

## *Yersinia kristensenii*: A New Species of Enterobacteriaceae Composed of Sucrose-Negative Strains (Formerly Called Atypical *Yersinia enterocolitica* or *Yersinia enterocolitica*-Like)

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**Abstract.** *Yersinia kristensenii* sp. nov. is defined biochemically and genetically. *Y. kristensenii* strains belong to a single DNA relatedness group. In 60°C reactions, the highest relatedness values of *Y. enterocolitica* strains to *Y. kristensenii* were 70% to as much as 79%, overlapping the lowest intraspecies *Y. kristensenii* relatedness values. *Y. kristensenii* was easily separable from *Y. enterocolitica* on the bases of percent divergence within related DNA sequences in 60°C reactions and relatedness in 75°C reactions. Strain 105 (=CIP 80-30) is proposed as the type strain for *Y. kristensenii*.

Trehalose-positive, sucrose-negative strains (S<sup>-</sup>) have been isolated from horses, frogs, sheep, wild rodents, earthworms, and monkeys, from human urine, blood, eye, and stool, and from food, water, sewage, and soil in Europe, Japan, Australia, and North America [1,3,6,10,16]. Most trehalose-positive, sucrose-negative strains are ornithine decarboxylase positive and rhamnose negative, but two other sucrose-negative groups have been described. These are *Yersinia* biotype X1, which is sucrose negative, ornithine decarboxylase negative, and *Yersinia* biotype X2, which is sucrose negative and rhamnose positive [3,8]. A fourth group of sucrose-negative yersiniae are the sucrose-negative, trehalose-negative strains in biotype 5 of *Yersinia enterocolitica* [4].

Knapp and Thal stated that the sucrose-negative, trehalose-positive strains were not *Yersinia enterocolitica* [11], and Wauters proposed a new biotype for these strains [17]. In this report, we examine the biochemical and genetic parameters of the sucrose-negative, trehalose-positive strains. We show that the trehalose-positive, ornithine decarboxylase-positive, rhamnose-negative group of sucrose-negative strains is a new species, *Yersinia kristensenii*, separable from biotype 5 of *Y. enterocolitica*, *Yersinia*

sucrose-negative biotypes X1 and X2, and the rhamnose-positive *Yersinia* species.

### Materials and Methods

The materials and methods and references to them are the same as those we reported for *Yersinia intermedia* [7] and *Yersinia enterocolitica* [4].

### Results and Discussion

Trehalose-positive, sucrose-negative, Voges-Proskauer-negative *Yersinia kristensenii* strains have the following properties that are characteristic of the family Enterobacteriaceae. They are Gram-negative, peritrichously flagellated rods that grow on ordinary media (nutrient agar), reduce nitrates to nitrites, ferment D-glucose, and are oxidase negative and catalase positive.

The physiology of these bacteria was detailed elsewhere [2]. They grow at temperatures from 4°C to 41°C, but they grow better at 28°C than at 37°C. Strains isolated from the environment hardly grow at 37°C when first subcultured. Compared to *Y. enterocolitica sensu stricto*, growth at 41°C is very scant. The size of the colonies after 24 h incubation at 28°C or 37°C on nutrient agar is similar to those of *Y. en-*

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Table 1. Biochemical characteristics of 115 *Yersinia kristensenii* strains.<sup>a</sup>

Test	Reaction	%+	%(+)	Type strain 105
Indole	V	61	7	+
Simmons' citrate (28°C)	-	0	8	-
Christensen's citrate	V	40	-	-
NO <sub>3</sub> reduction to NO <sub>2</sub> /Type	+ / B	100 / B99	-	+ / B
Tetrathionate reductase	V	83	-	+
$\beta$ -Galactosidase (ONPG) (37°C)	+	97	-	+
Lipase (Tween 80)	V	74	-	+
Deoxyribonuclease	-	1	2	-
Polypectate	(+)	-	100	(+)
Acid production from:				
D-Mannose	+	99	-	+
L-Sorbose	+	97	-	+
Lactose	V	0	60	(+)
D-Inositol	V	28	60	(+)
Esculin	-	0	V	-
Salicin	-	0	V	(+)
Amygdalin	-	0	V	(+)
Starch	(+)	-	100	(+)

