Yersinia ruckeri sp. nov., the Redmouth (RM) Bacterium

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Cultures of the redmouth (RM) bacterium, one of the etiological agents of redmouth disease in rainbow trout (*Salmo gairdneri*) and certain other fishes, were characterized by means of their biochemical reactions, by deoxyribonucleic acid (DNA) hybridization, and by determination of guanine-plus-cytosine (G+C) ratios in DNA. The DNA relatedness studies confirmed the fact that the RM bacteria are members of the family *Enterobacteriaceae* and that they comprise a single species that is not closely related to any other species of *Enterobacteriaceae*. They are about 30% related to species of both *Serratia* and *Yersinia*. A comparison of the biochemical reactions of RM bacteria and *Serratia* indicated that there are many differences between these organisms and that biochemically the RM bacteria are most closely related to *Yersinia*. The G+C ratios of RM bacteria were approximated to be between 47.5 and 48.5%. These values are similar to those of *Yersinia* but markedly different from those of *Serratia*. On the basis of their biochemical reactions and their G+C ratios, the RM bacteria are considered to be a new species of *Yersinia*, for which the name *Yersinia ruckeri* is proposed. Strain 2396-61 (= ATCC 29473) is designated the type strain of the species.

In 1966, Ross et al. (17) gave a description of a gram-negative, rod-shaped, oxidase-negative, peritrichous, fermentative bacterium that was isolated on numerous occasions from kidney tissues of rainbow trout (*Salmo gairdneri*) afflicted with redmouth disease. It was concluded that the redmouth (RM) bacteria were members of the family *Enterobacteriaceae*, but at that time it was not possible to determine with certainty their taxonomic position within the family. In addition, Ross et al. (17) reported the results of serological studies with cultures of the RM bacterium as well as transmission of the disease from infected to normal fish through the medium of water.

Redmouth disease syndrome in rainbow trout has been known for many years (18). The syndrome can be produced by certain aeromonads and pseudomonads, as well as by the bacterium reported on here (17, 18, 20). The disease is systemic, and its major gross characteristic is inflammation in the areas of the mouth and throat. It is also known as pink mouth and pink or red throat. Clinical and pathological aspects of the disease have been reviewed by others (e.g., see references 18 and 20). The disease is enzootic in some private, state, and federal hatcheries and has become epizootic on occasion. As an epizootic in hatcheries, the disease

constitutes an important economic problem. An outbreak of redmouth disease, in which the RM bacterium was incriminated, has been reported in Saskatchewan, Canada (20).

The purpose of this paper is to characterize the RM bacterium, to name and classify it properly, and to designate the type strain of the species.

MATERIALS AND METHODS

During the last 15 years, cultures of the RM bacterium (17) were isolated, by aseptic technique, from several hundred specimens of kidney tissue of rainbow and steelhead trout or sockeye and Chinook salmon. Most cultures were from rainbow trout. The 33 strains reported upon originated in fishes from hatcheries in Alaska, Arizona, California, Idaho, Ohio, Tennessee, and Washington (Table 1).

The methods used for determining the biochemical characteristics of the above-mentioned strains were the same as those described elsewhere (11, 12, 17). Over a period of more than 10 years, the strains were tested several times on all substrates. Incubation was at 35 to 37°C and at 22 to 25°C.

The techniques used in the isolation and purification of deoxyribonucleic acid (DNA), in DNA reassociation, and in the separation of single- and double-stranded DNA on hydroxyapatite have been described in detail (5, 8, 9). The guanine-plus-cytosine (G+C) percentages were approximated by carefully denaturing double-stranded DNA in a spectrophotometer at a wavelength of 260 nm. Sealed cuvettes contained 100 µg of DNA in 1 ml of 0.15 M sodium chloride-0.015 M sodium citrate. The temperature was raised 0.4°C per min from 40 to 95°C by means of a circulating

