Characterization of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* by Deoxyribonucleic Acid Hybridization and by Biochemical Reactions

DON J. BRENNER, ARNOLD G. STEIGERWALT, DEISE P. FALCÃO,¹ ROBERT E. WEAVER, AND
G. RICHARD FANNING²

Center for Disease Control, Atlanta, Georgia 30333

Strains of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* were characterized by deoxyribonucleic acid (DNA) hybridization, and the extent of DNA relatedness between the strains was assessed by hydroxyapatite chromatography. DNAs from 24 strains of *Y. pseudotuberculosis* were highly related, but *Y. pseudotuberculosis* was only 40 to 60% related to *Y. enterocolitica*. *Y. enterocolitica* strains formed three DNA relatedness groups, and the data strongly suggest a fourth relatedness group. Each relatedness group can be defined biochemically. One DNA relatedness group corresponds to typical *Y. enterocolitica*; the second is rhamnose positive; the third is rhamnose positive, melibiose positive, α -methyl glucoside positive, and raffinose positive. The suggested fourth hybridization group is sucrose negative. Although all of these DNA relatedness groups should remain in the genus *Yersinia*, only the first group is *Y. enterocolitica*. All of the yersiniae tested are distantly, but significantly, related to other members of *Enterobacteriaceae*. Yersiniae were more related to members of *Enterobacteriaceae* than to *Pasteurella multocida*.

The former *Pasteurella* species *P. pestis* and *P. pseudotuberculosis* have been placed in the new genus *Yersinia* within the family *Enterobacteriaceae* (9). A third species, *Yersinia enterocolitica*, has also been placed in this genus (9, 13, 25). *Y. enterocolitica* was first described in the United States in 1939 (30) and then in Europe some 10 years later (16). The organisms were human pathogens described as resembling *Yersinia pseudotuberculosis* or aberrant forms of *Y. pseudotuberculosis* (16, 30). A fourth species, *Yersinia philomiragia*, was proposed in 1969 (18), but it has not been generally accepted.

In the 1950s and 1960s, *Y. enterocolitica* was isolated from various animal species, including the chinchilla, cat, dog, galago, deer, cow, beaver, monkey, Canada goose, camel, racoon, robin, oyster, pig, and hare, and also from water (20, 24, 34, 37, 39). Until 1966 there were only 23 isolates of *Y. enterocolitica* from humans; by 1974 this figure had soared to more than 4,000 (34). Most of the isolates were from Europe; the first culturally confirmed case in the United States in 21 years was reported in 1970 (32). These organisms have been implicated in acute

gastroenteritis, acute terminal ileitis, mesenteric lymphadenitis, septicemia, conjunctivitis, meningitis, abscesses, polyarthritides, and Crohn's disease (1, 15, 17, 23, 27, 32, 34, 36, 39).

Y. pseudotuberculosis strains are biochemically homogeneous (24, 37, 38), whereas the biochemical reactions given by strains of *Y. enterocolitica* are quite variable (2, 10, 19, 26, 31, 33-36; G. Wauters, Ph.D. thesis, Catholic Univ. of Louvain, Vander, Louvain, Belgium, 1970). *Y. enterocolitica* strains separate into at least two groups when tested by numerical taxonomy (33). The species has been divided into four or five bio-groups on the basis of reactions for indole, xylose, esculin, and salicin (19, 26; G. Wauters, Ph.D. thesis). Several authors (2, 19, 25, 33-35) have discussed the possibility that strains of *Y. enterocolitica* should be split into two or more species. Among the atypical strains are rhamnose-positive organisms (2, 10, 19, 33, 36) and sucrose-negative organisms (10, 19, 26, 35, 36).

The International Committee on Systematic Bacteriology Subcommittee on the Taxonomy of *Pasteurella*, *Yersinia*, and *Franciscella* has recognized the need for genetic studies (25). Thus far, the guanine plus cytosine (G+C) contents of yersiniae have been determined (35), a partial genetic map of *Y. pseudotuberculosis* has been established by interrupted mating experiments (22), and preliminary deoxyribonu-

¹ Present address: Faculdade de Farmácia e Odontologia de Araraquara, Rua Expedicionários do Brasil, 1621-Caixa Postal, 331, Araraquara 14.800.S.P. Brazil.

² Present address: Walter Reed Army Institute of Research, Washington, D.C. 20012.

cleic acid (DNA) hybridization experiments have been carried out by using single strains of *Y. pseudotuberculosis*, *Y. pestis*, *Y. phloemiragia*, and *pasteurellae* (28).

The purpose of this study was to characterize *Y. enterocolitica* and *Y. pseudotuberculosis* both biochemically and by DNA hybridization.

DNA relatedness and the phenotypic basis of DNA relatedness are determined. The data are discussed with regard to biogrouping, speciation, and diagnostic identification of yersiniae.

MATERIALS AND METHODS

Organisms, media, and biochemical characterization. The bacteria used in this study are listed in Table 1. The organisms were maintained on either brain heart infusion agar or blood agar base. Biochemical tests were carried out as described by Edwards and Ewing (11). Deoxyribonuclease was assayed as described by Ewing and Davis (12). All tests were carried out at 35 C except that for gelatin, which was incubated at 22 or 35 C and then refrigerated to assay for liquefaction, and that for motility, which was carried out at 22 as well as 35 C. Except where contraindicated (11), tests were held and read for 7 days.

Bacteria were cultivated on brain heart infusion broth, and a modified C medium was used in the preparation of DNA labeled with $^{32}\text{PO}_4$ (5). The preparation of both labeled and unlabeled DNA has been described (5).

DNA reassociation. The conditions used for DNA reassociation, the subsequent separation of single- and double-stranded DNA on hydroxyapatite (HA), and thermal elution chromatography on HA have been described previously (6-8). DNA from reference organisms was labeled with $^{32}\text{PO}_4$. The labeled DNA at a concentration of 0.1 $\mu\text{g}/\text{ml}$ (specific activity of between 1×10^4 counts/min per μg and 1.2×10^5 counts/min per μg) was added to 150 μg of unlabeled DNA per ml from the homologous strain and other strains of interest. The 1,500-fold excess of unlabeled DNA insured maximum opportunity for the labeled DNA to reassociate with unlabeled DNA and little opportunity for complementary strands of labeled DNA to reassociate. The mixture of labeled and unlabeled DNA in 0.28 M phosphate buffer (PB; equimolar mixture of Na_2HPO_4 and NaH_2PO_4) was heat denatured and incubated at the desired temperature for 16 h (approximately 100 C_0t 's; reference 8). Self-reaction of labeled DNA was controlled by including a reaction containing only labeled DNA. After incubation, the mixture was diluted to 0.14 M PB and passed through HA equilibrated with 0.14 M PB + 0.4% sodium dodecyl sulfate. Reassociated (double-stranded, hybridized) DNA was adsorbed to HA and single-stranded DNA was eluted. The double-stranded DNA was eluted by either increasing the PB concentration to 0.4 M PB or by increasing the temperature in 5-degree increments. In the latter case, bound DNA became denatured and eluted from HA as the temperature exceeded the T_{me} (that temperature at which 50% of

a given double-stranded DNA was eluted from HA).

