Yersinia aldovae (Formerly *Yersinia enterocolitica*-Like Group X2): a New Species of *Enterobacteriaceae* Isolated from Aquatic Ecosystems

HERVÉ BERCOVIER,^{1†} ARNOLD G. STEIGERWALT,² ANNIE GUIYOULE,¹ GERALDINE HUNTLEY-CARTER,³
AND DON J. BRENNER^{2*}

Centre National des Yersinia, Institut Pasteur, 75724 Paris, Cedex 15, France,¹ and Molecular Biology Laboratory, Biotechnology Branch,² and Enteric Laboratory Section, Enteric Diseases Branch,³ Division of Bacterial Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia 30333

Previously, a group of 40 *Yersinia enterocolitica*-like strains that were isolated from water and fish were called group X2. These strains produced acid from L-rhamnose, did not ferment sorbose, cellobiose, melibiose, or raffinose, and rarely fermented sucrose (5% in 48 h, 10% in 7 days). This pattern of reactions separated group X2 strains from *Yersinia enterocolitica*, *Yersinia intermedia*, *Yersinia frederiksenii*, and *Yersinia kristensenii*. Positive reactions for acetoin production (Voges-Proskauer test), ornithine decarboxylase, and lack of acid production from melibiose distinguished group X2 strains from both *Yersinia pseudotuberculosis* and *Yersinia pestis*. Group X2 strains exhibited variable reactions only in tests for citrate utilization, hydrolysis of Tween 80, and acid production from maltose. Genetically, group X2 strains formed a single deoxyribonucleic acid hybridization group with an average level of relatedness of 86% or more (86% as determined by the S1 method at 60°C or by the hydroxyapatite method at 75°C and 92% as determined by the hydroxyapatite method at 60°C). The level of divergence among related sequences in 60°C reactions was 0.5%, as determined by the hydroxyapatite method. The relatedness of group X2 strains to other *Yersinia* species was 42 to 73% in 60°C reactions. The divergence in these reactions was 11.0 to 15.5%, and the relatedness to other yersiniae in 75°C reactions was 21 to 38%. Group X2 strains were 11 to 32% related to 67 species of the *Enterobacteriaceae* that belonged to genera other than *Yersinia*. On the basis of these biochemical and genetic data, we believe that group X2 represents a single new species in the genus *Yersinia*. The name *Yersinia aldovae* sp. nov. is proposed for this species. The type strain of *Y. aldovae* is strain CNY 6005 (= CDC 669-83 = ATCC 35236).

Strains that resembled *Yersinia enterocolitica* but were atypical by virtue of acid production from L-rhamnose and negative reactions for fermentation of sorbose, cellobiose, melibiose, and usually sucrose were described as group X2 strains by Bercovier et al. (3). Subsequently, Brenner et al. showed that strain CNY 6005^T (T = type strain), representing group X2, was substantially related to, but genetically distinct from, all *Yersinia* species, as determined by deoxyribonucleic acid (DNA) hybridization (8).

In this report we describe the biochemical characteristics and sources of 40 group X2 strains and the levels of DNA relatedness among 28 of these strains. We show that group X2 strains do, in fact, constitute a new species in the genus *Yersinia*. The name *Yersinia aldovae* sp. nov. is proposed for this new species.

MATERIALS AND METHODS

Bacterial strains. The 40 *Y. aldovae* strains which we studied were sent to the Centre National des Yersinia (H. H. Mollaret, Institut Pasteur, Paris, France) from Czechoslovakia and Norway as atypical *Y. enterocolitica*-like strains. The origins and serological and phage-typing characteristics of these strains are shown in Table 1. The strains representing other *Yersinia* species and other genera of the *Enterobacteriaceae* that were used in the DNA hybridization studies

have all been deposited in the American Type Culture Collection, Rockville, Md. All bacteria were cultivated either at 28°C (most *Yersinia* strains) or at 36°C and were maintained on nutrient agar.

Cultural and biochemical characteristics. The colonial morphology of *Y. aldovae* was observed after 2 days of aerobic growth at 28°C on petri plates containing nutrient agar. The flagella of *Y. aldovae* strain CNY 6005^T were studied by light microscopy after cells grown overnight at 28°C on semisolid nutrient agar were stained with Rhodes flagellum stain. The biochemical tests were done as previously described (1, 2, 12). Unless otherwise stated, the biochemical tests were done at 28°C. *Hafnia*-specific bacteriophage 1672 of Guinée and Valkenburg was used as described previously (11).

