Numerical Taxonomy of *Yersinia enterocolitica* and *Yersinia enterocolitica*-Like Bacteria

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We studied the taxonomic interrelationships of 332 *Yersinia* strains by using a numerical analysis that was based on 46 cultural and biochemical characters and involved both a hierarchical clustering procedure and a principal components analysis. *Yersinia pseudotuberculosis* and *Yersinia kristensenii* were recognized as relatively distinct phenotypic clusters. *Y. kristensenii* was further distinguishable from the remaining taxa by antigenic and enterotoxigenic parameters. These results supported the suggestion that *Y. kristensenii* deserves species status. On the other hand, *Yersinia enterocolitica* sensu stricto, *Yersinia frederiksenii*, and *Yersinia intermedia* constituted a phenotypic continuum. Each of these three taxa prevailed in different parts of a heterogeneous cluster of strains that were connected by intermediate phenotypes. This pattern of overlapping phenotypes was supported further by antigenic properties, habitat preferences, and pathogenic characteristics. Thus, we failed to find a basis for separating *Y. enterocolitica* sensu stricto, *Y. frederiksenii*, and *Y. intermedia* on phenetic, ecological, or pathogenic grounds. We suggest that the taxonomic relationships among these nomenspecies may require further evaluation. Two phenetic clusters contained strains not ascribable to any presently defined species. One of these clusters consisted mainly of rhamnose-positive, sucrose-negative strains and the other contained strains negative for sucrose or ornithine decarboxylase or both.

In *Bergey's Manual of Determinative Bacteriology*, 8th ed. (12), the genus *Yersinia* is placed in the family *Enterobacteriaceae* and contains three recognized species, *Yersinia pestis*, *Yersinia pseudotuberculosis*, and *Yersinia enterocolitica*. Two additional species, *Yersinia philomiragia* (19) and *Yersinia ruckeri* (15), have been proposed, but these species have not been accepted generally.

Y. enterocolitica as currently recognized is quite heterogeneous, both phenotypically and genetically. A broad diversity of strains are presently placed in this species. The need for a taxonomic revision with a more precise delineation of *Y. enterocolitica* has been recognized (6, 8, 10, 16, 21, 24, 27, 33, 34, 36, 39, 42, 43). The designation *Y. enterocolitica*-like bacteria has been applied to atypical variants.

In 1973, Knapp and Thal (27) expressed the opinion that the biochemical diversity of the strains designated *Y. enterocolitica* is sufficient to justify the establishment of two separate species, *Y. enterocolitica* and "*Yersinia enteritidis*" (names in quotation marks are not on the Approved Lists of Bacterial Names [37] and have not been validly published since January 1, 1980; hence, they are without standing in

nomenclature), based on indole production. Jantzen and Lassen (18) examined the fatty acid compositions of *Y. enterocolitica* and *Y. enterocolitica*-like bacteria. The genetic, cultural, and biochemical heterogeneity was not reflected by these fatty acid compositions. This is in accord with the observations of Bercovier and Carlier (3) and Sandhu et al. (K. K. Sandhu, E. J. Bottone, and M. A. Paisano, Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, I54, p. 93). The data per se do not suggest a subdivision of *Y. enterocolitica* as currently recognized.

Brenner (8) and Brenner et al. (10) considered genetic data obtained by deoxyribonucleic acid (DNA)-DNA hybridizations of *Yersinia* strains. These workers distinguished three DNA relatedness groups among *Y. enterocolitica* and related bacteria, and from the biochemical properties they defined a fourth entity. All groups were referred to the genus *Yersinia*, but only one deserved the name *Y. enterocolitica*. On the other hand, the genetic data of Moore and Brubaker (34) have suggested that the relationship of *Y. enterocolitica* to the genus *Yersinia* might require further evaluation. Just recently, Bercovier et al. (2, 4), Brenner et al. (9, 11), and Ursing et al. (40) described comprehensive stud-

ies of 175 *Y. enterocolitica* and related strains. These authors proposed the following four species, corresponding to distinct DNA relatedness groups: *Y. enterocolitica sensu stricto* (typical isolates), *Yersinia frederiksenii* (strains producing acid from rhamnose), *Yersinia intermedia* (strains producing acid from rhamnose and melibiose), and *Yersinia kristensenii* (strains not producing acid from sucrose).

One important question that needs to be answered is how phenotypic differentiation correlates with genetic subdivision. The numerical taxonomy study of Sakazaki et al. (36), which involved the simple matching coefficient and single-linkage clustering, distinguished four phenotypic clusters, three of which corresponded approximately to the three different DNA relatedness groups described by Brenner et al. (10). However, this clustering procedure is known to be less discriminating. Harvey and Pickett (16) examined 190 strains by using the simple matching coefficient and clustering by unweighted pair group analysis. These authors concluded that the numerical taxonomy relationships did not correlate highly with the results of DNA hybridizations between strains selected from their phenetic clusters. Actually, three strains, including the centrostrain, were selected from each of the main clusters. Two of these did show a good correlation between genetic classification and phenetic classification (namely, the centrostrain and one strain phenetically highly related to the centrostrain). The converse was true for strains distantly related to the centrostrain. This is not unexpected, however, since hierarchical numerical procedures do result in more heterogeneous clusters above certain similarity levels as the number of units to be grouped is increased. The validity of groups formed by hierarchical clustering procedures may be assessed by parallel application of several numerical grouping procedures. One efficient method which is supplementary to hierarchical clustering procedures is principal components analysis. This method projects the elements to be classified on two-dimensional coordinate plots based on an entirely different computational strategy than phenogram-generating procedures.

The aims of this study were (i) to study the taxonomic interrelationships of *Y. enterocolitica* and *Y. enterocolitica*-like bacteria by a numerical allocation analysis of cultural and biochemical characters, using both a hierarchical clustering procedure and principal components analysis, and (ii) to compare the phenetic relationships indicated by numerical taxonomy with the genetically defined relationships which have been proposed by Bercovier et al. (2, 4), Brenner et al. (9, 11), and Ursing et al. (40).

MATERIALS AND METHODS

Bacterial strains. We selected 332 strains belonging to the genus *Yersinia* for this study (Table 1). These strains were a priori referred to the following categories of Brenner et al. (11).

(i) *Y. enterocolitica sensu stricto*. A total of 148 strains were typical *Y. enterocolitica* isolates. All produced acid from sucrose but not from melibiose or rhamnose. Seven strains belonging to serogroup 3, biovar 4 (41) were isolated from feces of human patients with gastroenteritis in Norway (strains 32 through 38), and 119 strains were isolated from environmental sources in Scandinavian terrestrial and freshwater ecosystems (20, 21, 24, 25, 28, 29) (strains 39 through 157). The remaining 22 strains were received as reference strains from the Pasteur Institute, Paris, France (strains 1 through 3, 5 through 15, 20, 21, 25 through 29, and 31).

(ii) *Y. enterocolitica*-like bacteria. A total of 165 strains isolated from Scandinavian environmental sources (20, 21, 24, 25) deviated from the pattern of determinative characteristics typical of *Y. enterocolitica*. These were 51 *Y. kristensenii* strains that did not produce acid from sucrose (strains 158 through 208), 55 *Y. frederiksenii* strains that produced acid from rhamnose (strains 209 through 263), 25 *Y. intermedia* strains that produced acid from rhamnose and melibiose (strains 264 through 288), and 34 strains which could not be ascribed to any defined species (strains 289 through 322). In addition, five reference strains received from the Pasteur Institute, Paris, were classified as members of *Y. kristensenii* (strains 16 through 19 and 30), and four were classified as *Y. frederiksenii* (strains 4 and 22 through 24).

