The Biochemical Characteristics of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*

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July 1974

ABSTRACT. The Biochemical Characteristics of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*, 1973. Gary Darland, William H. Ewing, and Betty R. Davis. CDC Publication, Center for Disease Control, Atlanta, Ga. 30333. Data on the biochemical reactions given by 75 cultures of *Yersinia enterocolitica* and 20 of *Yersinia pseudotuberculosis* are presented in tabular form with percentages of positive, positive delayed, and negative results. Tests and substrates that are particularly useful for the differentiation of *Y. enterocolitica*, *Y. pseudotuberculosis*, and *Yersinia pestis* are enumerated. These isolants of *Yersinia* also were tested for their susceptibility to 12 antimicrobial agents.

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PUBLIC HEALTH SERVICE
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ATLANTA, GEORGIA 30333

January 1975

DHEW Publication No. (CDC) 75-8294

INTRODUCTION

In 1944 van Logham (see 49) proposed the transfer of *Pasteurella pestis* and *Pasteurella pseudotuberculosis* to a new genus, *Yersinia*, as *Yersinia pestis* and *Yersinia pseudotuberculosis*. Frederiksen (14) concluded that the biochemical characteristics of cultures now known as *Yersinia enterocolitica (Pasteurella X, Pasteurella enterocolitica, Bacterium enterocoliticum, P. pseudotuberculosis* b*)* were similar to those of the bacterium now called *Y. pseudotuberculosis* by most investigators (*bacillus* of Malassez and Vignal, *P. pseudotuberculosis*) but that the two were sufficiently different to warrant their designation as separate species of the genus *Yersinia* van Loghem. Further stated, in effect, that the genus *Yersinia* should be included in the family ENTEROBACTERIACEAE.

There appears to be general acceptance of the genus *Yersinia* with the three species *Y. pestis, Y. pseudotuberculosis,* and *Y. enterocolotica* (see citations given below). Moreover, many investigators now accept the addition of the genus to the family ENTEROBACTERIACEAE. One of the authors (WHE) studied 15 cultures of *Y. enterocolotica* for several years in an attempt to find ways to exclude these bacteria from the family ENTEROBACTERIACEAE. These efforts proved fruitless, however, and it now must be concluded that members of this species belong to the family. The same may be said of *Y. pseudotuberculosis* and *Y. pestis,* since it is clear that the three species are related. Members of these three species are gram-negative, rod-shaped bacteria that are fermentative in their metabolism of carbohydrates, are peritrichously flagellated when flagella are present, and are oxidase-negative. Dr. Don J. Brenner of the Division of Biochemistry, Walter Reed Army Institute of Research, Washington, D.C., thus far has examined 14 cultures of *Y. enterocolitica* and one of *Y. pestis* to determine the relatedness of their deoxyribonucleic acids (DNAs) to each other and to various members of the family ENTEROBACTERIACEAE. He found (personal communication, 1974) that the DNAs of the 14 strains of *Y. enterocolitica* are closely related to each other (87-100%) and that the DNAs of the single culture of *Y. pestis* are related to those of *Y. enterocolitica* (about 40%). Moreover, the DNAs of *Y. enterocolitica* were related to those of other ENTEROBACTERIACEAE at a level of 15 to 25 percent. Therefore it seems apparent, to the authors at least, that the three species comprise a new tribe, YERSINIEAE, within the family ENTEROBACTERIACEAE.

The authors have made no attempt to review the extensive literature that deals with *Y. enterocolitica* and *Y. pseudotuberculosis,* but we have included a bibliography that should be helpful to investigators who may wish to read more about yersiniae. Many of the publications cited contain extensive bibliographies.

Y. enterocolitica

Isolation and general characteristics: 15,20,38,43,62,65,73,75.

Biochemical characterization: 14,20,38-41,53,55,57,62,65,66,74,75,78,79.

Taxonomy: 14,19,43,62,65,67,69,75.

Antigens and serological characterization (including fluorescent antibody technics): 5,21,22,38,40,42,62,66,75-77,70,81.

Clinical and pathological aspects, outbreaks of disease, and mixed infections: 1,4,8,16-18,23,36,41,43,44,50,51,54,59,62,65,68,72,75,78, 82-84.

Bacteriophages: 61,75.

Antibiotic sensitivities and transferable resistance factors: 6,8,14,62,75.

Y. pseudotuberculosis

Isolation and general characteristics: 29,34,45,47.

Biochemical characteristics: 32,34,46,47,53,55,63,64,79.

Taxonomy: 14,19,45,67.

Antigens and serological characterization: 7,25,28,33,37,70,71.

Clinical and pathological aspects, occurrence and distribution: 24-27,35,45,48,49,52,56,58,60.

Bacteriophage and bacteriocins: 30-32.

Y. pestis

Biochemical characterization: 9,10,20,64,79.

Bacteriophage: 30.

The purpose of this paper is to report the biochemical reactions and antibiotic sensitivities of 75 cultures of *Y. enterocolitica* and 20 strains of *Y. pseudotuberculosis* that were tested in order to obtain numerical data that could be presented in the form used by the authors in other publications. Larger numbers of isolates of these two species have been reported upon by other investigators (references cited above), but numerical data in terms of percentages of positive, positive delayed, and negative results have not been given.

MATERIALS AND METHODS

Seventeen of the cultures of *Y. enterocolitica* were received from Dr. R. Sakazaki of the National Institute of Health, Tokyo, Japan, who obtained them from Dr. G. Wauters, Catholic University of Louvain, Louvain, Belgium. These strains were isolated in Europe, but their ultimate sources are not known. Forty-four isolants were supplied by Dr. L. Lafleur of the Hospital Sainte-Justine, Montreal, Canada. Forty-three of these were isolated from humans in Canada. The remaining 14 isolants were received from Mr. H.W. Tatum and Dr. R.E. Weaver, Bacterial Reference Laboratories, Center for Disease Control, Atlanta, Georgia. Six of these were isolated from humans (5 in the United States and 1 in Europe), and the remainder were from lower animals and were recovered in the United States or Europe.