DNA methods. DNA was isolated by the methods of Brenner et al. and Hickman et al. (7, 13). The guanine-plus-cytosine content of *Y. aldovae* strain CNY 6005^T DNA was determined by optical denaturation of high-molecular-weight DNA diluted in 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) (14). Using the equation of Owen et al. (15), we calculated the base compositions; these were determined in triplicate, and *Escherichia coli* B DNA was included as a control in each determination (the thermal denaturation midpoint of *Escherichia coli* B DNA in 0.1× SSC was 75.5°C). DNAs from *Y. aldovae* strains CNY 6005^T and CNY 7618 were labeled with ³²P_O₄ in vivo or with ³H in vitro (7, 13). DNA hybridization was done first at 60°C by using the S1 endonuclease method and diethylaminoethyl cellulose filters, as described by Popoff and Coynault (16). In these experiments DNA from *Y. aldovae* CNY 6005^T was labeled

* Corresponding author.

† Present address: Department of Clinical Microbiology, The Hebrew University, Hadassah Medical School, Jerusalem, Israel.

TABLE 1. Origins and serological and phage typing characteristics of the 40 *Y. aldovae* strains studied

Laboratory strain no.	CNY strain no. ^a	Habitat	Country ^b	Sent by:	Serogroup	Phage type
1	6005 ^T	Drinking water	C	E. Aldova	NT ^c	X _o
2	6679	Drinking water	C	E. Aldova	17	X _o
3	7089	Surface water	C	E. Aldova	17	X _o
4	7090	Drinking water	C	E. Aldova	17	X _o
5	7091	Drinking water	C	E. Aldova	17	X _o
6	7092	Drinking water	C	E. Aldova	NT	X _o
7	7096	River	C	E. Aldova	NT	XI
8	7109	Water	C	E. Aldova	NT	X _z
9	7111	Surface water	C	E. Aldova	17	X _o
10	7112	Surface water	C	E. Aldova	7,8	X _z
11	7328	Drinking water	C	E. Aldova	7,8	X _o
12	7330	Swimming pool	C	E. Aldova	NT	X _z
13	7331	Surface water	C	E. Aldova	NT	X _z
14	7332	Surface water	C	E. Aldova	NT	X _z
15	7618	Fish	N	G. Kapperud	NT	X _z
16	7622	Fish	N	G. Kapperud	NT	X _o
17	7632	Fish	N	G. Kapperud	NT	X _z
18	7683	Water	C	E. Aldova	22	X _z
19	7898	Water	N	G. Langeland	NT	X _o
20	7927	Water	N	G. Langeland	NT	X _o
21	7928	Water	N	G. Langeland	NT	X _o
22	7970	Water	N	G. Langeland	NT	X _z
23	7971	Water	N	G. Langeland	NT	X _o
24	8285	Water	N	G. Langeland	6,31	X _z
25	8286	Water	N	G. Langeland	6,31	X _z
26	8287	Water	N	G. Langeland	NT	X _z
27	8288	Water	N	G. Langeland	6,31	X _z
28	8290	Water	N	G. Langeland	NT	X _o
29	8616	Drinking water	C	E. Aldova	17	X _o
30	8619	Drinking water	C	E. Aldova	NT	X _o
31	8626	Drinking water	C	E. Aldova	NT	X _z
32	8631	Drinking water	C	E. Aldova	NT	X _o
33	8632	Drinking water	C	E. Aldova	NT	X _o
34	8780	Drinking water	C	E. Aldova	6,30	X _o
35	8784	Drinking water	C	E. Aldova	21	X _z
36	8789	Drinking water	C	E. Aldova	NT	X _o
37	8790	Drinking water	C	E. Aldova	17	X _o
38	8791	Drinking water	C	E. Aldova	17	X _z
39	8793	Drinking water	C	E. Aldova	6,30	X _o
40	9553	Drinking water	C	E. Aldova	NT	X _o

^a CNY, Centre National des Yersinia (H. H. Mollaret, Institut Pasteur, Paris, France).^b C, Czechoslovakia; N, Norway.^c NT, Untypable.

in vitro with [³H]thymidine. Subsequently, DNA hybridization was done by the hydroxyapatite method at 60 and 75°C, using DNAs from *Y. aldovae* strains CNY 6005^T and CNY 7618 that were labeled with ³²PO₄ in vivo.

RESULTS

Phenotypic characteristics. The 40 strains of *Y. aldovae* examined gave mostly homogeneous reactions in biochemical tests done at 28°C (Table 2). The characteristics common to all of the strains studied are listed below. A total of 36 strains did not ferment sucrose; 2 strains fermented sucrose within 48 h, and 2 strains fermented sucrose after 4 days. Utilization of citrate (Simmons), acid production from maltose, and hydrolysis of Tween 80 were independently variable reactions in the 40 *Y. aldovae* strains. Two strains were nonmotile at 28°C, and two other strains gave negative reactions for urease. A single strain gave a negative test for inositol. Like other *Yersinia* species, especially the L-rhamnose-fermenting species *Yersinia intermedia* and *Yersinia frederiksenii*, when *Y. aldovae* was incubated at 36 ± 1°C, it exhibited negative or delayed positive reactions in tests for

L-rhamnose, D-xylose, i-inositol, the Voges-Proskauer reaction, Simmons citrate, ornithine decarboxylase, beta-galactosidase (*o*-nitrophenyl-β-D-galactopyranoside), and, of course, motility. These data (except the motility, methyl red, Voges-Proskauer, beta-xylosidase, and Simmons citrate data) are not shown for all strains, but the results of the 36 ± 1°C reactions for representative strains CNY 6005^T and CNY 7618 are shown in Table 2.

