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Deoxyribonucleic Acid Relatedness of Serovars of *Yersinia ruckeri*, the Enteric Redmouth Bacterium

S. A. DE GRANDIS, P. J. KRELL, D. E. FLETT, AND R. M. W. STEVENSON*
Department of Microbiology, University of Guelph, Guelph, Ontario N1G 2W1, Canada

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The name *Yersinia ruckeri* was initially applied to a group of serologically and biochemically homogeneous bacteria from diseased salmonid fish. Subsequently, isolates that differed in serology and in their ability to ferment D-sorbitol were also called *Y. ruckeri*. Strains of serovars II, III, V, and VI showed a high degree of relatedness in dot-blot deoxyribonucleic acid hybridization assays on nitrocellulose filters. In reciprocal tests, serovar I and II isolates had relative binding ratios (RBRs) of greater than 70%. Serovar V isolates appeared more closely related to serovar II (85% RBR) than to serovar I (70% RBR) strains. Serovar III strains appeared closely related to both serovar I and II strains, with the exception of a single sorbitol-fermenting strain. The single serovar IV isolate was excluded from *Y. ruckeri* (8% RBR), as were other isolates that ferment L-arabinose, D-xylose, and L-rhamnose. *Y. ruckeri* had low levels of hybridization with *Hafnia alvei*, *Salmonella cholerae-suis* subsp. *arizonae*, and *Yersinia enterocolitica* in dot blots and colony hybridization. Overall, the description of *Y. ruckeri* can be broadened to include isolates that differ from previously studied strains in their serological reactions and ability to ferment D-sorbitol.

Yersinia ruckeri is the causative agent of enteric redmouth (ERM) disease of salmonid fish, which has produced significant losses of rainbow trout (*Salmo gairdneri*) in aquaculture operations (4). The disease was first a problem in the Hagerman Valley of Idaho, but later was found in other places where salmonids are intensively cultured (2, 3). Ross et al. (22) first characterized the disease agent as a member of the family *Enterobacteriaceae*, but it continued to be called the redmouth or ERM bacterium because it was not closely similar to any particular one of the described species and genera. Ewing et al. (10) eventually placed the redmouth bacterium in the genus *Yersinia*, as *Yersinia ruckeri*, on the basis of deoxyribonucleic acid (DNA) hybridization and guanine-plus-cytosine content. The 29 isolates examined in that work were from disease outbreaks in the western United States and appeared to be homogeneous in their biochemical reactions and serology (10, 22).

Since that study, new serological groups of the ERM bacterium have been found, notably the sorbitol-fermenting isolates from chinook salmon (*Oncorhynchus tshawytscha*) described by O'Leary et al. (21), which form serovar II. Bullock et al. (3) suggested that a group of isolates from Australian fish formed a third serological group (serovar III). Other Australian isolates, called the salmonid blood spot bacterium by Llewellyn (16), partially cross-reacted with serovar I isolates of *Y. ruckeri* and were designated serovar I' (23, 24). Three additional serovars, IV, V, and VI, have also been identified (6, 23, 24).

Isolates belonging to the new serovars were identified as *Y. ruckeri* largely on the basis of biochemical test reactions. Some isolates differed from the strains described by Ewing et al. (10) in one or more tests, particularly D-sorbitol fermentation. Serovar I strains are the most common isolates from diseased fish (19), although serovar II isolates have been associated with ERM in chinook salmon (5, 21). It was important to test the authenticity of the additional serovars, as many fish health protection regulations equate finding isolates of *Y. ruckeri* with detection of the ERM disease bacterium. In this study, DNA hybridization reactions were used to test relationships between the various serovars and strains, using filter-immobilized dot-blot and colony-blot methods (13, 15). These are rapid methods, with potential applications in diagnostic screening. We demonstrate here that the general description of the species *Y. ruckeri* can be broadened to include D-sorbitol-fermenting isolates and several additional serological groups.