<sup>a</sup> All incubations, except where indicated, were done at 28°C. +, 90.0% or more positive within 72 h; (+), 90.0% or more positive between 4 and 7 days; V, 10.1-89.9% positive; -, less than 10% positive after 72 h. For esculin, salicin, and amygdalin, all cultures were negative after 72 h of incubation; delayed positive reactions for these tests were extremely variable in different preparations of media, and are, therefore, of little or no significance. The following tests gave positive reactions for all strains tested: catalase, motility (28°C), urease, methyl red (28°C and 37°C), fermentation in O-F test, ornithine decarboxylase, and acid production from D-glucose, glycerol, L-arabinose, ribose, D-xylose, galactose, D-fructose, D-cellobiose, maltose, D-trehalose, D-mannitol, D-sorbitol, N-acetyl-glucosamine, arbutin, and dextrin. The following tests gave negative reactions for all strains tested: oxidase, motility (37°C), Voges-Proskauer (28°C and 37°C), Simmons' citrate (37°C), malonate, mucate, KCN, gas from D-glucose, H<sub>2</sub>S (Kliegler's), phenylalanine deaminase, tryptophan deaminase, lysine decarboxylase, arginine dihydrolase,  $\beta$ -xylosidase (PNPX; 37°C), gelatin (film), and acid production from erythritol, D-arabinose, L-xylose, adonitol, L-rhamnose, dulcitol, D-melibiose, sucrose, D-raffinose, D-melizitose,  $\alpha$ -methyl-xyloside,  $\alpha$ -methyl-D-mannoside,  $\alpha$ -methyl-D-glucoside, inulin, amylose, and glycogen.

*enterocolitica sensu stricto* strains [4]. They very often produce a strong odor characterized by Wauters [17] and Bottone and Robin [6] as "musty" or "cabbage-like."

Biochemical tests were done on 115 strains from the collection of the Centre National des Yersinia (Institut Pasteur, Paris), as shown in Table 1.

The reactions most useful in differentiating *Y. kristensenii* from other yersiniae are: sucrose, Voges-Proskauer, trehalose, rhamnose, melibiose, raffinose,  $\alpha$ -methyl-D-glucoside, sorbose, sorbitol, ornithine decarboxylase, and cellobiose. Its negative rhamnose reaction alone is sufficient to distinguish *Y. kristen-*

*senii* from *Y. intermedia*, *Y. frederiksenii*, *Yersinia* biotype X2, and *Y. pseudotuberculosis*. Negative reactions in melibiose,  $\alpha$ -methyl-D-glucoside and raffinose also separate *Y. kristensenii* from *Y. intermedia*; and negative sucrose and Voges-Proskauer reactions help to separate *Y. kristensenii* from *Y. frederiksenii*. Positive cellobiose and sorbose reactions, and a negative Voges-Proskauer reaction also distinguish *Y. kristensenii* from *Yersinia* biotype X2. Positive ornithine decarboxylase, cellobiose and sorbose reactions separate *Y. kristensenii* from *Y. pseudotuberculosis*. *Y. enterocolitica*, *Yersinia* biotype X1, and *Y. kristensenii* are all rhamnose negative. Its negative sucrose and Voges-Proskauer reactions serve to differentiate *Y. kristensenii* from biotypes 1 through 4 of *Y. enterocolitica*. Its reactions for trehalose (+), sorbitol (+), and Voges-Proskauer (-) separate *Y. kristensenii* from biotype 5 of *Y. enterocolitica*. Positive reactions for sorbose, sorbitol, and ornithine decarboxylase separate *Y. kristensenii* and *Yersinia* biotype X1.

Additional differential characters between *Y. kristensenii* and other *Yersinia* species were previously presented [4].

Seventy-six percent of the 115 *Y. kristensenii* strains were agglutinable in nine different serogroups of the Wauters scheme [17, 19]. Serogroups O12, O28, and O11 were most frequently encountered, with 26%, 12% and 12% of the strains belonging to the serogroups, respectively. The other serogroups found were O10 (0.8%), O10 and K1 (0.8%), O20 (0.8%), O16 (7%), O1,2a (15%), and O5 (1.6%). It is not surprising that 50% of the strains belong to serogroups O12, O11, and O28 because these serogroups were defined by strains which are trehalose positive and sucrose negative (strains 103, 105, and 1474). Wauters showed that four trehalose-positive, sucrose-negative strains had an H antigen pattern different from the H antigen pattern of *Y. enterocolitica sensu stricto* [17, 19]. Furthermore, Eylan and Barber compared protein determinants by the agar-gel diffusion technique and concluded that strain 103 (trehalose positive, sucrose negative, serogroup O12) is unrelated to strain 134 (*Y. enterocolitica sensu stricto*, serogroup O3) [9].