The 20 strains of *Y. pseudotuberculosis* were supplied by Prof. E. Thal, State Institute of Veterinary Medicine, Stockholm, Sweden. All were isolated from lower animals.

The methods used for determining the biochemical reactions of the above-mentioned cultures of *Yersinia* were the same as those used in previous studies (11-13). All strains were tested at room temperature (about 25 C) and at 35 to 37C. However, data are not given in the tables for both temperatures of incubation except in those instances when there was an important difference in the reactions obtained at the two temperatures. Unless otherwise indicated, the 35 to 37 C reactions are given in the tables.

Standard methods (2,3) were used for determination of the antibiotic sensitivities of the strains.

RESULTS AND COMMENTS

Biochemical reactions

Some of the literature on the indol reactions given by cultures of *Y. enterocolitica* is summarized in Table 1. It appears that most of the strains isolated thus far in Canada, Japan, and Europe have been indol-negative (8,14,53,62,83), whereas the majority of those recovered in the United States have been indol-positive (78).

The data on the biochemical reactions of *Y. enterocolitica* given in Table 2 are adapted from the text of the monograph by Nilehn (62). This summary is included for comparative purposes.

The results of the examination of 75 isolants of *Y. enterocolitica* are given in detail in Table 3 and these data are summarized in Table 4. Most of the information recorded in these tables is self-explanatory, but a few comments may be helpful.

None of the strains of *Y. enterocolitica* gave evidence of hydrogen sulfide production in triple sugar iron (TSI) agar medium or in Kligler's iron agar (Tables 3,4). Most cultures of this species yielded positive urease reactions in Christensen's medium within 1 to 4 hours. In general, these reactions were comparable to those given by cultures of *Proteus* in speed and intensity. A few, however, were delayed and did not become positive until the second or third day of incubation (Tables 3,4). Two isolants failed to yield evidence of urease production even upon prolonged incubation. Voges-Proskauer tests were negative when the growing cultures were incubated at 35 to 37 C, but the majority (86.7%) were positive when the cultures were incubated at 25 C. Motility similarly was temperature related, as reported by several investigators (references cited in Introduction). The bacteria were nonmotile when cultures in semisolid agar medium were incubated at 35 to 37 C, but 96% demonstrated motility when the inoculated medium was left at room temperature (Tables 3,4).

None of the 75 strains of *Y. enterocolitica* studied produced gas from glucose at either temperature of incubation, but a few isolants formed traces of gas during fermentation of other

carbohydrates such as sucrose, mannitol, or mannose. However, the amounts formed were small and production was erratic.

The majority (97.3%) of cultures of *Y. enterocolitica* were lipolytic as demonstrated by their reactions in medium containing corn oil (Tables 3,4). The ability of members of this species to hydrolyse certain lipids apparently has not been reported previously. However, Wauters (75) mentioned that some strains produced a lecithinase reaction in egg yolk medium.

The biochemical reactions given by 20 isolates of *Y. pseudotuberculosis* are summarized in Table 5. This information is largely self-explanatory. All cultures hydrolysed urea within 18 to 24 hours; nine gave positive reactions within 6 hours. Voges-Proskauer tests were negative when the growing cultures were incubated at either temperature used in the study (Table 5). All isolants were nonmotile at 35 to 37 C, but 95% were motile in semisolid agar medium incubated at 25C, although motility was not apparent in many (45%) until after 3 to 7 days. Ornithine was not decarboxylated, gas formation was not detected, and sucrose was not fermented.

Examination of the data presented in Tables 4 and 5 indicates that although cultures of *Y. enterocolitica* and *Y. pseudotuberculosis* are similar in many respects, they react quite differently on several substrates. The tests and substrates that are of greatest value for differentiation of *Y. enterocolitica* and *Y. pseudotuberculosis* are listed in Table 6.

Although the numbers of cultures of *Y. enterocolitica* and *Y. pseudotuberculosis* studied by the authors were small as compared to those reported by some other investigators (e.g., 38-40,46,47,62,76), the results were comparable in all important aspects.

The authors have not examined cultures of *Y. pestis*. The data given in Tables 7 and 8 concerning members of this species were derived from references 9,10,20, and 79. It should be noted that only 17 strains were tested for decarboxylation of lysine, arginine, and ornithine. These isolants failed to decarboxylate any of the three amino acids (R.E. Weaver, personal communication, 1974).

Antibiotic sensitivities

The 75 strains of *Y. enterocolitica* and 20 cultures of *Y. pseudotuberculosis* were examined for their susceptibility or resistance to 12 antimicrobial agents. The results obtained are summarized in Table 9. The antibiotic susceptibility patterns of the two species are similar. Two exceptions were the essentially uniform resistance of *Y. enterocolitica* to penicillin compared with the sensitivity of *Y. pseudotuberculosis* to the same antibiotic and the heterogeneous nature of the response of *Y. pseudotuberculosis* to colistin. These minor differences provide helpful ancillary information in the differentiation of the two species.