MATERIALS AND METHODS

Bacterial strains. The isolates of *Y. ruckeri* examined are listed in Table 1, with their sources and serovars indicated. The serovar designations were based on microtiter agglutination tests with antisera raised against Formalin-killed whole cells and on cross-absorbance tests, as described previously (24, 25). The serovar I strains include *Y. ruckeri* ATCC 29473^T and *Y. ruckeri* ATCC 29904, the DNA reference strain (10). The serovar II strains include the sorbitol-fermenting reference strain Big Creek 74 described by O'Leary et al. (20, 21). *Yersinia enterocolitica* CDC 2383 (RS31) was provided by P. Gemski, Walter Reed Army Institute of Research, Washington, D.C. Other bacteria used were obtained from the stock culture collection of the Department of Microbiology, University of Guelph, or were isolates identified by the Fish Health Laboratory, Department of Microbiology, University of Guelph. In general, cultures were grown on Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) or in Trypticase soy broth (BBL Microbiology Systems) at 25°C. Stock cultures were maintained both on tightly stoppered slants at 15°C and as lyophilized cultures.

Biochemical tests. Isolates were characterized and identified as before (25). Biochemical tests were performed by the conventional methods described by Ewing (9) and with API 20E test strips (Analytab Products, Montreal, Quebec, Canada), both incubated at 25°C. Fermentation tests were done in Hugh-Leifson basal medium, with filter-sterilized sugars added to a final concentration of 0.5% (wt/vol). A Steers multipoint replicator apparatus was used to inoculate plates

* Corresponding author.

TABLE 1. *Y. ruckeri* isolates used in this study

Laboratory code	Other designation	Geographic source	Serovar[a]	Fish source[b] and year isolated
RS1	ONT270	Ontario	IV	Brown trout, 1981
RS2	ONT258	Ontario	II	Rainbow trout,.1981
RS3	BC74	Oregon	II	Chinook salmon, 1974
RS4	11.4	Utah	I	Rainbow trout, 1968
RS6	ONT193	Ontario	II	Brook trout, 1980
RS7	R08-85	Ontario	I	Muskrat, 1978
RS8	ONT288	Ontario	II	Cisco, 1981
RS9	9-4-5	Nova Scotia	I	Rainbow trout, 1976
RS10	ONT339	Ontario	II	Brown trout, 1981
RS11	ATCC 29473[Tc]	Idaho	I	Rainbow trout
RS12	ATCC 29908	Washington	I	Rainbow trout
RS13	C75-199	British Columbia	I	Rainbow trout, 1975
RS14	ONT347	Ontario	II	Rainbow trout, 1981
RS20	0634	Australia	I′	Brook trout
RS21	0589	Australia	I′	Atlantic salmon
RS24	2.87	Colorado	V	Rainbow trout, 1978
RS26	Path 126-76	Idaho	I	Rainbow trout, 1977
RS27	2#C-M#8	Idaho	I	Rainbow trout, 1980
RS28	9-4-1	Unknown	I	Unknown
RS32	9-4-5	Nova Scotia	I[d]	Rainbow trout, 1976
RS33	YR12	New Mexico	I	Rainbow trout, 1978
RS34	YR20	California	I	Rainbow trout, 1978
RS35	JW10-76	Unknown	I	Unknown
RS36	JW8-78	Unknown	I	Unknown
RS37	JW76-78	Wisconsin	I	Rainbow trout, 1976
RS38	JW77-46	Indiana	I	Rainbow trout, 1977
RS39	JW77-71	Ohio	I	Rainbow trout, 1977
RS40	JW78-74	Wisconsin	I	Rainbow trout, 1978
RS41	JW79-52	Wisconsin	I	Rainbow trout, 1979
RS42	JW81-29	Missouri	I	Rainbow trout, 1981
RS43	JW81-48-4	Ohio	I	Rainbow trout, 1981
RS44	82.091	British Columbia	II	Rainbow trout, 1982
RS45	82.077	British Columbia	I	Steelhead trout, 1982
RS46	JW82-A	Ohio	I	Rainbow trout, 1982
RS47	JW82-B	Ohio	I	Rainbow trout, 1982
RS48	OS76	Oregon	I	Rainbow trout, 1976
RS49	11.54	Virginia	I	Rainbow trout, 1978
RS50	TH75	Oregon	I	Coho salmon, 1975
RS51	11.34	Colorado	I	Rainbow trout, 1978
RS52	11.40	North Carolina	I	Rainbow trout, 1978
RS53	82-75-3	Colorado	II	Rainbow trout, 1982
RS54	YR-40	British Columbia	I′	Dolly Varden trout, 1982
RS55	ONT487	Ontario	I	Rainbow trout, 1982
RS61	42/81	England	I	Rainbow trout, 1981
RS62	83-206-1	British Columbia	V	Whitefish, 1983
RS63	F.15.1/83	West Germany	UT	Rainbow trout, 1983
RS64	F.111/81	West Germany	I	Rainbow trout, 1981
RS66	ONT518	Ontario	II	Lake trout, 1983
RS68	83-106-7	British Columbia	I	Steelhead trout, 1983
RS69	ONT534	Ontario	I	Lake trout, 1983
RS72		Unknown	I	Unknown
RS74		Manitoba	UT	Whitefish, 1984
RS76	F979.T43/5	France	I	Rainbow trout
RS77	11.43	Australia	III	Rainbow trout, 1959
RS78	11.44	Australia	III	Rainbow trout, 1959
RS79	11.45	Australia	III	Rainbow trout, 1959
RS80	16A	Ontario	VI	Rainbow trout, 1984
RS81	84-024#3	British Columbia	V	Cutthroat trout, 1984
RS82	84-081	British Columbia	II	Chinook salmon, 1984
RS83	025#3	British Columbia	I	Steelhead trout, 1984
RS85		Saskatchewan	I	Unknown, 1984
RS86	84-247#1	British Columbia	I	Steelhead, 1984
RS88	8405-25 4/2	Denmark	I	Rainbow trout, 1984