Finally, Matthew, Cornelis, and Wauters [15] showed that the  $\beta$ -lactamase isoelectric focusing pattern of a sucrose-negative, trehalose-positive strain was different from the patterns of *Y. enterocolitica sensu stricto* strains. These data on the antigenic structure further support the separation of *Y. kristensenii* from *Y. enterocolitica sensu stricto*.

The 115 *Y. kristensenii* strains were isolated in Norway, Denmark, Great Britain, Czechoslovakia,

Table 2. Summary of intra- and interspecies *Yersinia kristensenii* DNA relatedness.<sup>a</sup>

Source of labeled DNA	Source of unlabeled DNA	RBR 60°C			%D			RBR 75°C		
		No.	Range	Avg	No.	Range	Avg	No.	Range	Avg
<i>Y. kristensenii</i> 5873	<i>Y. kristensenii</i>	27	72-100	84	5	0.5-3.5	2.5	9	63-97	82
	<i>Y. enterocolitica</i> biotypes 1-4	16	65-76	70						
	<i>Y. enterocolitica</i> biotype 5	8	58-73	65	†		7.0	1		37
<i>Y. kristensenii</i> 5920	<i>Y. kristensenii</i>	20	74-91	81	18	0.0-5.5	2.5	19	59-89	72
	<i>Y. enterocolitica</i> biotypes 1-4	9	52-63	59	9	7.5-9.5	8.5	11	35-47	39
	<i>Y. enterocolitica</i> biotype 5	5	58-63	61	5	7.5-10.0	9.0	5	32-38	34
<i>Y. kristensenii</i> 1474	<i>Y. kristensenii</i>	14	73-93	84	11	1.0-6.0	4.0	11	62-92	73
	<i>Y. enterocolitica</i> biotypes 1-4	21	57-79	69	19	7.5-11.5	9.0	19	36-53	48
	<i>Y. enterocolitica</i> biotype 5	8	64-70	68	8	8.0-10.0	9.0	8	43-52	48
<i>Y. kristensenii</i> 6048	<i>Y. kristensenii</i>	29	77-95	88	10	0.0-6.5	2.5	27	72-99	84
	<i>Y. enterocolitica</i> biotypes 1-4	19	66-78	74	17	7.5-11.5	9.5	19	36-55	52
	<i>Y. enterocolitica</i> biotype 5	8	64-77	72	7	8.0-9.0	8.5	8	46-53	50
<i>Y. enterocolitica</i> 498-70 biotype 1	<i>Y. enterocolitica</i> biotypes 1-4	53	73-100	86	8	0.5-3.5	2.5	33	68-97	82
	<i>Y. enterocolitica</i> biotype 5	16	74-87	79	16	1.0-3.5	2.5	16	67-83	75
<i>Y. enterocolitica</i> 1 biotype 5, sucrose <sup>-</sup>	<i>Y. enterocolitica</i> biotype 5	5	97-100	98	5	0.5-1.0	1.0	5	96-100	98
	<i>Y. enterocolitica</i> biotypes 1-4	12	77-92	84	9	0.0-2.0	1.0	12	80-98	88
<i>Y. enterocolitica</i> 498-70 biotype 1	<i>Y. kristensenii</i>	27	59-80	70	22	8.0-11.0	9.5	27	24-58	39
<i>Y. enterocolitica</i> 1 biotype 5, sucrose <sup>-</sup>	<i>Y. kristensenii</i>	19	58-69	64	12	7.5-9.5	8.5	10	44-49	47
<i>Y. kristensenii</i> 6048		6	61-68	64	3	10.5-11.5	11.0	6	26-35	31
<i>Y. kristensenii</i> 5873	<i>Y. intermedia</i>	2	58-63	61	1		8.5			
<i>Y. kristensenii</i> 5920		13	49-57	51	13	10.0-12.0	11.0	14	19-24	21
<i>Y. kristensenii</i> 1474		13	56-68	62	12	9.5-14.0	12.0	13	23-35	33
<i>Y. kristensenii</i> 5920	<i>Y. frederiksenii</i>	2	51-58	55	2	10.0-12.0	11.0	2	22-27	25
<i>Y. kristensenii</i> 1474		2	56-60	58	1		9.5	2	28-31	30
<i>Y. kristensenii</i> 6048		2	60-66	63	2	10.0-11.5	11.0	2	33-35	34
<i>Y. kristensenii</i> 5873	<i>Yersinia</i> biotype X2	2	57-61							
<i>Y. kristensenii</i> 1474		1		53	1		10.5			
<i>Y. kristensenii</i> 6048		2	65-66	66	2	11.5-12.0	12.0	2	33-37	35
<i>Y. kristensenii</i> 5873	<i>Yersinia</i> biotype X1	2	69	69						
<i>Y. kristensenii</i> 1474		2	58-59	59	2	10.5-11.0	11.0	2	31	31

<sup>a</sup> RBR, relative binding ratio; %D, percent divergence in related DNA sequences—calculated to the nearest 0.5%; No., number of strains tested. Homologous reactions are arbitrarily deemed to have 100% RBR and 0.0% D. Homologous reactions are not included in number, range, or average.