(iii) *Y. pseudotuberculosis*. Seven reference strains representing *Y. pseudotuberculosis* serogroups I through V were received from S. Winblad, Malmö, Sweden (strains 323 through 329).

(iv) *Y. pseudotuberculosis*-like bacteria. Three strains isolated from wild, small mammals in Denmark (21) exhibited biochemical properties consistent with the properties of *Y. pseudotuberculosis*, but they were antigenically atypical (strains 330 through 332).

All strains were maintained as stab cultures on meat extract agar at 4°C.

Cultural and biochemical determinants. For each strain, 46 cultural and biochemical properties were determined. Incubation was at 37°C unless otherwise stated. The tests were read daily for 4 days. Weakly positive or ambiguous tests were incubated further and were read for 7 days.

Acid from carbohydrates. Acidification of carbohydrate-containing media was studied in liquid media containing 0.5% carbohydrate in 1% peptone water and 0.0025% bromothymol blue as an indicator. The basal medium was heat sterilized before the carbohydrates were added. The carbohydrates were sterilized separately by filtration. The media were contained in tubes with cotton plugs.

The following carbohydrates were tested: adonitol, L-arabinose, arbutin, D-cellobiose, dextrin, dulcitol, esculin, D-fructose, D-galactose, glycerol, meso-inositol, inulin, lactose, sodium malonate, maltose, mannitol, D-mannose, D-melibiose, raffinose, L-rhamnose, salicin, D-sorbitol, L-sorbose, starch, sucrose, D-trehalose,

TABLE 1. *List of strains studied*

Strain no.		Serogroup ^a	Source
This study	Other		
Reference strains of <i>Y. enterocolitica</i> and <i>Y. enterocolitica</i> -like bacteria ^b			
1	IP 6	1	Chinchilla
2	IP 8	2	Hare
3	IP 85	3	Pig
4	IP 96	4,32	Chinchilla
5	IP 1476	4,33	Water
6	IP 123	5	Cow
7	IP 47	5,27	Monkey
8	IP 885	5,27	Dog
9	IP 102	6,30	Human
10	IP 1477	6,31	Water
11	IP 106	7,8	Guinea pig
12	IP 107	8	Human
13	IP 842	8,19	Human
14	IP 201	9	Human
15	IP 551	10	Human
16	IP 105	11,23	Human
17	IP 841	11,24	Human
18	IP 490	12,25	Hare
19	IP 103	12,26	Sheep
20	IP 553	13,7	Human
21	IP 480	14	Human
22	IP 614	15	Human
23	IP 1475	16	Water
24	IP 867	16,29	Human
25	IP 955	17	Water
26	IP 846	18	Human
27	IP 845	20	Human
28	IP 1110	21	Human
29	IP 1367	22	Human
30	IP 1474	28	Water
31	IP 1501	34	Human
<i>Y. enterocolitica</i> sensu stricto: human clinical isolates ^c			
32	501	3	Human
33	503	3	Human
34	505	3	Human
35	508	3	Human
36	509	3	Human
37	504	3	Human
38	502	3	Human
<i>Y. enterocolitica</i> sensu stricto: environmental isolates ^d			
39	11046	16	Small rodent
40	12027D	7,8	Small rodent
41	12027B	6	Small rodent
42	7029	NAG	Small rodent
43	9028	NAG	Small rodent
44	213	NT	Water
45	N59B	13,7	Fish
46	10096	4,18,21	Small rodent
47	B28	14	Fish
48	G29B	6	Water
49	G27A	15	Water
50	10016A	12	Shrew
51	10047	6	Small rodent
52	G30A	6	Water
53	φ1	4	Small rodent
54	10043	6	Small rodent

TABLE 1—*Continued*

Strain no.		Serogroup ^a	Source
This study	Other		
55	12025A	NAG	Small rodent
56	10012A	6	Small rodent
57	S14	16	Small rodent
58	φ2	6	Small rodent
59	V18	6	Small rodent
60	V31B	6	Small rodent
61	10005X	NAG	Small rodent
62	G6B	6	Water
63	5006A	NAG	Small rodent
64	Å4	1	Small rodent
65	5006B	6	Small rodent
66	5003B	4	Shrew
67	5003A	7,8	Shrew
68	5010A	3	Small rodent
69	10116C	6	Shrew
70	G33B	4	Water
71	11018	4	Small rodent
72	10129	1	Small rodent
73	R21A	6	Fox
74	11036	4	Small rodent
75	10063	3	Small rodent
76	201	5	Water
77	10143	4,16	Small rodent
78	G26C	4	Water
79	1011A	7,8	Small rodent
80	G23A	4,16	Water
81	R23	6	Fox
82	12013A	NAG	Small rodent
83	12013B	16	Small rodent
84	12025B	6	Small rodent
85	1005B	12	Shrew
86	10130	NAG	Small rodent
87	12001	6	Small rodent
88	1020A	7,8	Shrew
89	1016	6	Shrew
90	R24A	6	Fox
91	11005	3	Small rodent
92	7021	5	Small rodent
93	7024	7,8	Small rodent
94	7180	6	Small rodent
95	S30	4	Small rodent
96	S28	1	Small rodent
97	S18B	NAG	Small rodent
98	S18C	NAG	Small rodent
99	8018	3	Small rodent
100	8025	3	Small rodent
101	S18A	16	Small rodent
102	B118	NAG	Fish
103	B136B	4	Fish
104	10026	6	Small rodent
105	G33J	NAG	Water
106	B129C	17	Fish
107	11015	1	Small rodent
108	N59A	NAG	Fish
109	G29C	5	Water
110	1002	6	Small rodent
111	G30B	17	Water
112	B123A	14	Fish
113	G33I	18,IA,IB	Water
114	B138A	NAG	Fish

TABLE 1—Continued

Strain no.		Serogroup ^a	Source
This study	Other		
115	N10	NAG	Fish
116	N35B	4,13	Fish
117	G33H	NAG	Water
118	N24A	NAG	Fish
119	B25	NAG	Fish
120	N55	IIA	Fish
121	G34D	NAG	Water
122	B27	NAG	Fish
123	B115	NAG	Fish
124	G33F	NAG	Water
125	B94	16,21	Fish
126	B109	NAG	Fish
127	G23B	16,22	Water
128	LA37A	NAG	Fish
129	N36A	18,IA,IB	Fish
130	B125	NAG	Fish
131	B121B	NAG	Fish
132	B145	14	Fish
133	G33E	NAG	Water
134	11040	4	Small rodent
135	11030B	3	Small rodent
136	12034	6	Small rodent
137	214	NT	Water
138	217	NT	Water
139	202	NT	Water
140	208	4	Water
141	212	NT	Water
142	207	4	Water
143	203	NT	Water
144	204	NT	Water
145	218	NT	Water
146	N52	NAG	Fish
147	B22	16	Fish
148	B123B	16	Fish
149	B121A	NAG	Fish
150	215	NT	Water
151	G17B	6	Water
152	B113A	NAG	Fish
153	B117A	4,13	Fish
154	B138B	NAG	Fish
155	B139	NAG	Fish
156	B124A	4,13	Fish
157	2943	2	Goat
<i>Y. enterocolitica</i> -like bacteria: <i>Y. kristensenii</i> ^d			
158	11051A	28	Shrew
159	9019A	28	Shrew
160	9021A	NAG	Shrew
161	1003A	12	Shrew
162	1015A	NAG	Shrew
163	1015B	12	Shrew
164	G21B	12	Water
165	1001	28	Shrew
166	1017A	11	Shrew
167	1030	28	Small rodent
168	G9	11	Water
169	5008B	NAG	Small rodent
170	5010B	NAG	Small rodent
171	G14B	12	Water
172	11041	12	Small rodent
173	G23C	NAG	Water

