Five Biovars of *Yersinia enterocolitica* Delineated by Numerical Taxonomy

KEN-ICHI KANEKO AND NOBUO HASHIMOTO

Department of Veterinary Public Health, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060, Japan

A total of 52 strains (41 of *Yersinia enterocolitica* strains, 3 *Yersinia pseudotuberculosis* strains isolated from animals, and 8 reference strains) were scored for 210 characters (64 morphological, 63 growth, 8 antibiotic susceptibility, and 75 biochemical) which were determined at both 37 and 22°C. The results were analyzed by numerical taxonomy methods (simple matching coefficients and clustering by unweighted average). The phenograms of the *Y. enterocolitica* strains delineated four and five phenons from the 37 and 22°C data, respectively. The phenons at 22°C were as follows: (i) rhamnose-negative, lecithinase- and sucrose-positive strains; (ii) rhamnose-, lecithinase-, and sucrose-negative strains; (iii) rhamnose- and lecithinase-negative, sucrose-positive strains; (iv) rhamnose-, lecithinase-, and sucrose-positive strains; and (v) rhamnose-positive, lecithinase-negative, and sucrose-positive strains. These five phenons were designated biovars 1, 2, 3, 4, and 5, respectively. The phenograms of the *Y. pseudotuberculosis* strains delineated one phenon from both the 37°C data and the 22°C data. Three sucrose-negative strains of *Y. enterocolitica* had the same deoxyribonucleic acid base composition (guanine-plus-cytosine content) as a strain of serovar O3 of *Y. enterocolitica* and a strain of serovar IVA of *Y. pseudotuberculosis*.

Based on genetic data obtained by deoxyribonucleic acid (DNA)-DNA hybridization, the following four species of *Yersinia* are currently recognized by Bercovier et al. (3, 4), Brenner (6), Brenner et al. (7, 8), and Ursing et al. (33): *Yersinia enterocolitica* sensu stricto (rhamnose negative), *Yersinia frederiksenii* (rhamnose positive), *Yersinia intermedia* (rhamnose and melibiose positive), and *Yersinia kristensenii* (sucrose negative). *Y. enterocolitica* is quite heterogenous, both phenetically and genetically, and previously reported work has been aimed at obtaining a more precise delineation of *Y. enterocolitica* (12, 20).

Y. enterocolitica sensu stricto contains rhamnose- and lecithinase-negative strains, as well as rhamnose-negative, lecithinase-positive strains. The latter are more prevalent in small rodents than the former (1, 2, 15, 17-19). The taxonomic relationships of these strains have been examined in numerical analyses by Harvey and Pickett (12), Kapperud et al. (20), and Stevens and Mair (30). However, numerical taxonomy has not yet been applied to differentiating the rhamnose-negative, lecithinase-positive strains from the strains that are rhamnose and lecithinase negative.

The isolation of sucrose-negative *Y. enterocolitica* strains has been reported in Japan (17), Europe (2, 26), Canada (9), and the United

States (5). These strains were found in small rodents (2, 17).

The purpose of this study was to determine, by numerical analysis, the taxonomic relationships of the rhamnose- and lecithinase-negative strains and the sucrose-negative strains of *Y. enterocolitica* from small rodents.

MATERIALS AND METHODS

Bacterial strains. A total of 52 strains (41 *Y. enterocolitica* strains, 3 *Yersinia pseudotuberculosis* strains isolated from animals, and 8 reference strains) were examined in this study (Table 1). Of these, the 41 *Y. enterocolitica* strains (Table 1, serial no. 5 through 30 and 38 through 52) and 3 *Y. pseudotuberculosis* strains (serial no. 31 through 33) were our isolates (14-17). In addition, reference strains of *Y. enterocolitica* (serial no. 1), *Y. frederiksenii* (serial no. 2), *Y. intermedia* (serial no. 3), and *Y. kristensenii* (serial no. 4), and four reference strains of *Y. pseudotuberculosis* (serial no. 34 through 37) were studied. Of these, strain 2 was 64% related to the type strain of *Y. frederiksenii* (strain 6175) based on DNA-DNA hybridization (33). Strain 3 is the type strain of *Y. intermedia* (7). All cultures were maintained frozen at -80°C in equal volumes of calf serum and a 10% lactose-water solution.

Methods. In determining the morphological characters, the organisms were subcultured on Trypticase soy agar (BBL Microbiology Systems) for 40 to 48 h. Twenty morphological characters based on cell length, width, and shape and eight characters based on cell arrangement were examined by staining the cells with