[a] Serovars are as defined previously (3, 6, 24). Untyped (UT) strains did not react with antisera to any of the other strains.

[b] Fish sources are rainbow trout (*S. gairdneri*); lake trout (*Salvelinus namaycush*); steelhead trout (*S. gairdneri*); Dolly Varden trout (*Salvelinus malma*); whitefish (*Coregonus clupeaformia*); cutthroat trout (*Salmo clarkii*); chinook salmon (*O. tshawytscha*); brown trout (*Salmo trutta*); cisco (*Coregonus artedii*); brook trout (*Salvelinus fontinalis*); Atlantic salmon (*Salmo salmo*). Muskrat is *Ondatra zibethica*.

[c] T, Type strain.

[d] Isolation source is the same as RS9, but a minor antigenic difference occurs (24).

for testing citrate, malonate, and acetate utilization and reactions on casein, Tween, and blood agars.

Preparation of DNA. Chromosomal DNA was isolated by the method of Marmur et al. (18), with the modifications described by Stevenson and Airdrie (23) for lysing cells. That is, the cells were incubated in 2% (wt/vol) Triton X-100 for 5 min at 57°C before being lysed with 10% (wt/vol) sodium dodecyl sulfate for 10 min at 57°C. For DNA work, all the saline-sodium citrate (SSC) buffer concentrations are based on 1× SSC containing 0.15 M NaCl and 0.015 M sodium citrate. Before the last ethanol precipitation step, the DNA was treated with proteinase K (Sigma Chemical Co., St. Louis, Mo.) at a final concentration of 1 mg/ml and incubated at 37°C for 1 h followed by 18 h at room temperature. The DNA samples were considered to be free of protein contamination when the ratio of spectrophotometric readings at 260 and 280 nm gave values of 1.75 to 1.80. DNA from *Y. ruckeri* ATCC 29908 (RS12) and BC74 (RS3) and from *Y. enterocolitica* CDC 2383 (RS31) was radioactively labeled by using a nick translation kit and [^{32}P]dCTP (3,000 Ci/mmol; Amersham Corp., Arlington Heights, Ill.). Labeled DNA had specific activities of 10^7 to 10^8 cpm/μg. (Initially, DNA from *Y. ruckeri* RS12 was labeled in vivo by growing cells in defined medium [M9] with [^{3}H]thymidine. This procedure was abandoned when we found that DNA from *Y. ruckeri* RS3 could not be labeled in this way.)

Preparation of nitrocellulose filters. The dot hybridization procedures of Kafatos et al. (15) were used essentially as described previously. DNA at 100 μg/ml in 0.1× SSC buffer was sonicated for two 30-s bursts (Biosonic III; 0.375-in. [0.953-cm] probe used at an energy setting of 60). The resulting fragments were approximately 10^5 daltons, based on comparisons with size standards in agarose gel electrophoresis. DNA samples were denatured by adding sufficient 5 M NaOH to give a final concentration of 0.2 M and incubating for 10 min at 25°C. Denaturation was stopped by adding 10 M ammonium acetate at 0.1 of the reaction volume. The mixtures were gently vortexed and then stored on ice.