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TABLE 1. *Strains of Yersinia ruckeri* sp. nov. studied

Center for Disease Control Strain no.	BWF ^a strain no.	Source	Geographic origin
2396-61	H1	Rainbow trout	Idaho
2397-61	H31	Rainbow trout	Idaho
2398-61	H2	Rainbow trout	Idaho
2399-61	H30	Rainbow trout	Idaho
5972-61	H3	Rainbow trout	Idaho
2973-61	H12	Rainbow trout	Idaho
5974-61	H18	Rainbow trout	Idaho
5975-61	H28	Rainbow trout	Idaho
5976-61	H29	Rainbow trout	Idaho
5977-61	H35	Rainbow trout	Idaho
2514-63	H53	Rainbow trout	Washington
2515-63	H56	Rainbow trout	Washington
2516-63	H57	Rainbow trout	Washington
2517-63	H60	Rainbow trout	Washington
1558-68	H5	Rainbow trout	Washington
1559-68	H10	Rainbow trout	Washington
1560-68	H23	Rainbow trout	Washington
1561-68	H27	Rainbow trout	Washington
1562-68	H33	Rainbow trout	Washington
1563-68	H44	Rainbow trout	Washington
1564-68	H48	Rainbow trout	Washington
4535-69	DBE1	Trout	Washington
4536-69	DBB1	Trout	Washington
1846-73	600-16	Rainbow trout	Washington
1847-73	600-17	Rainbow trout	Washington
2848-73	600-18	Chinook salmon	Washington
1849-73	600-19	Sockeye salmon	Alaska
1850-73	600-25	Rainbow trout	Idaho
1851-73	600-25A	Rainbow trout	Idaho
1852-73	600-27	Chinook salmon	Washington
2674-73	600-28	Steelhead trout	Washington
2675-73	600-29	Fish	Tennessee
2676-73	600-30	Rainbow trout	Ohio

^a All strains were received from the Bureau of Wildlife and Fisheries (BWF), Seattle, Wash.

water bath that pumped water through heating coils within the cuvette holder.

RESULTS

The results of examination of the biochemical reactions given by cultures of the RM bacterium are given in detail in Table 2 and are summarized in Table 3. The data presented in the tables are largely self-explanatory and require little comment; they differ little from those reported earlier (17).

The RM bacterium grew better at 22 to 25°C than at 35 to 37°C (Table 2). In fact, growth may not occur on simple media when such media are incubated at the higher temperature. It may be noted that the biochemical reactions of the 33 strains were quite uniform despite the fact that the strains originated in several geographi-

cal locations. Detectable gas was formed infrequently from fermentable substrates (Tables 2 and 3). Most isolates yielded negative Voges-Proskauer tests, but 24% gave weakly positive reactions, most of which were delayed (4 days) when the cultures were incubated at 22 to 25°C (Tables 2 and 3). Some strains fermented lactose slowly (14 days). However, all gave positive tests for beta-galactosidase activity within 1 h with cultures incubated at 22 to 25°C, whereas strains incubated at 35 to 37°C were negative at 1 h but became positive upon continued incubation (18 to 24 h). A majority of isolates were lipolytic (at 22 to 25°C), but none was pectolytic or oxidase positive. All of the strains utilized glucose fermentatively (Hugh and Leifson medium), and all except two reduced nitrate to nitrite.

DNA from RM strain 4535-69 was labeled with ³²PO₄, sheared, denatured, and reacted with similarly prepared unlabeled DNA from other RM bacteria and representatives of *Enterobacteriaceae* (Table 4). DNAs from RM bacteria were 95% related, and the related sequences contained almost no unpaired bases (0.1 to 0.2% divergence [D]). The RM bacterium was 9% related to *Proteus mirabilis* and *Edwardsiella tarda* and 15 to 31% related to other species of *Enterobacteriaceae*. The related sequences showed 13 to 18% D. Of the species tested, *Serratia marcescens*, *S. liquefaciens*, and "Citrobacter-like" (19) microorganisms were the closest relatives of RM bacteria. DNA relatedness results reported elsewhere show that "Citrobacter-like" organisms are 50% related to species of *Serratia* and are, in all probability, members of the genus *Serratia* (19).