References

1. Arvastson, B., K. Damgaard, and S. Winblad. 1971. Clinical symptoms of infection with *Yersinia enterocolitica*. Scand. J. Infect. Dis. 3: 37-40.
2. Balows, A. Ed. 1974. Current technics for antibiotic susceptibility testing. Charles C. Thomas. Springfield, Illinois.
3. Bauer, A.H., W.W.M. Kirby, J.C. Sherris, and M. Turck. 1966. Antibiotic susceptibility testing by a standardized single disc method. Amer. J. Clin. Pathol. 45: 493-496.
4. Carter, P.B., C.F. Varga, and E.E. Keet. 1973. New strains of *Yersinia enterocolitica* pathogenic for rodents. Appl. Microbiol. 26: 1016-1018.
5. Cederberg, A. 1968. Demonstration of *Yersinia enterocolitica* by the fluorescent antibody technique. Acta Pathol. Microbiol. Scand. 73: 646-652.
6. Cornelis, G., G. Wauters, and G. Bruynoghe. 1973. Resistances transferables chez de souches sauvages de *Yersinia enterocolitica*. Ann. Microbiol. (Inst. Pasteur), 124A: 299-309.
7. Davies, D.A.L. 1958. The smooth and rough somatic antigens of *Pasteurella pseudotuberculosis*. J. Gen. Microbiol. 18: 118-128.
8. Delorme, J., M. Laverdiere, B. Martineau, and L. Lafleur. 1974. Yersiniosis in children: report of 35 cases in 1972. Canad. Med. Assoc. J. 110: 281-284.
9. Devignat, R. 1954. Comportement biologique et biochimique de *P. pestis* et de *P. pseudotuberculosis*. Bull. Wld. Hlth. Org. 10: 463-494.
10. Devignat, R., and A. Boivin. 1951. Sur la biochimie des souches Centro-Africaines de peste du Congo Belge. Bull. Soc. Pathol. Exot. 44: 279-284.
11. Edwards, P.R., and W.H. Ewing. 1972. Identification of Enterobacteriaceae. 3rd Ed. Burgess Publishing Co. Minneapolis, Minn.
12. Ewing, W.H. 1973. Differentiation of Enterobacteriaceae by biochemical reactions. Revised. CDC Publ. Center for Disease Control, Atlanta, Ga.
13. Ewing, W.H., and B.R. Davis. 1970. Media and tests for differentiation of Enterobacetriaceae. CDC Publ. Center for Disease Control, Atlanta, Ga.
14. Frederiksen, W. 1964. A study of some *Yersinia pseudotuberculosis*-like bacteria ("Bacterium enterocolitica" and "Pasteurella X"). Proc. XIV Scand. Cong. Pathol. and Microbiol. (Oslo). Norwegian Universities Press, Oslo.
15. Gilbert, R. 1940. An unidentified microorganism resembling *Actinobacillus lignieresi* and *Pasteurella pseudotuberculosis* and pathogenic for man. Ann. Rept. Div. Lab. Res., N.Y. State Dept. Health, pp. 50-51.
16. Graber, H., and W. Knapp. 1955. Die abscedierende reticulocytare Lymphadenitis mesenterialis (Masshoff) als Bestandteil eines enteralen Primarkomplexes und Folge einer Infektion mit Pasteurella pseudotuberculosis. Frankfurter Zeitschr. Pathol. 66: 399-415.
17. Graux, C., and H.H. Mollaret. 1968. Infection mixte a *Yersinia enterocolitica* et *Salmonella*. Louvain Med. 87: 437-443.
18. Gutman, L.T., E.A. Ottesen, T.J. Quan, P.S. Noce, and S.L. Katz. 1973. An inter-familial outbreak of *Yersinia enterocolitica* enteritis. New Engl. J. Med. 288: 1372-1377.
19. Haupt, H. 1966. Critique of Mollaret's proposals concerning the names Pasteurella pseudotuberculosis and *P. pestis*. Inter. J. Syst. Bacteriol. 16: 191-193.
20. Hudson, B.W., T.J. Quan, V.R. Sites, and J.D. Mitchell. 1973. An electrophoretic and bacteriologic study of *Yersinia pestis* isolates from Central Java, Asia, and Western Hemisphere. Amer. J. Trop. Med. Hyg. 22: 642-653.
21. Hurvell, B. 1973. Serological cross-reactions between different *Brucella* species and *Yersinia enterocolitica*. Thesis, National Veterinary Institute and the Department of Bacteriology and Epizootology, Royal Veterinalry College, Stockholm. Thule, Stockholm.