Nitrocellulose filters (BA 85/21; Schleicher & Schuell, Inc., Keene, N.H.) were prewet, first in water and then in 1.0 M ammonium acetate. Each filter was placed in a 96-well vacuum manifold (Minifold; Schleicher & Schuell), and DNA samples were aspirated onto the filter at 1 to 10 μg per spot, as indicated in the figure legends, and then rinsed twice with 500 ml of 1 M ammonium acetate. The filters were removed, rinsed gently for 2 min in 200 ml of 4× SSC, and then air dried for 1 h at 25°C before baking at 80°C for 2 h.

Filters were prehybridized in a buffer containing 5× SSC, 50% formamide, 50 mM sodium phosphate (pH 6.5), 0.1% glycine, 250 mg of denatured and sonicated calf thymus DNA per ml, and 5× Denhardt solution (17). This was replaced with the same buffer containing the radioactive DNA at a final concentration of 10^6 cpm/ml. The filters were hybridized for 48 h at 26°C (T_m − 45°C) 42°C (T_m − 29°C) or 60°C (T_m − 10°C) and then rinsed for 15 min in each of a series of SSC buffers (4×, 2×, 1×, 0.5×, and 0.1× SSC) containing 0.1% sodium dodecyl sulfate. The filters were dried and then exposed to Dupont x-ray film, with a Kodak X-Omat intensifying screen used when required.

Numerical values for hybridization were obtained by cutting each of the DNA dots from the filters and placing them in toluene-based scintillation fluid for counting. The relative binding ratios (RBRs) for the strains were calculated by dividing the radioactive counts bound by DNA from heterologous strains by the counts bound by DNA from the strain used as the source of the radioactive DNA probe and expressing the result as a percentage. Values were calculated with an average of five hybridization replicates for each sample, and confidence limits for the percent RBR values were calculated by an interval estimate equation (14).

Colony hybridization. Filters were prepared for colony hybridization by a modification of the method of Grunstein and Hogness (13). The wells of a microtiter plate were filled with 0.2 ml Trypticase soy broth, and each well was inoculated with a colony from a 24-h-old plate culture. After 18 h of incubation at 25°C, the bacteria were lysed by placing the filters, colony-side up, for 3 min on Whatman 3MM paper saturated with 0.5 M NaOH–1.5 M NaCl. The filters were neutralized with 0.5 M Tris hydrochloride (pH 7.5)–1.5 M NaCl for 5 min, rinsed in 95% ethanol, air dried, and then baked for 2 h at 80°C. Before hybridization, the filters were prerinsed at room temperature for 1 h in 500 ml of buffer (50 mM Tris hydrochloride [pH 8.0], 1 M NaCl, 1 mM EDTA, 0.1% sodium dodecyl sulfate). Hybridizations were done under the same conditions as for the dot hybridizations.

RESULTS

Biochemical tests. Only two isolates, RS1 (serovar IV) and RS74, produced acid from L-arabinose, D-xylose, and L-rhamnose. D-Sorbitol was fermented by all serovar II and serovar V isolates and by RS54 (serovar III). Serovar I isolates failed to ferment D-sorbitol. With a few exceptions, other biochemical test results for isolates of all serovars were as reported for serovar I strains by Ewing et al. (10). Strains did show variations in reactions for gelatin liquefaction, the Voges-Proskauer reaction (4 days at 25°C), Tween 80 hydrolysis, and production of a lecithin-dependent hemolytic activity on rabbit erythrocytes (data not shown). However, none of these differences correlated with serological groups. Gas production from glucose initially appeared to be common for serovar II strains and rare for serovar I strains, but when tubes were incubated at 18°C rather than 25°C, most strains of all serovars did produce gas.

Dot hybridization. Dot hybridization with ^{32}P-labeled DNA was somewhat specific for DNA homologous to the probe, as seen by comparison of the cross-reactions between DNA from *Y. ruckeri* ATCC 29908 and that from *Y. enterocolitica* RS31 (Fig. 1), even though some degree of cross-hybridization was observed. Under conditions of lower stringency (Fig. 1A and B), this specificity was not as apparent as at higher stringency (Fig. 1C and D). With ^{32}P-labeled DNA from *Y. enterocolitica* RS31 as the probe, there was much less binding to DNA from *Y. ruckeri* (Fig. 1B and D). The apparently stronger reaction between labeled DNA from *Y. ruckeri* and DNA from *Y. enterocolitica* compared with the reciprocal reaction may be due to differences in the specific activities of the two probes. Binding of both probes to homologous DNA remained strong at two higher levels of stringency, 42°C (T_m − 29°C) and 60°C (T_m − 10°C) (Fig. 1C and D; data not shown). Hybridizations between *Y. ruckeri* and *Y. enterocolitica* DNAs were significantly reduced at the higher temperatures (Fig. 1; data not shown).

