Hybridization of Deoxyribonucleotide Sequences of *Yersinia enterocolitica* and Other Selected Members of *Enterobacteriaceae*¹

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Using *Yersinia enterocolitica* Becht 18 as a standard reference, we obtained deoxyribonucleic acid base sequence homologies ranging from 79 to 100% for 11 additional isolates of the species representing 9 serotypes. These organisms, many of which exhibited dissimilar biochemical reactions, therefore belong to one and the same species. However, the degree of relatedness between *Y. enterocolitica* and the established members of the genus (*Yersinia pestis* and *Yersinia pseudotuberculosis*) was low (18 to 24%), approaching that obtained for some of 10 selected isolates of 7 other genera of *Enterobacteriaceae* (2 to 16%). These findings suggest that the proposal to include *Y. enterocolitica* within the genus *Yersinia* requires further evaluation.

The name *Bacterium enterocoliticum* was used by Schleifstein and Coleman (25) to refer to organisms that resembled *Yersinia pseudotuberculosis* (*Pasteurella pseudotuberculosis*) with respect to biochemical properties and pathology of disease in humans. Similar bacteria were subsequently described under a variety of designations, the most common being *Pasteurella pseudotuberculosis* b, *P. pseudotuberculosis*-like species, *Pasteurella* Y, and *Pasteurella* X (1, 2, 6, 7, 10). Although it was soon evident that these isolates did not belong to *Y. pseudotuberculosis* (6, 12-14), their superficial similarity to the latter prompted Frederiksen (9) to propose that they be referred to by the name *Yersinia enterocolitica*. The validity of this proposal, however, was questioned on the grounds that much diversity existed between strains of this newly described entity (15, 16). For example, certain isolates produced indole and fermented sugars (xylose, aesculin, and salicin), whereas others did not. These and other biochemical tests enabled Niléhn (22) and Wauters (Ph.D. thesis, Univ. of Vonder, Louvain, Belgium, 1970) to separate the organisms into five biotypes. Only strains of biotype 5, which differ markedly from the strains of the other biotypes, clustered separately in the similarity matrix constructed by Stevens and Mair (26). In a similar comparison with other members of *Enterobacteriaceae*, *Y. enterocolitica* was also resolved in an independent group (11).

To provide further information regarding the taxonomic position of *Y. enterocolitica*, we have compared the homology of polynucleotide se-

quences of deoxyribonucleic acid (DNA) prepared from standard reference and from atypical strains. In a similar study, the relationship between *Y. enterocolitica* and some selected members of *Enterobacteriaceae* was investigated.

MATERIALS AND METHODS

Bacteria. All of the strains of *Y. enterocolitica* used in this study were obtained through the courtesy of W. Knapp (Universität Erlangen-Nürnberg, Erlangen, W. Germany). The Pasteur Institute designations (Ye no.), synonyms, serotypes (29, 30), and selected properties of these strains are shown in Table 1. The strains of *Y. pseudotuberculosis* and *Y. pestis* used were from the Michigan State University collection. The other organisms studied were obtained from the American Type Culture Collection (Rockville, Md.), the Foothills Hospital (Calgary, Alberta), or the Provincial Laboratories (Edmonton, Alberta).

Media and cultivation. All cultures were aerated in 3% Trypticase soy broth with 0.1% yeast extract. *Y. pestis* and *Erwinia carotovora* cultures were grown at 30 C, and the remaining organisms were grown at 37 C.

Preparation of DNA. Purified DNA, prepared by the method of Marmur (19), was sheared by passage through a French pressure cell at 15,000 lb/in² to yield a molecular weight of approximately 300,000. This preparation was then dialyzed against three changes of distilled water and three changes of 0.1 M NaCl as outlined by Crosa et al. (5). Tritium-labeled DNA was prepared similarly from cells grown in the presence of [8-³H]adenine (27 Ci/mM, Amersham/Searle, Toronto, Ontario) and then further purified by chromatography on calcium hydroxyapatite (Bio-Rad Laboratories, Richmond, Calif.) and dialysis against three changes of 0.1 M NaCl.