22. Karlsson, K.A., and E. Thal. 1968. Experimentelle untersuchungen mit fluoreszierenden antikorpern (FA) im "Yersinia enterocolitica"-sera. Internat. Sympos. Pseudotuberculosis, Paris, 1967; Symp. Ser. Immunobiol. Stand., vol. 9, pp. 187-192. Karger, Basel/New York.
23. Kayser, F.H., W. Knapp, and A. Martenet. 1967. *Pasteurella pseudotuberculosis* (Syn. *Yersinia pseudotuberculosis*) als Ursache der Parinaudschen Conjunctivitis. Albrecht v. Graefes Arch. Klin. Exp. Ophthal. 173: 64-70.
24. Knapp, W. 1954. *Pasteurella pseudotuberculosis* als Erreger einer mesenterialen Lymphadenitis beim Menchen. Zentralbl. Bakteriol. 1 Orig. 161: 422-424.
25. Knapp, W. 1955. Die diagnostiche Bedeutung der antigenen Bezeihungen zwischen *Past. pseudotuberculosis* und der *Salmonella*-Gruppe. Zentralbl. Bakteriol. 1 Orig. 164: 57-59.
26. Knapp, W. 1956. Ein Beitrag zur Beweglichkeit von *Past. pseudotuberculosis*. Zeitschr. Hyg. 142: 219-226.
27. Knapp. W. 1958. Mesenteric adenitis due to *Pasteurella pseudotuberculosis* in young people. New Engl. J. Med. 259: 776-778.
28. Knapp. W. 1960. Uber weitere antigene Beziehungen zwischen *Pasteurella pseudotuberculosis* und der *Salmonella*-Gruppe. Zeitschr. Hyg. 146: 315-330.
29. Knapp, W. 1960. Die Laboratoriumsdiagnose von Infektionen mit *Pasteurella pseudotuberculosis*. Das Arztliche Laboratorium. 7: 197-206.
30. Knapp, W. 1962. Untersuchungen mit *Pasteurella pseudotuberculosis*- und *Pasteurella pestis*-Phagen. Zeitschr. Hyg. 148: 375-382.
31. Knapp, W. 1963. Uber unterschiedliches Verhalten von *Pasteurella*-Phagen. Zentralbl. Bakteriol. 1 Orig. 190: 36-46.
32. Knapp, W. 1965. Neuere experimentelle Untersuchungen mit *Pasteurella pseudotuberculosis* (Syn. *Yersinia pseudotuberculosis*). Arch. Hyg. Bakteriol. 149: 715-731.
33. Knapp, W. 1967. Serologische Kreuzreaktionen zwischen "Pasteurella pseudotuberculosis" (Syn. "Yersinia pseudotuberculosis"), "Escherichia coli" und "Enterobacter cloacae." Inter. Symposium on Pseudotuberculosis, Raris, pp. 179-186. Karger, Basel/New York.
34. Knapp, W. 1968. Pseudotuberculose. *In* Infektionskrankheiten, Vol. 2 Krankheiten durch Bakterein, pp. 367-383. Ed. O. Gsell und W. Mohr. Springer Verlag. Berlin/New York.
35. Knapp, W. 1968. Die Pseudotuberculose des Menchen. Therapeutische Umschau/Revue Therapeutique, 25: 195-200.
36. Knapp, W., J. Lysy, C. Knapp, W. Stille, and V. Goll. 1973. Enterale Infektionem beim Menchen durch *Yersinia enterocolitica* und ihre Diagnose. Zeitschr. Klin. Therap. Infekt. 1: 113-125.
37. Knapp, W., and W. Steuer. 1956. Untersuchungen uber den Nachweis komplementbindener und agglutinierender Antikorper gegen *Pasteurella pseudotuberculosis* in Sera infizierter und immunisierter Menchen und Tiere. Zeitsch. Immunitatforsch. Exper. Therap. 113: 370-374.
38. Knapp, W., and E. Thal. 1963. Untersuchungen uber die kult-urell-biochemischen, serologischen, tierexperimentellen und immunologischen Eigenschaften einer vorlaufig "Pasteurella X" nennannten Bakterienart. Zentralbl. Bakteriol. 1 Orig. 190: 472-484.
39. Knapp, W., and E. Thal. 1973. Differentiation of *Yersinia enterocolitica* by biochemical reactions. Contrib. Microbiol. Immunol. 2: 10-16.
40. Knapp, W., and E. Thal. 1973. Die biochemische Charakterisierung von *Yersinia enterocolitica* (syn. "Pasteurella X") als Grundlage eines verinfachten O-Antigenschemas. Zentralbl. Bakteriol. 1 Orig. A. 223: 88-105.
41. Lafleur, L., B. Martineau, and L. Chicoine. 1972. *Yersinia enterocolitica*. Aspects biologiques, epidemiologiques et

