Polynucleotide Sequence Relatedness in *Edwardsiella tarda*

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Relatedness among strains of *Edwardsiella tarda* was determined by using interspecific deoxyribonucleic acid reassociation followed by chromatography on hydroxyapatite. Relatedness was also assessed among *E. tarda* and representatives from all other established genera of the family *Enterobacteriaceae*. Twenty strains of *E. tarda* from five countries and 11 states, isolated from diseased human, normal human, and invertebrate sources, all formed one highly related hybridization group. *E. tarda* exhibited 20 to 29% relatedness to other enterobacteria. These data are consistent with the previous designation of a separate tribe, *Edwardsielleae*, for a single genus (*Edwardsiella*) and species (*E. tarda*).

The most recently described tribe of the family *Enterobacteriaceae* is *Edwardsielleae* (8). *Edwardsielleae* contains one genus, *Edwardsiella* Ewing and McWhorter, with a single species, *E. tarda* (8). This organism was first recognized in 1959 in Japan (11) and in the United States (8). Sakazaki (11) proposed the term Asakusa group for these organisms. King and Adler (9) independently isolated similar organisms and termed them the Bartholomew group. Ewing and his colleagues (8) named their organisms *Edwardsiella tarda*. The biochemical characteristics of all of these organisms are nearly identical (7, 11).

Ewing and colleagues (7) have identified 49 O antigen groups, 36 flagellar antigens, and 144 serotypes in 255 strains. In addition to antigenic heterogeneity, *E. tarda* strains show great diversity in geographical distribution and in source of isolation (7, 11). In humans, *E. tarda* has been isolated from wounds, urine, blood, feces, and spinal fluid (12).

In the present study, deoxyribonucleic acid (DNA) hybridization was used to assess molecular relatedness in a diverse sampling of *E. tarda* strains. DNA relatedness was also determined between *E. tarda* and representatives of all other tribes of *Enterobacteriaceae*.

MATERIALS AND METHODS

Bacterial strains. The *E. tarda* strains employed in this study are listed in Table 1. The *Proteus morganii* strain was obtained from S. Falkow. *Serratia rubidaea* 934-72, Red Mouth organism 2396-61, and *Citrobacter freundii* 4556-71 were obtained from W. H. Ewing. The other organisms studied were described previously (3, 5).

Media. Organisms were maintained on meat extract agar. Routine cultivation was carried out in brain heart infusion broth on a dry-air rotary shaker held at 37 C, except for pectobacteria and erwiniae, which were grown at 30 and 23 to 25 C, respectively. The medium used for labeling *Escherichia coli* K-12 with $^{32}\text{PO}_4$ has been described (1). None of the 20 strains of *E. tarda* used in this study grew on an unsupplemented medium. This finding is in agreement with that of D'Empaire (6), who found 26 strains of *E. tarda* to require cysteine, methionine, and nicotinamide for growth. To label *E. tarda*, the medium was supplemented with 150 μg of cysteine and methionine per ml and 2.2 μg of nicotinamide per ml (6). The specific activity of the labeled DNAs was between 3×10^5 and 1×10^6 counts per min per μg .

DNA reassociation. The preparation, purification, and shearing of both labeled and unlabeled DNA to an average single-strand fragment size of 1.2×10^5 daltons has been described (1, 3). The validity of the DNA hybridization data presented depends on the sensitivity and completeness of DNA reassociation in free solution and on the ability of hydroxyapatite (HA) to distinguish quantitatively between single-stranded DNA and DNA duplexes.