DNA base composition. The base compositions of

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TABLE 1. *Strains of Yersinia enterocolitica used in this investigation*

Ye no.	Additional strain designations	Sero-group ^a	Biochemical characteristics ^b					Mol% G+C content of DNA ^c
			Indole	Xylose	Aesculin	Salicin	Gas (glucose)	
62	Becht 18C ^d	1	0	—	0	0	0	47.6
64	Becht 51C	1	0	+	0	0	0	47.2
39	Daniels 905 ^d	2	0	+	0	0	0	47.6
128	Daniels 924	2	0	—	0	0	0	47.6
75	Dickinson 280/1	3	0	0	0	0	0	48.5
96	Fredericksen P76	4	+	+	0	0	0	47.3
91	Fredericksen P71	4	0	—	0	0	0	47.0
124	Jument	5A	+	—	+	+	+	47.3
102	Bojoson-Møller 70 ^e	6	+	+	+	+	+	47.6
106	Borg-Petersen P413	7	+	+	+	+	+	48.2
161	Schleifstein 33114	8	+	+	0	0	0	47.7
373	Ahvonon 246/68 ^d	9	+	+	0	0	0	48.3

^a According to Wauters (Ph.D. thesis) or Winblad (30).

^b According to Knapp and Thal (15); +, positive; 0, negative; —, not reported.

^c G+C, Guanine plus cytosine. Determined in this investigation.

^d Standard reference strains (27).

^e Synonymous with Fredericksen P 219.

the various preparations of DNA were determined on a Gilford 2400-S spectrophotometer by the thermal denaturation method of Mandel and Marmur (18).

DNA reassociation. Heat-disassociated DNA (150 µg) from the various bacterial strains was added to radioactive DNA (0.1 µg) from *Y. enterocolitica* Becht 18, *Y. pseudotuberculosis* PB1/+, or *Escherichia coli* B in 1 ml of 0.17 M NaCl. Near-optimal conditions for reassociation were obtained by incubating this mixture for 16 h at 64 C, a temperature which, at this concentration of salt, is about 25 C below the thermal denaturation of the DNA. Unpaired DNA fragments remaining after incubation were then hydrolyzed with S1 endonuclease (28). The enzyme mixture was prepared by adding 0.2 volume of standard enzyme preparation to 2.0 volume of heat-disassociated (Calbiochem) salmon DNA (200 µg/ml) and 8 volumes of a solution composed of 400 ml of 0.5 mM ZnSO₄, 400 ml of 0.15 M sodium acetate (pH 4.5), and 8.77 g of NaCl (5). Each sample received 1 ml of enzyme mixture and was then incubated at 50 C for 20 min. The samples were then precipitated with 0.25 ml of 50% trichloroacetic acid, filtered, and dried, and their radioactivity was measured in toluene liquid scintillation fluid in a Beckman LS-330 scintillation counter. In the absence of the 150 µg of nonlabeled DNA, no effect on double-stranded DNA was observed in assays carried out under these conditions while 97% of single-stranded DNA underwent hydrolysis. DNA homology values are expressed as the percentage of tritiated DNA complexed with heterologous DNA compared with that complexed with the homologous DNA (100%).

RESULTS AND DISCUSSION

DNA base composition. The DNA base compositions of the various strains of *Y. enterocolitica*

ranged from 47.0 to 48.5 mol% guanine plus cytosine (Table 1). These values were higher than the approximate 45 mol% guanine plus cytosine content reported for *Y. enterocolitica* by Domaradskij et al. (8) and exceeded the 46 to 47 mol% guanine plus cytosine content obtained for *Y. pseudotuberculosis* and *Y. pestis* (17, 20, 24). The average observed for *Y. enterocolitica* was significantly greater than the 36 to 43 mol% guanine plus cytosine content reported for members of the genus *Pasteurella* (*Bergey's Manual*, 8th ed.). No particular relationship between the base composition and the serotype of *Y. enterocolitica* was noted.

DNA base sequence homologies. Relatedness values among *Y. enterocolitica* ranged from 79 to 100% (Table 2). A narrow distribution of this nature, which is similar to that reported for a majority of *E. coli* isolates (4), indicates that all of the tested strains of *Y. enterocolitica* are of the same species. DNA preparations from strains of serogroups 1, 2, and 3 (29) were essentially homologous.

