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Yersinia enterocolitica: Biochemical, Serological, and Gas-Liquid Chromatographic Characterization of Rhamnose-, Raffinose-, Melibiose-, and Citrate-Utilizing Strains

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Thirteen atypical *Yersinia enterocolitica* isolates, all fermenting rhamnose, raffinose, and melibiose and utilizing sodium citrate within 24 to 48 h at 22°C (*Y.e.rh+*), were examined biochemically-serologically, and by gas-liquid chromatography. These data, as well as cultural, biochemical, and antibiotic susceptibility data gathered from two previous studies involving (i) these same atypical *Y.e.rh+* isolates, (ii) *Y. enterocolitica* serotypes O:1 through O:15 (rhamnose, raffinose, and citrate negative [*Y.e.rh−*]), (iii) *Y. enterocolitica* serotype O:16 (rhamnose positive but raffinose and citrate negative), and (iv) *Yersinia pseudotuberculosis* serogroups I through V were statistically compared. Both pre- and postabsorption agglutination studies demonstrated the serological distinctiveness of *Y.e.rh+* from *Y.e.rh−* and *Y. pseudotuberculosis*. At the same time, three immunological groups among the 13 *Y.e.rh+* strains were seen; 8 corresponded to *Y. enterocolitica* serotype O:17; 1 to *Y. enterocolitica* serotype O:16; and the remaining four were nontypable in antisera against known *Y. enterocolitica* antigen types. Each of the three *Yersinia* groups tested chromatographically produced acetic and lactic acids. Both *Y.e.rh−* and *Y.e.rh+* formed propionic acid, but only *Y.e.rh+* produced detectable amounts of succinic acid. Based on 49 variables, statistical analysis of the three *Yersinia* groups studied placed each of the *Y.e.rh+* strains in a homogeneous group separate from both *Y.e.rh−* and *Y. pseudotuberculosis*. These data, coupled with deoxyribonucleic acid homology studies of Brenner and co-workers (D. J. Brenner, A. G. Steigerwalt, D. F. Falcao, R. E. Weaver, and G. R. Fanning, Int. J. Syst. Bacteriol. **26**:180-194, 1976), support the distinctiveness of *Y.e.rh+* from typical *Y. enterocolitica* and *Y. pseudotuberculosis*.

The genus *Yersinia*, of the family *Enterobacteriaceae*, is composed of *Y. enterocolitica*, *Y. pseudotuberculosis*, and *Y. pestis*. Biochemically, the former two species are readily distinguished from the latter by their motility (22°C) and urease production (37°C, especially upon subculture) (12). Clinically, *Y. enterocolitica* and *Y. pseudotuberculosis* often present as acute mesenteric lymphadenitis, terminal ileitis, or gastroenteritis, whereas *Y. pestis* infections appear in pneumonic or bubonic forms and are accompanied by a high mortality rate (11).

Recently, essentially waterborne strains of rhamnose-fermenting *Y. enterocolitica* isolated from healthy animals and nonmesenteric human infections, usually of minor clinical importance (3-5), have been identified as *Y. enterocolitica* (3, 5-7), based on such features as: fermentation of cellobiose, sucrose, and sorbitol; inability to ferment adonitol; acetoin production at 22°C but not at 37°C; indole positivity; and production of ornithine decarboxylase.

Thirteen atypical *Y. enterocolitica* (12 recovered from clinical specimens submitted to the microbiology laboratory at The Mount Sinai Hospital) isolates, all fermenting rhamnose and raffinose and utilizing sodium citrate within 24 to 48 h at 22°C (*Y.e.rh+*), were examined serologically and by gas-liquid chromatography.

These strains were similarly compared to typical *Y. enterocolitica*, rhamnose, raffinose, and citrate negative (*Y.e.rh−*), of serotypes O:1 through O:15, *Y. enterocolitica* serotype O:16 (rhamnose positive, but raffinose and citrate negative), and *Y. pseudotuberculosis* serogroups I through IV. These data, as well as cultural, biochemical, and antibiotic susceptibility determinations gathered from two previous studies

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involving these same yersiniae (5, 7), were statistically compared.

This report comprises the results of these studies and stresses the distinctiveness of *Y.e.rh+* from typical *Y. enterocolitica* and *Y. pseudotuberculosis*.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains studied and their sources are listed in Table 1.