In all experiments, 0.1 μg of sheared, heat-denatured, $^{32}\text{PO}_4$ -labeled DNA/ml was reacted with 150 μg of similarly prepared unlabeled DNA per ml. The DNA reassociation reactions were carried out in 0.28 M PB (phosphate buffer, an equimolar mixture of NaH_2PO_4 and Na_2HPO_4) for 16 h at either 60 or 75 C. This exposure carried the reaction for unlabeled DNA to 100 C_0t 's (DNA concentration $\times$ time units; reference 4), sufficient for almost complete reassociation of labeled with unlabeled DNA. The C_0t for labeled DNA is small enough to preclude substantial reassociation of labeled fragments with one another. This is borne out by control reactions in which 0.1 μg of labeled DNA per ml was incubated in the absence of unlabeled DNA. Only 0.5 to 2% of the labeled DNA bound to the HA as double-stranded DNA.

After incubation, DNA mixtures were diluted to

TABLE 1. *Edwardsiella tarda*^a strains used

Strain	Place of isolation	Source	Antigenic formula
1483-59 ^b	Kentucky	Human stool	01483:h1
4226-59	Wisconsin	Head wound	04226:H2
3979-60	Equador	Diarrhea, child	03979:H3
4830-60	Illinois	Arm laceration	04830:H5
4862-60	Maryland	Stool, diarrhea	04862:H6
5343-61	Virginia	Human stool	05343:H9
3882-61	Florida	Human stool	03882:H10
5497-61 ^c	Japan	Reptile	05497:H31
6243-61	Louisiana	Human	06243:H11
3888-64 ^c	Japan	Reptile	03888:H10
3889-64 ^c	Japan	Reptile	03889:H6
3893-64 ^c	Japan		03893:H6
5159-63	Tennessee	Human foot	05159:H1
1795-62 ^d	Indiana	Infant stool	01456:H1
1126-64	Missouri	Fatal meningitis, blood, brain, spinal fluid	05765:H12
3592-64 ^c	Japan	Human	03592:H2, 31
2995-65	Maine	Turtle	01956:H18
4751-66	Congo	Stool, diarrhea	04751:H2
4411-68	Florida	Australian skink	04789:H2
2334-67	Israel	Unknown	05765:H2, 31

^a All *E. tarda* strains and their antigenic formulas were obtained from the Center for Disease Control, Atlanta, Ga.

^b Type strain of *E. tarda*.

^c Isolated by Sakazaki; members of the Asakusa group.

^d Original Bartholomew group strain.

TABLE 2. Relatedness of *Edwardsiella tarda* strains to *Escherichia coli* K-12

Source of unlabeled DNA	Relative (%) bindings, 60 C	Divergence (%), ^a 60 C	Relative (%) binding, 75 C
<i>Edwardsiella tarda</i> 1795-62	21	15.7	3.9
<i>E. tarda</i> 2334-67	18	14.3	2.2
<i>E. tarda</i> 5159-63	17	14.2	2.2
<i>E. tarda</i> 1483-59	22	10.1	9.3
<i>E. tarda</i> 1126-64	19	13.7	3.4
<i>E. tarda</i> 2995-65	20	13.4	2.7
<i>E. tarda</i> 4226-59	22	13.7	2.9
<i>E. tarda</i> 4862-60	24	13.3	3.7
<i>E. tarda</i> 5497-61	24	14.6	4.2
<i>E. tarda</i> 4411-68	25	13.9	6.0
<i>E. tarda</i> 3979-60	18	15.3	5.8
<i>E. tarda</i> 4751-66	19	13.3	6.9
<i>E. tarda</i> 3889-64	20	13.0	2.4
<i>E. tarda</i> 3893-64	18	13.1	2.5
<i>E. tarda</i> 3888-64	20	12.6	3.7
<i>E. tarda</i> 4830-60	23	11.9	6.1
<i>E. tarda</i> 5343-61	21	13.1	2.7
<i>Shigella flexneri</i> 25470	84	1.0	79
<i>Salmonella typhimurium</i> LT2	44	11.7	11
<i>Citrobacter freundii</i> P11-C	50	12.5	11
<i>Klebsiella pneumoniae</i> 2	38	14.2	7
<i>Enterobacter cloacae</i>	35	13.1	5
<i>Serratia marcescens</i>	24	14.1	2.7
<i>Enterobacter hafniae</i>	21	15.8	4
<i>Proteus morganii</i>	17	16.7	2.5
<i>P. mirabilis</i>	8	NT	1.5