The amount of relatedness between organisms was assessed by normalizing the amount of DNA bound to HA in heterologous reactions to that bound to HA in the homologous control reaction. Relatedness was expressed in percent and is conveniently referred to as the relative binding ratio (RBR). Percent divergence (D) of the related sequences can be calculated by comparing the thermal stability of the heterologous reactions to that of the homologous reaction. Approximately 1% unpaired bases was assumed to result in a 1-degree decrease in thermal stability (21). Reactions were carried out both at 60 C, where optimum reassociation occurs, and at 75 C, where only closely related sequences will reassociate. In these studies the average homologous DNA reassociation was between 75 and 90%, and the binding of a control containing only labeled DNA was 1 to 3%. This control value was subtracted from reactions exhibiting less than 10% reassociation. Closely or highly related strains were usually taken as those that yield an RBR of 70% or greater. Characteristic of closely related strains is the fact that the majority of DNA sequences that form duplexes at 60 C are stable at 75 C.

RESULTS

Strains of *Y. enterocolitica* and *Y. pseudotuberculosis* obtained from diverse geographical areas and from human, animal, and water sources were employed to obtain a representative sample (Table 1). Strains that were atypical biochemically were included in an attempt to define the taxonomic boundaries of these species. Approximately 65 biochemical tests were used to characterize most of the strains studied (Tables 2 and 3). *Y. enterocolitica* strains gave both positive and negative reactions in 31 tests (Table 2), whereas *Y. pseudotuberculosis* strains gave both positive and negative reactions in only 10 tests (Table 3).

DNA hybridization reactions were carried out to group these yersiniae genotypically, to determine genetic relatedness between these yersiniae and other members of *Enterobacteriaceae*, and to correlate phenotypic heterogeneity with genetic divergence. Table 4 shows the data obtained by using labeled DNA from *Y. enterocolitica* 501-70. In 60 C reactions, 13 strains of *Y. enterocolitica* had RBR values of 85 to 100% to strain 501-70. The percent D of the related sequences was 0 to 2.4. Further evidence of the close relatedness of these strains are the RBR values of 82 to 100% obtained in reactions carried out at the more restrictive 75 C criterion. A single strain of *Y. pestis* gave an RBR of 43% to strain 501-70. In this instance the related sequences showed a percent D of 15.2, and the RBR dropped to 11% at 75 C. Four strains of Red Mouth bacterium, a fish pathogen (29), were 30% related to *Y. enterocolitica*

TABLE 1. *Bacterial strains tested*

Strain	Origin	Received from
<i>Arizona hinshawii</i>		CDC ^a
<i>Citrobacter freundii</i> 80-58		CDC
<i>Edwardsiella tarda</i> 483-59		CDC
<i>E. tarda</i> 3979-60		CDC
<i>E. tarda</i> 4830-60		CDC
<i>Enterobacter aerogenes</i> 1627-66		CDC
<i>Enterobacter agglomerans</i> 1599-71		CDC
<i>E. agglomerans</i> 14589		ATCC ^b
<i>Enterobacter cloacae</i> 121-65		CDC
<i>E. cloacae</i> 256-4		V. Brenner
<i>E. cloacae</i> 1347-71		CDC
<i>Enterobacter hafniae</i> 932-68		CDC
<i>Erwinia amylovora</i> EA 178		ICPB ^c
<i>Escherichia coli</i> K-12		U. of Washington
<i>Klebsiella pneumoniae</i> 2		CDC
<i>Pasteurella multocida</i> 6530		ATCC
<i>P. multocida</i> 6535		ATCC
<i>P. multocida</i> 6587		ATCC
<i>P. multocida</i> 7707		ATCC
<i>P. multocida</i> 7773		ATCC
<i>P. multocida</i> 7960		ATCC
<i>P. multocida</i> 7987		ATCC
<i>P. multocida</i> 8369		ATCC
<i>P. multocida</i> 9556		ATCC
<i>P. multocida</i> 9658		ATCC
<i>P. multocida</i> 10544		ATCC
<i>P. multocida</i> 12947		ATCC
<i>P. multocida</i> 12948		ATCC
<i>P. multocida</i> 15742		ATCC
<i>P. multocida</i> 15743		ATCC
<i>Pectobacterium carotovorum</i> 495		ATCC
<i>Proteus mirabilis</i> 1		S. Falkow
<i>Proteus morganii</i>		S. Falkow
<i>Proteus vulgaris</i> PR 1		CDC
Red Mouth 2396-61		CDC
Red Mouth 2514-63		CDC
Red Mouth 4535-69		CDC
Red Mouth 5972-61		CDC
<i>Salmonella typhimurium</i> LT2		T. Theodore
<i>Serratia marcescens</i> SM6 red		WRAIR ^d
<i>Shigella flexneri</i> 24570		S. Falkow
<i>Shigella sonnei</i> virulent		WRAIR
<i>Yersinia enterocolitica</i> 17		B. Chester
<i>Y. enterocolitica</i> 39	Chinchilla, Holland	L. LeMinor
<i>Y. enterocolitica</i> 48		B. Chester
<i>Y. enterocolitica</i> 56		B. Chester
<i>Y. enterocolitica</i> 57		B. Chester
<i>Y. enterocolitica</i> 64	Chinchilla, Switzerland	L. LeMinor
<i>Y. enterocolitica</i> 75	Chinchilla, Switzerland	L. LeMinor
<i>Y. enterocolitica</i> 96	Chinchilla, Denmark	L. LeMinor
<i>Y. enterocolitica</i> 97	Chinchilla, Denmark	L. LeMinor
<i>Y. enterocolitica</i> 102	Human, Denmark	L. LeMinor
<i>Y. enterocolitica</i> 103	Sheep, Faroe Island	L. LeMinor
<i>Y. enterocolitica</i> 105	Human, Denmark	L. LeMinor
<i>Y. enterocolitica</i> 106	Guinea pig, Denmark	L. LeMinor
<i>Y. enterocolitica</i> 107	Human, United States	L. LeMinor
<i>Y. enterocolitica</i> 123	Cow, France	L. LeMinor
<i>Y. enterocolitica</i> 124	Horse, France	L. LeMinor
<i>Y. enterocolitica</i> 135	Chinchilla, Holland	L. LeMinor
<i>Y. enterocolitica</i> 160	Human, Switzerland	L. LeMinor
<i>Y. enterocolitica</i> 161	Human, United States	L. LeMinor
<i>Y. enterocolitica</i> 175	Human, Belgium	L. LeMinor
<i>Y. enterocolitica</i> 178	Hare, England	L. LeMinor
<i>Y. enterocolitica</i> 201-75	Human, United States	CDC
<i>Y. enterocolitica</i> 211	Human, Belgium	L. LeMinor
<i>Y. enterocolitica</i> 373	Human, Finland	L. LeMinor
<i>Y. enterocolitica</i> 480	Human, Belgium	L. LeMinor
<i>Y. enterocolitica</i> 490	Hare, France	L. LeMinor

