerogenes and Enterobacter hafniae¹
(Biochemical tests of particular usefulness)

Substrate or test	<u>E. aerogenes</u>		<u>E. hafniae</u>	
	Sign	% (%+)*	Sign	% (%+)*
Adonitol:				
acid	+	98.7	-	0
gas	+	98.7	-	0
Inositol:				
acid	+	100	-	0
gas	+	100	-	0
Sorbitol	+	100	-	0
Raffinose	+	96	-	0
Salicin	+	98.7(1.3)	d	13(8)
Alpha methyl glucoside	+	96(2)	-	0
Esculin	+	98	-	6(2)
Methyl red:				
37 C	-	0	+ or -	54
22 C			-	1
Voges-Proskauer:				
37 C	+	100	+ or -	65
22 C			+	99
Citrate (Simmons'):				
37 C	+	93.7	(+) or -	(58)
22 C			d	3(79)
Gelatin: 22 C	(+) or -	(77.3)	-	0
Mucate	+	94.7	-	0

¹ Adapted from Fife, Ewing, and Davis (1965)

* Numerals in parentheses indicate percentage of delayed reactions (3 or more days)

+, positive within 1 or 2 days' incubation (90 percent or more)

(+), positive reaction after 3 or more days

-, no reaction (90 percent or more)

+ or -, majority of strains positive, occasional cultures negative

- or +, majority of cultures negative, occasional strains positive

(+) or +, majority of reactions delayed, some occur within 1 or 2 days

d, different reactions: +, (+), -

Table 10

Differentiation of Serratia marcescens and Enterobacter liquefaciens

Substrate or test	<u>E. liquefaciens</u>			<u>S. marcescens</u>		
	Sign	%+	(%+)*	Sign	%+	(%+)*
Glucose:						
Acid	+	100		+	100	
Gas	+	94.1		+ ^b or -	61.3	
Inositol:						
Acid	+	97	(1.5)	d	73.8	(12.6)
Gas	d	1.5	(22)	-	0	0
Glycerol:						
Acid	+	98.5	(1.5)	+	94.6	(4.4)
Gas	d	45.6	(38.2)	-	0	0
Cellobiose:						
Acid	d	26.5	(44.1)	d	30.6	(40.2)
Gas	d	5.9	(33.8)	-	0	0
Esculin:						
Acid	d	75	(1.6)	d	71.3	(0.2)
Gas	or + ^a	37.5		-	0	0
Raffinose:						
Acid	d	86.8	(2.9)	-	0.9	(2.6)
Gas	d	17.6	(60.3)	-	0	0
Arabinose:						
Acid	+	92.6		-	0.2	(1.9)
Gas	d	23.5	(14.7)	-	0	0
Xylose:						
Acid	+	92.6	(1.5)	d	7.9	(18.6)
Gas	d	30.9	(23.5)	-	0	0
Erythritol (acid)	-	0		d	1.7	(22.8)
Alpha methyl glucoside (acid)	or +	21.7		-	0.9	(0.6)
Methyl red:						
(37 C)	+ or -	75		- or +	14	
(22 C)	- or +	33.3		- or +	8.8	
Voges-Proskauer:						
(37 C)	- or +	30.9		+	100	
(22 C)	+ or -	79.4		+	100	

¹ Adapted from Fife, Ewing, and Davis (1965)

^a Gas volumes: bubble to 10 percent

^b Gas volumes 10 percent or less

* Numerals in parentheses indicate percentage of delayed reactions(3 or more days)

+, positive within 1 or 2 days' incubation (90 percent or more)

(+), positive reaction after 3 or more days

Table 10 (cont'd)

Differentiation of Serratia marcescens and Enterobacter liquefaciens

- , no reaction (90 percent or more)
- + or - , majority of strains positive, occasional cultures negative
- or + , majority of cultures negative, occasional strains positive
- (+) or - , majority of reactions delayed, some occur within 1 or 2 days
- d, different reactions: +, (+), -