cliniques de 67 cas observes a l'Hospital Sainte-Justine (Montreal, Canada). L'union Medical Canada, 101: 2407-2413.
42. Lysy, J., and W. Knapp. 1973. Serological studies with *Y. enterocolitica.* Contrib. Microbiol. Immunol. 2: 42-53.
43. McIver, M. A., and R. M. Pike. 1934. Chronic glanders-like infection caused by an organism resembling *Flavobacterium pseudomallei* Whitmore. In: Clinical Miscellany. The Mary Imogene Basset Hospital, Cooperstown, New York. C. C. Thomas Co. Baltimore, Md.
44. Makulu, A., F. Gatti, H. H. Mollaret, and J. Vandepitte. 1969. Sur l'existance d'infections humaines a *Yersinia enterocolitica* en Reoublique Democratique du Congo. Bull. Soc. Pathol. Exotique 62. 62: 452-460.
45. Malassez, L., and W. Vignal. 1883. Tuberculose zooloeique. Forme ou espece de tuberculose sans bacilles. Arch. Physiol. Normal Pathol. Ser. 3. 2: 369-412.
46. Mollaret, H. H. 1961. Contribution a l'etude des characteres biochimique de *Pasteurella pseudotuberculosis.* Ann. Inst. Pasteur, 100:685-690.
47. Mollaret, H. H. 1962. Sur le bacille de Malassez et Vignal. Caracteres culturaux et biochimiques. Thesis medicine, Paris. Editions A. G. E. M. P., Paris.
48. Mollaret, H. H. 1965. Le laboratoire dans le diagnostic d'infection humaine a bacille de Mallassez et Vignal. Gazette Medicale, France, 2: 3457-3476.
49. Mollaret, H. H. 1966. Pasteurellose et Yersinioses. Gazette Medicale, France, 1: 3633-3644.
50. Mollaret, H. H. 1971. L'infection humaine a "Yersinia enterocolitica" en 1970. A la lumiere de 642 cas recents. Pathol-Biol. 19: 189-205.
51. Mollaret, H. H. 1972. *Yersinia enterocolitica* infection: a new problem in pathology. (Translated editorial) Ann. Biologie Clinique, No. VII-XI.
52. Mollaret, H. H., and P. Berthon. 1962. Une epidemie du au bacille de Mallassez et Vignal. Presse Med. 70: 2570-2572.
53. Mollaret, H. H., and A. Chevalier. 1964. Contribution a l'etude d'un nouveau groupe de germes proches du bacille de Malassez et Vignal. I. Caracteres culturaux et biochimiques. Ann. Inst. Pasteur. 107: 121-127.
54. Mollaret, H. H., and P. Destombes. 1964. Les germes "X" en pathologie humaine. Presse Med. 72: 2913-2915.
55. Mollaret, H. H., and L. LeMinor. 1962. Researche de la B-galactosidase chez les differentes *Pasteruella* et consequences quant a leur taxonomie. Ann. Inst. Pasteur, 102: 649-652.
56. Mollaret, H. H., J. LePennec. 1968. A propos d'un cas infection a bacille de Mallassez et Vignal chez le porc. Rec. Med. Vet. 144: 429-434.
57. Mollaret, H. H., and A. Lucas. 1965. Sur les particularites biochimiques des souches de *Yersinia enterocolitica* isolees chez les lievers.
58. Mollaret, H. H., H. Geoffroy, and M. A. Chaubaud. 1965. Sur presence du bacille de Malassez et Vignal en Afrique. Bull. Soc. Pathol. Exot. 58: 795-800.
59. Mollaret, H. H., T. Omland, S. D. Henriksen, P. R. Baeroe, G. Rykner, and M. Scavizzi. 1971. Les septicemies humaines a "Yersinia enterocolitica". A propos dix-sep cas recents. Presse Med. 79: 345-348.
60. Mollaret, H. H., J. Temkine, M. Prade, R. Pieron, P. Destombes, and M. C. Guillon. 1964. Les septicemies humaines a de Malassez et Vignal. Presse Med. 72: 2671-2674.
61. Nicolle, P. H. H. Mollaret, and J. Brault. 1968. Sur une parente lysotypique entre des souches humaines et des souches porcines de "Yersinia enterocolitica". International Symposium on Pseudotuberculosis, Paris, 1967. Symp. Ser. Immunobiol. Standard. 9: 357-360. Karger, Basel/New York.
62. Nilehn, B. 1969. Studies on *Yersinia enterocolitica* with special reference to bacterial diagnosis and occurrence in human acute enteric disease. Acta Pathol. Microbiol. Scand. Suppl. 206, pp 1-48.
63. Nilehn, B, and B. Sjostrom. 1967. Studies on *Yersinia enterocolitica.* Occurrence in groups of acute abdominal disease. Acta Pathol. Microbiol. Scand. 71: 612-628.
64. Parnas, J. 1961. L'epreuve esculinique dans le diagnostic de la peste et de la pseudotuberculose. Ann. Inst. Pasteur, 100: 691-692.
65. Schleifstein, I., and M. B. Coleman. 1939. An unidentified microorganism resembling *B. lignieresi* and *Past. pseudotuberculosis,* pathogenic for man. N.Y. State J. Med. 39: 1749-1753.
66. Sedgwick, A. K., and R. C. Tilton. 1971. Biochemical and serological characteristics of a *Yersinia enterocolitica* isolate. Appl. Microbiol. 21: 383-384.
67. Smith, J. E., and E. Thal. 1965. A taxonomic study of the genus *Pasteurella* using a numerical technique. Acta Pathol. Microbiol. Scand. 64: 213-233.
68. Sonnenwirth, A. 1970. Bacteremia with and without meningitis due to *Y. enterocolitica, E. tarda, C. terrigena,* and *P. maltophila.* Ann. N.Y. Acad. Sci. 174: 488-502.
69. Stephens, M., and N. S. Mair. 1973. A numerical taxonomic study of *Yersinia entercolitica* strains. Contributions to Microbiology, 2: 17-22 (Karger, Basel)
70. Thal, E. 1966. Weitere untersuchungen ueber die thermolabilen Antigene der *Yersinia pseudotuberculosis* (Syn. *Pasteurella pseudotuberculosis*). Zentralbl. Bakteriol. I Orig. 200: 56-65.
71. Thal, E., and W. Knapp. 1971. A revised antigenic scheme of "Yersinia pseudotuberculosis". International Symposium on Enterobacterial Vaccines, Bern, 1968. Symp. Ser. Immunobiol. Stand. 15: 219-222. Karger, Basel/New York.
72. Vandepitte, J., R. van Noyen, and A. Isebaert. 1970. *Yersinia enterocolitica:* its incidence in patients with infectious diarrhea. Proc. V Inter. Cong. Infect. Dis. 119-123.
73. van Noyen, R., and J. Vandepitte. 1968. L'isolement de *Yersinia enterocolitica* par une technique usuelle de coproculture. Ann. Inst. Pasteur, 114: 463-467.
74. van Noyen, R., A. Isebaert, and J. Vandepitte. 1969. Sur un biotype urease negatif de *Yersinia enterocolitica.* Ann. Inst. Pasteur, 117: 658-662.
75. Wauters, G. 1970. Contribution a l'etude de *Yersinia enterocolitica.* Thesis, Catholic University of Louvain, pp. 1-65. Vander, Louvain, Belgium.
76. Wauters, G., L. LeMinor, and A. M. Chalon. 1971. Antigenes somatiques et flagellaires des *Yersinia enterocolitica.* Ann. Inst. Pasteur, 120: 631-642.
77. Wauters, G., L. LeMinor, A. M. Chalon, and J. Lassen. 1972. Supplement au schema antigenique de *Yersinia enterocolitica.* Ann. Inst. Pasteur, 122: 951-956.
78. Weaver, R. E., and J. G. Jordan. 1973. Recent human isolates of *Yersinia enterocolitica* in the United States. Contrib. to Microbiol. Immunol. Karger, Basel.
79. Weaver, R. E., H. W. Tatum, and D. G. Hollis. 1972. The identification of unusual pathogenic gram-negative bacteria (Elizabeth O. King). CDC Publ. Center for Disease Control, Atlanta, Ga.
80. Winblad, S. 1967. Studies on serological typing of *Yersinia enterocolitica.* Acta Pathol. Microbiol. Scand. Suppl. 187: 115.
81. Winblad, S. 1968. Studies on O-antigen factors of *Yersinia enterocolitica.* International Symposium on Pseudotuberculosis, Paris, 1967. Symp. Ser. Immunobiol. Stand. 9: 337-342. (Karger, Basel/New York.
82. Winblad, S. 1969. Erythema nodosum associated with infection with *Yersinia enterocolitica.* Scand. J. Infect. Dis. 1: 11-16.
83. Zen-Yoji, H., and T. Maruyama. 1972. The first successful isolations and identification of *Yersinia enetrocolitica* from human cases in Japan. Japan. M. Microbiol. 16: 493-500.
84. Zen-Yoji, H., T. Maruyama, S. Sakai, S. Kimura, T. Mizuno, and T. Momose. 1973. An outbreak of enteritis due to *Yersinia enterocolitica* occurring at a junior high school. Japan. J. Microbiol. 17: 220-222.