Of the 40 *Y. aldovae* strains, 17 were typable in slide agglutination tests for O antigens by the scheme of Wauters et al. (19). Eight strains were serogroup O:17 strains, followed by decreasing numbers of strains in serogroups O:6,31, O:6,30, O:7,8, O:21, and O:22 (Table 1). All but 1 of the 40 strains belonged to either phage type X_z or phage type X_o; these phage types consist of bacteriophages that are isolated from sewage, not of specific temperate bacteriophages that are isolated from *Y. enterocolitica* (Table 1).

Genetic characteristics. The guanine-plus-cytosine content of DNA from *Y. aldovae* CNY 6005^T was 48 mol%. DNA relatedness studies were done by using radiolabeled DNAs from *Y. aldovae* CNY 6005^T and CNY 7618. The levels of

TABLE 2. Biochemical characteristics of *Y. aldovae* strains^a

Test	% of 40 <i>Y. aldovae</i> strains positive at 28°C within 72 h	Reaction of type strain CNY 6005 at:		Strains giving reaction opposite that of the type strain at 28°C	Reaction of sucrose-positive reference strain CNY 7618 at 36°C
		28°C	36°C		
Motility	95	+	—	22, 23 ^b	—
Urease	95	+	(+)	20, 21	+
Indole	0	—	—		—
Voges-Proskauer	100	+	—		—
Citrate (Simmons)	75	+	—	2, 8–10, 13, 22, 24, 28, 33, 34	—
Lysine decarboxylase	0	—	—		—
Ornithine decarboxylase	100	+	+		—
Beta-galactosidase (ONPG) ^c	100	+	—		(+)
Lipase (Tween 80)	60	+	—	7–10, 12, 15, 18, 19, 22, 24–26, 28, 31, 32, 40	—
Acid production from:					
Sucrose	5(5)	—	—	15, 16, 22, 23	+
L-Rhamnose	98(2)	+	(+)		—
D-Cellobiose	0	—	—		—
L-Sorbose	0	—	—		—
i-Inositol	98	+	(+)	19	—
Maltose	23	—	—	2, 15–17, 19–23	(+)

^a Unless indicated otherwise, incubation was at 28°C. Reactions were read after 72 h and 7 days. The 7-day readings are shown in parentheses when cultures were negative after 3 days and positive after 7 days. +, positive within 3 days; —, negative after 7 days; (+), positive between 3 and 7 days.

^b Strain numbers used in Table 1.

^c ONPG, *o*-Nitrophenyl- β -D-galactopyranoside.

DNA relatedness among 28 *Y. aldovae* strains are shown in Table 3. All of the *Y. aldovae* strains belonged to a single group, in which the average level of DNA relatedness was 85% or more. Specifically, the average level of relatedness of *Y. aldovae* strain CNY 6005^T to other *Y. aldovae* strains was 85% (range, 72 to 94%) in S1 hybridization experiments done at 60°C. In hydroxyapatite hybridization experiments, *Y. aldovae* CNY 6005^T showed 92% average relatedness to other *Y. aldovae* strains (range, 79 to 100%). The level of divergence in the related sequences was 0.5% (range, 0.0 to 1.5%), and the average level of relatedness at the stringent 75°C incubation temperature, at which only very closely related sequences can reassociate, was 85% (range, 78 to 92%). In hydroxyapatite experiments done with radiolabeled DNA from *Y. aldovae* CNY 7618, a sucrose-positive strain, the average level of relatedness to other *Y. aldovae* strains was 85% (range, 72 to 99%) at 60°C and 93% (range, 81 to 100%) at 75°C. Thus, the sucrose-negative and sucrose-positive strains are biogroups of the same species.