Probe DNA from the serovar II strain *Y. ruckeri* BC74 also hybridized strongly to DNA from strains belonging to serovars I, III, and V at the three levels of stringency (Fig. 2). The RBRs calculated for 37 strains of *Y. ruckeri* indicated good hybridization with labeled DNA from both *Y. ruckeri* ATCC 29908 (serovar I) and *Y. ruckeri* BC74 (serovar II)

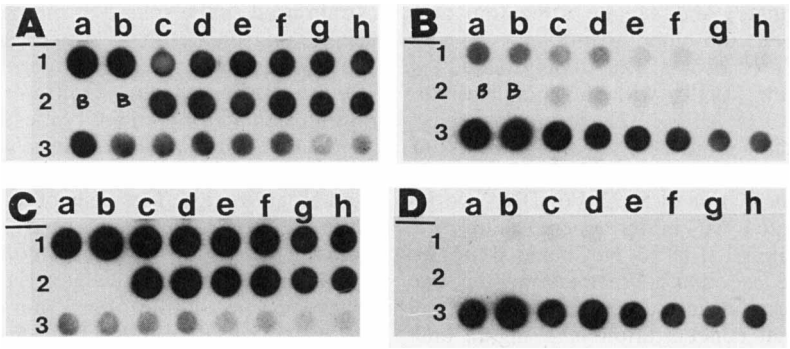

FIG. 1. Autoradiogram of dot-blot hybridizations using various amounts of DNA from *Y. ruckeri* ATCC 29908 (RS12) and *Y. enterocolitica* RS31. The probes used were ^{32}P-labeled DNA from *Y. ruckeri* ATCC 29908 (A and C) and ^{32}P-labeled DNA from *Y. enterocolitica* RS31 (B and D). Hybridizations were done at 26°C (A and B) or 42°C (C and D) with 50% formamide for 48 h. In each hybridization, row 1 contained DNA from *Y. ruckeri* ATCC 29908 as duplicate samples with the following amounts of DNA per dot: columns a and b, 10 μg; c and d, 5 μg; e and f, 2.5 μg; g and h, 1.25 μg. In row 2, columns a and b were calf thymus DNA controls, with the remaining columns as for row 1. Row 3 contained DNA from *Y. enterocolitica* RS31 in the same amounts as for row 1. B, Blank.

(Table 2). At lower levels of stringency (26°C), the general pattern was the same (data not shown). Plasmid DNA from *Y. ruckeri* ATCC 29908 had less than 1.5% hybridization with the serovar I or serovar II chromosomal DNA probes (data not shown); thus, the plasmids common to many serovar I strains (7) contribute little to relatedness values.

Some differences were observed among the percent RBR values for serovars, for example, with serovar V strains (Table 2). Also, the values for one of the serovar III strains, RS54, were lower than for other strains. Both this strain and the serovar V isolates showed stronger hybridization with serovar II DNA than with serovar I DNA. Variation between replicates was relatively high, ranging from 3 to 25% (average, 10.5%), making it difficult to assess the significance of less evident variations. RS1, the single isolate forming serovar IV, and the untyped strain RS74 showed low hybridization reactions (Table 2). The single serovar VI isolate, RS80, and an untyped isolate, RS63, did hybridize well with probe DNA from *Y. ruckeri* (Fig. 2, columns e and h; Table 2). Strains of *Y. enterocolitica*, *Salmonella cholerae-suis* subsp. *arizonae*, and *Hafnia alvei* gave RBRs of 15% or less at 42°C with either probe (Table 2; Fig. 2).

Colony hybridizations. When ^{32}P-labeled DNA from *Y. ruckeri* BC74 (serovar II) was used as a probe against colony blots, all strains except the previously excluded RS1 and RS74 gave a positive signal (Fig. 3). Colonies of five different strains of *Y. enterocolitica* and a variety of other members of the family *Enterobacteriaceae* and aeromonads also gave no significant reaction. Similar results were obtained when the labeled DNA was from serovar I strain ATCC 29908 and when the level of stringency was increased by hybridizing at 60°C (Fig. 3) rather than at 42°C (data not shown).