Preparation of antigen. Antigen was prepared from each of the strains by harvesting, with 10 ml of sterile phosphate-buffered saline (pH 7.2), growth from a Mueller-Hinton agar plate (150 mm) incubated for 48 h at 22°C, and centrifuging at 1,000 × *g* for 15 min. After washing the cells twice with phosphate-buffered saline, they were resuspended in 60 ml of phosphate-buffered saline to yield an optical density at 420 nm of 1.220. The individual suspensions were then autoclaved for 2.5 h at 121°C and 15 lb/in^2.

Production of antiserum. Two rabbits were each immunized with antigen suspensions prepared from *Y. enterocolitica* (*Y.e.rh−*) serotypes O:3 and O:8 and from *Y.e.rh+* strains 17 and 60 (suspended in 10 ml of phosphate-buffered saline and yielding an optical density at 420 nm of 2.661) by inoculating, initially, 0.5 ml into the marginal ear vein. Subsequent inoculations given 4 days apart were increased by 0.25 ml until an inoculum of 1.5 ml was reached. Thereafter, four 1.5-ml boosters were given 4 days apart. Each rabbit was bled 3, 4, and 5 weeks after the first inoculation. A final bleeding was performed after 2 months (16, 17).

Gas-liquid chromatography. The Virginia Polytechnic Institute chromatographic procedure was used for the analysis of acid products (9). Each strain was grown in chopped-meat–carbohydrate broth (CMC; Scott Laboratories, Fiskeville, R.I.) for 18 h at 22 and 37°C. Extraction of each broth followed the procedure

TABLE 1. *Source of Yersinia isolates studied*

Bacterium	Strain no.	Antigen type	Source	Contributor
Y. enterocolitica, rhamnose negative (*Y.e.rh−*)	1	O:1	Chinchilla	S. Winblad (Malmo, Sweden)
	2	O:2	Hare	
	3	O:3	Human	
	4	O:4	Chinchilla	
	5	O:5	Ox	
	6	O:6	Human	
	7	O:7	Guinea pig	
	8	O:8	Human	
	9	O:9	Human	
	10	O:10	Human	
	11	O:11	Human (urine)	
	12	O:12	Hare	
	13	O:13	Human	
	14	O:14	Human	
	15	O:15	Human	
Y. enterocolitica, rhamnose positive, but otherwise identical to *Y.e.rh−*	16	O:16	Water	
Y. enterocolitica, rhamnose positive (*Y.e.rh+*)	17	17	Water	S. Winblad
	48	17	Human (urine)	E. J. Bottone
	49	17	Human (eye)	
	50	17	Human (wound)	
	51	17	Human (eye)	
	53	17	Human (eye)	
	54	17	Human (stool)	
	55	NT[a]	Human (catheter)	
	56	NT	Human (throat)	
	57	16	Human (eye)	
	58	NT	Human (stool)	
	59	17	Human (urine)	
	60	NT	Human (stool)	
Y. pseudotuberculosis	286	III	Human	Analytab Products, Inc., (Plainview, N.Y.)
	287	I	Human	
	288	I	Human	
	289	II	Human	
	290	I	Human	
	291	I	Human	
	293	III	Human	
	294	IV	Human	
	295	V	Human	
	296	VI	Human	

[a] NT, Nontypable in antisera against existing *Y. enterocolitica* serotypes.

of Holdeman and Moore (9). Extracts were analyzed with a Dohrman model 15C-3 gas chromatograph (AnaBac Bacteriology System, Clinical Analysis Products, Sunnyvale, Calif.). Operating conditions were: 1× attenuation; 85-mA detector current; 140°C detector temperature; 135°C injection block temperature; 125°C column temperature. Helium was the carrier gas (Liquid Carbonic, Harrison, N.J.) at 120 ml/min. Column packing was 10% Resoflex LAC-1-296 on 30/60-mesh Chromosorb W-AW (Clinical Analysis Products). Before testing the CMC broth cultures, a sterile CMC broth incubated overnight at 35°C was examined for the presence of endogenous volatile and/or nonvolatile acids. Extraction of the control CMC broth for volatile acids showed an acetic acid peak, which was subsequently subtracted from corresponding peaks obtained with actual *Yersinia* CMC broth cultures. Although cultures were analyzed primarily for the presence or absence of acid products, some degree of quantitation was achieved by comparison of the obtained peaks with standards supplied by Clinical Analysis Products. This comparison showed that less than 1 meq of acetic, propionic, lactic, and succinic acids per 100 ml was produced by the organisms studied.