The levels of DNA relatedness of *Y. aldovae* to other *Yersinia* species and to other species in the family *Enterobacteriaceae* are shown in Table 4. *Y. aldovae* was 64 to 73% related to strains from various biogroups of *Y. enterocolitica*, including biogroup 5, which is usually sucrose negative. The level of divergence in the related sequences in these reactions was 11.0 to 13.0%, and the level of relatedness dropped to 38% in one case where the reaction was done at 75°C. *Y. aldovae* was also highly related to two strains of *Y. enterocolitica* biogroup X1, another sucrose-negative group (3). At 60°C these groups were 68 to 69% related, but the levels of relatedness fell to 32 to 34% in reactions done at 75°C. *Y. aldovae* was 45% related to *Y. frederiksenii* IP 867 and 64 to 67% related to three other *Y. frederiksenii* strains (these four *Y. frederiksenii* strains represented three different hybridization groups within this species [18]). The levels of divergence in these reactions were 12.0 to 15.0%, and the levels of relatedness in 75°C reactions were 21 to 29%. *Y. aldovae* was 59% related to the type

strains of both *Y. intermedia* and *Yersinia kristensenii*. The level of divergence in these reactions was 11.5%, and the levels of relatedness fell to 27 to 30% in 75°C reactions. Somewhat lower levels of relatedness (42 to 44%) were observed between *Y. aldovae* and strains of *Yersinia pseudotuberculosis* and *Yersinia ruckeri*. It was not necessary to test *Yersinia pestis* because this species is 95 to 100% related to *Y. pseudotuberculosis* (4). The levels of relatedness of *Y. aldovae* to other members of the *Enterobacteriaceae* were 11 to 32% (Table 4).

DISCUSSION

Our *Y. aldovae* strains corresponded to the previously described group X2, which was represented by strain CNY 6005^T (= IP 6005^T) (1, 3, 8). These *Y. aldovae* strains gave homogeneous reactions for most biochemical tests when they were grown at 25 to 28°C, but exhibited delayed, variable, or negative reactions in several key diagnostic tests when they were grown at 35 to 37°C (Table 2). These tests included the L-rhamnose, i-inositol, beta-galactosidase, Voges-Proskauer, Simmons citrate, ornithine decarboxylase, and motility tests. When done at 35 to 37°C, these reactions can confuse identification and may lead to misidentification of *Y. aldovae* as a metabolically inactive species, such as a *Shigella* species or metabolically inactive *Escherichia coli*. The presence of small colonies (and often medium-sized colonies on the same plate, obtained from streaking out of a single colony), a positive urease test, and the absence of phenylalanine deaminase, arginine dihydrolyase, and lysine decarboxylase direct identification toward the genus *Yersinia*. Differentiation of *Y. aldovae* from urease-positive *Hafnia alvei* strains (about 4% of *H. alvei* strains are urease positive [10, 17]) requires special mention. One of us (H.B.) has received strains that were misidentified as *Yersinia* and were, in fact, urease-positive *H. alvei* strains that gave negative lysine decarboxylase tests at 36°C. These *H. alvei* strains were distinguished from *Y. aldovae* at 36°C by production of gas from D-glucose, as well as by sensitivity

TABLE 3. Levels of intraspecies DNA relatedness of *Y. aldovae* strains^a

Source of unlabeled <i>Y. aldovae</i> DNA	Source of labeled DNA					
	<i>Y. aldovae</i> CNY 6005 ^T				<i>Y. aldovae</i> CNY 7618	
	RBR (%) at 60°C			RBR (%) at 75°C (HA method)	RBR (% HA method) at:	
	S1 method	HA method	D		60°C	75°C
CNY 6005 ^T	100	100	0.0	100	80	88
CNY 6679	89	ND	ND	ND	ND	ND
CNY 7089	85	94	0.0	91	ND	ND
CNY 7090	80	ND	ND	ND	ND	ND
CNY 7091	89	100	ND	89	81	91
CNY 7092	89	ND	ND	ND	ND	ND
CNY 7096	83	99	ND	83	74	96
CNY 7109	84	100	ND	84	86	98
CNY 7111	94	ND	ND	ND	ND	ND
CNY 7112	88	98	ND	83	72	92
CNY 7328	93	ND	ND	ND	ND	ND
CNY 7330	84	ND	ND	ND	ND	ND
CNY 7331	89	89	0.0	84	80	90
CNY 7332	87	ND	ND	ND	ND	ND
CNY 7618	88	94	0.0	84	100	100
CNY 7622	78	83	ND	89	88	92
CNY 7632	74	79	1.5	86	99	96
CNY 7683	89	ND	ND	ND	ND	ND
CNY 7898	78	83	1.5	78	92	94
CNY 7927	72	ND	ND	ND	ND	ND
CNY 7928	79	99	ND	80	ND	81
CNY 7970	87	100	ND	83	84	95
CNY 7971	74	ND	ND	ND	ND	ND
CNY 8285	89	ND	ND	ND	ND	ND
CNY 8286	92	83	ND	92	99	100
CNY 8287	92	ND	ND	ND	ND	ND
CNY 8288	88	ND	ND	ND	ND	ND
CNY 8290	89	ND	ND	ND	ND	ND