Germany, France, Japan, the United States, and Australia. Human isolates made up only 18% of these strains; 61% were from animals, 11% from water, 6% from soil, and 4% from vegetables. These figures may not accurately reflect the distribution of *Y. kristensenii*. In fact, Bercovier et al. [3] showed that trehalose-positive, sucrose-negative strains are commonly found in soil (70% of soil samples contained *Y. kristensenii*) and that they seem to be well adapted to this ecosystem.

With the exception of two stool isolates from patients with diarrhea, strains of *Y. kristensenii* were isolated only from extraintestinal human infections [5,6] (H. H. Mollaret, personal communication).

Other stool isolates, besides the two mentioned above, were found only after cold enrichment of stools taken from asymptomatic people. Thus, in epidemiological studies, a search for the source of human infection should be oriented towards an environmental source (e.g., soil, vegetables).

These data suggest that *Y. kristensenii* is usually an opportunistic pathogen. The clinical significance of finding such bacteria in human stools should be assessed in the light of symptomatology and whether cold enrichment is necessary for isolation. Only the future awareness of clinical bacteriologists will tell us if this organism has become common in human infections.

The G+C content in DNA was determined to be  $48.5 \pm 0.5$  mol% in six strains of *Y. kristensenii* (strains 103, 105, 1474, 1475, 5940, 7705).

Hybridization data obtained using DNA from *Y. kristensenii* strain 6048 were previously presented [8]. We here present data obtained using labeled DNA from *Y. kristensenii* strains 1474, 5873, and 5920. Both sets of results are summarized and compared in Table 2.

Thirty-four trehalose-positive, sucrose-negative strains were tested for relatedness to strain 6048 (Table 2). Thirty of these were very highly related (88% RBR in 60°C in reactions, 2.5% divergence [D], 84% RBR in 75°C reactions). These highly related strains were all trehalose positive, sucrose negative, Voges-Proskauer negative, rhamnose negative, and ornithine decarboxylase positive, and correspond to the S<sup>-</sup> biotype [8]. It is these strains that constitute *Y. kristensenii*. Similar results were obtained using labeled DNA from *Y. kristensenii* strains 5873, 5920, and 1474 (Table 2). The four strains that did not show species level relatedness to *Y. kristensenii* belonged to *Yersinia* biotypes X1 and X2 (Table 2).

Reciprocal DNA reactions between *Y. kristensenii* and *Y. enterocolitica* are shown in Table 2. Using labeled DNA from *Y. kristensenii* strains 5873, 5920, 1474, and 6048 and unlabeled DNA from *Y. enterocolitica* strains, relatedness at 60°C was between 52% and 79%. Average relatedness was 70%, 59%, 69%, and 74%, respectively for *Y. enterocolitica* biotype 1 through 4 strains and 65%, 61%, 68%, and 72%, respectively, for *Y. enterocolitica* biotype 5 strains. In reciprocal 60°C reactions, the range of relatedness of *Y. kristensenii* strains to labeled DNA from *Y. enterocolitica* strain 498-70 and strain 1 (biotype 5) was 58% to 80%, with an average relatedness of 70% and 64%, respectively. These values are substantially lower than relatedness values obtained between strains of *Y. enterocolitica* or between strains of *Y. kristensenii*. This especially true when data obtained using the same labeled DNA are compared; for instance, *Y. enterocolitica* strains were 74% related to *Y. kristensenii* strain 6048; *Y. kristensenii* strains were 88% related to strain 6048.

Nonetheless, the lowest intragroup *Y. kristensenii* or *Y. enterocolitica* relatedness values and the highest intergroup *Y. kristensenii*-*Y. enterocolitica* relatedness values overlap somewhat. Furthermore, relatedness between *Y. kristensenii* and *Y. enterocolitica* strains can be close to or even higher than the 70% minimum relatedness usually found between strains of the same species.