TABLE 1—*Continued*

Strain no.		Serogroup ^a	Source
This study	Other		
174	1018	NAG	Shrew
175	G26D	12	Water
176	11047B	12	Small rodent
177	1019B	NAG	Shrew
178	G2	28	Water
179	9036	28	Small rodent
180	S261	NAG	Fish
181	11049	NAG	Shrew
182	S264	NAG	Fish
183	9021B	28	Shrew
184	9019B	NAG	Shrew
185	9008	28	Small rodent
186	9020	11	Shrew
187	9017A	11	Shrew
188	9041A	6	Small rodent
189	12027C	11	Small rodent
190	12027A	NAG	Small rodent
191	12008	16	Small rodent
192	G7	11	Water
193	G13A	28	Water
194	G34C	NAG	Water
195	10016B	28	Shrew
196	G27B	NAG	Water
197	1020B	16	Shrew
198	1011C	16	Small rodent
199	9017B	NAG	Shrew
200	10012B	16	Small rodent
201	B21A	1	Fish
202	G26A	NAG	Water
203	8017	1	Small rodent
204	9041B	12	Small rodent
205	10050	1	Small rodent
206	10053	1	Small rodent
207	G14A	28	Water
208	11051B	11	Shrew
<i>Y. enterocolitica</i> -like bacteria: <i>Y. frederiksenii</i> ^d			
209	B19	4,18,21,IIB	Fish
210	B16	NAG	Fish
211	B21B	17	Fish
212	B30	NAG	Fish
213	B24	4	Fish
214	B34	4,18,21,IIB	Fish
215	G17A	14	Water
216	G10	14	Water
217	10128	NAG	Small rodent
218	G21A	17	Water
219	10139	18,21	Small rodent
220	10140	18,21	Small rodent
221	B18	17	Fish
222	G19B	18,21	Water
223	G19a	4	Water
224	N30	14	Fish
225	N47	IIA	Fish
226	G32A	4	Water
227	N2	4	Fish
228	N13B	NAG	Fish
229	G33D	NAG	Water
230	N42	4,13	Fish
231	N35A	14	Fish
232	N24B	NAG	Fish

TABLE 1—*Continued*

Strain no.		Serogroup ^a	Source
This study	Other		
233	N40B	4	Fish
234	B130B	4,13	Fish
235	B130A	NAG	Fish
236	N60	14	Bird
237	N54B	4,13	Fish
238	N13A	NAG	Fish
239	G18B	4,16,18,21	Water
240	B111	4,13	Fish
241	B113B	IV	Fish
242	B106	NAG	Fish
243	B97	16,21	Fish
244	B123C	14	Fish
245	B124B	NAG	Fish
256	B143	NAG	Fish
247	N38B	4,13	Fish
248	G33C	NAG	Water
249	N39	4,13	Fish
250	N56	IIA	Fish
251	N36B	16	Fish
252	10107	4,16,18,21	Small rodent
253	B39	4,18,21,IB,IIB	Fish
254	B128B	NAG	Fish
255	G18A	NAG	Water
256	B131	NAG	Fish
257	B128A	17	Fish
258	B136B	4	Fish
259	G34A	3	Water
260	G35A	IV	Water
261	B117B	4,13	Fish
262	B102	4	Fish
263	B129A	NAG	Fish
<i>Y. enterocolitica</i> -like bacteria: <i>Y. intermedia</i> ^d			
264	N48	13,7	Fish
265	B133	NAG	Fish
266	N51	13,7	Fish
267	10147	4,16,18,21	Small rodent
268	10119	4,16,18,21	Small rodent
269	10098	4,16,18,21	Small rodent
270	G19C	NAG	Water
271	7174	4	Small rodent
272	G36A	NAG	Water
273	F21	NAG	Fish
274	N6	NAG	Fish
275	N37	NAG	Fish
276	B122	4	Fish
277	LA37B	NAG	Fish
278	S295B	NAG	Fish
279	S292A	NAG	Fish
280	G35B	4,13	Water
281	S292B	NAG	Fish
282	B38	NAG	Fish
283	G32B	NAG	Fish
284	N23A	NAG	Fish
285	B129B	17	Fish
286	B126B	NAG	Fish
287	S260	IIA	Fish
288	S295A	NAG	Fish
<i>Y. enterocolitica</i> -like bacteria: miscellaneous ^e			
289	10005Y	NAG	Small rodent
290	G30C	NAG	Water

TABLE 1—Continued

Strain no.		Serogroup ^a	Source
This study	Other		
291	11016	12	Small rodent
292	G32C	NAG	Water
293	12017	NAG	Small rodent
294	12020	NAG	Small rodent
295	12036	NAG	Small rodent
296	12040	NAG	Small rodent
297	11050	17	Shrew
298	B156	NAG	Fish
299	LA58B	NAG	Fish
300	Co26	NAG	Fish
301	LA44A	13,7	Fish
302	LA58A	NAG	Fish
303	S267C	1	Fish
304	S272	NAG	Fish
305	G36B	NAG	Water
306	G26B	NAG	Water
307	G11	NAG	Water
308	1014	NAG	Shrew
309	G6A	NAG	Water
310	R21B	NAG	Fox
311	10115	NAG	Small rodent
312	117B	NAG	Shrew
313	S267A	NAG	Fish
314	S275	NAG	Fish
315	N27	NAG	Fish
316	1019A	NAG	Shrew
317	1004	NAG	Shrew
318	12012	NAG	Shrew
319	10148	NAG	Small rodent
320	J1	NAG	Soil
321	N54A	13,7	Fish
322	11048	NAG	Shrew
<i>Y. pseudotuberculosis</i> reference strains ^f			
323	402	IA	Unknown
324	403	IB	Unknown
325	407	IIA	Unknown
326	401	IIB	Unknown
327	405	III	Unknown
328	404	IV	Unknown
329	406	V	Unknown
<i>Y. pseudotuberculosis</i> -like bacteria ^g			
330	12024B	4,21,IIB	Small rodent
331	12027E	4,21,IIB	Small rodent
332	12035	4,21,IIB	Small rodent

^a Arabic figures indicate antigenic relationships to serogroups of *Y. enterocolitica*. Roman figures indicate antigenic relationships to serogroups of *Y. pseudotuberculosis*. NAG, Non-agglutinable; NT, not tested.

^b The study reported here was completed before the data of Bercovier et al. (2, 4), Brenner et al. (9, 11), and Ursing et al. (40) were published. Consequently, the type strains proposed by these authors could not be included in this study. The reference strains listed were received from the Pasteur Institute, Paris, as being representative of the taxonomic spectrum consisting of *Y. enterocolitica* and *Y. enterocolitica*-like bacteria.

^c According to Brenner et al. (11). Serogroup 3, biovar 4 strains (40). These strains were isolated at the National Institute of Public Health, Oslo, Norway, from human patients with gastroenteritis.