DISCUSSION

The dot-blot DNA hybridization results demonstrate that the description of *Y. ruckeri* given by Ewing et al. (10) can be

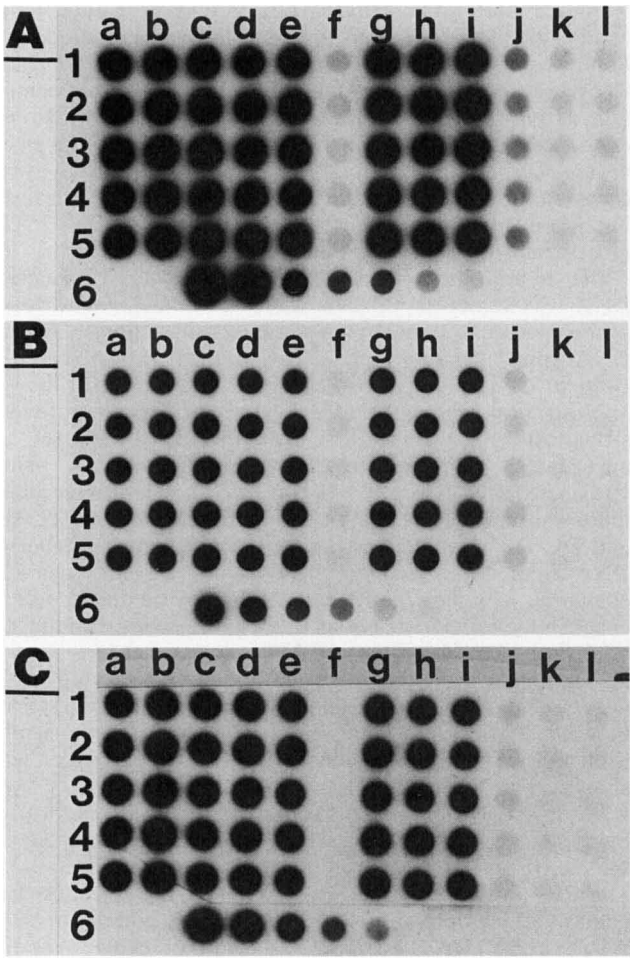

FIG. 2. Autoradiogram of dot-blot hybridizations of DNA from *Y. ruckeri* BC74 (RS3) with DNA from various serovars of *Y. ruckeri* and from other enteric bacteria. Hybridization was done at 26°C (A), 42°C (B), and 60°C (C) with 50% formamide for 48 h. DNA samples of 1 μg were spotted onto a nitrocellulose filter which was then hybridized with ^{32}P-labeled total chromosomal DNA from *Y. ruckeri* BC74. The columns represent DNA from the following bacterial strains: a, *Y. ruckeri* ATCC 29908 (serovar I); b, *Y. ruckeri* BC74 (serovar II, homologous reaction); c, *Y. ruckeri* RS77 (serovar III); d, *Y. ruckeri* RS24 (serovar V); e, untyped strain RS63; f, untyped strain RS74; g, untyped strain RS80; h, *Y. ruckeri* RS82 (serovar II); i, *Y. ruckeri* RS54 (serovar III); j, *Y. enterocolitica* RS31; k, *S. cholerae-suis* subsp. *arizonae* DMG 602; l, *Escherichia coli* DMG 566. Rows 1 to 5 are replicates of each sample. Row 6 shows the following controls: a and b, 10 μg of calf thymus DNA; c to i, doubling dilutions of the probe DNA, beginning with 2.5 μg per dot.

TABLE 2. Relative levels of DNA relatedness of serovars of *Y. ruckeri* and other species of *Enterobacteriaceae*