Statistical analysis. Inasmuch as the data used for the statistical analyses were nominal in nature, i.e., variables were not related (positive or negative), similarity measures were used to assess the proximity of two data items, i.e., organisms. As all data responses were either positive or negative, similarity coefficients were used (8, 13). The data were clustered by using minimization of pairwise stress (8, 14), a method that produced cluster groups by moving isolates from one group to another on the basis of maximum reduction of stress until a point was reached when no further reduction in stress occurred.

The data consisted of 48 variables: production or absence of nitrate reductase, oxidase, catalase, urease, beta-galactosidase, hydrogen sulfide, lysine and ornithine decarboxylase, phenylalanine deaminase, arginine dihydrolase, indole, acetic acid, lactic acid, propionic acid, and succinic acid (all at 37°C); acetoin and motility (22 and 37°C); citrate utilization (22 and 37°C); fermentation of glucose, lactose, maltose, sucrose, mannitol, arabinose, trehalose, esculin, salicin, dulcitol, xylose, rhamnose, raffinose, adonitol, sorbitol, melibiose (all at 37°C), and cellobiose (22 and 37°C); growth on salmonella-shigella, xylose-lysine-deoxycholate, and Hektoen-enteric agars (all at 22 and 37°C); and resistance to ampicillin (22 and 37°C) and cephalothin (37°C).

Absorption of antiserum. Absorption was accomplished by mixing 2 ml of antiserum with the cellular sediment obtained by centrifuging 60 ml of each of the 13 autoclaved antigens. The antiserum-antigen mixture was incubated at 37°C for 2 h and then refrigerated for 18 h. Clumped bacteria were removed by centrifugation for 15 min at 1,000 × *g* (2, 10).

Agglutination tests. An initial 1:10 dilution of each antiserum was prepared, after which a serial 10-fold dilution was carried out to a final dilution of 1:5,120. A 0.5-ml amount of the stock homologous antigen (optical density at 420 nm, 1,220) was added to 0.5 ml of each antiserum dilution, giving a final dilution range of 1:20 to 1:10,240. After shaking, the tubes were incubated at 37°C for 2 h, followed by incubation at 22°C for 18 h. After gentle mixing, each tube was examined with a hand lens (×4). The titer was recorded as the highest dilution of antiserum that produced distinct agglutination of the antigen. To determine the antigenic relationship among the three species of yersiniae studied, each of the four antisera was reacted with each of the 39 antigen preparations. Both pre- and postabsorption titers were determined.

RESULTS

Tube agglutination tests. Five weeks after sequential inoculation of *Yersinia* antigens, rabbit antisera demonstrated maximum titers to the homologous antigens ranging from 1:2,560 to 1:10,240 (Tables 2 and 3). Although in one instance *Y. enterocolitica* (*Y.e.rh*−) serotype O:1 antigen titered to as high as 1:640 with heterologous *Y.e.rh*− serotype O:3 antiserum, in no case was a titer higher than 1:80 obtained when *Y.e.rh*− antisera was reacted with any of the *Y.e.rh*+ antigen preparations. This indicated immunological relatedness among the *Y.e.rh*− serotypes but not to *Y.e.rh*+. Neither of the two *Y.e.rh*+ antisera showed cross-reactions against *Y.e.rh*− serotypes in dilutions greater than 1:80, thereby confirming a lack of significant antigenic relatedness between the two groups.