TABLE 1—Continued

Strain	Origin	Received from
<i>Y. enterocolitica</i> 495-70	Human, United States	CDC
<i>Y. enterocolitica</i> 496-70	Monkey, United States	CDC
<i>Y. enterocolitica</i> 497-70	Monkey, United States	CDC
<i>Y. enterocolitica</i> 498-70	Human, United States	CDC
<i>Y. enterocolitica</i> 499-70	Human, United States	CDC
<i>Y. enterocolitica</i> 500	Hare, France	L. LeMinor
<i>Y. enterocolitica</i> 500-70	Human, United States	CDC
<i>Y. enterocolitica</i> 501-70	Monkey, United States	CDC
<i>Y. enterocolitica</i> 502-70	Human, Belgium	CDC
<i>Y. enterocolitica</i> 503-70	Human, United States	CDC
<i>Y. enterocolitica</i> 504-70	Human, Sweden	CDC
<i>Y. enterocolitica</i> 505-70	Human, Sweden	CDC
<i>Y. enterocolitica</i> 506-70	Human, Sweden	CDC
<i>Y. enterocolitica</i> 551	Human, Belgium	L. LeMinor
<i>Y. enterocolitica</i> 614	Hare, Holland	L. LeMinor
<i>Y. enterocolitica</i> 842	Human, United States	L. LeMinor
<i>Y. enterocolitica</i> 845	Human, United States	L. LeMinor
<i>Y. enterocolitica</i> 846	Human, United States	L. LeMinor
<i>Y. enterocolitica</i> 867	Human, Belgium	L. LeMinor
<i>Y. enterocolitica</i> 955	Water, Norway	L. LeMinor
<i>Y. enterocolitica</i> 1110	Human, United States	L. LeMinor
<i>Y. enterocolitica</i> 1367	Human, Czechoslovakia	L. LeMinor
<i>Y. enterocolitica</i> 1474	Water, Norway	L. LeMinor
<i>Y. enterocolitica</i> 1475	Water, Norway	L. LeMinor
<i>Y. enterocolitica</i> 1477	Water, Norway	L. LeMinor
<i>Y. enterocolitica</i> C 1889	Water, United States	CDC
<i>Y. enterocolitica</i> D 2705	Human, United States	CDC
<i>Y. enterocolitica</i> C 3651	Human, United States	CDC
<i>Y. enterocolitica</i> C 3741	Human, United States	CDC
<i>Y. enterocolitica</i> C 5188	Human, United States	CDC
<i>Y. enterocolitica</i> C 5427	Human, United States	CDC
<i>Yersinia pestis</i>		L. Corwin
<i>Yersinia pseudotuberculosis</i> P7	Hare, Sweden	E. Thal
<i>Y. pseudotuberculosis</i> P25	Hare, Sweden	E. Thal
<i>Y. pseudotuberculosis</i> P27	Hare, Sweden	E. Thal
<i>Y. pseudotuberculosis</i> P32	Hare, Sweden	E. Thal
<i>Y. pseudotuberculosis</i> P43	Mink, Sweden	E. Thal
<i>Y. pseudotuberculosis</i> P48	Hare, Sweden	E. Thal
<i>Y. pseudotuberculosis</i> P52	Mink, Sweden	E. Thal
<i>Y. pseudotuberculosis</i> P65	Mink, Germany	E. Thal
<i>Y. pseudotuberculosis</i> P68	Mink, Germany	E. Thal
<i>Y. pseudotuberculosis</i> P105	Mink, Denmark	E. Thal
<i>Y. pseudotuberculosis</i> P198	Hare, Sweden	E. Thal
<i>Y. pseudotuberculosis</i> P262	Hare, Sweden	E. Thal
<i>Y. pseudotuberculosis</i> P279	Hare, Sweden	E. Thal
<i>Y. pseudotuberculosis</i> P281	Swallow, Sweden	E. Thal
<i>Y. pseudotuberculosis</i> P293	Human, Germany	E. Thal
<i>Y. pseudotuberculosis</i> P308	Human, Germany	E. Thal
<i>Y. pseudotuberculosis</i> P320	Human, Germany	E. Thal
<i>Y. pseudotuberculosis</i> P324	Human, Germany	E. Thal
<i>Y. pseudotuberculosis</i> P385	Hare, France	E. Thal
<i>Y. pseudotuberculosis</i> P407	Hare, France	E. Thal
<i>Y. pseudotuberculosis</i> P417	Guinea pig, England	E. Thal
<i>Y. pseudotuberculosis</i> P418	Guinea pig, England	E. Thal
<i>Y. pseudotuberculosis</i> P463	Guinea pig, Japan	E. Thal
<i>Y. pseudotuberculosis</i> P907	Guinea pig, England	ATCC
<i>Y. pseudotuberculosis</i> P913	Guinea pig, England	ATCC
<i>Y. pseudotuberculosis</i> 4284	Guinea pig, England	ATCC
<i>Y. pseudotuberculosis</i> 23207	Guinea pig, Germany	ATCC

^a CDC, Center for Disease Control.^b ATCC, American Type Culture Collection.^c ICPB, International Collection of Phytopathogenic Bacteria.^d WRAIR, Walter Reed Army Institute of Research.

with 15% D. RBR values of *Y. enterocolitica* 501-70 and all other members of *Enterobacteriaceae* tested were 10 to 25%. With tests at 75 C, these RBR values, as expected, decreased

to 5% or less in 75 C reactions. Relatedness between most members of *Enterobacteriaceae* and *Y. enterocolitica* was 15 to 20%. This is very similar to the "core" level of relatedness seen

[illegible][illegible]

TABLE 2—Continued

Biochemical Test	Strain																										
	500	504-70	505-70	551	842	845	846	1110	1367	480	1477	103	105	490	1474	1475	867	955	17	48	56	57	C3651	C3741	C5188	D2705	C1889
D-Mannose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ONPG	+			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+								
DNase ^b	+			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Corn oil	+			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Christensen citrate	-			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cetrimide	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Inulin	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Arbutin	+			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L-Sorbose	+			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Melibiose	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Celluliose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

^a*Y. enterocolitica* strains were all positive in the following tests: motility (22 C), glucose, mannitol, arabinose, maltose, glycerol, amygdalin, levulose, ribose, galactose. *Y. enterocolitica* strains were all negative in the following tests: H₂S, KCN, motility (37 C), gelatin (22 C), lysine, arginine, phenylalanine, mucate, malonate, dulcitol, adonitol, erythritol, pectate, alginate, D-arabinose, methyl xyloside, methyl-D-mannoside, melizitose, organic acids. A blank space indicates that the test was not carried out.

^b DNase, Deoxyribonuclease.

TABLE 3. Biochemical reactions of *Yersinia pseudotuberculosis*^a

Biochemical test	Strain																		
	P7	P25	P27	P32	P43	P48	P52	P65	P68	P105	P262	P279	P281	P293	P308	P320	P324	P385	P417
Nitrate	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
Sodium acetate	-	+	-	-	+	+	-	-	+	+	+	+	-	+	-	-	+	-	-
Raffinose	-	+	-	+	-	-	-	-	+	-	-	+	-	-	-	+	-	-	+
DNase ^b	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	-	-
Corn oil	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+
Christensen citrate	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Cetrimide	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Levulose	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
Ribose	+	-	+	-	-	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Galactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+

^a *Y. pseudotuberculosis* strains were all positive in the following tests: urease, methyl red, motility (22 C), glucose, mannitol, salicin, arabinose, rhamnose, maltose, xylose, trehalose, glycerol, esculin, mannose, o-nitrophenyl- β -D-galactopyranoside, arbutin. They were all negative in the following tests: H₂S, indole, Voges-Proskauer, Simmons citrate, KCN, motility (37 C), gelatin (22 C), lysine, arginine, ornithine, phenylalanine, mucate, malonate, lactose, sucrose, dulcitol, adonitol, inositol, sorbitol, cellibiose, α -methyl glucoside, erythritol, amygdalin, pectate, alginate, inulin, sorbose, D-arabinose, methyl xyloside, methyl D-mannoside, melizitose, organic acids. Blank space indicates that the test was not carried out.

^b DNase, Deoxyribonuclease.

between distantly related species of *Enterobacteriaceae* (4). Results similar to those shown in Table 4 were also obtained when two other typical strains of *Y. enterocolitica*, 497-70 and C5427 (see Table 2), were used as reference strains.