TABLE 1. List of strains studied

Serial no.	Species	Biochemical properties ^a			Strain designation	Source	Habitat
		Rham-nose	Leci-thinase	Su-crose			
1	<i>Y. enterocolitica</i>	—	—	+	CIP160	R. Sakazaki	Unknown
2	<i>Y. frederiksenii</i>	+	—	+	YE867	R. Sakazaki	Unknown
3	<i>Y. intermedia</i>	+	+	+	Bottone no. 48	R. Sakazaki	Human urine
4	<i>Y. kristensenii</i>	—	—	—	YE103	R. Sakazaki	Unknown
5	<i>Y. enterocolitica</i>	—	—	+	314-2	K. Kaneko	Dog
6	<i>Y. enterocolitica</i>	—	—	+	231-3	K. Kaneko	Dog
7	<i>Y. enterocolitica</i>	—	—	+	231-4	K. Kaneko	Dog
8	<i>Y. enterocolitica</i>	—	—	+	234-2	K. Kaneko	Dog
9	<i>Y. enterocolitica</i>	+	—	+	M824-2	K. Kaneko	Brown rat
10	<i>Y. enterocolitica</i>	+	—	+	M824-1	K. Kaneko	Brown rat
11	<i>Y. enterocolitica</i>	+	—	+	ZM433-1	K. Kaneko	Brown rat
12	<i>Y. enterocolitica</i>	+	—	+	M1245-1	K. Kaneko	Brown rat
13	<i>Y. enterocolitica</i>	+	—	+	ZS445-1	K. Kaneko	Brown rat
14	<i>Y. enterocolitica</i>	+	—	+	ZS564-2	K. Kaneko	Brown rat
15	<i>Y. enterocolitica</i>	+	+	+	FL-1	K. Kaneko	Fox
16	<i>Y. enterocolitica</i>	—	—	+	S1274-5	K. Kaneko	Brown rat
17	<i>Y. enterocolitica</i>	—	—	+	S1274-2	K. Kaneko	Brown rat
18	<i>Y. enterocolitica</i>	+	+	+	M1226-1	K. Kaneko	Brown rat
19	<i>Y. enterocolitica</i>	—	—	—	S1096-4	K. Kaneko	Large red-backed mouse
20	<i>Y. enterocolitica</i>	—	—	—	M1778-2	K. Kaneko	Old World wood mouse
21	<i>Y. enterocolitica</i>	—	—	—	M1779-1	K. Kaneko	Old World wood mouse
22	<i>Y. enterocolitica</i>	—	—	—	M2200-3	K. Kaneko	Large red-backed mouse
23	<i>Y. enterocolitica</i>	—	—	—	S2200-1	K. Kaneko	Large red-backed mouse
24	<i>Y. enterocolitica</i>	—	—	—	M1095-2	K. Kaneko	Large red-backed mouse
25	<i>Y. enterocolitica</i>	—	—	—	M1096-1	K. Kaneko	Large red-backed mouse
26	<i>Y. enterocolitica</i>	—	—	—	S1778-2	K. Kaneko	Old World wood mouse
27	<i>Y. enterocolitica</i>	—	—	—	S1779-2	K. Kaneko	Old World wood mouse
28	<i>Y. enterocolitica</i>	—	—	—	M2200-4	K. Kaneko	Large red-backed mouse
29	<i>Y. enterocolitica</i>	—	—	—	M2201-1	K. Kaneko	Large red-backed mouse
30	<i>Y. enterocolitica</i>	—	—	—	M2201-4	K. Kaneko	Large red-backed mouse
31	<i>Y. pseudotuberculosis</i>	+	—	—	S1186-1	K. Kaneko	Old World wood mouse
32	<i>Y. pseudotuberculosis</i>	+	—	—	EM1519-1	K. Kaneko	Brown rat
33	<i>Y. pseudotuberculosis</i>	+	—	—	M1909-2	K. Kaneko	Brown rat
34	<i>Y. pseudotuberculosis</i>	+	—	—	377	M. Tsubokura	Unknown
35	<i>Y. pseudotuberculosis</i>	+	—	—	83	M. Tsubokura	Unknown
36	<i>Y. pseudotuberculosis</i>	+	—	—	R16	M. Tsubokura	Unknown
37	<i>Y. pseudotuberculosis</i>	+	—	—	Nielson	M. Tsubokura	Unknown
38	<i>Y. enterocolitica</i>	—	+	+	M170-1	K. Kaneko	Large red-backed mouse
39	<i>Y. enterocolitica</i>	—	+	+	M1562-1	K. Kaneko	Large red-backed mouse
40	<i>Y. enterocolitica</i>	—	+	+	SD2182-1	K. Kaneko	Large red-backed mouse
41	<i>Y. enterocolitica</i>	—	+	+	SD2188-1	K. Kaneko	Large red-backed mouse
42	<i>Y. enterocolitica</i>	—	+	+	F38-1	K. Kaneko	Fox
43	<i>Y. enterocolitica</i>	—	+	+	M134-9	K. Kaneko	Brown rat
44	<i>Y. enterocolitica</i>	—	+	+	M694-3	K. Kaneko	Brown rat
45	<i>Y. enterocolitica</i>	—	+	+	S1044-4	K. Kaneko	Brown rat
46	<i>Y. enterocolitica</i>	—	+	+	S1784-11	K. Kaneko	Brown rat
47	<i>Y. enterocolitica</i>	—	+	+	M1949-1	K. Kaneko	Brown rat
48	<i>Y. enterocolitica</i>	—	+	+	ZM235-9	K. Kaneko	Brown rat
49	<i>Y. enterocolitica</i>	—	+	+	ZM389-9	K. Kaneko	Brown rat
50	<i>Y. enterocolitica</i>	—	+	+	ZM544-4	K. Kaneko	Brown rat
51	<i>Y. enterocolitica</i>	—	+	+	ZM734-1	K. Kaneko	Brown rat
52	<i>Y. enterocolitica</i>	—	—	+	SD1416-11	K. Kaneko	Brown rat

^aRhamnose and sucrose reactions, 3 days at 22°C; lecithinase reaction, 2 days at 22°C.

Loeffler methylene blue. The presence and location of flagella were determined by using Leifson stain. The Gram reaction was determined by the Hucker modification of the Gram stain. The staining reagents and methods used were those described by Paik and Suggs

(24). Fifteen characters involving colony size, shape (elevation and edge), and density were determined from cultures grown on Trypticase soy agar (BBL). Seventeen pigment formation characters were recorded from observations of growth on heart infusion agar

(Difco Laboratories) containing 0.2% (wt/vol) DL-phenylalanine. The morphological characters were coded as described by Colwell (10).

The abilities of the bacteria to utilize single carbon sources for growth were tested by the methods of Véron (35). The carbon sources tested were acetic acid, *N*-acetyl glucosamine, L-alanine, amidol, L-aspartic acid, *n*-butyl alcohol, *n*-butyric acid, *n*-caproic acid, *n*-caprylic acid, citric acid, L-citrulline, L-cysteine, ethyl alcohol, D-fucose, gluconate, D-glucosamine, D-glucose, L-glutamic acid, DL-glyceric acid, glycine, L-histidine, isobutyric acid, L-isoleucine, isopropyl alcohol, isovaleric acid, α -ketoglutaric acid, DL-lactic acid, L-leucine, D-malic acid, L-methionine, methyl alcohol, mucic acid, melezitose, nicotinic acid, oxalic acid, phenol, L-phenylalanine, L-proline, propionic acid, propylene glycol, pyruvic acid, D-ribose, L-serine, succinic acid, L-threonine, L-thyrosine, L-tartaric acid, L-tryptophane, *n*-valeric acid, valine, and urea.

Growth at pH 4, 7, and 9 was examined on nutrient agar (Difco) in which the pH was adjusted with trisaminomethane and citrate phosphate buffer after autoclaving. Tests for growth were made in or on the following media: nutrient broth containing 7.0% (wt/vol) NaCl; MacConkey agar (Eiken); *Salmonella*-shigella agar (Eiken); KCN broth; nutrient broth (Difco) containing 0.2% (wt/vol) 2,3,5-triphenyltetrazolium chloride; and nutrient agar (Difco) containing 1.0% oxbile (Oxoid) and 0.032% potassium tartrate. The ability to grow at 0 and 42°C was determined in nutrient broth (Difco).

Antibiotic susceptibilities were determined on heart infusion agar (Difco) containing one of the following antibiotics: penicillin (10 U/ml), dihydrostreptomycin (2.5 µg/ml), chloramphenicol (2.5 µg/ml), erythromycin (30 µg/ml), kanamycin (2.5 µg/ml), chlortetracycline (90 µg/ml), terramycin (2.5 µg/ml), and tetracycline (2.5 µg/ml).