Very low base sequence homology (20 to 23%) was observed between reference DNA of *Y. enterocolitica* and that of single strains of the established members of the genus (*Y. pestis* and *Y. pseudotuberculosis*). However, the latter exhibited a high degree of homology (83%) similar to the value reported previously by Ritter and Gerloff (23). As expected, essentially no homology was observed between reference DNA of *Y. enterocolitica* and a selected representative of the genus *Pasteurella* (*P. multocida*).

The low homology values found between

TABLE 2. DNA homology values determined between *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*, *Escherichia coli*, and other selected bacteria

Organism	Strain designation	DNA homology ^a		
		<i>Yersinia enterocolitica</i> Ye 62	<i>Yersinia pseudotuberculosis</i> PBI/+	<i>Escherichia coli</i> B
<i>Yersinia enterocolitica</i>	Ye 62	(100)	20	11
	Ye 64	103	— ^b	—
	Ye 39	100	20	—
	Ye 128	102	21	—
	Ye 75	99	23	—
	Ye 96	80	24	—
	Ye 91	90	—	—
	Ye 124	83	22	—
	Ye 102	79	—	—
	Ye 106	81	18	—
	Ye 161	81	—	—
	Ye 373	89	20	—
<i>Yersinia pseudotuberculosis</i>	PBI/+	22	(100)	9
<i>Yersinia pestis</i>	EV 76	23	83	9
<i>Escherichia coli</i>	B	13	9	(100)
<i>Serratia marcescens</i>	ES	16	11	14
<i>Shigella dysenteriae</i>	ATCC 13313	12	3	83
<i>Salmonella typhimurium</i>	LT-2	11	3	26
<i>Klebsiella pneumoniae</i>	298/53	9	8	—
<i>Erwinia carotovora</i>	ATCC 495	9	3	—
<i>Proteus morganii</i>	8169	8	4	—
<i>Proteus rettgeri</i>	ATCC 9918	4	—	—
<i>Proteus vulgaris</i>	ATCC 6380	3	—	—
<i>Proteus mirabilis</i>	D 799	2	—	—
<i>Pasteurella multocida</i>	ATCC 6524	0	1	—
<i>Neisseria subflava</i>		0.7	0.1	—
<i>Bacillus subtilis</i>		1	0	—

^a The results given are the averages of three determinations corrected for background by subtracting the value obtained with T4 coliphage DNA (5 to 6% of the input labeled DNA). The degree of homologous binding was 80 to 89% of the input labeled DNA. DNA of *Y. enterocolitica* Ye 62, *Y. pseudotuberculosis* PBI/+, and *E. coli* B was 52,000, 20,000, and 20,000 counts/min per μ g, respectively.

^b Dash indicates value not determined.

DNA of *Y. enterocolitica* and that of other enteric organisms indicate that there is only a distant relationship between these species, although this relationship is of the same order as that encountered among many genera of the family *Enterobacteriaceae* (3, 21). Of the latter, DNA of *Serratia marcescens* exhibited the greatest homology with that of *Y. enterocolitica*.

The relatedness found between *E. coli* and *Y. pestis* was lower than the earlier published value of 22% (23) due to differences in the method and conditions of reassociation. The homology between *E. coli* and *Y. enterocolitica* was only slightly higher than that between *E. coli* and the established yersiniae. The extent of

homology between DNA of *E. coli* and that of *S. marcescens*, *Shigella dysenteriae*, or *Salmonella typhimurium* was similar to values obtained previously (3–5, 21).

Together, these results support the hypothesis that the biochemically and serologically diverse strains currently grouped as *Y. enterocolitica* (9) constitute a valid species, at least with respect to biotypes 1 through 4 (22; Wauters, Ph.D. thesis). This species, however, bears only a distant genetic relationship to the accepted members of the genus *Yersinia*, namely, *Y. pestis* and *Y. pseudotuberculosis*. The assumption that *Y. enterocolitica* was closely related to *Y. pseudotuberculosis* was evidently based on expression of common, but superficial, biochem-

ical reactions, similar DNA base compositions, and ability to share the same environmental niche in nature.

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REPRINT REQUESTS

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