^d According to Brenner et al. (11). These strains were isolated from terrestrial and freshwater ecosystems in Scandinavia (20, 21, 24, 25, 28, 29).

^e These strains were isolated from terrestrial and freshwater ecosystems in Scandinavia (20, 21, 24, 25, 28, 29) and could not be placed in any presently defined species.

^f Received from S. Winblad, Malmö, Sweden.

^g These strains were isolated from terrestrial and freshwater ecosystems in Scandinavia (20, 21, 24, 25, 28, 29) and were biochemically ascribable to *Y. pseudotuberculosis*, but they had atypical antigenic properties (21, 24).

and D-xylose.

The oxidative-fermentative metabolism of lactose was evaluated by the method of Hugh and Leifson (17).

Miscellaneous tests. Acetoin production (Voges-Proskauer) and methyl red tests were performed in glucose phosphate peptone water (Clark and Lub medium). Acetoin formation was detected by the Barritt method (13); cultures were run in parallel and were incubated at 22 and 37°C. The ability of the strains to utilize citrate was tested on agar slants of Simmons citrate medium (31). Gelatin hydrolase activity was measured by using photographic film, as described by Le Minor (31). Urease activity and indole production were examined in a combined urea-indole medium, as described by Le Minor (31) and modified at the National Institute of Public Health, Oslo, Norway (30). Indole was detected by using Kovacs reagent. Motility and nitrate reductase activity were studied by using a combined mannitol-motility medium (30, 31). Motility was evaluated after incubation at both 22 and 37°C. Nitrate reductase activity was tested by adding sulfanilic acid and dimethyl- α -naphthylamine (31). The production of gas from glucose and H₂S formation were tested in a combined lactose-glucose-H₂S medium (30, 31). Examination of oxidase activity was by the Kovacs method, in which tetramethyl-*p*-phenylenediamine dihydrochloride was used. Arginine dihydrolase, lysine decarboxylase, and ornithine decarboxylase were detected by a method involving modified Falkow medium (31). Lecithinase activity was measured on agar plates containing egg yolk emulsion (type SR 47; Oxoid Ltd., London, England). β -Galactosidase activity was detected by the *o*-nitrophenyl- β -D-galactopyranoside test described by Le Minor (31).

Numerical procedures. All 46 characters determined were coded in binary form. The two approaches used for numerical analyses of the bacterial strains were as follows.

(i) Hierarchical procedure. The hierarchical clustering procedure was performed by using a program developed at the Norwegian Computing Center, Oslo. This program involves calculating the squared Euclidean distance as a measure of similarity between each pair of strains being grouped (the original numerical units [ONUs]) and subsequent clustering by the Ward procedure (1). This procedure bases successive clusterings on the distances between the centrostrains of individual clusters. The program was constructed to enable efficient handling of a large similarity matrix with a low demand for computer capacity. Each clustering cycle combines ONUs within a narrow range of variance limits. For large similarity matrices, this leads to less detailed differentiation at each clustering cycle compared with the high accuracy achieved by unweighted pair group analysis, in which clustering is based upon exact similarity values between phenons (5, 38).

(ii) Principal components analysis. The principal components analysis was performed by using a program which was developed at the Norwegian Computing Center and has been described previously (14). This approach conceives the cultural and biochemical

characters as vectors in Euclidean hyperspace and involves calculating the ability of these characters to differentiate between ONUs. To do this, eigenvectors representing the characters are computed, and the variance of each character is calculated on the basis of correlation coefficients. The ONU collection is projected on the principal components as orthogonal coordinates in order of decreasing differentiation between ONUs. The eigenvectors defining the principal components show the relative contribution of every character in defining each component. Thus, the eigenvectors form the basis of an assessment of what bacterial properties contribute most to differentiation among the strains studied.

RESULTS

Clustering by hierarchical procedure. The results of the hierarchical clustering were represented by a phenogram (Fig. 1). Four main clusters (clusters A through D) were distinguished.

(i) Cluster A. Cluster A could be subdivided into four subclusters (subclusters A1 through A4). All seven *Y. pseudotuberculosis* reference strains constituted subcluster A1 (strains 323 through 329). Subcluster A2 contained the three *Y. pseudotuberculosis*-like strains (strains 330 through 332). Subclusters A3 and A4 encompassed *Y. enterocolitica*-like bacteria which could not be placed in any presently defined species. Subcluster A3 consisted of 10 strains, 9 of which produced acid from rhamnose but not from sucrose (strains 289 through 298), and subcluster A4 consisted of 17 strains, most of which were negative for ornithine decarboxylase or sucrose or both (strains 299 through 315).

(ii) Cluster B. Cluster B contained 70 strains, 50 of which belonged to *Y. kristensenii*. Five of these *Y. kristensenii* strains were among the reference strains received from the Pasteur Institute (strains 16 through 19 and 30). The remaining 20 strains in this cluster belonged to *Y. enterocolitica* sensu stricto. Among these 20 strains were all 7 human clinical isolates of serogroup 3, biovar 4 (strains 32 through 38) and the two isolates of serogroup 2, biovar 5 (strains 2 and 157) included in this study.

(iii) Cluster C. Cluster C consisted predominantly of strains of *Y. enterocolitica* sensu stricto. Of the 97 strains in this cluster, 66 were *Y. enterocolitica* sensu stricto, 15 were *Y. frederiksenii*, 10 were *Y. intermedia*, and 2 were *Y. kristensenii*. The remaining four strains were *Y. enterocolitica*-like bacteria which could not be placed in any presently defined species (strains 316 through 319).

(iv) Cluster D. Cluster D was the most heterogeneous of the four main clusters. Of the 128 strains in this cluster, 65 were *Y. enterocolitica*

sensu stricto, 42 were *Y. frederiksenii*, 15 were *Y. intermedia*, and 3 were *Y. kristensenii*. Three additional strains were *Y. enterocolitica*-like isolates that differed from all previously recognized species (strains 320 through 322).

Clustering by principal components analysis. Figure 2 shows the results of the principal components analysis. The 332 *Yersinia* strains are represented as two-dimensional projections on the first and second principal components (Fig. 2A) and the first and third principal components (Fig. 2B). The clearest separation of the species proposed by Bercovier et al. (2, 4), Brenner et al. (9, 11), and Ursing et al. (40) was obtained in Fig. 2B. For reference purposes Fig. 2B was subdivided into four quadrants (quadrants QI through QIV).

The strains belonging to phenogram cluster A (Fig. 1) appeared in quadrants QIII and QIV and were reasonably separated from the remaining strains. A tendency toward subclustering was found with the strains belonging to phenogram subclusters A1 through A4. The subcluster A1, A2, and A3 strains were in quadrant QIV. The subcluster A4 strains formed a separate group in quadrant QIII.

Most of the *Y. kristensenii* strains formed a distinct, relatively tight cluster in quadrant QII, which corresponded approximately to phenogram cluster B. This included the seven human clinical isolates belonging to serogroup 3, biovar 4. However, the two strains belonging to serogroup 2, biovar 5 were grouped together with the strains of phenogram subcluster A4 in quadrant QIII.