Table 11
Differentiation within the tribe PROTEEEAE¹

Substrate or test	Proteus								Providencia			
	<u>vulgaris</u>		<u>mirabilis</u>		<u>morganii</u>		<u>rettgeri</u>		<u>alcalifaciens</u>		<u>stuartii</u>	
	Sign	% (%)*	Sign	% (%)*	Sign	% (%)	Sign	% (%)*	Sign	% (%)*	Sign	% (%)*
Indol	+	98.2	-	(1.9)	+	100	+	100	+	99.5	+	98.7
Voges- 37 C	-	0	-or+	15.6	-	0	-	0	-	0	-	0
Proskauer 22C	-or+	11.3	+or-	51.6	-	0	-	0	-	0	-	0
Simmons' citrate	d	10.5(14.1)	+or(+)	58.7(37.1)	-	0	+	95.6(3.3)	+	97.9(1.3)	+	95.6(1.3)
Hydrogen sul- fide(TSI)	+	94.7	+	94.2	-	0	-	0	-	0	-	0
Urease	+	94.7	+or(+)	88.4(1.9)	+	98.2(0.9)	+	100	-	0	-	0
Gelatin 22 C	+	90.6(9.4)	+	91.8(5.7)	-	0	-	(2.3)	-	(1.4)	-	(6.8)
Lysine decar- boxylase	-	0	-	0	-	(1 ^w)	-	0	-	(0.9 ^w)	-	0
Ornithine decar- boxylase	-	0	+	99.2	+	97.1	-	0	-	1.4 ^w	-	0
Glucose	+	100	+	100	+	98.2(2.8)	+	100	+	100	+	100
gas	+or-	86	+	93.4(0.4)	d	84.9(0.9)	-or+	12.2	d	85.8(0.6)	-	0
Sucrose	+	94.7	d	18.9(63.3)	-	1(2.9)	d	13.3(56.7)	d	13(74.2)	(+)or+	26(65.8)
Mannitol	-	0	-	0	-	0	+or-	88.5	-	2(0.2)	d	13.3(1.3)
Adonitol	-	0	-	0	-	0	d	80.9(5.6)	+	94.5(0.2)	-	3.8
Inositol	-	0	-	0	-	0	+	93.3(4.5)	-	0.6	+	97.5(2.5)
Maltose	+	96.2(1.9)	-	0.9(0.4)	-	0	-	2.3(2.4)	-	0.7(0.7)	-	3.2
Salicin	d	58.2(10.9)	d	0.8(29.8)	-	0	d	30(6.6)	-	0.6(0.3)	-	1.9
Alpha methyl glucoside	d	79.5(5.1)	-	0	-	0	-	2.2	-	0	-	(2.6)

Table 11 (cont'd)
Differentiation within the tribe PROTEAE¹

Substrate or test	Proteus						Providencia					
	<u>vulgaris</u>		<u>mirabilis</u>		<u>morganii</u>		<u>rettgeri</u>		<u>alcalifaciens</u>		<u>stuartii</u>	
	Sign	% (%)*	Sign	% (%)*	Sign	% (%)*	Sign	% (%)*	Sign	% (%)*	Sign	% (%)*
Erythritol	-	2.6	-	0	-	0	d	78.3(6.5)	-	0	-	0
Esculin	d	59(2.6)	-	(0.9)	-	0	d	30.4(8.7)	-	0	-	0
Xylose	+or(+)	88.7(1.9)	+ 96.2(3.8)	-	0	-or+	15.1	-	0.8	d	10(1.7)	
Cellobiose	(+)-or-	(58.7)	d 1.6(46.6)	-	(1.9)	d	3.7(30.4)	-	1.5(3)	d	12.5(68.7)	

¹ Adapted from Ewing et al. (1960), Ewing (1962) and Ewing, Davis, and Martin (unpublished data)

* Figures in parentheses indicate percentages of delayed reactions (3 days or more)

N.B. Volumes of gas are small, i.e., a bubble to 15 percent

+, positive within 1 or 2 days' incubation (90 percent or more)

(+), positive reaction after 3 or more days

-, no reaction (90 percent or more)

+ or -, majority of strains positive, occasional cultures negative

- or +, majority of cultures negative, occasional strains positive

(+) or +, majority of reactions delayed, some occur within 1 or 2 days

d, different reactions: +, (+), -

w, weakly positive reaction

Table 12

The proposed tribe EDWARDSIELLEAE
 Summary of differential reactions (200 cultures)¹
Edwardsiella tarda

Substrate or test	Sign	%+	(%+)*	Substrate or test	Sign	%+	(%+)*
Hydrogen sulfide (TSI)	+	99.5	(0.5)	Malonate	-	0	
Urease	-	0		Mucate	-	0	(0.5)
Indol	+	98.5	(1)	Jordan's tartrate	d	16	(9)
Methyl red (37 or 22 C)	+	100		Acetate	-	0	
Voges-Proskauer (37 or 22 C)	-	0		Lipase (corn oil)	-	0	
Citrate (Simmons')	-	0		Maltose	+	99	(0.5)
KCN	-	0		Xylose	-	0	
Motility	+	98.5		Trehalose	-	0	
Gelatin (22 C)	-	0		Cellobiose	-	0	
Lysine decarboxylase	+	100		Glycerol	d	35	(61)
Arginine dihydrolase	-	0	(0.5)	Beta galactosidase	-	0	
Ornithine decarboxylase	+	100		Organic acids **			
Phenylalanine deaminase	-	0		citrate	(+)	0	(100)
Glucose acid	+	100		D-tartrate	-	0	
gas	+	99					
Lactose	-	0					
Sucrose	-	0.5					
Mannitol	-	0					
Dulcitol	-	0					
Salicin	-	0	(0.5)				
Adonitol	-	0					
Inositol	-	0					
Sorbitol	-	0					
Arabinose	d	10.5	(0.5)				
Raffinose	-	0					
Rhamnose	-	0					

¹Based upon Ewing et al. (1965) and unpublished data

*Figures in parentheses indicate percentages of delayed reactions (3 days or more)

** Media of Kauffmann and Petersen (1956)

+, 90 percent or more positive within 1 or 2 days

-, 90 percent or more negative

d, different biochemical reactions +, (+), "

(+), delayed reaction (3 days or more)

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