Production of acid from carbohydrates was detected in peptone water (Difco) containing 0.002% (wt/vol) bromthymol blue and 1% (wt/vol) filter- or heat-sterilized carbohydrate. Acid formation was scored after 24 h and 3 days. The following carbohydrates were tested: adonitol, arabinose, arbutin, cellobiose, dextran, dulcitol, erythritol, esculin, fructose, galactose, glycerin, glycogen, inositol, inulin, maltose, mannitol, mannose, melibiose, α -methyl glucoside, raffinose, rhamnose, salicin, sorbitol, sorbose, starch, sucrose, trehalose, and xylose.

The following tests were performed by the methods described by Cowan and Steel (11). Hemolysis was detected on heart infusion agar (Difco) containing 2% washed sheep erythrocytes. To determine gelatin liquefaction, stabs in nutrient broth (Difco) containing 12% (wt/vol) gelatin were observed for 10 days. Other characters determined included: the mode of lactose metabolism by means of the oxidation-fermentation medium of Hugh and Leifson; production of dextran and levan on nutrient agar (Difco) containing 5% (wt/vol) sucrose; methyl red and Voges-Proskauer reactions in methyl red-Voges-Proskauer broth (Eiken); oxidase by the method of Kovacs; catalase activity (addition of 3% hydrogen peroxide to growth on heart infusion agar [Difco]); phosphatase production by the method of Baird-Parker; and reduction of nitrate and

nitrite (determined in peptone water [Difco] containing 0.1% potassium nitrate or potassium nitrite). Also tested were the following: reactions in purple milk; hydrolysis on skim milk agar made from heart infusion agar (Difco) containing 1% skim milk; ammonia production from Trypticase peptone water (BBL); hydrogen sulfide production on triple sugar iron agar (Eiken); urease production on Christensen urease agar (BBL); indole production (determined in lysine-indole-motility medium [Nissui] by using Kovac reagent); hydrolysis of Tweens 20, 40, 60, and 80 on Sierra medium; citrate utilization on Simmons medium; phenylalanine deaminase production on phenylalanine agar (Difco); production of decarboxylases for L-lysine, L-ornithine, L-arginine, and L-glutamic acid in Møller medium; arginine hydrolysis on arginine medium; esculin hydrolysis on nutrient agar (Difco) containing 0.1% ferric citrate and 0.1% esculin; deoxyribonuclease production on deoxyribonuclease medium (BBL); utilization of the organic acids of D-tartrate, mucate, and citrate on the medium of Kauffmann and Petersen; lecithinase production on heart infusion agar (Difco) containing 0.5% egg yolk; oxidation of gluconate in gluconate broth and hippurate hydrolysis on hippurate agar; and decomposition of tyrosine and xanthine on tyrosine and xanthine agars. The *o*-nitrophenyl- β -D-galactopyranoside test was performed with *o*-nitrophenyl- β -D-galactopyranoside broth.

Acetoin formation on nutrient broth containing 0.5% (vol/vol) 2,3-buteneglycol was also determined by using the Voges-Proskauer reagent. The production of indole pyruvic acid from tryptophane was determined as described previously (29). The production of lipase

TABLE 2. Characters for which all 52 strains were positive at both 37 and 22°C

Cell shape straight, coccobacillary, round-ended, tapered ^a
Gram stain reaction (negative)
Cell arrangement (single cells) ^a
Colony size (<2 mm)
Colony elevation (convex)
Colony edge (entire)
Density of colony (translucent)
Pigment formation (off-white to gray)
Utilization of glucose ^b
Susceptibility to chlortetracycline
Growth at pH 7
Grown on MacConkey agar
Acid from:
Fructose
Galactose ^a
Glycerin
Mannitol
Mannose
Trehalose
Production of:
Urease
Catalase
Acetoin from 2,3-buteneglycol
Reduction of nitrate ^a

^a All 52 strains were positive only at 37°C.

^b All 52 strains were positive only at 22°C.

TABLE 3. Characteristics for which all 52 strains were negative at both 37 and 22°C

Cell length	Utilization of— <i>continued</i>
≤0.5 µm	Ethyl alcohol
>3 µm ^a	DL-Glyceric acid ^b
Variable	Glycine
Cell width	Isobutyric acid
Oval	L-Isoleucine
Variable	Isopropyl alcohol
Cell shape	Isovaleric acid ^a
Curved	DL-Lactic acid ^a
Spiral	L-Leucine
Coccus	D-Malic acid ^a
Branching	Melezitose ^a
Pleomorphic	L-Methionine
Square-ended	Methyl alcohol ^b
Fusiform ^a	Nicotinic acid ^b
Spores present	Phenol ^a
Gram stain reaction	L-phenylalanine
Gram positive	Propionic acid
Variable	Propylene glycol ^a
Peritrichous cells ^a	L-Tartaric acid
Cell arrangement	L-Tryptophane ^a
Paired cells	n-Valeric acid
Chains of cells with ≤5 cells per chain ^a	Valine ^a
Chains of cells with >5 cells per chain	Urea ^a
Clumping of coccal forms	Growth on potassium tartrate agar
Packet formation of coccal forms	Growth at pH 4 ^a
Filaments	Acid from:
Variable	Adonitol ^a
Colony size	Erythritol ^a
2–5 mm	Dulcitol
>5 mm	Glycogen
Colony elevation	Inulin
Flat	Production of:
Raised	Indolepyruvic acid
Umbonate	Hydrogen sulfide
Colony edge	Ammonia
Crenated	Oxidase
Erose	Decarboxylase for lysine
Fimbriate	Decarboxylase for arginine
Spreading	Decarboxylase for glutamic acid ^b
Colony density	Phenylalanine deaminase
Transparent	Phosphatase
Opaque	Arginine hydrolysis
Pigment formation	Gelatin liquefaction ^a
White	Hydrolysis on skim milk agar ^a
Red	Clotting in purple milk ^a
Pink	Diffusible pigments
Orange	Yellow
Yellow	Green
Green	Brown
Violet-purple	Black
Brown	Fluorescent under ultraviolet light
Black	Luminous
Blue	Hemolysis
Utilization of:	Reduction of nitrite
L-Alanine ^b	Utilization of D-tartrate in Kauffmann-Petersen
Amidol	(KP) medium
n-Butyl alcohol	Hippurate hydrolysis
n-Butyric acid ^a	Decomposition of:
n-Caproic acid	Tyrosine
n-Caprylic acid	Xanthine
L-Cysteine ^b	

^a All 52 strains were negative only at 37°C.^b All 52 strains were negative only at 22°C.