Subsequent experiments with labeled DNA from species of *Serratia* and *Yersinia* (Table 5) showed that RM bacteria were 24 to 30% related to species of *Serratia* (16.5 to 17% D) and 30% related to two species of *Yersinia* (15% D). Only closely related sequences can reassociate at 75°C. At this temperature, relatedness of RM bacteria to *serratiae* and *yersiniae* decreased by three- to fourfold.

G+C determinations were done spectrophotometrically (Fig. 1). The G+C contents of the RM bacteria were approximately 47.5 to 48.5%, quite close to the values established for species of *Yersinia* (16).

DISCUSSION

Ross et al. (17) considered the RM bacteria to be members of the family *Enterobacteriaceae* but were unable to assign them to any of the genera or species then contained in that family. They pointed out that in some respects the

TABLE 2. *Biochemical characteristics of Yersinia ruckeri* sp. nov.

Test or substrate	No. of strains giving result ^a indicated							
	Incubation at 22 to 25°C (33 strains)				Incubation at 35 to 37°C (23 strains)			
	+	(+)	(+)	—	+	(+)	(+)	—
	1-2 ^b	3-7	8-14		1-2 ^b	3-7	8-14	
Hydrogen sulfide ^c	0			33	0			23
Urease	0			33	0			23
Indole	0			33	0			23
Methyl red	32			1	22			1
Voges-Proskauer	1	9		23	0			23
Citrate (Simmons)	1	31		1	0			23
KCN	8			25	4			19
Motility	27			6	0	6		17
Gelatin liquefaction	17	14		2	NT	NT		
Lysine decarboxylase	29	4		0	1	20		2
Arginine dihydrolase	1			32	1	4		18
Ornithine decarboxylase	33			0	23			0
Phenylalanine deaminase	0			33	0			23
Glucose								
Acid	33			0	23			0
Gas	3 ^d			30	1			22
Lactose	0		4 ^e	29	0			23
Sucrose	0			33	0			23
Mannitol	33			0	23			0
Dulcitol	0			33	0			23
Salicin	0			33	0			23
Adonitol	0			33	0			23
Inositol	0			33	0			23
Sorbitol	0			33	1 ^w			22
Arabinose	0			33	1 ^w			22
Raffinose	0			33	2			21
Rhamnose	0			33	0			23
Malonate	0			33	0			23
Mucate	0			33	0			23
Christensen citrate	25	8		0	3	6		14
Sodium acetate	0	8		25	0			23
Ammonium salts-glucose agar	1	13		0	NT	NT		
Sodium alginate	0			33	0			23
Lipase (corn oil)	18	7		8	0			23
Maltose	33			0	22			1
Xylose	0			33	0			23
Trehalose	32			1	21			2
Cellobiose	0			33	1 ^w			22
Glycerol	23	5		5	0	5		18
Alpha-methylglucoside	0			33	0			23
Erythritol	0			33	0			23
Esculin	0			33	0			23
Mannose	32	1		0	23			0
Melibiose	0			33	0			23
Amygdalin	0			33	0			23
Beta-galactosidase	33			0	20			3
Deoxyribonuclease	0			33	0			23
Nitrate to nitrite	28	4		1	17			6
Oxidation-fermentation	33F				23F			
Oxidase	0			33	0			23
Pectate	0			33	0			23
Cetrimide	0	8 ^w		25	0			23
Pigment	0			33	0			23
Organic acids ^f								
Citrate	0		31	0	0			23
D-Tartrate	0			33	0			23

^a Symbols: w, weakly positive reaction; F, fermentative; +, positive reaction within 48 h; (+), positive reaction between 3 and 14 days; —, negative reaction after 14 days; NT, not tested.