*Y. kristensenii* can easily be separated biochemi-

cally from *Y. enterocolitica* biotypes 1 through 5. These organisms also differ antigenically, in pathogenicity for humans and animals, and in sources of isolation. One can still argue that, based upon DNA relatedness at 60°C, *Y. kristensenii* should remain in *Y. enterocolitica* as a nonpathogenic, environmental biotype. However, when one compares the thermal stability (%D) of *Y. kristensenii* reactions (regardless of which strain was labeled) to that of intraspecies *Y. kristensenii* or *Y. enterocolitica* reactions, the related portions of *Y. kristensenii* and *Y. enterocolitica* are significantly less stable (show greater divergence) than intraspecies DNA duplexes (Table 2). Divergence among *Y. kristensenii* strains averages 2.5% with three of the labeled *Y. kristensenii* preparations and 4% with a fourth preparation (strain 1474). Average divergence among *Y. enterocolitica* strains was less than 2%. Interspecies divergence showed a range of 7.0–11.5%, with an average of 9%. This degree of divergence is rarely found among strains of a given species. Where it has been found and further investigated, among strains of *Serratia liquefaciens*, the "divergent" strains were subsequently shown to be not *S. liquefaciens*, but *S. plymuthica* [10,15].

Further evidence that *Y. kristensenii* and *Y. enterocolitica* are different species is gained from 75°C reactions (Table 2). For *Y. kristensenii* strains, interrelatedness remained quite high (72–84% averages with four different labeled strains). The range of relatedness among *Y. enterocolitica* strains in 75°C reactions was 67–100%, with an average of approximately 81%. Interspecies relatedness between *Y. kristensenii* and *Y. enterocolitica*, regardless of source of six labeled DNA preparations, averaged between 30% and 52% (Table 2). Differences in %D and relatedness in 75°C reactions, where only closely related DNA sequences can reassociate, biochemical differences, and differences in ecology are considered ample justification to propose *Y. kristensenii* as a new species.

It is important to note that the trehalose-negative, sucrose-negative strains within biotype 5 of *Y. enterocolitica* show the same level of relatedness to *Y. kristensenii* as do members of *Y. enterocolitica* biotypes 1 through 4 [8].

The separation of *Y. kristensenii* from *Y. enterocolitica* is demonstrated graphically in Fig. 1. Two separate relatedness clusters are present for DNA from strains of *Y. kristensenii* reacted with labeled DNA from *Y. kristensenii* strain 5920. Strain 5920 belongs to *Yersinia* serotype O12, as do all of the *Y. kristensenii* strains that are most highly related to strain 5920. The *Y. kristensenii* strains in the cluster

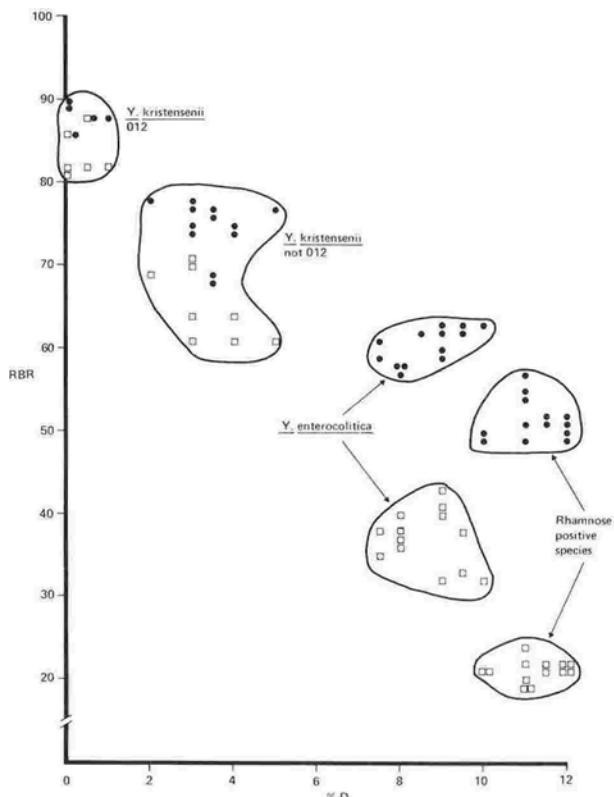


Fig. 1. Relatedness clusters of *Yersinia kristensenii* strains and other *Yersinia* species to *Y. kristensenii* strain 5920. Labeled DNA from *Y. kristensenii* strain 5920 (serotype O12) was reacted with unlabeled DNA from *Y. kristensenii*, *Y. enterocolitica*, *Y. intermedia*, and *Y. frederiksenii* strains at 60°C and 75°C. Thermal stability profiles were done at 60°C to determine percent divergence (%D). Relative binding ratio (RBR;% relatedness) was plotted against %D. Circles represent relatedness values obtained in 60°C reactions, and squares represent relatedness values obtained in 75°C reactions. The clusters labeled "Rhamnose-positive species" represent reactions with strains of both *Y. intermedia* and *Y. frederiksenii*.