TABLE 4. Characters for which the 52 strains gave different results at 37°C

Character	No. of strains positive	Strain(s) that gave the less common result
Utilization of D-glucosamine	51	1
Acid from arabinose	51	35
Growth on agar containing 1% ox bile	51	4
<i>o</i> -Nitrophenyl- β -D-galactopyranoside	51	1
Utilization of:		
D-Glucose	50	1, 35
N-Acetyl glucosamine	50	1, 14
Methyl red reaction	50	31, 35
Growth on salmonella-shigella agar	50	3, 35
Growth at 0°C	50	4, 46
Acid from:		
Dextran	49	11, 13, 32
Maltose	49	1, 6, 7
Xylose	47	1, 6-8, 52
Ornithine decarboxylase	45	31-37
Acid from:		
Arbutin	44	1, 4-8, 16, 52
Sorbitol	44	26, 31-37
Utilization of D-ribose	43	1, 31-37, 50
Acid from sorbose	42	1, 16, 17, 31-37
Utilization of:		
Gluconate	41	1, 5-7, 16, 17, 22, 29, 33, 36, 52
Pyruvic acid	40	1, 25, 27-29, 32-34, 36, 37, 44, 48
Susceptibility to kanamycin	40	1, 2, 5-8, 11, 17, 23, 35, 39, 52
Utilization of L-histidine	39	5-8, 19, 28-30, 32-34, 36, 37
Acid in purple milk	39	1, 6, 7, 11, 13, 31-37, 46
Acid from cellobiose	38	11, 13, 19, 24, 25, 31-38, 40
Utilization of acetic acid	37	1, 25-27, 30, 31, 33-37, 40, 43, 44, 52
Acid from salicin	36	4-8, 19, 20, 22-26, 28-30, 52
Indole production	34	1, 6, 7, 16, 17, 19, 20, 22, 23, 28, 30, 31, 33-37, 52
Susceptibility to erythromycin	34	2, 9-11, 13, 19-30, 51
Cell width (slender)	34	2, 3, 5-9, 16, 19, 31, 33, 36, 38, 46, 48, 49, 51, 52
Esculin hydrolysis	33	1, 4-8, 16, 17, 19, 20, 22-26, 28-30, 52
Cell length (1.3-3 μ m)	33	2, 3, 5-9, 15, 16, 19, 31, 33, 36, 38, 46, 48, 49, 51, 52
Acid from sucrose	32	4, 19-37
Utilization of L-glutamic acid	31	1, 8-10, 19, 20, 25-27, 29-34, 36, 37, 40, 41, 45, 46
Susceptibility to tetracycline	29	16-27, 29, 30, 38-40, 44-46, 48, 50, 52
Utilization of L-serine	26	1, 2, 7, 8, 15, 17, 19, 24-37, 39, 40, 45, 46, 50 ^a
Utilization of citrate in KP medium	25	2, 3, 9, 11, 13, 15, 18, 22, 23, 28-30, 38-46, 48-51
Utilization of L-aspartic acid	24	2, 4-7, 10-18, 21, 23, 24, 38, 39, 42, 44, 50-52
Voges-Proskauer reaction	22	2, 3, 5-8, 15, 18, 38-48, 50-52
Utilization of L-proline	21	1-3, 9, 11, 14, 18, 21-25, 28-31, 36, 37, 43, 45, 52
Acid from:		
Esculin	20	2, 3, 9, 10, 15, 18, 38-51
Inositol	19	2, 3, 9, 10, 12, 14, 15, 38, 39, 41-46, 48-51
Cell length (0.5-1.2 μ m)	19	2, 3, 5-9, 15, 16, 19, 31, 33, 36, 38, 46, 48, 49, 51, 52
Oxidation of lactose	18	2, 4, 8, 17, 19, 38-50
Cell width (short)	18	2, 3, 5-9, 16, 19, 31, 33, 36, 38, 46, 48, 49, 51, 52
Utilization of L-threonine	17	1-3, 9, 12, 15, 16, 20-25, 30, 31, 43, 52

on lipase medium supplemented with corn oil was determined by the method of Vera and Dumoff (34). Reduction of trimethylamine oxide was determined by the method of Wood and Baird (38).

With the exception of the determinations of temperature growth ranges, all tests were done at both 22 and 37°C.

Numerical analysis. The simple single-linkage method was used for the numerical analysis. The data were coded in binary notation by using 1 for positive and 0 for negative. All characters were assigned equal

weight, and the association coefficients between pairs of bacteria were obtained by computation, using the program of Quadling (27). The similarities between pairs of strains were calculated by using the simple matching coefficient, including negative matches in the similarity computation. Pairs of strains were arranged in order of their similarity values by computation. Phenograms were constructed from the computation data by using the method described of Lessel and Holt (21). The unweighted pair group method was used.

G+C content of DNA. The DNAs were extracted

TABLE 4—Continued

Character	No. of strains positive	Strain(s) that gave the less common result
Acid from rhamnose	17	2, 3, 9–15, 18, 31–37
Lipase of corn oil	15	8, 38–51
Utilization of L-alanine	14	3–5, 38–40, 42–44, 47, 49–52
Susceptibility to dihydrostreptomycin	14	3, 9, 10, 12–14, 16, 18, 31–34, 36, 37
Hydrolysis of:		
Tween 60	14	3, 15, 38–40, 43–51
Tween 40	13	3, 15, 39, 40, 43–51
Utilization of α -ketoglutaric acid	12	3, 10, 11, 13, 16, 22, 23, 28–31, 42
Acid from melibiose	12	3, 15–18, 31–37
Utilization of mucic acid	11	3, 9, 10, 12–16, 19, 22, 23
Growth in KCN broth	11	3, 9, 13–15, 18, 22, 23, 28, 29, 46
Hydrolysis of Tween 80	11	38–40, 42–48, 51
Growth at pH 9	10	2, 9–14, 25, 38, 39
Growth at 42°C	10	38–43, 45, 46, 49, 51
Growth in 7% NaCl broth	9	22, 23, 28, 29, 38, 39, 44, 46, 47
Susceptibility to penicillin	8	1, 31–37
Lecithinase	8	38, 40, 42–45, 48, 51
Acid from raffinose	7	15–18, 20, 31, 44
Citrate utilization on Simmons medium	7	3, 9, 11–14, 18
Utilization of citric acid	6	2, 3, 9, 12, 17, 18
Reduction of trimethylamine oxide	6	2, 16, 17, 45, 50, 51
Utilization of D-fucose	5	25, 26, 30, 33, 37
Susceptibility to chloramphenicol	5	1, 4, 31, 32, 35
Fermentation of lactose	5	9, 20, 21, 26, 27
Utilization of mucate in KP medium	4	4, 10, 12, 14
Hydrolysis of Tween 20	4	3, 15, 48, 51
Utilization of:		
L-Cysteine	3	11, 24, 52
L-Tyrosine	3	22–24
Nicotinic acid	3	7, 13, 22
Acid from:		
α -Methyl glucoside	3	3, 15, 18
Starch	3	29, 30, 35
Utilization of oxalic acid	2	9, 12
Glutamic acid decarboxylase	2	39, 40
Utilization of:		
L-Citrulline	1	7
DL-Glyceric acid	1	10
Methyl alcohol	1	6
Succinic acid	1	4
Susceptibility to terramycin	1	35
Deoxyribonuclease	1	32
Growth in TTC broth ^b	1	14
Oxidation of gluconate	1	18
Levan	1	29
Dextran	1	1