Table 1. Indol reactions of *Yersinia enterocolitica*

No. of cultures	Indol				Where isolated	References
	No. +	% +	No. –	% –		
82	occ +		most –		mostly in Europe	Mollaret and Chevalier (53)
330	15	4.5	315	95.5	do	Niléhn (62)
12	1	83	11	92	Japan	Zen-Yoji and Maruyama (83)
55	3	5.5	52	95	mostly in Europe	Frederiksen (14)
29	24	82.8	5	17	United States	Weaver and Jordan (78)
11	8	73	3	27	do	Herein
20	10	50	10	50	Europe	do
44	1	2.3	43	97.7	Canada	do, Deloreme et al. (8)

Table 2. The biochemical reactions of *Yersinia enterocolitica* according to Niléhn (62)[a]

Test or substrate	Sign	%+ 1-3 days	(%+) 4-30 days
Hydrogen sulfide	–	0	
Urease	+	99.7	
Indol	–	4.5	
Methyl red	+	98.2	(1.8) (4-7 days)
Voges-Proskauer 37 C	–	0	
25 C	+ or –	82.7	
Citrate (Koser's)	–	0	
Motility 37 C	–	0	
25 C	+ or (+)	51.8	(48.2) (3-10 days)
Gelatin	–	0	
Lysine decarboxylase	–	0	
Arginine dihydrolase	–	0	
Ornithine decarboxylase	+	92.7	
Phenylalanine deaminase	–	0	
Glucose	+	100.0	
Lactose	–	0	(2.4^{w})
Sucrose	+	94.9	(2.1)
Mannitol	+	100.0	
Dulcitol	–	0	
Salicin[b]	–	2.4	(0.3)
Adonitol	–	0	
Inositol	d	2.1	(28.5)
Sorbitol	+	97.6	(0.9)
Arabinose	+	96.4	(3.6)

Table 2 (cont'd.)

Test or substrate	Sign	%+	(%+)
Raffinose	–	0	
Rhamnose	–	0	
Malonate	–	0	
Maltose 37 C	(+) or +	15.8	(78.5)
25 C	+	95.8	(4.2)
Trehalose	+	92.7	
Cellobiose	+	100.0	
Glycerol 37 C	(+) or +	47.3	(52.7)
Erythritol	–	0	
Mannose	+	100	
Melibiose	–	0	
Amygdalin	(+) or –	0	(87.3)
Fructose	+	100	
Inulin	–	0	
Galactose	+ or (+)	81.5	(18.5)
Sorbose	+	95.2	(2.1)
Beta galactosidase 37 C	d	83.3	(5.5)
25 C	+	91.2	
Nitrate to nitrate	+	92.4	
Oxidase	–	0	
Catalase	+	100.0	

[a] Based upon results obtained with 330 cultures (reference 62).

[b] A total of 321 cultures were recorded as negative or weakly positive after 20-30 days. Ninety strains fermented xylose in 1-3 days, and 240 were characterized as negative or weak and irregularly positive. Eighteen hydrolysed esculin within 1-7 days; the remainder were weak and irregularly positive.

Table 3. The biochemical reactions given by 75 cultures of *Yersina enterocolitica*

Test or substrate	Number			
	+ 1-2[a]	(+) 3-7	(+) 8-14	–
Hydrogen sulfide (TSI)	0			75
Urease	68	5		2
Indol 37 C	20			55
25 C	21			54
Methyl red 37 or 25 C	75			0
Voges-Proskauer 37 C	0			75
25 C	65			10
Citrate (Simmons') 37 or 25 C	0			75
KCN	0			75
Motility 37 C	0			75
25 C	72			3
Gelatin 22 C	0			75
Lysine decarboxylase	0			75
Arginine dihydrolase	0			75
Ornithine decarboxylase	68	5		2
Phenylalanine deaminase	0			75
Glucose acid 37 or 25 C	75			0
gas	0			75
Lactose 37 C	0	4		71
25 C	0	1	1	73
Sucrose	72			3
Mannitol	75			0
Dulcitol	0			75
Salicin	10	10	1	54
Adonitol	0			75
Inositol 37 C	2	13		60
25 C	7	21	4	43
Sorbitol	74			1
Arabinose	74	1		0
Raffinose	5			70

Table 3. (cont'd.)

Test or substrate	+	(+)	(+)	–
Rhamnose	1			74
Malonate	0			75
Mucate	0			75
Christensen's citrate	8	16		51
Jordan's tartrate	75			0
Sodium pectate	0			75
Sodium acetate 37 C	0			75
25 C	5	33		37
Sodium alginate, nutrient	0			75
utilization	0			75
Lipase, corn oil 37 C	55	18		2
25 C	54	15		6
Maltose 37 C	32	31	7	5
25 C	74	1		0
Xylose	27	2		46
Trehalose	67	7		1
Cellobiose	66	9		0
Glycerol	49	21		5
Alpha methyl glucoside	0			75
Erythritol	0			75
Esculin	11	12	4	48
Mannose	75			0
Melibiose	0			75
Amygdalin	72	3		0
Beta galactosidase 37 C	18			57
25 C	70			5

Table 3. (cont'd.)

Test or substrate	+	(+)	(+)		–
Nitrate to nitrite	74				1
Oxidation-fermentation	74F	1F			
Oxidase	0				75
Dnase	2				73
Cetrimide	3				72
Pigment	0				75
Organic acids (12)[b]	1[a]	2	5	14	–
citrate	0	0	0	3	9
D-tartrate	0				12
i-tartrate	0				12
l-tartrate	0				12

[a] Days of incubation.
[b] Number tested if different from total.

Table 4. The biochemical reactions of 75 cultures of *Yersinia enterocolitica*

Test or substrate	Sign	% +	(%+)[a]
Hydrogen sulfide (TSI)	–	0	
Urease	+	90.7	(6.7)
Indol	*b*		
Methyl red[c] 37 or 25 C	+	100.0	
Voges-Proskauer 37 C	–	0	
25 C	+ or –	86.7	
Citrate (Simmons') 37 or 25 C	–	0	
KCN	–	0	
Motility 37 C	–	0	
25 C	+	96.0	
Gelatin 22 C	–	0	
Lysine decarboxylase	–	0	
Arginine dihydrolase	–	0	
Ornithine decarboxylase	+	90.7	(6.7)
Phenylalanine deaminase	–	0	
Glucose acid 37 or 25 C	+	100.0	
gas	–	0	
Lactose 37 C	–	0	(5.3)
25 C	–	0	(2.6)
Sucrose	+	96.0	
Mannitol	+	100.0	
Dulcitol	–	0	
Salicin	d	13.3	(14.6)

Table 4. (cont'd.)