The principal components analysis did not clearly separate *Y. enterocolitica* sensu stricto, *Y. frederiksenii*, and *Y. intermedia*. These strains formed a large, heterogeneous cluster located in quadrant QI and the upper part of quadrant QII, together with a few *Y. kristensenii* isolates and seven strains which could not be placed in any of the presently defined species (strains 316 through 322). This cluster corre-

FIG. 1. Phenogram for 332 *Yersinia* strains obtained by hierarchical clustering based on cultural and biochemical determinants. The numbers correspond to the strain numbers in Table 1. Symbols: *, *Y. pseudotuberculosis* reference strains; x, *Y. pseudotuberculosis*-like bacteria; ○, *Y. enterocolitica* sensu stricto; ⊙, *Y. enterocolitica* sensu stricto serogroup 3, biovar 4; ⊖, *Y. enterocolitica* sensu stricto serogroup 2, biovar 5; ●, *Y. kristensenii*; ▲, *Y. frederiksenii*; △, *Y. intermedia*; ■, *Y. enterocolitica*-like bacteria—rhamnose positive and sucrose negative; ▽, *Y. enterocolitica*-like bacteria—sucrose negative or ornithine decarboxylase negative or both; □, *Y. enterocolitica*-like bacteria—miscellaneous.

sponded to phenogram clusters C and D. However, the different taxa were not randomly scattered in this cluster. Strains of *Y. enterocolitica sensu stricto* dominated the upper part of quad-

rant QII and the lower part of quadrant QI, as in phenogram cluster C. *Y. frederiksenii* became increasingly more prevalent with increasing distance from quadrant QII, whereas *Y. intermedia*

FIG. 2. Principal components analysis. The 332 *Yersinia* strains are presented in two-dimensional projections on the first and second principal components (A) and the first and third principal components (B) according to their cultural and biochemical properties. For an explanation of the symbols, see the legend to Fig. 1.

prevailed toward the upper part of quadrant QI (appearing in phenogram cluster D).

Cultural and biochemical characteristics. The cultural and biochemical properties of the main groups are shown in Table 2. The characters shared by all strains included lack of oxidase activity, H₂S production, and formation of acid from fructose, malonate, and mannose. With few exceptions, the strains were positive for acid production from arabinose, galactose, maltose, mannitol, and trehalose, for β -galactosidase, for motility at 22°C, for the methyl red reaction, for nitrate reductase, and for urease. Furthermore, most strains were negative for acetoin production at 37°C, for arginine dihydrolase, for gelatin hydrolase, for lysine decarboxylase, and for motility at 37°C.

***Y. kristensenii*.** *Y. kristensenii* was defined a priori as those strains which did not produce acid from sucrose. Three additional features distinguished this species. *Y. kristensenii* was frequently negative for acetoin production at 22°C (86%), for esculin (86%), and, to a lesser degree, for salicin (55%). High percentages (74 to 100%) of the *Y. enterocolitica* sensu stricto, *Y. frederiksenii*, and *Y. intermedia* strains were positive for these features.

***Y. frederiksenii*, *Y. intermedia*, and *Y. enterocolitica* sensu stricto.** The results with *Y. frederiksenii*, *Y. intermedia*, and *Y. enterocolitica* sensu stricto were tabulated both separately and collectively (Table 2) since the numerical analysis did not clearly separate these taxa. *Y. frederiksenii* and *Y. intermedia* seemed to be biochemically more similar to *Y. enterocolitica* sensu stricto than to *Y. kristensenii*. However, except for the production of acid from melibiose, rhamnose, and sucrose, which served to define these species, no further distinction was evident.

Phenogram subclusters A1 and A2. The *Y. pseudotuberculosis* reference strains (subcluster A1) and the *Y. pseudotuberculosis*-like bacteria (subcluster A2) were biochemically similar. All strains produced acid from rhamnose and melibiose (one exception) but not from cellobiose, sorbose, or sucrose; all lacked ornithine decarboxylase.

Phenogram subcluster A3. Of the 10 strains in phenogram subcluster A3, 9 produced acid from rhamnose but not from cellobiose, sorbose, or sucrose. All were positive for lysine decarboxylase.

Phenogram subcluster A4. Subcluster A4 consisted of two biochemically different subgroups. A core of eight strains was negative for cellobiose, melibiose, ornithine decarboxylase (six showed delayed reactions), rhamnose, sorbose, and sucrose. The remaining nine strains

were positive for sorbitol and sucrose but negative for melibiose (one exception), ornithine decarboxylase (two strains showed delayed reactions), rhamnose, and sorbose; six strains were negative for cellobiose.

Other strains. Seven *Y. enterocolitica*-like bacteria which did not belong to any defined species (strains 316 through 322) were distributed between phenogram clusters C and D and appeared in quadrant QI in Fig. 2B. These strains constituted a heterogeneous group of strains that were distinguishable from *Y. enterocolitica* sensu stricto by at least two of the following characters: acid production from melibiose and rhamnose; no acid from cellobiose, sorbose, or sucrose; and absence of ornithine decarboxylase.

Differential characters. The relative contribution of each cultural and biochemical character in defining the first three principal components was derived from the eigenvectors representing each particular principal component. This indicated objectively the relative contributions of the cultural and biochemical properties in differentiating among the strains examined and formed the basis for a rational selection of a minimum set of key properties enabling efficient species identification by routine bacteriology laboratories. The list of key properties derived in this way was compared with the results in Table 2, and Table 3 shows the characters that differentiate among the main taxa.

Ecology, antigenicity, and pathogenicity factors. The cultural and biochemical characteristics described above formed the basis for taxonomic classification, characterization of taxa, and identification of individual strains. Some of the integral properties of a microorganism are its habitat, pathogenicity, and antigenic specificity. To supplement the description of the four nomenspecies constituting *Y. enterocolitica* and the *Y. enterocolitica*-like bacteria, the above-mentioned properties are summarized in Table 4. The data are based on Scandinavian isolates included in this and previous studies (20–26, 28, 29).

A majority of the *Y. kristensenii* strains studied belonged to three serogroups (serogroups O:11, O:12, and O:28), which were found only infrequently among the other taxa (24). *Y. kristensenii* is the only *Yersinia* species for which enterotoxin production at 37°C has been demonstrated at any noticeable frequency (22). Ecologically, *Y. kristensenii* was comparable to *Y. enterocolitica* biovar 1; the highest occurrence of these organisms was among terrestrial vertebrates (24). On the other hand, *Y. frederiksenii* and *Y. intermedia* were characterized by anti-