^a These strains were negative.^b TTC, 2,3,5-Triphenyltetrazolium chloride.

from three sucrose-negative strains of *Y. enterocolitica*, one rhamnose-negative *Y. enterocolitica* serovar O3 strain, one *Y. pseudotuberculosis* serovar IVA strain, and one *Aeromonas salmonicida* strain (ATCC 14174) as described by Marmur (22). The guanine-plus-cytosine (G+C) content of each DNA preparation was calculated from the thermal melting point by using the method of Marmur and Doty (23). The thermal melting point was determined with a Hitach model 124 spectrophotometer. The experiment was repeated three times for each of the *Yersinia* strains.

RESULTS

Characters of the strains tested. The characters of the strains are listed in Tables 2 through 5. At 37°C, 24 characters were all positive, 100 were all negative, and there were 86 characters in which one or more of the strains differed (Table 4). At 22°C, 21 characters were all positive, 86 were all negative, and there were 103 characters in which one or more of the strains differed (Table 5).

TABLE 5. Characteristics for which the 52 strains gave different results at 22°C

Character	No. of strains positive	Strain(s) that gave the less common result
Acid from:		
Arabinose	51	35
Maltose	51	1
Reduction of nitrate	51	4
Growth on agar containing 1% ox bile	51	4
<i>o</i> -Nitrophenyl- β -D-galactopyranoside	51	1
Growth on salmonella-shigella agar	51	35
Cell shape (tapered)	51	4
Cell arrangement (single cells)	51	4
Utilization of:		
<i>N</i> -Acetyl glucosamine	50	1, 44
D-Glucosamine	50	1, 44
Growth at 0°C	50	4, 46
Methyl red reaction	48	38, 40-42
Utilization of pyruvic acid	47	28, 44, 46, 50, 51
Acid from:		
Dextran	47	24, 38-41
Xylose	47	1, 6-8, 52
Galactose	46	6, 7, 18, 33, 35, 37
Utilization of:		
L-Glutamic acid	46	1, 8, 19, 21, 24, 34
L-Histidine	45	5-8, 10, 36, 37
Ornithine decarboxylase	45	31-37
Acid from sorbitol	44	26, 31-37
Utilization of citrate in KP medium	44	1, 19-21, 24, 26, 27, 47
Peritrichous cells	44	1, 6, 7, 31-34, 52
Utilization of D-ribose	42	1, 31-38, 50
Growth at pH 9	42	1, 4, 6-8, 17, 34, 35, 37, 52
Acid from sorbose	41	1, 16, 17, 24, 31-37
Utilization of gluconate	39	1, 2, 5-7, 9, 11, 13, 28, 32, 36, 46, 52
Acid from cellobiose	38	11, 13, 19, 24, 25, 31-38, 40
Cell width (slender)	37	2, 6-9, 21, 22, 24, 27, 32, 33, 36, 45, 49, 52
Indole production	36	1, 6, 7, 16, 17, 22, 23, 28-31, 33, 35-37, 52
Acid in purple milk	36	1, 6, 7, 11-13, 31-37, 46, 49, 52
Cell length (1.3-3 μ m)	36	2, 4, 8, 14, 19, 21, 22, 24, 27, 29, 32, 33, 36, 45, 49, 52
Acid from arbutin	35	1, 4-8, 19, 20, 22-26, 28-30, 52
Utilization of L-asparatic acid	33	1, 2, 5-8, 10, 11, 13, 15-21, 23, 24, 35
Growth in TTC broth ^a	33	1, 3-5, 8, 11, 12, 16, 17, 26, 27, 31-37, 50
Reduction of trimethylamine oxide	33	4, 10-14, 19, 20, 24-27, 31-37
Acid from:		
Sucrose	32	4, 19-37
Salicin	31	4-8, 16, 17, 19-23, 25-30, 35, 37, 52
Esculin hydrolysis	31	1, 4-8, 16, 17, 19-30, 52
Utilization of acetic acid	30	1, 2, 10, 26, 28, 32-36, 38-44, 46-49, 50, 52
Growth in KCN broth	29	1, 2, 4-8, 19-21, 26, 27, 32-37, 41, 43, 44, 51, 52
Growth in 7% NaCl broth	27	1-7, 16, 18, 21, 24-26, 28, 31-33, 35-37, 43, 45, 51, 52
Utilization of L-serine	26	2-4, 6, 9-14, 16, 18, 21, 22, 24, 25, 27, 31, 33, 34, 42-45, 48, 51 ^b
Susceptibility of tetracycline	25	1-9, 15-18, 32-37, 44-47, 49, 52
Acid from:		
Esculin	24	1-3, 9, 10, 12-15, 18, 38-51
Inositol	23	2-5, 8-15, 39, 41-46, 48-51
Utilization of L-proline	23	2-5, 9-11, 15-18, 22, 28, 29, 30-37, 46
Voges-Proskauer reaction	23	1-3, 5-8, 15-18, 38-48, 50-52
Hydrolysis of:		
Tween 20	22	3, 15, 18, 22, 23, 28-30, 38-51
Tween 40	22	3, 15, 18, 22, 23, 28-30, 38-51
Tween 60	22	3, 15, 18, 22, 23, 28-30, 38-51
Tween 80	22	3, 15, 18, 22, 23, 28-30, 38-51