^b Days of incubation.

^c Triple sugar-iron or peptone-iron agar.

^d Tiny bubble to 5%.

^e Fermentation required more than 14 days.

^f Method of Kauffmann and Petersen.

TABLE 3. *Summary of the biochemical reactions at 22 to 25°C of 33 strains of Yersinia ruckeri sp. nov. including the type strain^a*

Test or substrate	Reaction	%+	Type strain ^b	Strains that gave the less common result
Hydrogen sulfide ^c	—	0	—	
Urease	—	0	—	
Indole	—	0	—	
Methyl red	+	97	+	1561-68
Voges-Proskauer	— or (+ ^w)	3 (27) ^d	—	1563-68
Citrate (Simmons)	(+)	3 (94)	+ ₄	5974-61
KCN	d	27 (27)	+ ₂	
Motility	+ or —	82	+ ₂	5976-61 2516-63 4535-69 4536-69 1846-73 2674-73
Gelatin liquefaction	+ or (+)	52 (14)	+ ₄	
Lysine decarboxylase	+ or (+)	88 (12)	+	
Arginine dihydrolase	—	3	—	1564-68
Ornithine decarboxylase	+	100	+	
Phenylalanine deaminase	—	0	+	
Glucose				
Acid	+	100	+	
Gas	— ^e	9	— ^e	1564-68 1848-73 1852-73
Lactose	— or (+)	0 (12)	—	
Sucrose	—	0	—	
Mannitol	+	100	+	
Dulcitol	—	0	—	
Salicin	—	0	—	
Adonitol	—	0	—	
Inositol	—	0	—	
Sorbitol	—	0	—	
Nitrate to nitrite	+ or (+)	85 (12)	+	1560-68
Oxidation-fermentation	F	100	F	
Oxidase	—	0	—	
Pectate	—	0	—	
Cetrimide	— or (+)	0 (24)	—	
Pigment	—	0	—	
Organic acids ^f				
Citrate	(+)	0 (100)	+ ₁₄	
D-Tartrate	—	0	—	

^a Symbols: +, 90% or more strains positive within 1 or 2 days; (+), positive reaction after 3 or more days (decarboxylase tests: 3 or 4 days); —, negative reaction (90% or more); + or —, most cultures positive, some strains negative; — or +, most strains negative, some cultures positive; + or (+), most reactions occur within 1 or 2 days, some are delayed; d, different reactions [+ , (+), or —]. F, Fermentative; w, weakly positive reaction.

^b Ross number H1 (= 2396-61 = ATCC 29473). Subscript numerals indicate day on which reaction occurred.

^c Triple sugar-iron or peptone-iron agar.

^d Figures in parentheses indicate percentages of delayed reactions (3 days or more).

^e Tiny bubble to 5%.

^f Method of Kauffmann and Petersen.

biochemical reactions of RM bacteria resembled those of some cultures of *S. marcescens* (particularly the *kiliensis* biotype), *S. liquefaciens*, and *Arizona hinshawii*. At that time the genus *Yersinia* was not included in the family *Enterobacteriaceae*.

Representative strains of the RM bacterium were sent to Rudolph Hugh, Department of Microbiology, George Washington University, Washington, D.C., who made flagella stains (Leifson method) and reported that the bacteria were peritrichous (17). This observation was confirmed by electron photomicrography (see Fig. 1 in reference 17). All strains of RM bacteria were composed of gram-negative, rod-shaped cells. All were fermentative and all yielded negative oxidase tests. Erwin Neter of Children's Hospital, Buffalo, N.Y., reported (personal communication, 1974) that 12 strains of RM bacteria

examined possessed the antigen common to the *Enterobacteriaceae* (15).