TABLE 2. *Cultural and biochemical characteristics of the main taxa*

Character ^a	% of positive strains					No. of positive strains				
	<i>Y. enterocolitica</i> (<i>n</i> = 148)	<i>Y. frederiksenii</i> (<i>n</i> = 59)	<i>Y. intermedia</i> (<i>n</i> = 25)	<i>Y. kristensenii</i> (<i>n</i> = 56)	<i>Y. enterocolitica</i> plus <i>Y. frederiksenii</i> plus <i>Y. intermedia</i> (<i>n</i> = 232)	<i>Y. enterocolitica</i> plus <i>Y. frederiksenii</i> (<i>n</i> = 207)	Subcluster ^b A1 (<i>n</i> = 7)	Subcluster A2 (<i>n</i> = 3)	Subcluster A3 (<i>n</i> = 10)	Subcluster A4 (<i>n</i> = 17)
Acid from:										
Adonitol	18	2	12	14	13	13	0	0	0	1
Arabinose	99	98	100	98	99	99	5	3	10	16
Arbutin	95	100	100	88	97	97	7	3	0	0
Cellobiose	99	98	96	100	99	99	0	0	0	3
Dextrin	29	14	28	36	25	25	0	1	0	0
Dulcitol	6	0	12	5	5	4	0	0	0	0
Esculin	85	100	100	14	91	89	0	3	1	6
Fructose	100	100	100	100	100	100	7	3	10	17
Galactose	98	100	100	96	99	99	6	3	10	16
Glycerol	83	64	72	84	77	78	0	3	10	9
Inositol	53	44	64	27	52	51	0	0	0	1
Inulin	14	7	4	5	11	12	0	0	0	0
Lactose (oxidative)	40	36	36	61	38	39	0	0	0	12
Lactose (fermentative)	24	22	48	16	26	24	0	0	0	1
Malonate	0	0	0	0	0	0	0	0	0	0
Maltose	97	100	100	100	98	98	7	3	10	5
Mannitol	99	98	100	100	99	99	7	3	8	17
Mannose	100	100	100	100	100	100	7	3	10	17
Melibiose	7	0	100	4	15	5	6	3	3	1
Raffinose	7	12	36	0	12	9	0	0	2	5
Rhamnose	0	100	100	0	38	32	7	3	9	0
Salicin	86	100	100	45	91	90	4	3	0	0
Sorbitol	99	100	100	100	99	99	0	0	0	17
Sorbose	99	92	84	100	95	97	0	0	0	0
Starch	44	31	28	68	39	40	0	1	2	1
Sucrose	100	100	100	0	100	100	0	0	1	9
Trehalose	99	100	100	100	99	99	7	3	10	17
Xylose	41	20	12	23	33	35	0	0	0	2
Acetoin production (22°C)	74	95	92	14	81	80	0	1	10	10
Acetoin production (37°C)	1	2	0	0	1	1	0	0	5	0
Arginine dihydrolase	1	0	0	0	0	0	0	0	0	0
Citrate utilization	1	0	0	9	0	0	0	0	0	0
β-Galactosidase	98	100	100	96	99	99	7	2	6	16
Gas from glucose ^c	39	34	24	57	36	38	0	0	10	7
Gelatin hydrolase	1	0	0	0	0	0	0	0	0	0
H ₂ S production	0	0	0	0	0	0	0	0	0	0
Indole production	68	73	76	43	70	70	0	0	0	0
Lecithinase	52	32	20	20	44	46	0	0	0	0
Lysine decarboxylase	2	0	4	2	2	1	0	0	10	1
Methyl red	100	98	100	100	100	100	7	3	10	17
Motility (22°C)	96	98	100	100	97	97	6	1	10	15
Motility (37°C)	0	2	0	0	0	0	0	0	0	0
Nitrate reductase	97	100	96	100	98	98	7	3	10	17
Ornithine decarboxylase	93	92	100	95	93	92	0	0	10	8 ^d
Oxidase	0	0	0	0	0	0	0	0	0	0
Urease	99	100	100	93	100	100	7	3	9	17

^a Incubation was at 37°C unless otherwise stated.^b Subclusters A1 through A4 refer to the phenogram in Fig. 1, as follows: subcluster A1, *Y. pseudotuberculosis* reference strains; subcluster A2, *Y. pseudotuberculosis*-like bacteria; subclusters A3 and A4, *Y. enterocolitica*-like bacteria that cannot be placed in any presently defined species.^c Small, but definite amounts of gas were produced.^d Delayed reaction (positive after 6 to 7 days).

TABLE 3. *Differential characteristics of the main taxa*

Differential characteristic ^a	Reaction of:						No. of strains positive/total no.			
	<i>Y. enterocolitica</i>	<i>Y. frederiksenii</i>	<i>Y. intermedia</i>	<i>Y. kristensenii</i>	<i>Y. enterocolitica</i> , <i>Y. frederiksenii</i> , and <i>Y. intermedia</i> combined	<i>Y. enterocolitica</i> and <i>Y. frederiksenii</i> combined	Sub-cluster ^b A1	Sub-cluster A2	Subcluster A3	Subcluster A4
Cellobiose	+	+	+	+	+	+	0/7 ^d	0/3	0/10	3/17
Lysine decarboxylase	—	—	—	—	—	—	0/7	0/3	10/10	1/17
Melibiose ^d	—	—	+	—	V	V	6/7	3/3	3/10	1/17
Rhamnose ^d	—	+	+	—	V	V	7/7	3/3	9/10	0/17
Sorbitol	+	+	+	+	+	+	0/7	0/3	0/10	17/17
Sorbose	+	+	V	+	+	+	0/7	0/3	0/10	0/17
Sucrose ^d	+	+	+	—	+	+	0/7	0/3	1/10	9/17
Ornithine decarboxylase	+	+	+	+	+	+	0/7	0/3	10/10	8/17 ^e

^a Incubation was at 37°C.^b Subclusters A1 through A4 refer to the phenogram in Fig. 1.^c +, >90% of the strains positive; —, <10% of the strains positive; V, 10 to 90% of the strains positive.^d Characters selected a priori to differentiate *Y. enterocolitica sensu stricto*, *Y. frederiksenii*, *Y. intermedia*, and *Y. kristensenii*.^e Delayed reaction (positive after 6 to 7 days).

genic, ecological, and pathogenic properties which did not clearly distinguish them from *Y. enterocolitica sensu stricto*. However, it is notable that *Y. frederiksenii* and *Y. intermedia* were ecologically more confined to aquatic ecosystems (fish and water) than *Y. enterocolitica sensu stricto*, which was prevalent in both terrestrial and aquatic habitats (24). Furthermore, strains of each of these three taxa produced enterotoxin at 22°C, but this property was rare in *Y. frederiksenii* and *Y. intermedia* compared with *Y. enterocolitica sensu stricto* (22). The highest prevalence of enterotoxin production (91%) was found among human clinical isolates belonging to serogroup 3, biovar 4. Invasiveness for HeLa cells was displayed by serogroup O:3, biovar 4 and serogroup O:2, biovar 5 strains (23), the only recognized pathogens included in Table 4.

DISCUSSION

Bercovier et al. (2, 4), Brenner et al. (9, 11), and Ursing et al. (40) divided *Y. enterocolitica* and *Y. enterocolitica*-like bacteria into four species by using genotypic parameters. All of these species could be defined phenotypically by using acid production from melibiose, rhamnose, and sucrose as key characters. In our study, this classification was adopted a priori, and the taxonomic relationships of the strains were examined by a numerical clustering analysis based on cultural and biochemical characters. This approach offered an opportunity to compare the genotypically based taxonomy with the phenotypic classification.