^a Calculated on the assumption that each 1 C decrease in the $T_{m(e)}$ of a DNA heteroduplex [compared to the $T_{m(e)}$ of a homologous DNA duplex] reflects 1% unpaired nucleotide bases or divergence (10). $T_{m(e)}$ is the thermal elution midpoint, i.e., that temperature at which 50% of the DNA bound to hydroxyapatite is eluted. NT, Not tested.

0.14 M PB and applied to HA that was equilibrated in 0.14 M PB + 0.4% sodium dodecyl sulfate and held at 60 C. Unhybridized DNA was eluted in 0.14 M PB + 0.4% sodium dodecyl sulfate by use of a batch procedure (3). Reassociated DNA is adsorbed to HA. It is eluted at higher salt (0.4 M PB) or in a series of 0.14 M PB washes at increasing 5 C increments. The latter procedure yields a thermal elution profile, since duplexed DNA fragments elute when the temperature is high enough to denature them.

RESULTS AND DISCUSSION

Members of *E. tarda* are gram-negative, peritrichous, rod-shaped bacteria that conform to the biochemical definition of *Enterobacteriaceae*. They exhibit a large number of serotypes based on their O and H antigens, but are quite uniform in their biochemical reactions (9, 11). They seem to resemble *E. coli* in

TABLE 3. Reassociation of DNA from *Edwardsiella tarda* 3592-64

Source of unlabeled DNA	Relative (%) binding, 60 C	Divergence (%), ^a 60 C	Relative (%) binding, 75 C
<i>Edwardsiella tarda</i> 3592-64	100	NT	100
<i>E. tarda</i> 5343-61	85	1.1	84
<i>E. tarda</i> 3979-60	90	0.6	88
<i>E. tarda</i> 3889-64	93	0.8	93
<i>E. tarda</i> 1126-64	86	2.8	86
<i>E. tarda</i> 6243-61	95	0.3	NT
<i>E. tarda</i> 1795-62	84	+0.3	82
<i>E. tarda</i> 3888-64	88	4.8	NT
<i>E. tarda</i> 3882-61	93	2.1	NT
<i>E. tarda</i> 2334-67	84	3.9	81
<i>E. tarda</i> 5159-63	86	+0.5	NT
<i>E. tarda</i> 1483-59	91	+0.1	NT
<i>E. tarda</i> 2995-65	82	0.5	84
<i>E. tarda</i> 4226-59	87	1.2	NT
<i>E. tarda</i> 4862-60	87	0.7	NT
<i>E. tarda</i> 5497-61	85	0.5	84
<i>E. tarda</i> 4411-68	86	0.1	NT
<i>E. tarda</i> 4751-66	88	0.8	NT
<i>E. tarda</i> 4830-60	96	2.4	90
<i>E. tarda</i> 3893-64	89	NT	92
<i>Escherichia coli</i> K-12	25	NT	7
<i>Salmonella typhimurium</i> LT2	25	NT	7
<i>S. phoenix</i>	25	NT	NT
<i>Arizona hinshawii</i> 1	25	NT	NT
<i>A. hinshawii</i> 62	25	NT	NT
<i>Citrobacter freundii</i> 460-61	22	NT	7
<i>Serratia marcescens</i> 868-57	28	NT	11
<i>Citrobacter freundii</i> lysine+ 4556-71	28	NT	NT
<i>Serratia liquefaciens</i> 446-68	25	NT	NT
<i>Serratia rubidaea</i> 934-72	25	NT	NT
<i>Enterobacter aerogenes</i> 1627-66	26	NT	8
<i>Enterobacter cloacae</i> 1347-71	25	NT	NT
<i>Enterobacter cloacae</i> 256-4	23	NT	NT
<i>Enterobacter agglomerans</i> EH103	24	NT	NT
<i>Enterobacter hafniae</i>	29	NT	7
<i>Klebsiella rhinoscleromatis</i> 895-68	24	NT	NT
<i>Erwinia nimipressuralis</i> EN1	25	NT	NT
<i>Erwinia amylovora</i> EA178	22	NT	7
<i>Erwinia nigrifluens</i> EN101	25	NT	NT
<i>Pectobacterium carotovorum</i> 495	20	NT	5
<i>Pectobacterium cypripedii</i> EC155	22	NT	NT
<i>Pectobacterium carnegieana</i> EC186	21	NT	NT
<i>Proteus morgani</i>	16	NT	NT
<i>Proteus mirabilis</i>	8	NT	NT