Test or substrate	Sign	%+	(%+)[a]
Adonitol	–	0	
Inositol 37 C	d	2.7	(17.3)
25 C	d	9.3	(32.3)
Sorbitol	+	98.7	
Arabinose	+	98.7	(1.3)
Raffinose	–	6.7	
Rhamnose	–	1.3	
Malonate	–	0	
Mucate	–	0	
Christensen's citrate	d	10.7	(21.3)
Jordan's tartrate	+	100.0	
Sodium pectate	–	0	
Sodium acetate 37 C	–	0	
25 C	d	6.7	(44.0)
Sodium alginate, nutrient	–	0	
utilization	–	0	
Lipase, corn oil 37 C	+ or (+)	73.3	(24.0)
25 C	+ or (+)	72.0	(20.0)
Maltose 37 C	d	42.7	(50.6)
25 C	+	98.7	(1.3)
Xylose	d	36.0	(2.7)
Trehalose	+ or (+)	89.3	(9.3)
Cellobiose	+ or (+)	88.0	(12.0)
Glycerol	+ or (+)	65.3	(28.0)
Alpha methyl glucoside	–	0	

Table 4. (cont'd.)

Test or substrate	Sign	%+	(%+)
Erythritol	–	0	
Esculin	d	14.7	(21.3)
Mannose	+	100.0	
Melibiose	–	0	
Amygdalin	+	96.0	(4.0)
Beta galactosidase 37 C	– or +	24.0	
25 C	+	93.3	
Nitrate to nitrite	+	98.7	
Oxidation-fermentation	F	98.7	(1.3)
Oxidase	–	0	
DNAse	–	2.7	
Cetrimide	–	4.0	
Pigment	–	0	
Growth on			
MacConkey agar 37 C	+	89.3	
25 C	+	90.7	
SS agar 37 or 25 C	+ or –	89.3	

Footnote Table 4

[a] Figures in parentheses indicate percentages of delayed reactions (3 days or more).

[b] Of these cultures, 26.7% produced indol. However, the majority of strains isolated in the United States have been indol-positive, whereas most of those isolated in Europe have been indol-negative (see also Tables 1 and 2).

[c] Most methyl red tests done with cultures incubated at 37 C yielded weakly positive reactions.

Key: +, 90% or more positive within 1 or 2 days; (+), positive reaction after 3 or more days (decarboxylase tests: 3 or 4 days); –, no reaction (90% or more); + or –, most cultures positive, some strains negative; – or +, most strains negative, some cultures positive; + or (+), most reactions occur within 1 or 2 days, some are delayed; d, different reactions: +, (+), or –. F, fermentative.

Table 5. The biochemical reactions of 20 cultures of *Yersinia pseudotuberculosis*

Test or substrate	Sign	%+	(%+)[a]
Hydrogen sulfide (TSI)	–	0	
Urease	+	100	
Indol 37 or 25 C	–	0	
Methyl red 37 C	$+^{w}$	100	
25 C	+	100	
Voges-Proskauer 37 or 25 C	–	0	
Citrate (Simmons')	–	0	
KCN	–	0	
Motility 37 C	–	0	
25 C	(+) or +	40	(45)
Gelatin 22 C	–	0	
Lysine decarboxylase	–	0	
Arginine dihydrolase	–	0	
Ornithine decarboxylase	–	0	
Phenylalanine deaminase	–	0	
Glucose acid 37 or 25 C	+	100	
gas	–	0	
Lactose	–	0	
Sucrose	–	0	
Mannitol	+	100	
Dulcitol	–	0	
Salicin	(+)	5	(95)
Adonitol	–	0	
Inositol 37 or 25 C	–	0	
Sorbitol	–	0	
Arabinose	+ or (+)	50	45
Raffinose	– or +	20	
Rhamnose	+	100	
Malonate	–	0	
Mucate	–	0	
Christensen's citrate	– or $(+^{w})$	0	(30)
Jordan's tartrate	+ or –	80	
Sodium pectate	–	0	

Table 5. (cont'd.)

Test or substrate	Sign	%+	(%+)
Sodium acetate 37 or 25 C	–	0	
Sodium alginate, nutrient	–	0	
utilization	–	0	
Lipase, corn oil 37 C	–	0	(5^{w})
25 C	–	0	
Maltose 37 C	+	90	
25 C	+	100	
Xylose	+	100	
Trehalose	+	100	
Cellobiose	–	0	
Glycerol	+ or (+)	80	(20)
Alpha methyl glucoside	–	0	
Erythritol	–	0	
Esculin	+	100	
Mannose	+	100	
Melibiose	+	90	(10)
Amygdalin	–	0	
Beta galactosidase 37 C	+ or –	70	
25 C	+ or –	80	
Nitrate to nitrite	+	95	
Oxidation-fermentation	F	100	
Oxidase	–	0	
DNAse	– or (+)	0	(30)
Pigment	–	0	
Growth on			
MacConkey agar 37 or 25 C	+	100	
SS agar 37 C	– or +	25	
25 C	+ or –	65	

[a] Figures in parentheses indicate percentages of delayed reactions (3 days or more).

Key: +, 90% or more positive within 1 or 2 days; (+), positive reaction after 3 or more days (decarboxylase tests: 3 or 4 days); –, no reaction (90% or more); + or –, most cultures positive, some strains negative; – or +, most strains negative, some cultures positive; + or (+), most reactions occur within 1 or 2 days, some are delayed; d, different reactions: +, (+), or –. F, fermentative. w, weakly positive reaction.