TABLE 5—Continued

Character	No. of strains positive	Strain(s) that gave the less common result
Oxidation of lactose	21	2, 4, 5, 8, 19, 20, 24–26, 38–49
Acid from rhamnose	17	2, 3, 9–15, 18, 31–37
Oxidation of gluconate	17	3, 15, 18, 38–48, 50–52
Lecithinase	17	3, 15, 18, 38–51
Lipase of corn oil	16	8, 15, 38–51
Cell width (short)	15	2, 6–9, 21, 22, 24, 27, 32, 33, 36, 45, 49, 52
Susceptibility to erythromycin	15	1, 3, 4, 6–8, 15–18, 31, 32, 35, 37, 52
Utilization of citrate on Simmons medium	14	3, 9, 11–18, 31–33, 36
Cell length (0.5–1.2 µm)	14	2, 8, 19, 21, 22, 24, 27, 29, 32, 33, 36, 45, 49, 52
Growth at pH 4	14	2, 10, 19–21, 24–27, 29, 38, 48, 49, 51
Utilization of citric acid	13	3, 6, 9, 11–18, 20, 23
Clotting in purple milk	13	18, 20, 21, 26, 27, 38–42, 44, 47, 50
Utilization of L-threonine	12	2–5, 8, 9, 11, 15, 16, 18, 20, 22
Acid from melibiose	12	3, 15–18, 31–37
Utilization of mucate in KP medium	12	2–4, 9–16, 18
Utilization of:		
Mucic acid	11	2–4, 9–15, 18
Urea	10	2, 4, 9, 11, 13, 18, 20–22, 24
Growth at 42°C	10	38–43, 45, 46, 49, 51
Utilization of α-ketoglutaric acid	9	3, 4, 6, 7, 12–14, 22, 29
Acid from starch	9	23, 30, 31, 34–36, 43, 50, 51
Susceptibility to:		
Penicillin	8	1, 31–37
Kanamycin	8	1, 9, 12, 31–33, 36, 37
Utilization of oxalic acid	7	3, 6, 9, 13, 15, 18, 23
Acid from:		
Adonitol	7	19–21, 24–27
Raffinose	6	3, 15–18, 31
Utilization of:		
D-Fucose	5	10, 11, 18, 31, 32
n-Butyric acid	4	6, 18, 22, 23
DL-Lactic acid	3	2, 9, 24
Succinic acid	3	2, 4, 8
Acid from α-methyl glucoside	3	3, 15, 18
Levan	3	2, 13, 29
Susceptibility to:		
Dihydrostreptomycin	3	31, 36, 37
Chloramphenicol	3	1, 4, 35
Fermentation of lactose	2	21, 27
Susceptibility to terramycin	2	1, 35
Cell length (>3 µm)	2	4, 14
Deoxyribonuclease	2	11, 14
Utilization of:		
L-Citrulline	2	31, 45
Isovaleric acid	2	4, 21
D-Malic acid	2	3, 39
Propylene glycol	2	4, 21
L-Threonine	2	10, 31
Valine	2	31, 45
Melezitose	1	46
Phenol	1	17
L-Tryptophane	1	35
Gelatin liquefaction	1	18
Acid from erythritol	1	24
Hydrolysis on skim milk agar	1	18
Dextran	1	1
Cell shape (fusiform)	1	4
Cell arrangement (chains of cells with ≤5 cells per chain)	1	4

^a TTC, 2,3,5-Triphenyltetrazolium chloride.^b These strains were positive.

FIG. 1. 37°C phenogram.

Numerical taxonomy. The phenograms constructed from the computation data are shown in Fig. 1 and 2.

At a similarity level of 93%, the 37°C matrix distinguished five clusters (Fig. 1). All seven strains in cluster 1 belonged to *Y. pseudotuberculosis*. The 11 strains in cluster 2 were sucrose, rhamnose, and lecithinase negative. Five of the six strains in cluster 3 were sucrose positive and rhamnose and lecithinase negative; the other strain in cluster 3 was sucrose, rhamnose, and lecithinase negative. A total of 14 of the strains in cluster 4 were sucrose positive, rhamnose negative, and lecithinase positive. Six of the nine strains in cluster 5 were sucrose and rhamnose positive and lecithinase negative, and the other three strains in cluster 5 were sucrose, rhamnose, and lecithinase positive. Five strains (strains 1, 4, 9, 16, and 17) were not clustered.

At a similarity level of 92%, the 22°C matrix distinguished six clusters (Fig. 2). The strains in clusters 1, 2, 3, and 6 were found in clusters 4, 2, 3, and 1 of the 37°C matrix, respectively. The strains in clusters 4 and 5 were in cluster 5 of the

37°C matrix. The three strains in cluster 4 were sucrose, rhamnose, and lecithinase positive, and seven strains in cluster 5 were sucrose and rhamnose positive and lecithinase negative. Three of the five unclustered strains of the 37°C matrix were clustered in the 22°C matrix; two strains (strains 16 and 17) were in cluster 3, and one strain (strain 9) was in cluster 5. Two strains (strains 1 and 4) were not clustered.

The biochemical characters which differentiated clusters 1 through 5 of the 22°C phenogram are shown in Table 6. The 14 strains in cluster 1 were rhamnose negative and lecithinase and sucrose positive; the 12 strains in cluster 2 were rhamnose, lecithinase, and sucrose negative; the 7 strains in cluster 3 were rhamnose and lecithinase negative and sucrose positive; the 3 strains in cluster 4 were rhamnose, lecithinase, and sucrose positive; and the 7 strains in cluster 5 were rhamnose positive, lecithinase negative, and sucrose positive.

G+C contents of the sucrose-negative strains of *Y. enterocolitica*. Table 7 shows the G+C contents of the DNAs of the sucrose-negative

FIG. 2. 22°C phenogram.

strains of *Y. enterocolitica* isolated from rodents; all of the G+C values were about 49 mol%. The G+C content of the DNA of the *A. salmonicida* strain which was used as a control was 57.2 mol%.

DISCUSSION

In our study, the typical rhamnose-negative *Y. enterocolitica* strains were divided into two clusters, lecithinase-positive and lecithinase-negative strains (Fig. 1 and 2). Lecithinase-positive strains have been isolated frequently from rodents in Japan (15, 17) and Europe (1, 18, 19). Lecithinase-negative strains, which are recognized as human enteric pathogens, have seldom been detected in these animals (15). The reservoirs of the human pathogens are pigs (13, 25, 26, 31, 32, 37) and dogs (16). Thus, lecithinase-negative and lecithinase-positive strains of *Y. enterocolitica* seem to have different host species. Wauters (36) placed the lecithinase-positive and lecithinase-negative strains in different biovars: the lecithinase-positive strains in biovar

1 and the lecithinase-negative strains in biovars 2 through 5. In our study, biovar 1 of Wauters belonged to a cluster different from that of the lecithinase-negative biovars (Fig. 1 and 2). These findings support the suggestion that the rhamnose-negative strains of *Y. enterocolitica* comprise two taxonomic subgroups.

The rhamnose- and lecithinase-negative strains of cluster 3 (Fig. 2) were biochemically heterogeneous (Table 2). Sakazaki et al. (28) distinguished two phenotypic clusters among such strains. Therefore, it is possible that there is a subgroup among the rhamnose- and lecithinase-negative *Y. enterocolitica* strains.