The reactions for indole, D-xylose, esculin, and salicin, respectively, were designated as positive (+) or negative (-) for the *Y. enterocolitica* strains used in Tables 4 through 6. Many authors (19, 26; G. Wauters, Ph.D. thesis) placed strains of *Y. enterocolitica* into either four or five biogroups based primarily on these reactions, but other investigators (10, 36; J. Feeley, personal communication) did not use this biogrouping scheme. Knapp and Thal (19) recognized four biogroups, within which were the following patterns for indole, xylose, esculin, and salicin, respectively: ----, +---, +++-, +---, and ----. We obtained a greater variety in the patterns of indole, xylose, esculin, and salicin reactions, observing -+---, -++-, -+++, -+++, -+++,

++++, +++-, +++-, +---, and ++++ (Tables 2 and 3). Many of the strains employed by Knapp and Thal (19) and those used in the present study were the same, having come from the culture collection at the Pasteur Institute. The reactions we observed in many of these strains were different from those recorded by Knapp and Thal (19). The reasons for these differences will be discussed below.

Because of these biochemical differences, we chose reference strains of *Y. enterocolitica* known to belong to biogroups 1, 2, and 3 of Knapp and Thal (19). The results obtained with labeled DNA from *Y. enterocolitica* 211 (biogroup 2) are shown in Table 5. All strains of *Y. enterocolitica* are very closely related (RBR, 60 C = 90 to 100%; percent D = 0.0 to 1.2; RBR, 75 C = 84 to 99%). Several strains from each of the six serogroups of *Y. pseudotuberculosis* gave RBR values of 45 to 64% with *Y. enterocolitica* 211. The percent D in these reactions was usually between 8 and 13, and RBR values at 75 C decreased significantly. Relatedness be-

TABLE 4. Relative binding ratios and divergences of DNA from *Y. enterocolitica* 501-70

Source of unlabeled DNA ^a	RBR, ^b 60 C	D, ^c 60 C	RBR, 75 C
<i>Yersinia enterocolitica</i> 501-70 - + - + ^d	100	0.0	100
<i>Y. enterocolitica</i> 495-70 + + - -	99	1.2	96
<i>Y. enterocolitica</i> 496-70 - + - +	98	0.5	93
<i>Y. enterocolitica</i> 497-70 + + - -	92	0.8	100
<i>Y. enterocolitica</i> 498-70 + + - +	96	0.6	
<i>Y. enterocolitica</i> 499-70 + + - +	99	0.8	90
<i>Y. enterocolitica</i> 500-70 - + - +	88	2.0	83
<i>Y. enterocolitica</i> 502-70 - - - +	85	1.0	82
<i>Y. enterocolitica</i> 503-70 + - + +	90	0.0	
<i>Y. enterocolitica</i> 504-70 + + + +	87	2.4	83
<i>Y. enterocolitica</i> 505-70 + + + +	87	1.6	
<i>Y. enterocolitica</i> 506-70 + + - -	98	0.0	94
<i>Y. enterocolitica</i> C1889 + + - +	100	0.2	
<i>Y. enterocolitica</i> C5427 + + - +	94	0.0	93
<i>Y. pestis</i>	43	15.2	11
Red Mouth 2396-61	31	15.5	
Red Mouth 2514-63	31	15.1	8
Red Mouth 4535-69	31	14.1	
Red Mouth 5972-61	29	15.5	6
<i>Serratia marcescens</i> SM6 red	25		6
<i>Salmonella typhimurium</i> LT2	21		2
<i>Escherichia coli</i> K-12	20	12.7	3
<i>Enterobacter aerogenes</i> 1627-66	20		5
<i>Klebsiella pneumoniae</i> 2	19		4
<i>Enterobacter hafniae</i> 932-68	19		3
<i>Pectobacterium carotovorum</i> 495	19		
<i>Shigella sonnei</i> virulent	18		
<i>Enterobacter cloacae</i> 1347-71	18		
<i>E. cloacae</i> 256-4	18		3
<i>Citrobacter freundii</i> 80-58	18		4
<i>Enterobacter agglomerans</i> 14589	17		
<i>Proteus morganii</i>	17		1
<i>Arizona hinshawii</i> 1	15		
<i>Erwinia amylovora</i> EA178	15		
<i>Edwardsiella tarda</i> 1483-59	15		
<i>E. tarda</i> 3979-60	10		
<i>Proteus mirabilis</i> 1	10		

^a The + or - symbols indicate positive or negative reactions for indole, xylose, esculin, and salicin, respectively.

^b RBR, Relative binding ratio = % (heterologous DNA bound to HA)/(homologous DNA bound to HA) × 100. RBR is a convenient way to express percent relatedness.

^c D, Percent divergence. D is calculated on the assumption that a 1-degree C decrease in thermal stability of a heterologous DNA duplex compared to that of the homologous DNA duplex is caused by each 1% of the bases within the duplex that are unpaired (21). For example, consider organisms A and B which are 50% related (RBR = 50%). The thermal stability of an A-A duplex is 92 C, and the thermal stability of an A-B duplex is 77 C; the percent divergence (D) of the related DNA is 15.0.

^d Binding of DNA from *Y. enterocolitica* 501-70 to HA was 79% after incubation at 60 C and 78% after incubation at 75 C. The thermal stability of DNA bound to HA at 60 C was 92.3 C.

tween *Y. enterocolitica* and 15 strains of *Pasteurella multocida* was between 6 and 13%. Similar patterns of relatedness between *Y. enterocolitica*, *Y. pseudotuberculosis*, and *P. multocida* were observed when *Y. enterocolitica* strains 614 (biogroup 1) and 106 (biogroup 3) were used as reference strains.

We next employed two grossly atypical strains of *Y. enterocolitica* as the sources of reference DNA. These are strain 178 (- - + +), which is nitrate negative, ornithine negative,

sorbitol negative, trehalose negative, and sorbose negative, and strain 48 (+ + + +, biogroup 4), which is methyl red positive, citrate positive, rhamnose positive, cellibiose negative, and melibiose positive. We tested relatedness between these strains and members of all four biogroups (Table 6). Strain 178 was highly related to all typical (by hybridization) *Y. enterocolitica* (Table 6, starting with strain 135), but had RBR values of only 55 to 67% with all strains from biogroup 4 (strain 48 through

TABLE 5. Relative binding ratios and divergences of DNA from *Y. enterocolitica* 211