^a S1 method, Diethylaminoethyl cellulose S1 nuclease hybridization assay; HA method, hydroxyapatite hybridization assay; RBR, relative binding ratio; D, divergence. For the S1 nuclease assay the following formula was used: relative binding ratio = (percentage of heterologous DNA bound to diethylaminoethyl cellulose filter)/(percentage of homologous DNA bound to diethylaminoethyl cellulose filter) × 100. For the hydroxyapatite assay the formula used was: relative binding ratio = (percentage of heterologous DNA bound to hydroxyapatite)/(percentage of homologous DNA bound to hydroxyapatite) × 100. Divergence was calculated on the assumption that each 1°C decrease in the stability of a heterologous DNA duplex compared with the stability of a homologous DNA duplex was caused by approximately 1% unpaired nucleotide bases within the heteroduplex. Divergence is expressed to the nearest 0.5%. ³H-labeled DNA from *Y. aldovae* CNY 6005^T was used in the S1 nuclease experiments. Each reaction was done twice on duplicate filters. The values given are averages of four values, which did not differ by more than 2%. The average level of homologous binding was 81%. Approximately 3% of the labeled DNA bound to diethylaminoethyl cellulose filters in the absence of unlabeled DNA. This control value was subtracted from the heterologous binding values before normalization. DNAs from *Y. aldovae* strains CNY 6005^T and CNY 7618, labeled in vivo with ³²P₄, were used in the hydroxyapatite hybridization experiments. The average level of binding in homologous reactions was 75% for both labeled DNA preparations. Each reaction was done twice. Approximately 5% of the labeled DNA bound to the hydroxyapatite in the absence of unlabeled DNA. This control value was subtracted from the heterologous binding values before normalization.

^b ND, Not done.

to a *Hafnia*-specific bacteriophage (11). At 28°C, the reactions described above, as well as a positive lysine decarboxylase test and a negative test for acid production from D-sorbitol, distinguished *H. alvei* from *Y. aldovae*.

The variability in biochemical test results for *Y. aldovae* obtained at 36 ± 1°C compared with the results obtained at 28°C is found in most *Yersinia* species (2, 3, 5, 6, 8, 18). These phenotypic characteristics, especially motility at 25 to 28°C but not at 36 ± 1°C, strengthen the phenotypic resemblance of *Y. aldovae* to other *Yersinia* species. Practical biochemical tests that are useful for differentiating *Y. aldovae* from other *Yersinia* species are shown in Table 5. *Y. aldovae* is easily separated from the other *Yersinia* species that are sucrose negative or that contain sucrose-negative strains. *Y. aldovae* is separated from *Y. kristensenii* by its positive L-rhamnose reaction, negative reactions for cellobiose and sorbose, and positive Voges-Proskauer reaction. *Y. aldovae* is distinguished from *Y. pseudotuberculosis* on the

bases of its positive ornithine decarboxylase and D-sorbitol reactions and its negative melibiose reaction and from *Y. pestis* by the same tests plus its positive urease reaction. It is separated from biogroup 5 of *Y. enterocolitica* (which contains sucrose-negative strains) by its positive D-sorbitol and L-rhamnose reactions and its negative cellobiose reaction. *Y. aldovae* is differentiated from *Y. ruckeri* by its positive reactions for urease and D-sorbitol. It is also simple to differentiate *Y. aldovae* from the sucrose-positive *Yersinia* species by its negative sucrose reaction, as well as its negative reactions for sorbose and cellobiose (from *Y. enterocolitica* biogroups 1 through 4, *Y. intermedia*, and *Y. frederiksenii*), negative indole reaction (from *Y. intermedia*, and *Y. frederiksenii*), negative melibiose reaction (from *Y. intermedia*), and positive L-rhamnose reaction (from *Y. enterocolitica* biogroups 1 through 4).

DNA hybridization studies showed that the *Y. aldovae* strains are highly interrelated, forming a good genospecies

TABLE 4. Levels of DNA relatedness of *Y. aldovae* to species of *Yersinia* and other genera in the *Enterobacteriaceae*^a