Both the principal components analysis and the hierarchical clustering procedure identified *Y. kristensenii* as a relatively distinct phenotypic cluster (Fig. 1 and 2B). On the other hand, *Y. enterocolitica sensu stricto*, *Y. frederiksenii*, and *Y. intermedia* were grouped together without distinct delineations. These taxa constituted a continuum of intergrading phenotypes. The degree of apparent overlapping between different species depends on and increases with the number of strains included in the study. This is particularly true with dendrogram-generating procedures. Consequently, the principal components analysis is especially important when a large number of organisms are studied. In this procedure, each ONU is positioned more independently in relation to the other strains and more by virtue of its own properties than in dendrograms. Therefore, considerable emphasis must be put on the interpretations of the principal components analysis. The principal components analysis showed that *Y. enterocolitica sensu stricto* and *Y. frederiksenii* overlapped completely (Fig. 2B). *Y. intermedia* demonstrated a trend toward separation from the *Y. enterocolitica*-*Y. frederiksenii* cluster. However, certain *Y. intermedia* strains were phenetically closely related to the other two nomenclatures. Likewise, a few strains seemed to represent intermediate forms between *Y. enterocolitica* and *Y. kristensenii*. Some sucrose-negative strains were grouped together with *Y. enterocolitica* and vice versa. This observation agrees with the observations of Bercovier et al. (4) and Brenner et al. (11), who reported that the high-

TABLE 4. *Characteristics of Yersinia taxa isolated from environmental and human sources in Scandinavia*

Taxon ^a	Serogroup(s) ^b	Biovar ^c	Ecological origin(s) ^d	Enterotoxin production at the following temp (°C) ^e	Invasiveness for HeLa cells ^f
<i>Y. enterocolitica</i> sensu stricto	2	5	Goat, gastroenteritis (F)	None ^g	+ ^h
	3	4	Humans, gastroenteritis (C)	22 (91)	+
		4	Swine, healthy carriers	22 ^h	+ ^h
		1	Mammals, healthy carriers (F)	22	
	4, 6, miscellaneous	1	Mammals, healthy carriers (C); humans, healthy carriers (F); birds, healthy carriers (F); water (F); minced meat (F)	22 (20), 4 ^h	—
	Miscellaneous	N ⁱ	Mammals, healthy carriers (C) fish, healthy carriers (C); humans, healthy carriers (F); water (C)	22 (20)	—
<i>Y. kristensenii</i>	11, 12, 28, miscellaneous	N	Mammals, healthy carriers (C); fish, healthy carriers (F); humans, healthy carriers (F); birds, healthy carriers (F); water (C)	22 and 37 (49), 4 ^h	—
<i>Y. frederiksenii</i>	4, miscellaneous	N	Fish, healthy carriers (C); mammals, healthy carriers (F); humans, healthy carriers (F); water (C)	22 (<5)	—
<i>Y. intermedia</i>	Miscellaneous	N	Fish, healthy carriers (C); mammals, healthy carriers (F); water (F)	22 (<5)	—
A3	NAG ^j	N	Mammals, healthy carriers (C); fish, healthy carriers (F); humans, healthy carriers (F); water (F)	None	—
A4	NAG	N	Fish, healthy carriers (C); mammals, healthy carriers (F); water (F)	None	—

^a Species as proposed by Bercovier et al. (2, 4), Brenner et al. (9, 11), and Ursing et al. (40). A3 and A4 refer to the phenogram subclusters shown in Fig. 1, as follows: A3, sucrose-negative and rhamnose-positive strains; A4, strains negative for sucrose or ornithine decarboxylase or both.

^b See references 24, 28, and 29.

^c According to Wauters (41).

^d See references 24, 28, and 29. The letters in parentheses indicate the relative importance of each ecological source as a reservoir of the different *Yersinia* taxa, as follows: C, common; F, a few strains isolated.

^e See references 22 and 26. The numbers in parentheses indicate the approximate percentages of enterotoxigenic strains.

^f See reference 23.

^g Only one strain was examined.

^h Only 20 strains were examined for enterotoxin production at 4°C (26); 3 of 6 *Y. kristensenii* strains and 1 of 13 *Y. enterocolitica* sensu stricto strains were positive.

ⁱ N, Most strains could not be placed in any of the biovars proposed by Wauters (41).

^j NAG, Non-agglutinable.

est interspecific DNA relatedness values obtained between these two taxa overlapped the lowest intraspecific values. In contrast to our results, however, *Y. frederiksenii*, *Y. intermedia*, and *Y. enterocolitica* sensu stricto formed distinct DNA relatedness groups. Harvey and Pickett (16) recognized only one DNA related-

ness group among rhamnose-positive strains, indicating that the taxonomic positions of *Y. frederiksenii* and *Y. intermedia* may require further evaluation. However, these authors did not mention whether any melibiose-positive strains were represented among their rhamnose-positive isolates.

The Scandinavian isolates of *Y. kristensenii* could be distinguished reasonably well from the remaining taxa by antigenic and enterotoxigenic parameters (Table 4). Together with the phenetic distinctness indicated by our work, these observations support the genetic data which suggests that *Y. kristensenii* deserves separate species status. On the other hand, the pattern of overlapping phenotypes displayed by *Y. frederiksenii* and *Y. intermedia* in relation to *Y. enterocolitica* sensu stricto was supported further by the antigenic properties, habitat preferences, and pathogenic characteristics of these organisms (Table 4). Thus, we failed to find a basis for separating *Y. frederiksenii* or *Y. intermedia* from *Y. enterocolitica* sensu stricto on either phenetic, antigenic, ecological, or pathogenic grounds. These observations may have implications for the ability of diagnostic microbiologists to recognize the proposed species, and they question the practical advantage of giving these taxa separate species rank.

The ability to distinguish pathogens from non-pathogens is of considerable practical importance. The typical clinical manifestations of yersiniosis in humans and other animals are associated with only a few distinct serogroup-biovar combinations (6, 32). A broad diversity of strains that are antigenically and biochemically distinguishable from the pathogens are omnipresent in nature (6, 24, 32). Such strains have been isolated occasionally in connection with atypical clinical syndromes (6, 32). This has permitted reliable identification of the typical agents of yersiniosis by antigenic and biochemical means. Both *Y. intermedia* and *Y. kristensenii* occasionally have been associated with atypical clinical syndromes, but so have various environmental variants of *Y. enterocolitica* sensu stricto (6, 32). The clinical distinctiveness of *Y. intermedia* and *Y. kristensenii* has not been proven yet.

In this present study we included two metabolically inactive, biovar 5, serogroup 2 clinical isolates. These were strain 2 (IP 8), which was isolated from a hare, and strain 157, which was obtained from a goat (28). Both of these strains fell outside the *Y. enterocolitica* sensu stricto cluster (Fig. 1 and 2B). Furthermore, the seven human clinical isolates belonging to biovar 4, serogroup O:3 were placed in the *Y. kristensenii* cluster. The conclusion which we drew from this somewhat surprising observation was that these clinical isolates were more related in overall similarity to *Y. kristensenii* than to the bulk of the *Y. enterocolitica* sensu stricto isolates. However, most of the latter were nonclinical isolates from environmental sources (Table 1). Furthermore, both serogroup O:2, biovar 5 and sero-

group O:3, biovar 4 strains were characterized by ecological and pathogenic properties which clearly distinguished them from all of the remaining taxa examined (Table 4). These observations indicated that the taxonomy of serogroup O:2, biovar 5 and serogroup O:3, biovar 4 strains in relation to the majority of apparently nonpathogenic *Y. enterocolitica* sensu stricto strains prevailing in nature deserves further assessment. On the contrary, these taxa belonged to the same DNA relatedness group (11). Thus, the strict genotypic considerations failed to distinguish the pathogens but did distinguish among *Y. enterocolitica* sensu stricto, *Y. frederiksenii*, and *Y. intermedia*, which is of dubious practical value.

Brenner (8) felt that given a choice between genotypic and phenotypic correctness at the genus level, one may normally choose the phenotypic taxonomy because a taxonomic scheme must be practical (i.e., have identification of strains in mind). However, a classification system which is practical in one context (e.g., medical microbiology) is not necessarily practical in another field (e.g., vertebrate ecology). There is considerable interest in *Y. enterocolitica* and related bacteria in fields other than medical microbiology. Some of these bacteria are ubiquitous in terrestrial and aquatic ecosystems (6, 24, 32) and may represent adaptations to different habitats (24). The possible wildlife disease implications are not known completely. These circumstances should also be taken into account when a final decision is made concerning the taxonomy of the genus *Yersinia*.