Source of unlabeled DNA ^a	RBR, ^a 60 C	D, ^a 60 C	RBR, 75 C
<i>Yersinia enterocolitica</i> 211 - + + - ^b	100	0.0	100
<i>Y. enterocolitica</i> 373 - + - -	100		
<i>Y. enterocolitica</i> 175 - - + +	99		99
<i>Y. enterocolitica</i> 135 - - + +	97		98
<i>Y. enterocolitica</i> 160 - + + +	97	0.2	
<i>Y. enterocolitica</i> 97 + + + -	96	0.4	98
<i>Y. enterocolitica</i> 75 - - + +	95	0.2	
<i>Y. enterocolitica</i> 614 - - + +	94		98
<i>Y. enterocolitica</i> 39 - + - -	94	0.0	
<i>Y. enterocolitica</i> 64 - + + -	94		97
<i>Y. enterocolitica</i> 96 - + + +	92		
<i>Y. enterocolitica</i> 161 + + + +	92	1.2	87
<i>Y. enterocolitica</i> 107 + + + -	90		
<i>Y. enterocolitica</i> 501-70 - + - +	90		84
<i>Yersinia pseudotuberculosis</i> P25 (5) ^c	64	8.6	
<i>Y. pseudotuberculosis</i> P62 (2)	62	5.4	41
<i>Y. pseudotuberculosis</i> P43 (3)	61	10.4	33
<i>Y. pseudotuberculosis</i> P417 (5)	61		
<i>Y. pseudotuberculosis</i> P262 (1)	59		
<i>Y. pseudotuberculosis</i> P324 (4)	59		26
<i>Y. pseudotuberculosis</i> P65 (2)	58		
<i>Y. pseudotuberculosis</i> P279 (4)	57		
<i>Y. pseudotuberculosis</i> P418 (5)	56		16
<i>Y. pseudotuberculosis</i> P320 (1)	55	10.6	17
<i>Y. pseudotuberculosis</i> P7 (2)	55		16
<i>Y. pseudotuberculosis</i> P48 (2)	55	8.6	
<i>Y. pseudotuberculosis</i> P293 (1)	54		
<i>Y. pseudotuberculosis</i> P68 (2)	54		13
<i>Y. pseudotuberculosis</i> P32 (4)	54	12.6	28
<i>Y. pseudotuberculosis</i> P463 (6)	54		
<i>Y. pseudotuberculosis</i> P105 (3)	53		
<i>Y. pseudotuberculosis</i> P27 (3)	52	12.9	
<i>Y. pseudotuberculosis</i> 23207 (?)	52		
<i>Y. pseudotuberculosis</i> P281 (1)	49	11.1	11
<i>Y. pseudotuberculosis</i> 4284 (?)	47	13.7	11
<i>Y. pseudotuberculosis</i> 907 (?)	47	13.6	10
<i>Y. pseudotuberculosis</i> 913 (?)	45	13.3	
<i>Salmonella typhimurium</i> LT2	26	16.8	2
<i>Escherichia coli</i> K-12	24	17.0	2
<i>Proteus mirabilis</i> 1	19		3
<i>Proteus vulgaris</i> PR1	13		1
<i>Pasteurella multocida</i> 6530	13		4
<i>P. multocida</i> 10544	12		
<i>P. multocida</i> 12948	12		2
<i>P. multocida</i> 6587	11		
<i>P. multocida</i> 15742	11		
<i>P. multocida</i> 15743	10		
<i>P. multocida</i> 7987	10		1
<i>P. multocida</i> 12947	9		
<i>P. multocida</i> 9658	8		2
<i>P. multocida</i> 8369	8		
<i>P. multocida</i> 6535	7		
<i>P. multocida</i> 7707	6		0
<i>P. multocida</i> 7773	6		
<i>P. multocida</i> 7960	6		
<i>P. multocida</i> 9556	6		

^a See footnotes a, b, c of Table 4 for explanation.^b The binding of DNA from *Y. enterocolitica* 211 to HA was 79% in 60 C reactions and 82% in 75 C reactions. The thermal stability of DNA bound to HA at 60 C was 90.6 C.^c The numbers in parentheses next to the *Y. pseudotuberculosis* strains indicate the serogroups to which the strains belong.

strain 105). Strain 48 (biogroup 4) had high RBR values (83 to 98%) with 6 of the 10 biogroup 4 strains but had RBR values of only 40 to 60% with the four remaining biogroup 4 strains (biogroup 4 strains are strain 48 through strain 105). Strain 48 was 60% related

to typical strains of *Y. enterocolitica*, 46 to 52% related to *Y. pseudotuberculosis*, and 7 to 13% related to *P. multocida*.

The biochemical profiles of biogroup 4 strains fell into three groups (Table 2). One of these groups was sucrose negative (strains 103, 105,

TABLE 6. Relative binding ratios and divergences of DNA from *Y. enterocolitica* 178 and *Y. enterocolitica* 48

Source of unlabeled DNA ^a	<i>Y. enterocolitica</i> 178		<i>Y. enterocolitica</i> 48		
	RBR, ^a 60 C	D, ^a 60 C	RBR, 60 C	D, 60 C	RBR, 75 C
<i>Yersinia enterocolitica</i> 178 --++ ^b	100	0.0			
<i>Y. enterocolitica</i> 48 ++++ ^b	61	12.8	100	0.0	100
<i>Y. enterocolitica</i> 17 ++++	58	11.9	95	0.5	97
<i>Y. enterocolitica</i> 57 ++++	56		98	0.0	100
<i>Y. enterocolitica</i> 56 ++++	55	12.5	91	1.1	87
<i>Y. enterocolitica</i> 955 ++++	63	10.5	92	1.7	
<i>Y. enterocolitica</i> 867 ++++	62	10.1	60	11.4	19
<i>Y. enterocolitica</i> C3741 ++++			93		94
<i>Y. enterocolitica</i> C3651 ++++			85		90
<i>Y. enterocolitica</i> C5188 ++++			83		75
<i>Y. enterocolitica</i> D2705 ++++			60		31
<i>Y. enterocolitica</i> 103 -+++	67		48	12.6	17
<i>Y. enterocolitica</i> 490 ++++	59	8.9			
<i>Y. enterocolitica</i> 105 ++++	55	9.4	42	15.0	17
<i>Y. enterocolitica</i> 135 -+--	97				
<i>Y. enterocolitica</i> 175 --++	96	0.0			
<i>Y. enterocolitica</i> 373 -+--	94	0.4			
<i>Y. enterocolitica</i> 75 --++	93	0.0			
<i>Y. enterocolitica</i> 106 ++++	88	2.3			
<i>Y. enterocolitica</i> 107 +++-	88	1.7			
<i>Y. enterocolitica</i> 1477 ++++	87				
<i>Y. enterocolitica</i> 842 ++++	86				
<i>Y. enterocolitica</i> 123 ++++	85	0.5			
<i>Y. enterocolitica</i> 500 -+--	85	1.0			
<i>Y. enterocolitica</i> 845 ++++	81	3.9			
<i>Y. enterocolitica</i> 846 ++++			56		
<i>Y. enterocolitica</i> 495-70 +-+-			58 ¹		
<i>Y. enterocolitica</i> 496-70 -+--			61	10.9	
<i>Y. pseudotuberculosis</i> P262			52		
<i>Y. pseudotuberculosis</i> P320			50		
<i>Y. pseudotuberculosis</i> P25			49		
<i>Y. pseudotuberculosis</i> P62			48		
<i>Y. pseudotuberculosis</i> P105			46		
<i>Pasteurella multocida</i> 10544			13		
<i>P. multocida</i> 6530			11		
<i>P. multocida</i> 7960			8		
<i>P. multocida</i> 7773			7		

^a See footnotes a, b, c of Table 4 for explanation.

^b The binding of DNA from *Y. enterocolitica* 178 to HA in 60 C reactions was 77%, and the thermal stability of DNA bound to HA was 91.3 C. The binding of *Y. enterocolitica* 48 to HA averaged 65% in 60 and 75 C reactions. The thermal stability of this bound DNA was 91.6 C.

490, 1474, 1475). The other two groups were rhamnose positive. One of the rhamnose-positive groups (strains 867, D2705) was raffinose negative, melibiose negative, and α -methyl glucoside negative; the other was melibiose positive, α -methyl glucoside positive, and usually delayed positive for raffinose (strains 17, 48, 56, 57, C1889, C3651, C3741). DNA relatedness data indicated that the two groups of rhamnose-positive strains belong to different hybridization groups, each of which was distinct from typical *Y. enterocolitica* strains. The sucrose-negative strains are in a hybridization group separate from both typical *Y. enterocolitica* and from strains positive for rhamnose, α -methyl glucoside, and melibiose. Since neither a sucrose-negative strain nor a rham-

nose-positive, melibiose-negative, α -methyl glucoside strain has been used as a reference strain, we cannot yet assign them to different hybridization groups.