Comparison of the biochemical reactions of RM bacteria with those given by members of other genera of *Enterobacteriaceae* (11) indicates that the RM bacteria are more closely related to *Serratia* (13, 14) and *Yersinia* (1, 10) than to any other members of the family. However, close examination shows that there are many more biochemical differences between RM bacteria and members of the three species of *Serratia* than there are between the RM bacteria and members of the three species of *Yersinia* (Table 6). Examination of the aggregate biochemical reactions given by RM bacteria (Tables 2 and 3) and data reported for *Yersinia pestis*, *Y. pseudotuberculosis*, and *Y. enterocolitica* (1, 10) reveals many similarities, which are apparent in Table 6. Although the RM bac-

TABLE 4. DNA relatedness of RM bacteria and members of the family *Enterobacteriaceae* to RM strain 4535-69

Source of unlabeled DNA	RBR ^a (60°C)	% D ^b	Source of unlabeled DNA	RBR ^a (60°C)	% D ^b
RM 4535-69	100	0.0	"Citrobacter-like" 4554-71	27	
RM 5972-61	95	0.2	"Citrobacter-like" 4557-71	24	
RM 2396-61	94	0.1	<i>Hafnia alvei</i>	24	15.7
RM 2514-63	94	0.1	<i>Erwinia nigrifluens</i> EN101	21	
<i>Serratia marcescens</i> 868-56	31	13.0	<i>Pectobacterium carotovorum</i>		
<i>S. marcescens</i> SM6 W2	31	14.8	EC495	21	18.3
<i>S. marcescens</i> 866-67	29	15.2	<i>Citrobacter freundii</i> 876-58	21	
<i>S. marcescens</i> 1201-65	28		<i>Enterobacter cloacae</i> 1347-71	20	16.0
<i>S. marcescens</i> (biotype <i>kiliensis</i>)	26		<i>Pectobacterium carnegieana</i>		
<i>S. marcescens</i> 863-57	25	15.0	EC186	19	
<i>S. marcescens</i> 864-57	25	14.7	<i>Enterobacter agglomerans</i> EH103	19	16.4
<i>Serratia liquefaciens</i> 1107-57	29		<i>Citrobacter freundii</i> 460-61	19	14.6
<i>S. liquefaciens</i> 1284-57	28	14.4	<i>Salmonella typhimurium</i> LT2	19	13.3
<i>S. liquefaciens</i> 1286-57	27		<i>Escherichia coli</i> K-12	18	16.1
<i>S. liquefaciens</i> 446-68	27		<i>Enterobacter aerogenes</i> 1627-66	18	14.6
<i>S. liquefaciens</i> (biotype <i>lipolyticus</i>)	26	14.8	<i>Klebsiella rhinoscleromatis</i> 895-68	18	12.8
<i>Serratia rubidaea</i> 5474-68	24		<i>Erwinia amylovora</i> EA178	18	16.7
<i>S. rubidaea</i> 4445-64	23	14.1	<i>Pectobacterium cypripedii</i>	17	
<i>S. rubidaea</i> 4057-71	21		<i>Enterobacter cloacae</i> (yellow) 256-4	17	
<i>S. rubidaea</i> 934-72	21	14.2	<i>Proteus morganii</i>	15	
"Citrobacter-like" 4553-71	29		<i>Proteus mirabilis</i> PM1	9	
"Citrobacter-like" 4556-71	28	15.8	<i>Edwardsiella tarda</i> 3592-64	9	
"Citrobacter-like" 4552-71	27				

^a RBR, Relative binding ratio. Relative binding ratio is a convenient way to express percent DNA relatedness.

$$\text{RBR} = \frac{\% \text{ heterologous DNA reaction}}{\% \text{ homologous DNA reaction}} \times 100$$

^b % D, Percent divergence, calculated on the assumption that a 1-C degree decrease in thermal stability of a DNA duplex is caused by each 1% of unpaired bases within that duplex (3). For example, consider organisms A and B that are 50% related. An A-A duplex has a mean thermal stability of 90°C, and an A-B duplex has a mean thermal stability of 80°C. The D in related DNA is 10%. Reactions were done two to four times. DNA reassociation in the homologous RM reaction averaged 83%. DNA reassociation in the homologous RM reaction averaged 83%. DNA reassociation in control reactions that contained labeled DNA in the absence of unlabeled DNA were 2% or less.