Source of unlabeled DNA	RBR (%) at 42°C to labeled DNA from[a]:	
	Y. ruckeri ATCC 29908 (RS12; serovar I)	*Y. ruckeri* BC74 (RS3; serovar II)
Y. ruckeri serovar I		
RS12	100[b]	95 ± 10
RS7	103 ± 20	88 ± 17
RS9	102 ± 13	77 ± 10
RS11[T]	113 ± 12	100 ± 11
RS13	112 ± 12	91 ± 11
RS26	103 ± 12	84 ± 9
RS33	100 ± 12	72 ± 16
RS39	106 ± 12	76 ± 9
RS41	108 ± 12	83 ± 9
RS45	104 ± 8	83 ± 14
RS48	115 ± 6	88 ± 15
RS61	100 ± 13	94 ± 19
RS76	91 ± 10	86 ± 11
Y. ruckeri serovar II		
RS3	97 ± 10	100[b]
RS2	91 ± 5	86 ± 13
RS6	90 ± 4	78 ± 11
RS8	99 ± 6	89 ± 11
RS10	106 ± 16	95 ± 11
RS14	106 ± 16	90 ± 11
RS44	88 ± 16	82 ± 12
RS53	79 ± 6	79 ± 9
RS66	94 ± 13	83 ± 9
RS82	86 ± 3	76 ± 11
Y. ruckeri serovar III		
RS20	86 ± 8	101 ± 7
RS21	97 ± 11	111 ± 8
RS54	54 ± 8	73 ± 6
RS77	82 ± 9	91 ± 9
RS78	86 ± 13	94 ± 5
RS79	88 ± 13	99 ± 6
Y. ruckeri serovar IV		
(RS1)	15 ± 5	8 ± 5
Y. ruckeri serovar V		
RS24	71 ± 9	86 ± 5
RS62	75 ± 10	95 ± 5
RS81	NT[c]	90 ± 4
Untyped isolates		
RS63	79 ± 5	84 ± 8
RS74	3 ± 3	7 ± 5
RS80	92 ± 5	82 ± 7
Y. enterocolitica		
RS31	8 ± 4	16 ± 6
S. cholerae-suis subsp. *arizonae*	3 ± 4	7 ± 5
H. alvei	3 ± 4	6 ± 6

[a] RBR were measured by dot-blot hybridization on nitrocellulose followed by liquid scintillation counting, as described in Materials and Methods.
[b] Homologous reaction for this column.
[c] NT, Not tested.

broadened to include isolates that differ from the previously studied strains in serological reactions. The sorbitol-fermenting serovar II strains described by O'Leary et al. (20, 21) are clearly shown to be closely related to the original serovar I strains (Table 2), as are isolates of serovars III, V, and VI. DNA hybridization results confirm that the salmonid blood spot bacteria (16), represented by strains RS20 and RS21, are authentic isolates of *Y. ruckeri*, as suggested previously by serological and biochemical tests (23, 24).

The DNA hybridization results clearly disqualify the single isolate representing serovar IV, RS1, and the untyped strain RS74 from membership in the species *Y. ruckeri*. The phenotypic characters of *Y. ruckeri* were compared with those of the excluded strains to identify tests of diagnostic value. Fish isolates which ferment both arabinose and rhamnose can reasonably be assumed not to be *Y. ruckeri*. Isolates with these biochemical characteristics are frequent false-positives in fish disease detection and may be *H. alvei*. This species is similar to *Y. ruckeri* in many biochemical reactions, particularly those represented on the API 20E test strip system, and can cross-react with diagnostic antisera for *Y. ruckeri* (24). Besides the sugar reactions, tests of malonate utilization (positive for *H. alvei*) and gelatinase and Tween 80 lipase (positive for *Y. ruckeri*) (10, 21) are also useful in distinguishing these species, although variants do occur. As gas production from glucose by *Y. ruckeri* is dependent on growth temperature, this does not distinguish the species.

With the exception of one strain, RS54, the authentic strains of *Y. ruckeri* had RBR values of greater than 70% at two levels of stringency. By the DNA relatedness criteria used for members of the *Enterobacteriaceae*, isolates of *Y. ruckeri* appear to form a single genetic species, regardless of serovar. Variation between test replicates in the dot-blot procedure was often fairly high (Table 2), making it difficult to determine whether differences between serovars were significant. Serovar V strains may be more closely related to serovar II than to serovar I.