When unabsorbed antisera prepared against *Y.e.rh*+ strains 17 (serotype O:17) and 60 (nontypable) were reacted against the 13 *Y.e.rh*+ antigen preparations, three distinct agglutination patterns emerged. The first consisted of the homologous *Y.e.rh*+ strain 17 (serotype O:17) and strains 48, 49, 50, 51, 53, 54, and 59, which agglutinated in dilutions of *Y.e.rh*+ serotype O:17 antiserum ranging from 1:1,280 to 1:5,120. All of these strains were shown to be serotype O:17 by Mollaret (personal communication). When tested in antiserum produced against *Y.e.rh*+ strain 60, however, these same strains did not agglutinate in antiserum dilutions greater than 1:80. The second antigenic group contained *Y.e.rh*+ strains 55, 56, 58, and 60, which agglutinated in *Y.e.rh*+ strain 60 antiserum dilutions of 1:1,280 to 1:2,560. These strains were essentially nonreactive (titer less than 1:20) in antiserum against *Y.e.rh*+ strain 17 (serotype O:17). The third antigenic group contained only *Y.e.rh*+ 57, which failed to agglutinate in either *Y.e.rh*+ antisera more dilute than 1:80 (Table 3). No serological relatedness could be established between *Y. pseudotuberculosis* and either *Y.e.rh*− or *Y.e.rh*+. Antisera prepared against *Y.e.rh*− serotypes O:3 and O:8, and against *Y.e.rh*+ isolates 17 and 60, showed only minimal (1:20) agglutinins against the 10 *Y. pseudotuberculosis* antigens.

Absorption of samples of antiserum prepared

TABLE 2. *Agglutinin titers of rabbit antisera prepared against four Yersinia antigens and reacted with Y. enterocolitica serotype O:1 through O:16 antigens*

Antigen prepared from *Y.e.rh*− serotype:	Agglutinin titer of rabbit antiserum against:			
	Y.e.rh− serotype 3 antigen	*Y.e.rh*− serotype 8 antigen	*Y.e.rh*+ isolate 17 antigen[a]	*Y.e.rh*+ isolate 60 antigen[b]
1	640	40	20	20
2	160	160	40	40
3	10,240	40	80	80
4	40	40	40	20
5	40	80	20	20
6	40	80	20	20
7	80	80	20	40
8	80	5,120	80	40
9	40	40	<20	20
10	80	320	40	20
11	20	40	20	20
12	40	20	20	<20
13	160	80	80	40
14	40	80	80	20
15	80	160	40	40
16[c]	20	40	40	40

[a] Homologous titer, 1:5,120.
[b] Homologous titer, 1:2,560.
[c] Rhamnose positive but otherwise identical to *Y.e.rh*−.

TABLE 3. *Agglutinin titers of rabbit antisera prepared against four Yersinia antigens and reacted with the 13 Y.e.rh+ isolates*

Antigen prepared from *Y.e.rh*+ isolate	Agglutinin titer of rabbit antiserum against:			
	Y.e.rh− serotype 3 antigen[a]	*Y.e.rh*− serotype 8 antigen[b]	*Y.e.rh*+ isolate 17 antigen	*Y.e.rh*+ isolate 60 antigen
17	40	40	5,120	40
48	40	40	1,280	40
49	40	40	1,280	80
50	40	40	2,560	80
51	40	40	2,560	40
53	40	40	2,560	80
54	40	40	5,120	80
55	20	20	20	1,280
56	20	20	20	2,560
57	80	80	80	40
58	40	40	20	2,560
59	80	80	2,560	80
60	40	40	20	2,560

[a] Homologous titer, 1:10, 240.
[b] Homologous titer, 1:5, 120.

against *Y.e.rh*+ strain 17 with antigen from each of 13 *Y.e.rh*+ isolates caused a drop in the homologous titer from 1:5,120 to less than 1:20 with strains 17, 48, 49, 50, 51, 53, 54, and 55 and a one- to twofold drop in titer with strains 55, 56, 57, 58, and 60 (Table 4).

Absorption of *Y.e.rh*+ strain 60 antiserum with antigen from each of the 13 *Y.e.rh*+ strains reduced the homologous titer from 1:2,560 to 1:20 when the absorbing antigen was strain 55, 56, 58, or 60. Titers were reduced two- to fourfold when the absorbing antigen was *Y.e.rh*+ strain 17, 48, 49, 50, 51, 53, 54, 57, or 59. These data again distinguished three immunological groups, as represented by strains 17, 48, 49, 50, 51, 53, 54, and 59; 55, 56, 58, and 60; and strain 57.