showing somewhat lower relatedness to strain 5920 were from serotypes O1,2a, O11, O15, O16, and O28. Similar results were obtained with another O12 strain: 6048 (not shown). The O12 strains were isolated from human stool, rodents, hare, sheep, and soil. Most of the isolates were from a terrestrial ecosystem in France [3], but one isolate was from Australia and another from the Faroe Islands. It seems, therefore, that O12 isolates are somewhat preferentially related to another, regardless of their source or geographical origin. Strains of serotype O1,2a were also somewhat preferentially related to *Y. kristensenii* strain 5873, which is serotype O1,2a.

Two biotype 1 strains of *Y. enterocolitica* gave aberrant DNA relatedness reactions (Table 3). *Y. en-*

Table 3. Strains giving aberrant DNA relatedness reactions.<sup>a</sup>

Source of unlabeled DNA	Source of labeled DNA	RBR	
		60°C	75°C
<i>Y. enterocolitica</i> 6553	<i>Y. enterocolitica</i> 498-70	90	86
	<i>Y. kristensenii</i> 6048	93	91
	<i>Y. kristensenii</i> 5873	89	81
<i>Y. enterocolitica</i> 6554	<i>Y. enterocolitica</i> 498-70	87	85
	<i>Y. kristensenii</i> 6048	82	58
	<i>Y. kristensenii</i> 5873	80	60
<i>Y. kristensenii</i> 5587	<i>Y. enterocolitica</i> 498-70	86	79
	<i>Y. kristensenii</i> 6048	94	90
	<i>Y. kristensenii</i> 5873	90	82

<sup>a</sup> See Table 2 for definitions of RBR and %D.

*terocolitica* 6553 (biotype 1, serotype O1,2a) was, as expected, highly related to *Y. enterocolitica* strain 498-70, but it was just as highly related to *Y. kristensenii* strains 6048 and 5873. *Y. enterocolitica* strain 6554 (biotype 1, serotype O1,2a) also showed higher relatedness than expected to *Y. kristensenii* strains, but, in this case, it was clearly more closely related to *Y. enterocolitica* than to *Y. kristensenii*. Serotype similarity cannot account for the unexpectedly high relatedness of strains 6553 and 6554 to *Y. kristensenii* because both of the labeled *Y. kristensenii* DNAs were from serotype O12 strains. Data obtained from these strains were not included in averages for either *Y. enterocolitica* or *Y. kristensenii*. We cannot explain these data.

*Y. kristensenii* strain 5587 was more than 90% related to labeled DNA from each of two *Y. kristensenii* strains, but showed almost as much relatedness to labeled *Y. enterocolitica* DNA (Table 3). We cannot explain this result.

*Y. kristensenii* strains are 53–63% related to two sucrose-negative, rhamnose-negative strains from *Yersinia* biotype X2, and 59–69% related to two sucrose-negative, ornithine decarboxylase-negative strains from *Yersinia* biotype X1 (Table 2). The %D in these 60°C reactions was 10.5 to 12, and relatedness fell to between 30% and 35% in 75°C reactions. *Yersinia* biotypes X1 and X2 do not belong to any of the named *Yersinia* species. We would predict that X1 and X2 each represent a new species, but each of these groups requires further study, including a study of DNA relatedness.

Data presented elsewhere [7,16] and in Table 2 indicated that *Y. intermedia* and *Y. frederiksenii* were each about 55–60% related to *Y. kristensenii*. *Y. kristensenii* strain 6048 was shown to be 50% related to *Y. pseudotuberculosis* and 11–29% related to representative species from most of the genera within Enterobacteriaceae [8].

*Yersinia kristensenii* was named in honor of the Danish microbiologist M. Kristensen, who first isolated this organism. The suggested pronunciation is "kris-tin-sin'-ee". The type strain of *Y. kristensenii* is 105 (=CIP 80-30) = Frederiksen P226 = Kristensen MK 104 isolated from human urine in Denmark [12]. The biochemical reactions of strain 105 are given in Table 1. It is a serogroup O11 strain [18].

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