Source of unlabeled DNA	Relatedness to ³² PO ₄ -labeled <i>Y. aldovae</i> CNY 6005 ^T DNA			Source of unlabeled DNA	Relatedness to ³² PO ₄ -labeled <i>Y. aldovae</i> CNY 6005 ^T DNA		
	RBR (%) at 60°C	D (%)	RBR (%) at 75°C		RBR (%) at 60°C	D (%)	RBR (%) at 75°C
<i>Y. enterocolitica</i> 2525-75 (biogroup unknown)	73	12.5		<i>Enterobacter amnigenus</i> 1325-79	23		
<i>Y. enterocolitica</i> 497-70 (biogroup 1)	70	11.0	38	<i>Kluyvera ascorbata</i> 408-78	23		
<i>Y. enterocolitica</i> 500-70 (biogroup 3)	70	11.0		<i>Erwinia salicis</i> ES102	22		
<i>Yersinia</i> biogroup X1 strain IP 7706	69		32	<i>Erwinia amylovora</i> EA178	22		
<i>Yersinia</i> biogroup X1 strain IP 7702	68		34	<i>Enterobacter agglomerans</i> 219-71	22		
<i>Y. enterocolitica</i> IP 178 (biogroup 5)	68	12.5		<i>Enterobacter agglomerans</i> 4388-71	22		
<i>Y. frederiksenii</i> IP 5960	67		29	<i>Enterobacter agglomerans</i> 3482-71	22		
<i>Y. frederiksenii</i> IP 6175	67	14.5		<i>Enterobacter agglomerans</i> 3123-70	22		
<i>Y. frederiksenii</i> 2851-77	64	15.0		<i>Serratia marcescens</i> 868-57	22		
<i>Y. enterocolitica</i> IP 211 (biogroup 2)	64	13.0		<i>Citrobacter amalonaticus</i> ATCC 25406	22		
<i>Y. kristensenii</i> IP 1474	59	11.5	30	<i>Cedecea neteri</i> 621-75	22		
<i>Y. intermedia</i> IP 3953	59	11.5	27	<i>Salmonella typhimurium</i> LT2	22		
<i>Y. frederiksenii</i> IP 867	45	12.0	21	<i>Enterobacter agglomerans</i> 1741-71	21		
<i>Y. ruckeri</i> 4535-69	44	15.5		<i>Serratia ficaria</i> 1165-77	21		
<i>Y. pseudotuberculosis</i> P62	42	14.0		<i>Cedecea davisae</i> 3278-77	21		
<i>Serratia fonticola</i> 4556-71	32			<i>Cedecea lapagei</i> 485-76	21		
<i>Serratia liquefaciens</i> 446-68	30			<i>Erwinia mallotivora</i> NCPPB 2851	20		
<i>Erwinia cypripedii</i> EC155	28			<i>Enterobacter agglomerans</i> 6070-69	20		
<i>Erwinia rhapontici</i> ER106	28			<i>Erwinia quercina</i> EQ102	19		
<i>Erwinia carotovora</i> ATCC 495	27			<i>Klebsiella pneumoniae</i> 2	19		
<i>Enterobacter aerogenes</i> 1627-66	27			<i>Shigella boydii</i> C13 1610-55	19		
<i>Erwinia nigrifluens</i> EN104	25			<i>Edwardsiella tarda</i> 3592-64	19		
<i>Enterobacter agglomerans</i> 2780-70	25			<i>Escherichia vulneris</i> 2898-73	19		
<i>Enterobacter agglomerans</i> 1429-71	25			<i>Proteus vulgaris</i> PR1	19		
<i>Serratia rubidaea</i> 934-72	25			<i>Enterobacter skazakii</i> 4562-70	18		
<i>Serratia plymuthica</i> 392	25			<i>Escherichia hermannii</i> 980-73	18		
<i>Rahnella aquatilis</i> 1327-79	25			<i>Citrobacter diversus</i> 1066-71	18		
<i>Kluyvera cryocrescens</i> 409-78	25			<i>Providencia rustigianii</i> 132-68	18		
<i>Escherichia blattae</i> 9005-74	25			<i>Enterobacter gergoviae</i> CIP 7601	17		
<i>Obesumbacterium proteus</i> 4302-74	24			<i>Hafnia alvei</i> III 329-73	17		
<i>Erwinia carnegieana</i> EC186	24			<i>Enterobacter agglomerans</i> 1645-71	16		
<i>Enterobacter agglomerans</i> 6003-71	24			<i>Providencia alcalifaciens</i> 3370-67	15		
<i>Enterobacter agglomerans</i> 1600-71	24			<i>Providencia stuartii</i> 2896-68	15		
<i>Enterobacter agglomerans</i> 5378-71	24			<i>Providencia rettgeri</i> 1163	15		
<i>Enterobacter cloacae</i> 1347-71	24			<i>Hafnia alvei</i> II 4510-75	14		
<i>Klebsiella oxytoca</i> ATCC 13182	24			<i>Erwinia chrysanthemi</i> SR32	14		
<i>Enterobacter agglomerans</i> 5422-69	23			<i>Escherichia coli</i> K-12	12		
<i>Citrobacter freundii</i> 460-61	23			<i>Proteus myxofaciens</i> ATCC 19692	12		
<i>Hafnia alvei</i> I 5632-72	23			<i>Proteus mirabilis</i> PR14	11		
<i>Morganella morganii</i> ATCC 25830	23			<i>Erwinia rubrifaciens</i> ER105	11		

^a RBR, Relative binding ratio; D, divergence. See Table 3, footnote a, for experimental conditions.