TABLE 5. DNA relatedness of *serratiae* and *yersinia* to RM bacteria

Source of labeled DNA	Relatedness to RM bacteria		
	RBR ^a (60°C)	% D ^b (60°C)	RBR ^a (75°C)
<i>Serratia marcescens</i>			
868-57	26 (4) ^c	17.0	7 (2)
<i>S. liquefaciens</i> 446-68	30 (2)	17.2	10 (2)
<i>S. rubidaea</i> 934-72	24 (4)	16.6	
<i>Yersinia enterocolitica</i>			
501-70	30 (4)	15.1	8 (1)
<i>Y. pseudotuberculosis</i>			
P105	30 (3)	15.1	8 (2)

^{a, b} See footnotes to Table 4.

^c The numbers in parentheses indicate the number of strains tested. All reactions were done at least twice. Average DNA reassociation in homologous reactions was: *S. marcescens*, 78% at 60°C and 86% at 75°C; *S. liquefaciens*, 74% at 60°C and 75% at 75°C; *S. rubidaea*, 75% at 60°C; *Y. enterocolitica*, 78% at 60°C and 74% at 75°C; *Y. pseudotuberculosis*, 69% at 60°C and 65% at 75°C. DNA reassociations in control reactions that contained labeled DNA in the absence of unlabeled DNA were 3% or less.

FIG. 1. Spectrophotometric determination of G+C composition in RM bacteria. The increase in ultraviolet absorption at 260 nm with increased temperature was measured for four strains of RM bacteria and several strains of *Enterobacteriaceae* of known G+C composition. T_m is temperature at which one-half of the maximal increase in ultraviolet absorption (hyperchromicity) has occurred. The T_m values for RM bacteria (●) were 73.7, 73.5, and 73.2°C (two strains).

teria biochemically are most closely related to *yersinia*, they are easily differentiable from all three established species of *Yersinia* (Table 6) and should be recognized as a new species within that genus.

DNAs from strains of a given species are usually at least 70% related (4, 6). The four RM strains certainly belong to the same species on the basis of DNA relatedness. Except for most protei, species of *Enterobacteriaceae* share a "core" DNA relatedness of 15 to 20%. RM bacteria exhibit this level of core relatedness. They are not closely related to any species of *Enterobacteriaceae* but show about 30% relatedness to species of *Serratia* and *Yersinia*. Thus, from DNA data, one can argue that RM bacteria can be put in either of these genera.

The G+C ratios in *Enterobacteriaceae* are from 37% in some protei to almost 60% in strains of *Serratia* and *Klebsiella*. Definitive classification based on G+C rarely is possible. Similar G+C ratios cannot be equated with species similarity (humans, *Bacillus subtilis*, and *Proteus rettgeri* have similar G+C ratios), but organisms with markedly different G+C ratios cannot belong to the same species. The G+C ratios of *serratiae* (58%) and *yersinia* (46 to 48%) are significantly different. The G+C ratios in four RM bacteria were approximated to be between 47.5 and 48.5%. These results indicate that RM bacteria do not belong to the genus *Serratia* and are consistent with those for the genus *Yersinia*.

The name *Yersinia ruckeri* is proposed for the RM bacterium (ruck'er.i. M. L. gen. n. *ruckeri* of Rucker, named in honor of Robert R. Rucker, who spent many years studying red-mouth disease and its etiological agents). Strain 2396-61 (= American Type Culture Collection [ATCC] 29473) is the type strain of the species.