Y. pseudotuberculosis strains formed the only highly related hybridization group (Table 7). This was not surprising in view of the extremely similar biochemical profiles of these strains (Table 3). RBR values between *Y. pseudotuberculosis* strain P105 and typical strains of *Y. enterocolitica* were about 40 to 50%.

Two ranges of RBR and D values occurred between strain P105 and strains of *P. multocida* (Table 7). One group of strains exhibited RBR values of 7 to 13% with P105. RBR values fell to 3% or less in 75 C reactions. The D in these reactions was greater than 10%. The sec-

TABLE 7. Relative binding ratios and divergences of DNA from *Y. pseudotuberculosis* P105

Source of unlabeled DNA	RBR, ^a 60 C	D, ^a 60 C	RBR, 75 C
<i>Yersinia pseudotuberculosis</i> P105 ^b	100	0.0	100
<i>Y. pseudotuberculosis</i> P281	96	1.1	85
<i>Y. pseudotuberculosis</i> P7	95	0.0	97
<i>Y. pseudotuberculosis</i> P262	94	0.0	88
<i>Y. pseudotuberculosis</i> P62	93	0.3	92
<i>Y. pseudotuberculosis</i> P65	93	0.2	93
<i>Y. pseudotuberculosis</i> 23207	93		
<i>Y. pseudotuberculosis</i> P43	92	0.0	90
<i>Y. pseudotuberculosis</i> P293	92	0.3	96
<i>Y. pseudotuberculosis</i> P320	92	0.0	90
<i>Y. pseudotuberculosis</i> P48	91	0.6	89
<i>Y. pseudotuberculosis</i> P68	91	0.3	90
<i>Y. pseudotuberculosis</i> P385	89		
<i>Y. pseudotuberculosis</i> P463	89		99
<i>Y. pseudotuberculosis</i> P27	88		97
<i>Y. pseudotuberculosis</i> P418	88		88
<i>Y. pseudotuberculosis</i> P407	87		
<i>Y. pseudotuberculosis</i> P417	87	0.0	92
<i>Y. pseudotuberculosis</i> P32	85	0.5	84
<i>Y. pseudotuberculosis</i> P324	85	0.0	80
<i>Y. pseudotuberculosis</i> 4284	83	0.7	96
<i>Y. pseudotuberculosis</i> 913	83		
<i>Y. pseudotuberculosis</i> P25	81	1.3	71
<i>Y. pseudotuberculosis</i> P279	81	1.0	85
<i>Y. enterocolitica</i> 175	53	9.7	19
<i>Y. enterocolitica</i> 64	48	10.8	13
<i>Y. enterocolitica</i> 373	46	12.1	13
<i>Y. enterocolitica</i> 75	45	13.0	18
<i>Y. enterocolitica</i> 135	43	9.9	14
<i>Y. enterocolitica</i> 107	42	10.5	15
<i>Y. enterocolitica</i> 37	42	11.7	14
<i>Pasteurella multocida</i> 12947	32		
<i>P. multocida</i> 6587	27	2.9	18
<i>P. multocida</i> 6530	26	1.7	17
<i>P. multocida</i> 12948	26	2.7	19
<i>P. multocida</i> 10544	24	4.5	13
<i>P. multocida</i> 15742	17	2.7	13
<i>P. multocida</i> 7987	17		
<i>P. multocida</i> 8369	13		
<i>P. multocida</i> 6535	11		3
<i>P. multocida</i> 7773	10		
<i>P. multocida</i> 7987	9		1
<i>P. multocida</i> 7707	7	13.2	
<i>P. multocida</i> 9658	7		
Red Mouth 2514-63	30		7
Red Mouth 2396-61	29	15.1	8
Red Mouth 5972-61	29	15.0	7
<i>Enterobacter cloacae</i> 121-65	22		4
<i>Citrobacter freundii</i> 80-58	22		3
<i>Escherichia coli</i> K-12	21	14.0	3
<i>Serratia marcescens</i> SM6, red	19		1
<i>Shigella flexneri</i> 2a 24570	18		5
<i>Klebsiella pneumoniae</i> 2	17		4
<i>Enterobacter agglomerans</i> 1599-71	17		3
<i>Salmonella typhimurium</i> LT2	15		2
<i>Edwardsiella tarda</i> 3979-60	15		3
<i>Enterobacter aerogenes</i> 1627-66	14		4
<i>Arizona hinshawii</i> 1	14		1
<i>Erwinia amylovora</i> EA 178	11		4
<i>Proteus mirabilis</i> 1	11		

^a See footnotes b and c of Table 4 for explanation.^b The binding of DNA from *Y. pseudotuberculosis* P105 to HA in 60 C and 75 C reactions was 71%. The thermal stability of bound DNA was 89.7 C.

ond group of *P. multocida* strains had RBR values of 17 to 32% with P105. What is particularly noteworthy about these strains is that RBR values remained relatively high in 75 C

reactions (13 to 18%) and that D values were only 1.7 to 4.5%. The indication is that the related sequences between *Y. pseudotuberculosis* and this second group of *P. multocida*

strains contain only a small percentage of unpaired bases.

Other enteric bacteria exhibit the same level of relatedness to *Y. pseudotuberculosis* (mainly 15 to 20%) as seen previously to *Y. enterocolitica* (Table 4). RBR values between Red Mouth bacteria and *Y. pseudotuberculosis* (30%) are also similar to those observed between Red Mouth bacteria and *Y. enterocolitica* (Table 4).

To assess the potential application of DNA hybridization in a diagnostic laboratory, we tested three suspected *Y. enterocolitica* cultures sent to the Center for Disease Control, Atlanta, Ga., for identification. The cultures were grown and DNA was isolated by a rapid modification of our standard techniques (3). All three were confirmed as *Y. enterocolitica* in reactions with a typical strain of *Y. enterocolitica* (RBR = 70% or higher in 75 C reactions). Control reactions with strains of *Escherichia coli*, *Salmonella*, *Klebsiella*, and *Citrobacter* were negative (RBR = 10% or lower).

DISCUSSION

In 1972 the Subcommittee on the Taxonomy of *Pasteurella*, *Yersinia*, and *Francisella* (25) designated *Y. enterocolitica* strains 39, 62, 160, 161, and 373 as reference strains. Their report stated that "strains whose biochemical characteristics are in good agreement with those reference strains are to be considered as belonging to *Yersinia enterocolitica*." These strains (62 was not tested) were all highly related and were in the hybridization group composed of typical *Y. enterocolitica* strains (Table 5).

Knapp and Thal (19) placed *Y. enterocolitica* and *Y. enterocolitica*-like organisms into four groups according to reactions for indole, xylose, esculin, and salicin. Group 1 is ---- or -+-- for these reactions. Group 2 is ++--, and group 3 is +++. Group 4 is composed of atypical strains. The patterns given are +++, lactose positive and rhamnose positive; ---, ornithine decarboxylase negative; and -+--, lactose positive, sucrose negative. They stated that groups 3 and 4 should not be included in *Y. enterocolitica*. They also stated that, if indole-positive variants belong to a separate species, *Y. enteritidis* may be used for indole-negative strains and *Y. enterocolitica* for indole-positive strains.