Table 6. Differentiation of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*

Test or substrate	*Y. enterocolitica*[a]			*Y. pseudotuberculosis*[b]		
	Sign	%+	(%+)	Sign	%+	(%+)
Indol	c			–	0	
Voges-Proskauer 37 C	–	0		–	0	
25 C	+ or –	86.7		–	0	
Ornithine decarobxylase	+	90.7	(6.7)	–	0	
Sucrose	+	96.0		–	0	
Salicin	d	13.3	(14.6)	(+)	5	(95.0)
Inositol 37 C	d	2.7	(17.3)	–	0	
25 C	d	9.3	(32.3)	–	0	
Sorbitol	+	98.7		–	0	
Rhamnose	–	1.3		+	100.0	
Xylose	d	36.0	(2.7)	+	100.0	
Cellobiose	+ or (+)	88.0	(12.0)	–	0	
Esculin	d	14.7	(21.3)	+	100.0	
Melibiose	–	0		+	90.0	(10.0)
Amygdalin	+	96.0	(4.0)	–	0	
Beta galactosidase 37 C	– or +	24.0		+ or –	70.0	
25 C	+	93.3		+ or –	80.0	
Lipase, corn oil	+ or (+)	73.3	(24.0)	–	0	(5.0[w])

[a] Based on data given in Table 4.

[b] Based on data given in Table 5.

[c] Of the cultures studied, 26.7% produced indol. However, the majority of strains isolated in the United States have been indol-positive, whereas most of those isolated in Europe have been indol-negative.

Key: +, 90% or more positive within 1 or 2 days; (+), positive reaction after 3 or more days (decarboxylase tests: 3 or 4 days); –, no reaction (90% or more); + or –, most cultures positive, some strains negative; – or +, most strains negative, some cultures positive; + or (+), most reactions occur within 1 or 2 days, some are delayed; d, different reactions: +, (+), or –. F, fermentative. w, weakly positive reaction.

Table 7. Differentiation of *Yersinia enterocolitica* and *Yersinia pestis*

Test or substrate	*Y. enterocolitica*[a]			*Y. pestis*[b]		
	Sign	%+	(%+)	Sign	%+	(%+)
Urease	+	90.7	(6.7)	–	0	
Indol	c			–	0	
Motility 37 C	–	0		–	0	
25 C	+	96.0		–	0	
Ornithine decarboxylase	+	90.7	(6.7)	–	0	
Sucrose	+	96.0		–	0	
Sorbitol	+	98.7		– or +	30.5	
Cellobiose	+ or (+)	88.0	(12.0)	–	0	
Glycerol	+ or (+)	65.3	(28.0)	–	1.9	
Esculin	d	14.7	(21.3)	+	100.0	
Amygdalin	+	96.0	(4.0)	–	0	
DNAse	–	2.7		(+)	0	(100)
Growth on SS agar	+ or –	89.3		– or (+)	0	(rare +)

[a] Based on data given in Table 4.

[b] Adapted from results published by Devignat (9), Devignat and Boivin (10), Hudson et al. (20), and Weaver et al. (79).

[c] Of the cultures studied, 26.7% produced indol. However, the majority of strains isolated in the United States have been indol-positive, whereas most of those recovered in Europe have failed to produce indol.

Key: +, 90% or more positive within 1 or 2 days; (+), positive reaction after 3 or more days (decarboxylase tests: 3 or 4 days); –, no reaction (90% or more); + or –, most cultures positive, some strains negative; – or +, most strains negative, some cultures positive; + or (+), most reactions occur within 1 or 2 days, some are delayed; d, different reactions: +, (+), or –. F, fermentative. w, weakly positive reaction.

Table 8. Differentiation of *Yersinia pseudotuberculosis* and *Yersinia pestis*

Test or substrate	*Y. pseudotuberculosis*[a]			*Y. pestis*[b]		
	Sign	%+	(%+)	Sign	%+	(%+)
Urease	+	100.0		–	0	
Motility 37 C	–	0		–	0	
25 C	(+) or +	40.0	(45.0)	–	0	
Salicin	(+)	5.0	(95.0)	– or +	19.0	
Sorbitol	–	0		+ or –	50.0	
Arabinose	–	0		– or +	30.5	
Rhamnose	+	100.0		–	0	
Xylose	+	100.0		*c*		
Glycerol	+ or (+)	80.0	(20.0)	–	1.9	
Melibiose	+	90.0	(10.0)	–	0	
DNAse	–	0	(6.0)	(+)	0	(100.0)

[a] Based on data given in Table 5.

[b] Adapted from results published by Devignat (9), Devignat and Boivin (10), Hudson et al. (20), and Weaver et al. (79).

[c] The value of this substrate for this particular differentiation cannot be ascertained with certainty. Hudson et al. (20) indicate that all of their strains (105) failed to ferment xylose, whereas Devignat (8) and Weaver et al. (79) list all cultures as xylose-positive. Whether this is a biotypic difference, the result of using different methods, or a difference in the form of the carbohydrate employed cannot be determined. The authors have used only the naturally occurring form of xylose d (–) in the past and in the present work. This form also is used by Dr. Weaver.

Key: +, 90% or more positive within 1 or 2 days; (+), positive reaction after 3 or more days (decarboxylase tests: 3 or 4 days); –, no reaction (90% or more); + or –, most cultures positive, some strains negative; – or +, most strains negative, some cultures positive; + or (+), most reactions occur within 1 or 2 days, some are delayed; d, different reactions: +, (+), or –. F, fermentative, w, weakly positive reaction.

Table 9. Comparison of antibiograms given by cultures of *Yersinia enterocolitica* (N=75) and *Yersinia pseudotuberculosis* (N=20)

Antibiotic	Percentage of Isolates					
	Y. enterocolitica			*Y. pseudotuberculosis*		
	Sensitive[a]	Intermediate	Resistant	Sensitive	Intermediate	Resistant
Colistin	100.0			45.0	10.0	45.0
Nalidixic acid	100.0			100.0		
Sulfadiazine	98.7	1.3		95.0	5.0	
Gentamycin	100.0			100.0		
Streptomycin	96.0	4.0		100.0		
Kanamycin	100.0			100.0		
Tetracycline	98.7		1.3	90.0	10.0	
Chloramphenicol	100.0			100.0		
Penicillin	1.3		98.7	80.0	20.0	
Ampicillin	17.3	6.7	76.0	100.0		
Carbenicillin	10.7[b]		89.3	100.0		
Cephalothin	66.7	26.7	6.6	100.0		

[a] Interpretation of zone sizes was made according to standard methods (2).
[b] Based on sensitivity of *Proteus* and *Escherichia coli* to carbenicillin (2).