Our results support the suggestion that the sucrose-negative strains of *Y. enterocolitica* do indeed belong to the genus *Yersinia*. The G+C contents of the DNAs of these strains coincide with those of *Y. enterocolitica* serovar O3 and *Y. pseudotuberculosis* serovar IVA. Our sucrose-negative isolates were clustered differently from the typical rhamnose-negative strains of *Y. enterocolitica*. This is in accord with the results of

TABLE 6. Biochemical differentiation of *Y. enterocolitica* clusters obtained by using data from cultures incubated at 22°C

Test	Reaction of:				
	Cluster 1 (n = 14)	Cluster 2 (n = 12)	Cluster 3 (n = 7)	Cluster 4 (n = 3)	Cluster 5 (n = 7)
Acid from:					
Rhamnose	— ^a	—	—	+	+
α-Methyl glucoside	—	—	—	+	—
Sucrose	+	—	+	+	+
Raffinose	—	—	—(5) ^b	+	—
Melibiose	—	—	—(5)	+	—
Trehalose	+	+	+	+	+
Lecithinase	+	—	—	+	—
Ornithine decarboxylase	+	+	+	+	+
Nitrate reduction	+	+	+	+	+

^a No number indicates that all strains showed the homologous reaction.^b The numbers in parentheses indicate the number of strains that showed the reaction.

Bercovier et al. (4), who proposed the new name *Yersinia kristensenii* for sucrose-negative strains. The fact that one sucrose-negative strain was clustered together with *Y. enterocolitica* sensu stricto strains at 37°C may have been due to some genetic overlap between *Y. kristensenii* and *Y. enterocolitica*, as revealed by the DNA-DNA hybridization data of Bercovier et al. (4).

The frequency of recovery of atypical rhamnose-positive strains from freshwater ecosystems (7, 33) is apparently high. In our study, these strains were divided into the following groups: (i) the rhamnose-positive and raffinose- and melibiose-negative group and (ii) the rhamnose-, raffinose-, and melibiose-positive group (clusters 4 and 5). This is in accord with the DNA-DNA hybridization data (6, 7, 33). The new name *Yersinia frederiksenii* was proposed by Ursing et al. (33) for the former group, and for the latter group the name *Yersinia intermedia*

was offered by Brenner et al. (7).

All of the *Y. pseudotuberculosis* strains were in one cluster according to either the 22°C phenogram or the 37°C phenogram.

Harvey and Pickett (12) reported that a 30°C *Y. enterocolitica* matrix differentiated more clusters than a 35°C matrix because the enzymes of the organisms are more active at the lower temperature. In our study, the lecithinase activities of the positive strains tested were generally weak, and six of the strains were negative in this character at 37°C. Our study also showed that more clusters were delineated at the lower temperature and that the biochemical characters of the strains within each cluster were more homogenous at 22°C. Therefore, we prefer the five clusters derived from the 22°C data to the four clusters derived from the 37°C data in our study. Furthermore, we apply five biovars to the five clusters as follows: (i) biovar 1, the rhamnose-negative and lecithinase- and sucrose-positive strains; (ii) biovar 2, the rhamnose-, lecithinase-, and sucrose-negative strains; (iii) biovar 3, the rhamnose- and lecithinase-negative, sucrose-positive strains; (iv) biovar 4, the rhamnose-, lecithinase-, and sucrose-positive strains; and (v) biovar 5, the rhamnose-positive, lecithinase-negative, and sucrose-positive strains.

Two reference strains (rhamnose- and lecithinase-negative, sucrose-positive strain 1 and rhamnose-, lecithinase-, and sucrose-negative strain 4) were not clustered in our study. These strains differed from the other strains with respect to utilization of single carbon sources and antibiotic susceptibilities. The fact that these two strains did not cluster may be due to their sources and place of isolation, small rodents in Japan. Therefore, it appears that taxonomic studies on strains collected from diverse origins are necessary.

TABLE 7. G+C contents of the DNAs of sucrose-negative *Y. enterocolitica* strains

Organism	Strain no.	G+C content (mol%) ^a
Sucrose-negative <i>Y. enterocolitica</i>	20	49.0, 49.0, 49.6 ^b
Sucrose-negative <i>Y. enterocolitica</i>	25	48.2, 48.2, 49.0
Sucrose-negative <i>Y. enterocolitica</i>	29	49.3, 49.3, 49.0
<i>Y. enterocolitica</i> serovar O3	6	48.5, 48.5, 48.2
<i>Y. pseudotuberculosis</i> serovar IVA	32	49.0, 49.0, 49.3
<i>A. salmonicida</i> ATCC 14174		57.2

^a Calculated from thermal melting temperatures.^b Results of three experiments.

ACKNOWLEDGMENTS

We thank T. Seya, I. Furuta, K. Fukawa, and K. Nakano for technical assistance. We also thank T. Kimura, Department of Food Science and Technology, Faculty of Fisheries, Hokkaido University, Hakodate Branch, for help in determining the thermal denaturation temperatures of DNAs. All computations were done in the Hokkaido University Computing Center.

This work was supported in part by a grant-in-aid for special project research from the Ministry of Education, Science and Culture, Japan.

REPRINT REQUESTS

Address reprint requests to: Ken-ichi Kaneko, Department of Veterinary Public Health, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060, Japan.