Groups 1, 2, and 3 (Tables 4-6) all belonged to the same DNA relatedness group. Therefore, we saw no reason to separate indole-positive and indole-negative strains and no basis for excluding group 3 strains from *Y. enterocolitica*. The group 4 strains tested were less closely related to typical *Y. enterocolitica* (Table 6). In

fact, they definitely formed two (and probably a third) relatedness groups separate from that of typical *Y. enterocolitica*. Sucrose-negative strains varied in the ability to ferment lactose (Tables 2 and 3). These strains are acetoin negative (35). Only one of the three sucrose-negative strains used in hybridization experiments was lactose positive. The same held true for rhamnose-positive strains where both positive and negative lactose reactions were observed (Tables 2 and 3). The rhamnose-positive strains formed two relatedness groups (Table 6), one of which could be differentiated by α -methyl glucoside, melibiose, and usually raffinose tests.

All strains of *Y. pseudotuberculosis* belonged to one DNA relatedness group. This finding correlates well with the rather homogeneous biochemical profiles of these strains (Tables 2 and 3; references 24, 37, 38). *Y. pseudotuberculosis* (Table 7) was 40 to 60% related to *Y. enterocolitica*. Both *Yersinia* species showed low but significant relatedness to other members of *Enterobacteriaceae*. It is difficult to use hybridization data for taxonomic purposes at the family level; suffice it to say that this level of relatedness seems to be present as a "core" within distantly related members of *Enterobacteriaceae* (4).

Y. enterocolitica strains had RBR values of 6 to 13% to *P. multocida* (Tables 5 and 6). *Y. pseudotuberculosis* exhibited RBR values of 7 to 32% to strains of *P. multocida* (Table 7). These reactions appeared to fall into two ranges of relatedness (7 to 13% and 17 to 32%), or even three ranges of relatedness (7 to 11%, 13 to 17% 24 to 32%). Of particular interest was that, in reactions where the RBR was 17 to 32%, percent D was only 1.7 to 4.5. The low amount of unpaired bases in related sequences was confirmed by the RBR values from 75 C reactions. These values showed that at least two-thirds of the sequences related at 60 C remained stable at the stringent 75 C reassociation criterion. We have not previously seen evidence of closely related sequences in species where overall relatedness was 30% or less.

Possible explanations for these results are as follows. (i) DNA from the *P. multocida* strains exhibiting 17 to 32% relatedness to *Y. pseudotuberculosis* was contaminated with *Y. pseudotuberculosis* DNA. (ii) The labeled *Y. pseudotuberculosis* DNA was contaminated with DNA from *P. multocida*. (iii) The results are valid and indicate that *P. multocida* strains belong to more than one DNA relatedness group, one of which contains about 15% of DNA which is conserved with respect to *Y. pseudotuberculosis* DNA.

The first two possibilities cannot be ruled out, but there is no evidence to support them. If the labeled *Y. pseudotuberculosis* DNA preparation had been contaminated, we would have expected lower relatedness of unlabeled *Y. pseudotuberculosis* strains to the reference strain. If the *P. multocida* strains that have RBR values of 17 to 32% to *Y. pseudotuberculosis* are contaminated with *Y. pseudotuberculosis* DNA, we should have also seen somewhat higher relatedness between these strains and *Y. enterocolitica*. This was not the case (Table 5).

One piece of evidence may support the validity of these data. Fredericksen (14) reported that *P. multocida* strains contain different percentages of guanine plus cytosine (G+C) in their DNA. G+C values of 37, 40, and 43 mol% occur. The G+C content of *Y. pseudotuberculosis* is 43 to 46 mol%. It is attractive to theorize that those *P. multocida* strains exhibiting 17 to 32% relatedness to *Y. pseudotuberculosis* are the 43 mol% G+C strains.

During this study, biochemical profiles were obtained that were not listed by Knapp and Thal (19). A search of the literature revealed that several groups of investigators have commented upon the difficulty of interpreting certain biochemical reactions given by *Y. enterocolitica* at 37 C. This is especially true for indole, xylose, esculin, and salicin for which reactions have been reported as weak, weakly positive, and irregularly positive (2, 10, 26). A second difficulty is that many positive reactions are not evident at 37 C until 5 to 10 days have elapsed. If tests are discarded after 48 h, the number of negatives will be falsely high (or, if one prefers, cultures held for 1 to 2 weeks will yield a disproportionately high number of positive reactions). It is known that *Y. enterocolitica* is motile at 22 C but not at 37 C. The same is true for many biochemical reactions. Therefore, the exact temperature of incubation may well be critical to obtaining a positive or negative reaction. One would expect more positive reactions in a laboratory where the incubator was 33 to 35 C than in a laboratory where the incubator fluctuated between 36 and 38 C. In addition, some cultures left at room temperature for an hour or longer might be expected to yield spurious "37 C" positive reactions. Finally, the method used to carry out a given test may affect the results obtained (2, 37).

In 1966 Ritter and Gerloff (28) did hybridizations on single strains of *Pasteurella novicida*, *P. multocida*, *Francisella tularensis*, *Y. pestis*, *Y. pseudotuberculosis*, *Y. philomiragia*, and *E. coli*. The *Yersinia* strains were 6 to 14% related to the strains of *P. multocida*. *Y. pseu-*

dotuberculosis and *Y. pestis* strains were 87% related. The *Y. philomiragia* strain was 24% related to the *Y. pestis* strain, and the *E. coli* strain was 22% related to the *Y. pestis* strain. We tested a single strain of *Y. pestis*, which was 43% related to *Y. enterocolitica* 501-70 (Table 4). Further work must be done on *Y. philomiragia*, *Pasteurella*, and *Francisella*.

ACKNOWLEDGMENTS

We are grateful to W. H. Ewing for fruitful discussions and encouragement during this study. We thank J. Feeley for his help and encouragement.

We thank E. Cadet for competent technical assistance.

D. P. Falcão was supported by the Fundação de Ampara a Pesquisa do Estado de São Paulo.

ADDENDUM

After the submission of this manuscript, a study on DNA hybridization in *Yersinia enterocolitica* was published (R. L. Moore and R. R. Brubaker, Int. J. Syst. Bacteriol. 25:336-339, 1975). These authors found 18 to 24% relatedness between *Y. enterocolitica* and *Y. pseudotuberculosis*, and 2 to 16% relatedness between *Y. enterocolitica* and seven genera other than *Yersinia* within *Enterobacteriaceae*. These experiments were carried out in 0.17 M NaCl at 64 C. Their results are in good agreement with our 75 C data obtained in 0.14 M PB (0.21 M sodium concentration).

REPRINT REQUESTS

Address reprint requests to: Don J. Brenner, Bldg. 1, Room B311, Center for Disease Control, Atlanta, Ga. 30333.