The phenotypic continuum revealed by the numerical clustering procedures was reflected by the cultural and biochemical data from which the numerical analysis was originally derived (Table 2). Compared with the other taxa, *Y. kristensenii* contained a relatively high percentage of strains negative for acetoin production, esculin, and salicin. Likewise, *Y. intermedia* contained a high proportion of raffinose-positive strains. However, none of these characters would be useful diagnostically. With the exception of acid production from melibiose, rhamnose, and sucrose, which a priori defined *Y. enterocolitica* sensu stricto, *Y. frederiksenii*, *Y. intermedia*, and *Y. kristensenii*, none of the cultural or biochemical characters used in this study would serve to differentiate clearly among these taxa (Table 3). Brenner et al. (9, 11) used a positive α -methyl-D-glucoside reaction and growth on Simmons citrate to distinguish *Y. intermedia*. The former reaction was not tested in our study, and the latter was negative. Furthermore, Bercovier et al. (4) claimed that *Y. kristensenii* did

not produce acetoin, but we found that 14% of the *Y. kristensenii* strains which we studied produced this compound.

However, we should note that most of the cultural and biochemical observations of Brenner and his co-workers were based on incubations at 28°C, in contrast to our incubation temperature of 37°C. We chose this incubation temperature to be in accord with standard methods used in medical microbiology. Certain cultural and biochemical properties of *Y. enterocolitica* and related bacteria display a pronounced temperature dependency; generally, the properties are negative at 37°C and positive at incubation temperatures lower than 30°C (6, 35, 41). At present, however, we have no reason to suggest that phenotypic data obtained at lower temperatures are better indicators of underlying genetic differences. Harvey and Pickett (16) argued that incubation temperature is a critical factor in determining the relative arrangement of *Yersinia* strains within phenograms generated by hierarchical clustering. More work will be needed to determine whether numerical classification systems based on phenotypic expressions at lower temperatures reflect the reported genetic discontinuities between *Y. enterocolitica sensu stricto* and related species.

Another factor which may influence taxonomic interpretations is the particular collection of strains selected for a taxonomic study. Thus, the incomplete separation of *Y. enterocolitica sensu stricto*, *Y. frederiksenii*, and *Y. intermedia* in this work may be attributable to the broad ecological range covered by our strains. Most of these strains were isolated from nonhuman, non-clinical sources from Scandinavian terrestrial and aquatic ecosystems, representing numerous microhabitats (20, 21, 24, 25, 29). When such a broad range of related habitats is examined, a corresponding spectrum of intermediate strains spanning the gaps between clusters of markedly distinct phenotypes may result. The limited number of habitats traditionally examined for routine diagnostic purposes by medical microbiologists predominantly uncover strains adapted to these habitats and do not represent the complete ecological ranges of these bacteria. This may give a false impression of discontinuous taxa. Clinical isolates were more prevalent among the strains investigated by Brenner and co-workers (2, 4, 9, 11, 40) than in our study. In addition, their strains represented a very broad geographic range. This also might have contributed to dissimilar taxonomic interpretations. Ideally, a taxonomic scheme should allow a classification of strains representing the total ecological and geographic ranges.

Bercovier et al. (2, 4), Brenner et al. (9, 11), and Ursing et al. (40) used a suitable technique to study the genetic relationships among *Yersinia* strains and thereby have contributed considerably to an understanding of the taxonomy of this bacterial group. The studies of these authors were prompted by the phenotypic heterogeneity in *Y. enterocolitica* and related bacteria. First, the strains were grouped on the basis of biochemical parameters, and then these biochemical groups were tested for intra- and intergroup DNA relatedness by using one or two strains from each group as a source of reference DNA. These workers concluded that most of the phenotypic groups defined a priori formed distinct genotypic clusters. However, the reported differences in intragroup DNA relatedness values might indicate that selection of a different set of reference DNAs and inclusion of more diverse strain material could have resulted in a higher degree of overlap between intra- and intergroup DNA relatedness. It may be that selection of only one or two reference DNAs from each of the proposed species constitutes too narrow a basis for formal assessment of genetic diversity within the genus *Yersinia*. At present, we would tend to be a little restrained in accepting the clear genetic discontinuities documented as necessarily justifying a taxonomic subdivision since the degree of genetic overlap has not been clarified fully. Our data indicate that the subdivision may be pending and that the distinctions among *Y. enterocolitica sensu stricto* and the related nomenspecies have not been resolved yet.

In this work we distinguished two phenotypic clusters that could not be placed in any recognized species (phenogram subclusters A3 and A4) (Fig. 1). Subcluster A3 consisted mainly of strains which were positive for lysine decarboxylase and rhamnose but negative for cellobiose, sorbose, and sucrose (Table 3). Knapp and Thal (27) were the first to suggest that rhamnose-positive, sucrose-negative strains did not belong to *Y. enterocolitica*. A DNA relatedness group containing rhamnose-positive, sucrose-negative strains was recognized by Harvey and Pickett (16). Likewise, Brenner et al. (11) distinguished a DNA relatedness group with biochemical properties corresponding approximately to the properties of subcluster A3; they designated this group *Yersinia* biotype X2. However, subcluster A3 strains differed from biotype X2 strains by being negative for citrate utilization (Simmons) and acid production from sorbitol. These differences may be attributable to the dissimilar incubation temperatures used (see above). All strains in subcluster A3 were positive for lysine decarboxylase and maltose. The presence of ly-

sine decarboxylase is typical of *Y. ruckeri*, one of the agents of red-mouth disease in trout and salmon (8, 15). Subcluster A3 strains differed from *Y. ruckeri* by being positive for rhamnose, urease, and acetoin production (Table 2).

Subcluster A4 included eight strains that were negative for ornithine decarboxylase and sucrose. This group corresponded approximately to *Yersinia* biotype X1, a distinct DNA relatedness group recognized by Brenner et al. (11). The eight subcluster A4 strains differed from biotype X1 strains by being positive for sorbitol and occasionally producing acetoin.

The remaining nine subcluster A4 strains were positive for sorbitol and sucrose but negative for melibiose (one exception), ornithine decarboxylase (two showed delayed reactions), rhamnose, and sorbose. Similar strains have not been distinguished by DNA hybridization studies.

Subclusters A3 and A4 strains seem to have different ecological properties, occurring most frequently in terrestrial and aquatic ecosystems, respectively (Table 4). Enterotoxin production was not detected in these strains. However, a study of a more extensive collection of strains will be necessary to allow definite conclusions concerning the ecology and enterotoxigenicity of subclusters A3 and A4 strains.

Phenotypically, both subcluster A3 and subcluster A4 formed relatively distinct groups (Fig. 1 and 2B). Both seemed more similar to *Y. pseudotuberculosis* than to *Y. enterocolitica sensu stricto*, but further work will be needed to evaluate the taxonomic rank of these taxa.

The three *Y. pseudotuberculosis*-like isolates (strains 330 through 332) were phenetically closely related to the *Y. pseudotuberculosis* reference strains (Fig. 1 and 2). All were invasive for HeLa cells (23), which is a recognized feature of *Y. pseudotuberculosis* (7). We suggest that these strains should be classified as antigenically atypical variants within this species.

REPRINT REQUESTS

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