Gas-liquid chromatography. All of the *Y.e.rh*+, *Y.e.rh*−, and *Y. pseudotuberculosis* strains produced acetic acid. Propionic acid, the only other volatile acid detected, was produced by most of the *Y.e.rh*+ and *Y.e.rh*− strains but not by *Y. pseudotuberculosis* (Table 5).

Although only two nonvolatile acids were detected in the methylated extracts prepared from growth of the *Yersinia* strains in CMC broth, a significant difference among the three *Yersinia*

TABLE 4. *Homologous agglutinin titer of rabbit antisera prepared against Y. enterocolitica (Y.e.rh+) isolates 17 and 60 before and after absorption with antigens prepared from the 13 Y.e.rh+ isolates*

Absorbing antigen prepared from Y.e.rh+ isolates	Homologous titer of antiserum against Y.e.rh+ strain:			
	17		60	
	Before absorption	After absorption	Before absorption	After absorption
17	5,120	<20		1,280
48		<20		1,280
49		<20		1,280
50		<20		640
51		<20		1,280
53		<20		1,280
54		<20		2,560
55		2,560		<20
56		1,280		<20
57		2,560		1,280
58		2,560		<20
59		<20		640
60		2,560	2,560	<20

TABLE 5. *Production of volatile and nonvolatile acids by Y. enterocolitica (Y.e.rh+), Y. enterocolitica (Y.e.rh−), and Y. pseudotuberculosis grown in CMC broth at 37°C for 18 h*

Organism	Volatile acid		Nonvolatile acid	
	Acetic	Propionic	Lactic	Succinic
Y.e.rh+	+[a]	+[b]	+	+[c]
Y.e.rh−	+	+[d]	+	−
Y. pseudotuberculosis	+	−	+	−

[a] +, Positive; −, negative.
[b] Not produced by *Y.e.rh+* isolates 51 and 53.
[c] Not produced by *Y.e.rh+* isolates 50, 51, and 53.
[d] Not produced by *Y.e.rh−* serotypes 2 and 5.

groups was established. All strains of *Y.e.rh+*, *Y.e.rh−*, and *Y. pseudotuberculosis* tested produced lactic acid, whereas only *Y.e.rh+* strains produced detectable amounts of succinic acid. Peaks of approximately 2 mm in height were obtained within 9 min and 30 s after injection of the methylated extracts of *Y.e.rh+*. The three *Yersinia* groups, therefore, could be differentiated from each other on the basis of the production of propionic and succinic acid; most of the *Y.e.rh+* isolates produced both propionic and succinic acids, whereas *Y.e.rh−* produced only propionic acid and *Y. pseudotuberculosis* produced neither propionic nor succinic acid in detectable amounts (Table 5).

Temperature appeared to have no effect on the production of volatile or nonvolatile acid by any of the strains tested, since the results were the same with cultures grown at 22 or 37°C.

Statistical analysis. Based on 48 variables, the statistical analysis of the 13 *Y.e.rh+*, 16 *Y.e.rh−*, and 10 *Y. pseudotuberculosis* strains indicated three distinct groups. The first group consisted of *Y.e.rh+* strains 17, 48, 49, 50, 51, 53, 54, 55, 56, 57, 58, 59, and 60; the second group included the 10 *Y. pseudotuberculosis* strains; and the third group contained *Y.e.rh−* serotypes O:1 through O:16.

Those isolates that demonstrated greatest stress, i.e., similarity, with members of the two other groups are shown in Table 6. Certain variables of *Y.e.rh+* strains 50 and 53 caused them to cluster with *Y.e.rh−* strain 6, whereas *Y. pseudotuberculosis* strains 286 and 288 showed relatedness to *Y.e.rh−* strain 2. Based on the computed stresses, group one, consisting of the 13 *Y.e.rh+* strains, and group two, containing the 10 *Y. pseudotuberculosis* strains, were homogeneous within themselves, whereas group three, containing the 16 *Y.e.rh−* strains, was heterogeneous. However, the stress between *Y.e.rh−* strains 2 and 6 was not sufficient to shift them into another group, but, rather, caused them to stand out within group three.

The 24 principal variables used in distinguishing the three groups are analyzed in Table 7. The remaining 24 variables, although included in the statistical analysis, were uninformative in that all isolates in all groups gave uniform responses.