^a See Table 2. NT, Not tested.

certain reactions, but many biochemical characteristics differ in *E. tarda* and *E. coli*. There seems to be complete acceptance of their placement as one species in a separate genus and tribe of *Enterobacteriaceae*.

The indole, methyl red, Voges-Proskauer, and citrate reactions of *E. tarda* are similar to those exhibited by *E. coli* (8). For this reason, DNA from strains of *E. tarda* was reacted with labeled DNA from *E. coli* K-12. Optimal relatedness between *E. coli* and strains of *E. tarda* is approximately 20% (Table 2). The related sequences contain about 14% unpaired nucleotide bases. When reactions are carried out at 75 C, where only highly related sequences can reassociate, relatedness falls to less than 5%. Thus, *E. tarda* is significantly less related to *E. coli* than are representatives of *Shigella*, *Salmonella*, *Citrobacter*, *Klebsiella*, and *Enterobacter cloacae*. *Serratia marcescens*, *Enterobacter hafniae*, and *Proteus morganii* are related to *E. coli* at a level similar to that shown by *E. tarda*. Only *Proteus mirabilis* shows less relatedness to *E. coli* than does *E. tarda*.

Relatedness in a diverse assortment of *E. tarda* strains was determined in reactions using labeled DNA from *E. tarda* 3592-64 (Table 3). The average relatedness of *E. tarda* strains in 60-C reactions is 88%. There is evidence of only about 1% average divergence in related sequences. Conservation of *E. tarda* is also obvious from 75-C reactions, where average relatedness is 86%. Representatives of established genera from the tribes *Escherichieae*, *Salmonelleae*, and *Klebsielleae* exhibit 20 to 29% relatedness to *E. tarda* 3592-64. An approximate threefold decrease in duplex formation is observed in 75-C reactions. Relatedness of *E. tarda* to *P. morganii* and *P. mirabilis* at 60 C is 16 and 8%, respectively.

These data leave little doubt that the strains of *E. tarda* examined thus far are extremely similar in overall polynucleotide base sequence. Furthermore, *E. tarda* exhibits only 20 to 25% relatedness to most members of all other genera. As pointed out in previous studies (1), most members of *Enterobacteriaceae* exhibit a minimum of about 20% relatedness. That *E. tarda* shows no more than this core level of relatedness to other enteric bacteria is consistent with its classification as a single genus in a separate tribe.

All 20 strains of *E. tarda* studied are highly interrelated. This sample includes the type strain, 1483-59, the Bartholomew group isolate, 1795-62, and five of the Asakusa group strains. The strains were isolated from clinical and

nonclinical human sources, as well as from invertebrates from 11 states and five widely separated countries. Furthermore, these strains contain 19 different O antigens and 11 different H antigens. Thus, the single species designation seems valid for all strains of *E. tarda* thus far isolated.

ACKNOWLEDGMENT

We are indebted to William J. Martin for his kindness in selecting and providing the wide variety of *Edwardsiella tarda* strains used in this study.

REPRINT REQUESTS

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