(Table 3). Although strain CNY 7618 produced acid from sucrose, the levels of relatedness between this strain and other *Y. aldovae* strains were similar to the levels of relatedness between the more typical (sucrose-negative) strain CNY 6005^T and other *Y. aldovae* strains. This proves that the sucrose-positive biogroup is part of *Y. aldovae*. The results of DNA hybridization studies done with labeled DNAs from other *Yersinia* species and unlabeled DNAs from group X2 strains CNY 6005^T and IP 5850 have been published previously (8). In these studies *Y. enterocolitica* was 53 to 65% related to *Y. aldovae* at 60°C and 28 to 36% related at 75°C; *Y. kristensenii* was 60 to 66% related to *Y. aldovae* at 60°C and 33 to 35% related at 75°C; *Y. frederiksenii* was 43 to 56% related to *Y. aldovae* at 60°C and 14 to 23% related at 75°C; and *Y. intermedia* was 51 to 57% related to *Y. aldovae* at 60°C and 29 to 31% related at 75°C. Where determined, the levels of divergence in related sequences were 10 to 14% (8).

In the present study the reciprocal hybridization experiments yielded similar results (Table 4). The levels of diver-

gence in related sequences were 11 to 15%; the levels of relatedness in 75°C reactions were 21 to 38%, and the levels of relatedness in 60°C reactions were 59% (to *Y. kristensenii*), 45 to 67% (to *Y. frederiksenii*), 59% (to *Y. intermedia*), 42% (to *Y. pseudotuberculosis*), and 64 to 73% (to *Y. enterocolitica*). The level of relatedness between *Y. aldovae* and *Y. enterocolitica* (as determined with labeled *Y. aldovae* DNA) is close to the lower level of relatedness observed between strains of a single species (70% or more). The higher levels of relatedness observed between these species when *Y. aldovae* DNA rather than labeled *Y. enterocolitica* DNA was used (64 to 74% compared with 53 to 65%) might have been due to differences in genome size between strains of these two species (*Y. enterocolitica* is 12 to 14% smaller than *Y. aldovae*). The fact that *Y. aldovae* and *Y. enterocolitica* are separate species was proven by the substantial levels of divergence present in DNA heteroduplexes (10 to 15%) compared with the levels observed in reactions between strains of *Y. aldovae* (0.0 to 1.5% [Table 3]) or *Y. enterocolitica* (0.0 to 4.0% [8]) and by the substantially lower levels of

TABLE 5. Biochemical differentiation of *Y. aldovae* from other *Yersinia* species^a

Test	Reaction of:								
	<i>Y. aldovae</i>	<i>Y. pseudo-tuberculosis</i>	<i>Y. pestis</i>	<i>Y. kristensenii</i>	<i>Y. ruckeri</i>	<i>Y. enterocolitica</i>		<i>Y. intermedia</i>	<i>Y. frederiksenii</i>
						Biogroup 5	Biogroups 1 through 4		
Voges-Proskauer	+	—	—	—	—	+	+	+	+
Sucrose	—	—	—	—	—	V	+	+	+
Cellobiose	—	—	—	+	—	+	+	+	+
Indole	—	—	—	V	—	—	V	+	+
Sorbitol	—	—	—	+	ND	V	+	+	+
L-Rhamnose	+	+	—	—	—	—	—	+	+
Melibiose	—	+	V	—	—	—	—	+	—
D-Sorbitol	+	—	—	+	—	—	+	+	+
Ornithine decarboxylase	+	—	—	+	+	V	+	+	+
Urease	+	+	—	+	—	+	+	+	+

^a The data for *Y. aldovae* are from Table 2, the data for *Y. ruckeri* are from reference 9, and the data for the other yersiniae are from references 3 through 6 and 18. All of the data are for 3 days of incubation at 28°C (2 days of incubation at 25°C for *Y. ruckeri*). +, 90% or more of the strains positive; —, less than 10% positive; V, 10 to 89% positive; ND, test not done.

relatedness observed in 75°C reactions between these species (24 to 36% [Table 4] [8]) than in reactions among strains of *Y. aldovae* (78 to 100% [Table 3]) or *Y. enterocolitica* (68% to 97% [8]). For that matter, the level of relatedness among strains of either species at 60°C (average for *Y. aldovae*, 85 to 92% [Table 3]; average for *Y. enterocolitica*, 85% [8]) was substantially higher than the levels obtained in reactions between strains of *Y. aldovae* and *Y. enterocolitica* (69% average relatedness at 60°C with labeled *Y. aldovae* DNA and unlabeled *Y. enterocolitica* DNAs [Table 4]; 60% average relatedness at 60°C with labeled *Y. enterocolitica* DNA and unlabeled *Y. aldovae* DNAs [8]).