DISCUSSION

The 13 atypical, rhamnose-positive *Y. enterocolitica* isolates (*Y.e.rh+*) possess the basic biochemical profile of *Y. enterocolitica* (*Y.e.rh−*)

TABLE 6. *Strainwise pairs determined for cluster analysis of 49 variables of 13 Y. enterocolitica (Y.e.rh+), 10 Y. pseudotuberculosis, and 16 Y. enterocolitica (Y.e.rh−) serotypes*[a]

Bacterium	Group	Bacterium	Group
Y.e.rh+, isolate 50	1	*Y.e.rh−*, serotype 6	3
Y.e.rh+, isolate 53	1	*Y.e.rh−*, serotype 6	3
Y. pseudotuberculosis, isolate 286	2	*Y.e.rh−*, serotype 2	3
Y. pseudotuberculosis, isolate 288	2	*Y.e.rh−*, serotype 2	3
Y.e.rh−, serotype 2	3	*Y.e.rh−*, serotype 6	3

[a] Each bacterium in the left-hand column was determined to have greater similarity to the bacterium in the right-hand column than the other organisms tested but insufficient similarity to place it into the same cluster group. 1, Cluster group containing the 13 *Y.e.rh+* isolates; 2, cluster group containing the 10 *Y. pseudotuberculosis* isolates; 3, cluster group containing the 16 *Y.e.rh−* serotypes.

TABLE 7. *Differential responses used in statistical analysis of yersiniae studied*

Variable	Temp (°C)	% Positive in group[a]:		
		1	2	3
Fermentation of:				
Esculin	37	100	100	44
Salicin	37	100	100	44
Lactose	37	46	0	0
Cellobiose	22	100	0	100
	37	77	0	100
Raffinose	22	100	10	0
Rhamnose	22	100	100	6
Melibiose	22	100	100	0
Adonitol	37	0	100	0
Sucrose	37	100	0	82
Sorbitol	37	100	0	100
Indole	37	100	0	63
Citrate	22	100	0	0
Acetoin	22	100	0	82
Ornithine	37	100	0	94
Growth on agar[b]				
SS	37	23	80	100
XLD	22	100	100	100
	37	39	100	100
Hektoen	22	7	90	100
	37	7	40	100
Resistance to:				
Ampicillin	22	44	0	81
Cephalothin	37	0	0	75
Succinic acid	37	77	0	0
Propionic acid	37	85	0	88

[a] Group 1, *Y. enterocolitica* isolates (*Y.e.rh+*) 17, 48, 49, 50, 51, 53, 54, 55, 56, 57, 58, 59, and 60; 2, *Y. pseudotuberculosis* isolates 286, 287, 288, 289, 290, 291, 293, 294, 295, and 296; 3, *Y. enterocolitica* serotypes 1 through 16 (*Y.e.rh−*).

[b] SS, Salmonella-shigella; XLD, xylose-lysine-deoxycholate.

and *Y. pseudotuberculosis*: motility at 22°C but not at 37°C; production of urease and beta-galactosidase; fermentation of arabinose; lack of H_2S production; and failure to deaminate phenylalanine or decarboxylate lysine. As shown in two previous reports (5, 7), these same 13 *Y.e.rh+* strains (i) share features of *Y.e.rh−* not found among *Y. pseudotuberculosis*, namely, production of acetylmethylcarbinol at 22°C but not at 37°C, decarboxylation of ornithine, production of indole, and fermentation of sucrose, sorbitol, and cellobiose; and (ii) possess features in common with *Y. pseudotuberculosis* but not with *Y.e.rh−*, such as agglutination of several of these isolates in anti-*Y. pseudotuberculosis* group II serum (3) and fermentation of rhamnose and melibiose. More important, however, these isolates have characteristics possessed by neither *Y.e.rh−* nor *Y. pseudotuberculosis*, among which the temperature-dependent utilization of citrate as sole carbon source and fermentation of raffinose, both at 22°C within 24 h, serve as notable examples (5, 7).

The fermentation of rhamnose does not, in itself, classify an apparent *Y. enterocolitica* as *Y.e.rh+*, since rhamnose-positive variants of otherwise typical *Y. enterocolitica* strains, although rare, apparently do exist. Alonso et al. (3) found only 36 rhamnose-positive *Y. enterocolitica* strains among 4,783 strains in the collection of the Centre National des Yershinia in Paris. The majority of these rhamnose-positive strains, however, were biochemically compatible with *Y.e.rh+*, whereas the remainder conformed to otherwise typical *Y.e.rh−*.