LITERATURE CITED

- Aldová, E., and D. Lim. 1974. *Yersinia enterocolitica* in small rodents. I. Pilot study in two wildlife areas. Zentrbl. Bakteri. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A 226:491-496.
- Bercovier, H., J. Brault, N. Barre, M. Treignier, J. M. Alonso, and H. H. Mollaret. 1978. Biochemical, serological and phage typing characteristics of 459 *Yersinia* strains isolated from a terrestrial ecosystem. Curr. Microbiol. 1:353-357.
- Bercovier, H., D. J. Brenner, J. Ursing, A. G. Steigerwalt, G. R. Fanning, J. M. Alonso, G. P. Carter, and H. H. Mollaret. 1980. Characterization of *Yersinia enterocolitica sensu stricto*. Curr. Microbiol. 4:201-206.
- Bercovier, H., J. Ursing, D. J. Brenner, A. G. Steigerwalt, G. R. Fanning, G. P. Carter, and H. H. Mollaret. 1980. *Yersinia kristensenii*: a new species of *Enterobacteriaceae* composed of sucrose-negative strains (formerly called atypical *Yersinia enterocolitica* or *Yersinia enterocolitica*-like). Curr. Microbiol. 4:219-224.
- Bottone, E. J. 1978. Atypical *Yersinia enterocolitica*: clinical and epidemiological parameters. J. Clin. Microbiol. 7:562-567.
- Brenner, D. J. 1979. Speciation in *Yersinia*. Contrib. Microbiol. Immunol. 5:33-43.
- Brenner, D. J., H. Bercovier, J. Ursing, J. M. Alonso, A. G. Steigerwalt, G. R. Fanning, G. P. Carter, and H. H. Mollaret. 1980. *Yersinia intermedia*: a new species of *Enterobacteriaceae* composed of rhamnose-positive, melibiose-positive, raffinose-positive strains (formerly called *Yersinia enterocolitica* or *Yersinia enterocolitica*-like). Curr. Microbiol. 4:207-212.
- Brenner, D. J., A. G. Steigerwalt, D. P. Falcao, R. E. Weaver, and G. R. Fanning. 1976. Characterization of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* by deoxyribonucleic acid hybridization and by biochemical reaction. Int. J. Syst. Bacteriol. 26:180-194.
- Caprioli, T., A. J. Drapeau, and S. Kasatiya. 1978. *Yersinia enterocolitica*: serotypes and biotypes isolated from humans and the environment in Quebec, Canada. J. Clin. Microbiol. 8:7-11.
- Colwell, R. R. 1970. Collecting the data, p. 4-21. In W. R. Lockhart and J. Liston (ed.), Methods for numerical taxonomy. American Society for Microbiology, Washington, D.C.
- Cowan, S. T., and K. J. Steel. 1974. Identification of medical bacteria. Cambridge University Press, London.
- Harvey, S., and M. J. Pickett. 1980. Comparison of adansonian analysis and deoxyribonucleic acid hybridization results in the taxonomy of *Yersinia enterocolitica*. Int. J. Syst. Bacteriol. 30:86-102.
- Hurvell, B., V. Glatthard, and E. Thal. 1979. Isolation of *Yersinia enterocolitica* from swine at an abattoir in Sweden. Contrib. Microbiol. Immunol. 5:243-248.
- Kaneko, K., S. Hamada, Y. Kasai, and N. Hashimoto. 1979. Smoldering epidemic of *Yersinia pseudotuberculosis* in barn rats. Appl. Environ. Microbiol. 37:1-3.
- Kaneko, K., S. Hamada, Y. Kasai, and E. Kato. 1978. Occurrence of *Yersinia enterocolitica* in house rats. Appl. Environ. Microbiol. 36:314-318.
- Kaneko, K., S. Hamada, and E. Kato. 1977. Occurrence of *Yersinia enterocolitica* in dogs. Jpn. J. Vet. Sci. 39:407-414.
- Kaneko, K., and N. Hashimoto. 1981. Occurrence of *Yersinia enterocolitica* in wild animals. Appl. Environ. Microbiol. 41:635-638.
- Kapperud, G. 1975. *Yersinia enterocolitica* in small rodents from Norway, Sweden and Finland. Acta Pathol. Microbiol. Scand. Sect. B 83:335-342.
- Kapperud, G. 1977. *Yersinia enterocolitica* and *Yersinia*-like microbes isolated from mammals and water in Norway and Denmark. Acta Pathol. Microbiol. Scand. Sect. B 85:129-135.
- Kapperud, G., T. Bergan, and J. Lassen. 1981. Numerical taxonomy of *Yersinia enterocolitica* and *Yersinia enterocolitica*-like bacteria. Int. J. Syst. Bacteriol. 31:401-419.
- Lessel, E. F., and J. G. Holt. 1970. Presenting and interpreting the results, p. 50-58. In W. R. Lockhart and J. Liston (ed.), Methods for numerical taxonomy. American Society for Microbiology, Washington, D.C.
- Marmur, J. 1961. A procedure for the isolation of deoxyribonucleic acid from microorganisms. J. Mol. Biol. 3:208-218.
- Marmur, J., and P. Doty. 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. J. Mol. Biol. 5:109-118.
- Paik, G., and N. T. Suggs. 1974. Reagents, stains and miscellaneous test procedures, p. 930-950. In E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), Manual of clinical microbiology, 2nd ed. American Society for Microbiology, Washington, D.C.
- Pedersen, D. B. 1979. Occurrence of *Yersinia enterocolitica* in the throat of swine. Contrib. Microbiol. Immunol. 5:253-256.
- Pedersen, K. B., and S. Winblad. 1979. Studies on *Yersinia enterocolitica* isolated from swine and dogs. Acta Pathol. Microbiol. Scand. Sect. B 87:137-140.
- Quadling, D. 1970. Analyzing the data, p. 34-49. In W. R. Lockhart and J. Liston (ed.), Methods for numerical taxonomy. American Society for Microbiology, Washington, D.C.
- Sakazaki, R., K. Tamura, and T. Shimada. 1979. Numerical classification of *Yersinia enterocolitica* and relationship between its subdivision and pathogenicity. Contrib. Microbiol. Immunol. 5:23-32.
- Singer, J., and B. E. Volcani. 1955. An improved ferric chloride test for differentiating *Proteus* Providence group from other *Enterobacteriaceae*. J. Bacteriol. 69:303-306.
- Stevens, M., and N. S. Mair. 1973. A numerical taxonomic study of *Yersinia enterocolitica* strains. Contrib. Microbiol. Immunol. 2:17-22.
- Tsubokura, M., T. Fukuda, K. Otsuki, M. Kubota, K. Itagaki, K. Yamaoka, and M. Wakatsuki. 1976. Studies on *Yersinia enterocolitica*. II. Relationship between detection from swine and seasonal incidence, and regional distribution of the organism. Jpn. J. Vet. Sci. 38:1-6.
- Tsubokura, M., K. Otsuki, and K. Itagaki. 1973. Studies on *Yersinia enterocolitica*. I. Isolation of *Y. enterocolitica* from swine. Jpn. J. Vet. Sci. 35:419-424.
- Ursing, J., D. J. Brenner, H. Bercovier, G. R. Fanning, A. G. Steigerwalt, J. Brault, and H. H. Mollaret. 1980. *Yersinia frederiksenii*: a new species of *Enterobacteriaceae* composed of rhamnose-positive strains (formerly called atypical *Yersinia enterocolitica* or *Yersinia enterocolitica*-like). Curr. Microbiol. 4:213-218.
- Vera, H. G., and M. Dumoff. 1974. Culture media, p. 881-929. In E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), Manual of clinical microbiology, 2nd ed. American Society for Microbiology, Washington, D.C.

35. **Véron, M.** 1975. Nutrition et taxonomie des *Enterobacteriaceae* et bactéries voisines. I. Méthods d'étude des auzanogrammes. *Ann. Microbiol.* **126A**:267-273.
36. **Wauters, G.** 1973. Correlation between ecology, biochemical behaviour and antigenic properties of *Yersinia enterocolitica*. *Contrib. Microbiol. Immunol.* **2**:38-41.
37. **Wauters, G.** 1979. Carriage of *Yersinia enterocolitica* serotype 3 by pigs as a source of human infection. *Contrib. Microbiol. Immunol.* **5**:249-252.
38. **Wood, A. J., and E. A. Baird.** 1943. Reduction of trimethylamine oxide by bacteria. I. The *Enterobacteriaceae*. *J. Fish. Res. Board Can.* **6**:194-201.