The levels of relatedness of *Y. aldovae* to other members of the *Enterobacteriaceae* were substantially lower than the levels of relatedness to yersiniae, but nonetheless were significant (11 to 32% [Table 4]). The guanine-plus-cytosine content of *Y. aldovae* strain CNY 6005^T DNA was 48 mol%, a value which is similar to the guanine-plus-cytosine contents of other *Yersinia* species. Therefore, we conclude that, on the basis of phenotypic and genetic characteristics, *Y. aldovae* is a new species of the *Enterobacteriaceae* and does indeed belong in the genus *Yersinia*.

It must be emphasized that, as with most other yersiniae, biochemical characterization is more definitive when test preparations are incubated at 25 to 28°C (the optimal growth temperature) than when they are incubated at 36 ± 1°C. The results of biochemical tests done at 36 ± 1°C are frequently misleading, because several important diagnostic tests give false-negative or delayed positive results. These false-negative findings can result in misidentification at the species level or even at the genus level. Results from this study and our previous studies have clearly shown that all *Yersinia* species are readily identified when they are studied at 28°C (3–6, 9, 18).

The 40 strains of *Y. aldovae* which we studied were all isolated from aquatic ecosystems in Czechoslovakia or Norway. One previously studied strain (strain IP 5850) was isolated from soil in France (8). *Y. aldovae* has not been implicated in animal infections and has not been isolated from humans. It may be isolated from many of the same water sources as *Y. enterocolitica*. Therefore, clinical and environmental microbiologists should be able to distinguish these species from one another.

We propose the name *Yersinia aldovae* sp. nov. (al. do'vae. M. L. gen. n. *aldovae* in honor of Eva Aldova, the

Czechoslovakian microbiologist who first isolated this bacterium) for the *Yersinia* group formerly referred to as group X2. The type strain of *Y. aldovae* is strain CNY 6005 (= ATCC 35236 = IP 6005 = AI 19955 = CDC 669-83); it was isolated by E. Aldova from drinking water in Czechoslovakia.

Description of *Yersinia aldovae* sp. nov. The phenotypic characteristics of *Y. aldovae* fit the definition of the *Enterobacteriaceae*. *Y. aldovae* cells are gram negative, nonspore-forming, non-encapsulated, straight, and rod shaped and have peritrichous or degeneratively peritrichous flagella when they are grown at 28°C, but not when they are grown at 37°C; 2 to 10 flagella are usually present. *Y. aldovae* CNY 6005^T grown for 48 h at 28°C aerobically on nutrient agar produces both small (diameter, 0.5 mm) and medium-sized (diameter, 2 mm) colonies. The colonies are translucent and have smooth, entire edges.

All *Y. aldovae* strains give positive results in tests for beta-galactosidase (*o*-nitrophenyl-β-D-galactopyranoside), catalase, methyl red (28 and 36°C), nitrate reduction to nitrite by type B nitrate reductase, ornithine decarboxylase, the Voges-Proskauer reaction at 28°C, and acid production from L-arabinose, esculin, D-fructose, galactose, *N*-acetyl-glucosamine, D-glucose, glycerol, D-mannitol, D-mannose, L-rhamnose, ribose, D-sorbitol, trehalose, and D-xylose. All *Y. aldovae* strains give negative results in tests for arginine dihydrolase, citrate (Simmons) (36°C), deoxyribonuclease, gas from D-glucose, gelatin liquefaction (film), H₂S (Kligler), indole, lysine decarboxylase, malonate, motility at 36°C, oxidase, phenylalanine deaminase, tetrathionate reductase, tryptophan deaminase, the Voges-Proskauer reaction at 36°C, beta-xylosidase (36°C), and acid production from adonitol, amygdalin, D-arabinose, arbutin, D-cellobiose, dulcitol, erythritol, lactose, melibiose, melizitol, alpha-methyl-D-glucoside, alpha-methyl-D-mannoside, alpha-methyl-xyloside, raffinose, salicin, L-sorbitol, and L-xylose. At least 95% of the strains are positive in tests for i-inositol, motility at 28°C, and urease. All but two of our strains are sucrose negative after 48 h.

The guanine-plus-cytosine content of the DNA of the type strain is 48 mol%.

All of our *Y. aldovae* strains except strain IP 5850 were isolated from aquatic ecosystems (drinking water, river water, fish); strain IP 5850 was isolated from soil. None was associated with disease in humans or animals.

The type strain is CNY 6005 (= ATCC 35236 = IP 6005 = AI 19955 = CDC 669-83).

Description of the type strain. Some characteristics of the type strain are given in Table 2. Other characteristics are as given above for the species. This strain is serologically untypable and belongs to phage type X₀.

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