Winblad (personal communication) listed several rhamnose- and lactose-positive, but citrate-, raffinose-, and melibiose-negative, strains of *Y. enterocolitica* (serotypes O:4, O:14, and O:16). Therefore, to identify an organism as *Y.e.rh+* biochemically, the demonstration of growth on citrate at 22°C and fermentation of rhamnose, raffinose, and melibiose at 22°C (usually within 24 h) is of paramount importance.

Deoxyribonucleic acid (DNA) relatedness studies conducted by Brenner and colleagues (6) have indicated two distinct hybridization groups among the rhamnose-positive *Y. enterocolitica* strains. One of these groups contained, among others, *Y.e.rh+* strains 17, 48, 56, and 57 from our laboratory. The other group consisted of organisms that, although rhamnose positive, were both raffinose and melibiose negative and either citrate or lactose negative.

On the basis of a relative binding ratio (which expresses DNA relatedness in percentages) of 70% or greater as indicating closely or highly related strains, each of the four *Y.e.rh+* strains tested was found to be unrelated to either *Y.e.rh−* or *Y. pseudotuberculosis* and yielded relative binding ratio values at 60°C of 50 to 65% and 40 to 55%, respectively. The instability of these DNA complexes at 75°C was further evidence of the unrelatedness of *Y.e.rh+* with *Y.e.rh−* or *Y. pseudotuberculosis*, since it is characteristic of closely related strains that the DNA complexes formed at 60°C are stable at 75°C (6; D. J. Brenner, personal communication) (Table 8).

The immunological basis for separating *Y.e.rh+* from *Y.e.rh−* and *Y. pseudotuberculosis* was demonstrated by agglutination tests, which grouped the former strains apart from the latter two species and further indicated the presence of at least three distinct immunological subgroups among the 13 *Y.e.rh+* isolates. Eight were identified as *Y. enterocolitica* serotype O:17, and one strain was similar to *Y. enterocolitica* serotype O:16, whereas the remaining four isolates were nontypable in existing antisera against *Y. enterocolitica*. Alonso et al. (3) reported that most of our 13 *Y.e.rh+* strains sub-

TABLE 8. *DNA relatedness of Y. enterocolitica (Y.e.rh+), Y. enterocolitica (Y.e.rh−), and Y. pseudotuberculosis*[a]

Source of labeled DNA	Source of unlabeled DNA	Relative binding (%)[b] at:	
		60°C	75°C
Y.e.rh−[c]	*Y.e.rh−*	88–100	80–90
Y.e.rh−	*Y.e.rh+* 17	50–65	15–25
Y.e.rh−	*Y.e.rh+* 48	50–65	15–25
Y.e.rh−	*Y.e.rh+* 57	50–65	15–25
Y.e.rh−	*Y.e.rh+* 60	50–65	15–25
Y. pseudotuberculosis[d]	*Y. pseudotuberculosis*	75–100	75–100
Y. pseudotuberculosis	*Y.e.rh−*	40–55	10–20
Y. pseudotuberculosis	*Y.e.rh+* 17	40–55	10–20
Y. pseudotuberculosis	*Y.e.rh+* 48	40–55	10–20
Y. pseudotuberculosis	*Y.e.rh+* 57	40–55	10–20
Y. pseudotuberculosis	*Y.e.rh+* 60	40–55	10–20

[a] DNA homologies were performed by D. Brenner of the Center for Disease Control, Enterobacteriology Branch, Atlanta, Ga.

[b] Percentage of DNA base pairings.

[c] Center for Disease Control culture 178.

[d] Center for Disease Control culture P105.

mitted to him agglutinated in anti-*Y. pseudotuberculosis* group II serum; however, titers were not stated. Additionally, they showed that a bacteriophage of *Y. pseudotuberculosis* could not lyse these *Y.e.rh+* strains. Although antiserum against *Y. pseudotuberculosis* was not reacted against *Y.e.rh+* antigen, none of the *Y. pseudotuberculosis* antigen preparations agglutinated with *Y.e.rh+* antisera that were more dilute than 1:20.