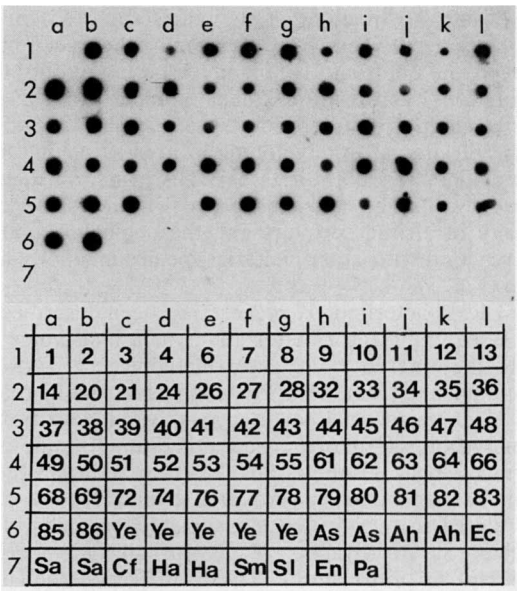

FIG. 3. Colony hybridizations with ^{32}P-labeled total chromosomal DNA from *Y. ruckeri* BC74 (RS3). Hybridization was done at 60°C in 50% formamide for 48 h. Colony positions on the nitrocellulose filter are indicated by the RS code numbers for *Y. ruckeri* strains and by the following initials for the other species: Ye, *Y. enterocolitica*; Ae, *Aeromonas salmonicida*; Ah, *Aeromonas hydrophila*; Ec, *E. coli*; Sa, *S. cholerae-suis* subsp. *arizonae*; Cf, *Citrobacter freundii*; Ha, *H. alvei*; Sm, *Serratia marcescens*; Sl, *Serratia liquefaciens*; En, *Enterobacter cloacae*; Pa, *Pseudomonas aeruginosa*.

The characteristics of serovar I strains of *Y. ruckeri* are as described by Ewing et al. (10). We previously noted that serovar I strains can be subdivided on the basis of growth at 37°C and sensitivity to polymyxin B and bacteriophages (8, 23). Serovar II strains all ferment sorbitol, grow at 37°C, and are resistant to polymyxin B (MICs of 128 to 512 μg/ml) (8). Serovar V isolates share these serovar II characteristics, but they are distinguished by serology (23) and cell wall lipopolysaccharides (D. E. Flett and R. M. W. Stevenson, unpublished observations). Serovar III isolates have an Australian origin and do not ferment D-sorbitol, with the exception of the anomalous D-sorbitol-fermenting strain from British Columbia, RS54 (25). The salmonid blood spot isolates (16) are included in serovar III as they are serologically identical to the Australian serovar III strains (RS77, RS78, RS79) mentioned by Bullock et al. (3) (data not shown). Isolates of *Y. ruckeri* from diseased rainbow trout most often belong to serovar I (19), but serovar II can also be significant, particularly in chinook salmon (5). The relative virulence of other serovars is not clear, but there is as yet no basis for excluding them as potential pathogens in monitoring fish health.

Despite phenotypic similarities, *Y. ruckeri* does not show close genetic relationship with *H. alvei*. Also, there was minimal hybridization between *S. cholerae-suis* subsp. *arizonae* and *Y. ruckeri* (Table 2; Fig. 3), which conflicts with the suggestion by Green and Austin (12) that these organisms are related, based on the results of numerical phenetics. Under our conditions, relatedness of *Y. ruckeri* to other members of the *Enterobacteriaceae*, including *H. alvei*, was low, generally less than 10% (Table 2). In comparison, Ewing et al. (10) reported an RBR value of 24% at 60°C between labeled DNA from *Y. ruckeri* and DNA from *H. alvei*. Also, in the dot-blot tests, there was little hybridization between DNA from *Y. ruckeri* and *Y. enterocolitica* RS31 (Table 2), in contrast to values reported for DNA hybridization between *Y. ruckeri* and other yersiniae (1). However, the different kinetics involved in the dot-blot and free-solution hybridizations make comparisons of results difficult. The dot-blot procedure was chosen for this work because of interest in developing a rapid screening test for use in diagnostic laboratories. A DNA probe for diagnostic purposes would be feasible, based on the close genetic similarity of all the serovars and the negligible amount of cross-reaction with other bacterial species in colony hybridization (Fig. 3).

The ERM bacterium, *Y. ruckeri*, has been described as "a species searching for a better genus as a final home" (11). Besides the differences in DNA relatedness, *Y. ruckeri* can be distinguished from other *Yersinia* species by its reactions in the biochemical tests for urease, L-lysine decarboxylase, gelatin hydrolysis, indole, citrate utilization (Simmons), and patterns of sugar fermentations (1, 9). In addition, the large plasmid carried by many serovar I strains (7) is not genetically related to the virulence-associated plasmids of other yersiniae (S. De Grandis, P. Krell, and R. Stevenson, manuscript in preparation). These differences and the increased diversity among the isolates suggest that the position of the ERM bacterium in the genus *Yersinia* should be reevaluated.

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