According to the proposals made, the nomenclature of the members of the tribe *Yersinieae* is as follows:

Tribe *Yersinieae* Martinevskij

Genus *Yersinia* van Loghem

1. *Yersinia pestis* (Lehmann and Neumann) van Loghem
2. *Yersinia pseudotuberculosis* (Pfeiffer) van Loghem
3. *Yersinia enterocolitica* (Schleifstein and Coleman) Frederiksen (species will be subdivided in the future [8]).
4. *Yersinia ruckeri* sp. nov.

REPRINT REQUESTS

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TABLE 6. Major biochemical differences between *Y. ruckeri*, *serratiae*, and other yersiniae

Test or substrate	<i>Y. ruckeri</i> (22–25°C) ^a	<i>S. marcescens</i> (35–37°C)	<i>S. liquefaciens</i> (35–37°C)	<i>S. rubidaea</i> (35–37°C)	<i>Y. pestis</i> (35–37°C)	<i>Y. pseudotuberculosis</i> (22–25°C)	<i>Y. enterocolitica</i> (22–25°C)
Urease	0 ^b	40 ^w (22) ^c				100	91 (7) _d
Indole	0						
Methyl red	97	18	37	24			
Voges-Proskauer	3 (21)	99	86	94			87
Citrate (Simmons)	3 (94)	98 (1)	94 (6)	88 (2)			
KCN	24	99	92				
Motility (35°C)	85	95	93	88		0	0
Gelatin liquefaction (22°C)	52 (42)				0	0	0
Lysine decarboxylase	88 (12)				0	0	0
Ornithine decarboxylase	100		0	0	0	0	
Malonate	0			86			
Sodium acetate	0 (24)	84	38 (38)	67 (14)	ND ^e	0	7 (44)
Gas from glucose	12	53	73 (1)	35 (4)			
Lactose	0 (12)		16 (21)	100			
Sucrose	0	99	98	96			96
Salicin	0	95 (2)	96	88 (4)	19	5 (95)	13 (15)
Adonitol	0	47 (14)		88 (2)			
Inositol	0	77 (6)	64 (26)	35 (16)			9 (32)
Sorbitol	0	99	97				99
Arabinose	0		97	100	30	50 (45)	99 (1)
Raffinose	0		91 (5)	96		20	
Rhamnose	0					100	
Xylose	0	7 (17)	99 (1)	98	100	100	36 (3)
Cellobiose	0	14 (25)	27 (36)	90			88 (12)
Erythritol	0	8 (25)					
Esculin	0	73	95	90	100	100	15 (21)
Melibiose	0		74 (8)	96		90 (10)	
Amygdalin	0	100	100	94			95 (4)
Deoxyribonuclease	0	97	88	100	(100)	0 (30)	
Pigment	0	16		61			
Organic acids ^f							
Citrate	0 (100)	100	65	2 (68)	ND		0 (25)
D-Tartrate	0	2 (19)	22				

^a The results given for *Y. ruckeri* (from Table 2), *Y. pseudotuberculosis*, and *Y. enterocolitica* (references 1 and 10) were obtained at 22 to 25°C, whereas those given for the species of *Serratia* (11, 13, 14) and for *Y. pestis* (1, 10), were for cultures incubated at 35 to 37°C. *S. liquefaciens* is known to be more reactive at 22 to 25°C than at 35 to 37°C (11, 13, 14), but reactions given by *S. marcescens*, *S. rubidaea*, and *Y. pestis* are not known to be affected significantly by incubation at temperatures between 22 and 37°C. Therefore, it is believed that the data given for the various bacteria listed in Table 5 can be compared.

^b Percent positive within 1 or 2 days.

^c Percent positive in 3 or more days.

^d The majority (67 to 83%) of cultures isolated in the United States have been indole positive (2, 7, 10).

^e ND, Not determined.

^f Method of Kauffmann and Petersen.

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