The production of few volatile and nonvolatile acids by the *Yersinia* species studied was not unexpected inasmuch as a similar paucity of such acids among other members of the *Enterobacteriaceae* was found in our laboratories (unpublished data). The production, however, of detectable amounts of succinic acid by most of the *Y.e.rh+* strains served as another parameter of their distinctiveness from *Y.e.rh−* and *Y. pseudotuberculosis*, neither of which produced detectable amounts of succinic acid.

A comparison of *Y.e.rh+*, *Y.e.rh−*, and *Y. pseudotuberculosis* with regard to critical biochemical reactions, growth on enteric media, antimicrobial susceptibility, and production of volatile and nonvolatile acids is shown in Table 9. Parallel testing at 22 and 37°C allows for easy and rapid differentiation among these species. It is essential that laboratory tests be carried out at 22°C and that biochemical tests be incubated for at least 10 days before being reported as negative in order to prevent misleading results (7). For example, Alonso et al. (3) failed to show fermentation of inositol and raffinose by 11 of our 13 *Y.e.rh+* strains, or fermentation of sorbitol and cellobiose by 7 of 13 and 10 of the 13 *Y.e.rh+* strains, respectively, when using 30°C rather than 22°C as the incubation temperature. Similarly, Brenner et al. (6) failed to show fermentation of raffinose, cellobiose, or lactose by three of the four *Y.e.rh+* strains when the strains were incubated at 35°C for 7 days.

Although many *Y.e.rh+* isolates have probably gone undetected due to failure to incubate cultures at 22°C, or as a result of misidentification due to seemingly aberrant results for *Y.e.rh−*, it appears that *Y.e.rh+*, at least the 13 isolates examined (as well as six more isolated to date), is an organism of moderate virulence when compared with *Y.e.rh−* or *Y. pseudotuberculosis*. None of the 13 *Y.e.rh+* strains studied were isolated from the more pronounced infections associated with *Y.e.rh−* and *Y. pseudotuberculosis* such as acute mesenteric lymphadenitis, terminal ileitis, or septicemia but were recovered from sites such as eye, throat, urine, and stools, in which mild infections were noted (4, 5). Alonso et al. (3) found that of the 36 rhamnose-positive strains of *Y. enterocolitica* in the *Yersinia* collection at the Pasteur Institute, only 13 were of human origin, none of which came from seriously ill patients.

The combined results of biochemical, cultural, serological, and gas-liquid chromatographic studies presented in this and two previous reports involving the 13 *Y.e.rh+* (5, 7), coupled with the DNA homology studies of Brenner and colleagues (6), and the restricted pathogenicity of *Y.e.rh+* (E. J. Bottone, submitted for publication) support the distinctiveness of *Y.e.rh+* from *Y.e.rh−* and *Y. pseudotuberculosis*. The possibility therefore exists that a new species designation may be warranted for these *Y.e.rh+* strains. Clinically, a change in nomenclature

TABLE 9. *Characteristics differentiating Y.e.rh+ from Y.e.rh− and Y. pseudotuberculosis*[a]

Test or characteristic	*Y.e.rh+*	*Y.e.rh−*	*Y. pseudotuberculosis*
Biochemicals			
Citrate			
22°C	+	−	−
37°C	−	−	−
Raffinose			
22°C	+	−	−
37°C	(+)	−	−
Lactose			
22°C	(+)	−	−
37°C	(+)	−	−
Growth on agar			
Salmonella-shigella			
22°C	d	+	+
37°C	−	+	+
Hektoen enteric			
22°C	−	+	+
37°C	−	+	d
Xylose-lysine-deoxycholate			
22°C	+	+	+
37°C	d	+	+
Ampicillin			
22°C	V	V	S
37°C	S	V	S
Nonvolatile acid			
Succinic			
22°C	+	−	−
37°C	+	−	−

[a] +, 90% or more positive within 2 days; −, 90% or more negative within 2 days; (+), 90% or more positive within 14 days, but not before 3 days; d, different reactions, +, (+), −; S, sensitive; V, some strains sensitive, some resistant.

would seem to be preferable to the usage of such terms as "atypical *Y. enterocolitica*" or even "*Y. enterocolitica*-like" organisms, which do not impart an insight into the pathogenic potential of the isolate.

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