LITERATURE CITED

1. Ahvonen, P. 1972. Human yersiniosis in Finland. I. Bacteriology and serology. Ann. Clin. Res. 4:30-38.
2. Bottone, E. J., B. Chester, M. S. Malowany, and J. Allerhand. 1974. Unusual *Yersinia enterocolitica* isolates not associated with mesenteric lymphadenitis. Appl. Microbiol. 27:858-861.
3. Brenner, D. J. 1974. DNA reassociation for the clinical differentiation of enteric bacteria. Publ. Health Lab. 32:118-130.
4. Brenner, D. J., and S. Falkow. 1971. Molecular relationships among members of the Enterobacteriaceae. Adv. Genet. 16:81-118.
5. Brenner, D. J., G. R. Fanning, K. E. Johnson, R. V. Citarella, and S. Falkow. 1969. Polynucleotide sequence relationships among members of the Enterobacteriaceae. J. Bacteriol. 98:637-650.
6. Brenner, D. J., G. R. Fanning, A. Rake, and K. E. Johnson. 1969. A batch procedure for thermal elution of DNA from hydroxyapatite. Anal. Biochem. 28:447-459.
7. Brenner, D. J., A. G. Steigerwalt, G. V. Miklos, and G. R. Fanning. 1973. Deoxyribonucleic acid relatedness among erwiniae and other Enterobacteriaceae: the soft-rot organisms (genus *Pectobacterium* Waldee). Int. J. Syst. Bacteriol. 23:205-216.
8. Britten, R. J., and D. E. Kohne. 1966. Nucleotide sequence repetition in DNA. Carnegie Inst. Wash. Yearb. 65:78-106.
9. Buchanan, R. E., and N. E. Gibbons (ed.). 1974. Bergey's manual of determinative bacteriology, 8th ed. The Williams and Wilkins Co., Baltimore.
10. Darland, G., W. H. Ewing, and B. R. Davis. 1975. The

- biochemical characteristics of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. CDC Publication, Center for Disease Control, Atlanta, Ga.
11. Edwards, P. R., and W. H. Ewing. 1972. Identification of *Enterobacteriaceae*, 3rd ed. Burgess Publishing Co., Minneapolis, Minn.
 12. Ewing, W. H., and B. R. Davis. 1970. Media and tests for differentiation of *Enterobacteriaceae*. CDC Publication, Center for Disease Control, Atlanta, Ga.
 13. Frederiksen, W. 1964. A study of some *Yersinia pseudotuberculosis*-like bacteria ("*Bacterium enterocoliticum*" and "*Pasteurella* X"). Scand. Congr. Pathol. Microbiol. Proc. XIV:103-104.
 14. Frederiksen, W. 1973. *Pasteurella* taxonomy and nomenclature. Contrib. Microbiol. Immunol. 2:170-176.
 15. Gurry, J. F. 1974. Acute terminal ileitis and *Yersinia* infection. Br. Med. J. 2:264-266.
 16. Hassig, A., J. Karrer, and F. Pusterla. 1949. Über Pseudotuberkulose beim Menschen. Schweiz. Med. Wochenschr. 79:971-973.
 17. Jansson, E., G. R. Wallgren, and P. Ahvonen. 1968. *Yersinia enterocolitica* as a cause of acute mesenteric lymphadenitis. Acta Paediatr. Stockholm 57:448-450.
 18. Jensen, W. I., C. R. Owen, and W. J. Jellison. 1969. *Yersinia philomiragia* sp. n., a new member of the *Pasteurella* group of bacteria, naturally pathogenic for the muskrat (*Ondatra zibethica*). J. Bacteriol. 100:1237-1241.
 19. Knapp, W., and E. Thal. 1973. Differentiation of *Yersinia enterocolitica* by biochemical reactions. Contrib. Microbiol. Immunol. 2:10-16.
 20. McClure, H. M., R. E. Weaver, and A. F. Kaufmann. 1971. Pseudotuberculosis in nonhuman primates: infection with organisms of the *Yersinia enterocolitica* group. Lab. Anim. Sci. 21:376-382.
 21. McConoughy, B. L., and B. J. McCarthy. 1970. Related base sequences in the DNA of simple and complex organisms. VI. The extent of base sequence divergence among the DNAs of various rodents. Biochem. Genet. 4:425-446.
 22. McMahon, P. C. 1973. Mapping the chromosome of *Yersinia pseudotuberculosis* by interrupted mating. J. Gen. Microbiol. 77:61-69.
 23. Mair, N. S., H. J. Mair, E. M. Stirk, and J. G. Corson. 1960. Three cases of acute mesenteric lymphadenitis due to *Pasteurella pseudotuberculosis*. J. Clin. Pathol. 13:432-439.
 24. Mollaret, H. H. 1961. Contribution à l'étude des caractères biochimiques de *Pasteurella pseudotuberculosis* (Bacille de Malassez et Vignal). Ann. Inst. Pasteur (Paris) 100:685-690.
 25. Mollaret, H. H., and W. Knapp. 1972. International Committee on Systematic Bacteriology Subcommittee on the Taxonomy of *Pasteurella*, *Yersinia*, and *Francisella*. Int. J. Syst. Bacteriol. 22:401.
 26. Niléhn, B. 1969. Studies on *Yersinia enterocolitica* with special reference to bacterial diagnosis and occurrence in human acute enteric disease. Acta. Pathol. Microbiol. Scand. Suppl. 206:1-48.
 27. Niléhn, B., and B. Sjöström. 1967. Studies on *Yersinia enterocolitica*: occurrence in various groups of acute abdominal disease. Acta Pathol. Microbiol. Scand. 71:612-628.
 28. Ritter, D. B., and R. K. Gerloff. 1966. Deoxyribonucleic acid hybridization among some species of the genus *Pasteurella*. J. Bacteriol. 92:1838-1839.
 29. Ross, A. J., R. R. Rucker, and B. H. Ewing. 1966. Description of a bacterium associated with redmouth disease of rainbow trout (*Salmo gairdneri*). Can. J. Microbiol. 12:763-770.
 30. Schleifstein, J., and M. B. Coleman. 1939. An unidentified microorganism resembling *B. lignieresii* and *Pasteurella pseudotuberculosis*, pathogenic for man. N. Y. State J. Med. 39:1749-1753.
 31. Smith, J. E., and E. Thal. 1965. A taxonomic study of the genus *Pasteurella* using a numerical technique. Acta. Pathol. Microbiol. Scand. 64:213-223.
 32. Sonnenwirth, A. C. 1970. Bacteremia with and without meningitis due to *Yersinia enterocolitica*, *Edwardsiella tarda*, *Comamonas terrigena* and *Pseudomonas maltophilia*. Ann. N. Y. Acad. Sci. 174:488-502.
 33. Stevens, M., and N. S. Mair. 1973. A numerical taxonomic study of *Yersinia enterocolitica* strains. Contrib. Microbiol. Immunol. 2:17-22.
 34. Toma, S., and L. Lafleur. 1974. Survey on the incidence of *Yersinia enterocolitica* infection in Canada. Appl. Microbiol. 28:469-473.
 35. Wauters, G. 1973. Correlation between ecology, biochemical behaviour and antigenic properties of *Yersinia enterocolitica*. Contrib. Microbiol. Immunol. 2:38-41.
 36. Weaver, R. E., and J. G. Jordan. 1973. Recent human isolates of *Yersinia enterocolitica* in the United States. Contrib. Microbiol. Immunol. 2:120-125.
 37. Wetzler, T. F., and W. T. Hubbert. 1968. *Yersinia enterocolitica* in North America. Int. Symp. Pseudotuberculosis 9:343-356.
 38. Wetzler, T. F., M. N. Rosen, and J. D. Marshall, Jr. 1970. The *Pasteurella*, the *Yersinia* and the *Francisella*, p. 67-81. In C. D. Graber (ed.), Rapid diagnostic methods in medical microbiology. The Williams and Wilkins Co., Baltimore.
 39. Winblad, S., B. Niléhn, and N. H. Sternby. 1966. *Yersinia enterocolitica* (*Pasteurella* X) in human enteric infections. Br. Med. J. 2:1363-1366.