Numerical Taxonomy and Emended Description of *Renibacterium salmoninarum*

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Forty-four strains of *Renibacterium salmoninarum* and 12 representative cultures of *Actinomyces*, *Arthrobacter*, *Lactobacillus*, *Microbacterium*, *Micrococcus*, *Planococcus*, *Rothia* and *Listeria denitrificans* were compared using numerical taxonomic techniques based upon 86 unit characters. Data were examined using the simple matching (S_{SM}) and Jaccard (S_J) coefficients, and clustering was achieved using the unweighted pair group method with arithmetic averages (UPGMA) technique. Cluster composition was barely affected by the statistics used, or by the test error, which was estimated at 0.36%. The renibacteria formed a distinct and homogeneous cluster and were distinguished from the marker cultures by a characteristic API-ZYM profile. An emended description of *R. salmoninarum* is presented, based on the numerical data and the results of recent chemical and microbiological studies.

INTRODUCTION

Bacterial kidney disease (BKD; Dee disease; corynebacterial kidney disease) is an important infectious disease of hatchery-reared salmonid fish (Fryer & Sanders, 1981). The causal organism has been associated with *Corynebacterium* (Ordal & Earp, 1956; Smith, 1964; Vladík *et al.*, 1974; Sanders & Fryer, 1978), *Listeria* (Bullock *et al.*, 1975) and with coryneform bacteria generally (Bullock & Stuckey, 1975), but is currently classified in the monospecific genus *Renibacterium*, mainly on the basis of chemical properties derived from an analysis of a few strains (Sanders & Fryer, 1980). Subsequent chemotaxonomic studies (Collins, 1982; Embley *et al.*, 1983; Kusser & Fiedler, 1983) have supported the integrity of *Renibacterium salmoninarum*, which has been tentatively assigned to the actinobacteria (Goodfellow & Cross, 1984), an aggregate group of actinomycetes encompassing fermentative actinomycetes and established coryneform taxa.

Little is known about the relationship of renibacteria to other Gram-positive organisms that have DNA relatively rich in guanine (G) plus cytosine (C) and lysine as the diamino acid of the wall peptidoglycan (Goodfellow & Cross, 1984), though an affinity with *Listeria denitrificans* has been postulated on chemotaxonomic grounds (Collins *et al.*, 1983). In the present study representatives of *R. salmoninarum*, including the type strain, were the subject of numerical phenetic analyses designed to determine the homogeneity of the taxon and its affinity, if any, with representative actinobacteria and lactobacilli.

METHODS

Cultures and maintenance. Forty-four *R. salmoninarum* and related strains and 12 marker cultures were obtained from either private or public culture collections (Table 1). The organisms were maintained as frozen glycerol

Table 1. *Bacterial strains*

Lab. no.	Name as received	Source and collection no.†	Isolated from
127	* <i>Actinomyces viscosus</i>	ATCC 15987	Hamster; peridontal disease
K114	* <i>Arthrobacter aureus</i>	NCIB 8912	
K112	* <i>Arthrobacter globiformis</i>	NCIB 8907	
K116	* <i>Arthrobacter nicotianae</i>	NCIB 9458	
K28 to K32	<i>Corynebacterium</i> sp. KD	Baudin-Laurençin; BL3, BL4, BL7, BL8	<i>Oncorhynchus kisutch</i> (France)
K81 to K83	<i>Corynebacterium</i> sp. KD	Baudin-Laurençin; BL1, BL11, BL12	Salmonid fish (France)
K96	* <i>Lactobacillus brevis</i>	NCDO 1749	Human faeces
K95	* <i>Lactobacillus casei</i>	NCDO 161	Cheese
K102	* <i>Lactobacillus curvatus</i>	NCIB 9710	Milk
K91	* <i>Listeria denitrificans</i>	ATCC 14870	
K104	* <i>Microbacterium lacticum</i>	NCIB 8450	
K92	* <i>Micrococcus luteus</i>	NCIB 9278	
K93	* <i>Planococcus citreus</i>	NCMB 1493	Water
K52	* <i>Renibacterium salmoninarum</i>	ATCC 33209	<i>Oncorhynchus tshawytscha</i>
K53, K54	<i>R. salmoninarum</i>	J. L. Fryer; RB173, Cole river isolate	Salmonid fish (USA)
K56 to K63, K70, K72, K73, K84, K86, K87	<i>R. salmoninarum</i>	Laboratory isolates	<i>Salmo salar</i> (England)
K89	<i>R. salmoninarum</i>	Laidler; M213	<i>Salmo salar</i> (England)
128	* <i>Rothia dentocariosa</i>	ATCC 17931	Human; dental caries
K25	KDM (kidney disease micro-organism)	Evelyn; KDB-897	<i>Oncorhynchus nerka</i> (Canada)
K26	KDM	NCMB 1113	<i>Salmo gairdneri</i>
K35	KDM	Spacey	<i>S. gairdneri</i> (England)
K42	KDM	Paterson; AP-75	<i>S. salar</i> (Canada)
K43	KDM	Groman; 6.0	<i>O. nerka</i> (Canada)
K44	KDM	Groman; 6.12	Salmonid fish (USA)
K46	KDM	NCMB 1111	<i>Salmo</i> sp.
K47	KDM	NCMB 1112	<i>Salvelinus fontinalis</i>
K48	'Dee Disease'	NCMB 1114	<i>S. salar</i>
K49	'Dee Disease'	NCMB 1115	<i>S. salar</i>
K50	KDM	Hastein; A57/80	Salmonid fish (Norway)
K75	KDM	Souter; WS-1	<i>Salmo</i> sp. (Canada)
K76	KDM	Souter; FQ-1.75	<i>Salvelinus fontinalis</i> (Canada)
K77, K79, K80	KDM	Souter; KLAV-75, NISQ-80, FO-RBT-12	<i>S. gairdneri</i> (Canada)
K78	KDM	Souter; SOC-3	<i>O. nerka</i> (Canada)
K90	KDM	Bruno; LF	Salmonid fish (Scotland)

* Type strain.

† Sources: ATCC, American Type Culture Collection, Rockville, Md., USA; NCDO, National Collection of Dairy Organisms, National Institute for Research in Dairying, Shinfield, Reading, UK; NCIB and NCMB, now National Collection of Industrial and Marine Bacteria, Aberdeen, UK; Dr F. Baudin-Laurençin, Laboratoire National de Pathologie des Animaux Aquatiques, Brest, France; Dr D. Bruno, Department of Agriculture and Fisheries for Scotland, Aberdeen, UK; Dr T. P. T. Evelyn, Department of Fisheries and the Environment, Pacific Biological Station, Nanaimo, Canada; Dr J. L. Fryer, Department of Microbiology, Oregon State University, USA; Dr D. Groman, College of Forestry, Wildlife and Range Sciences, University of Idaho, USA; Dr T. Hastein, National Veterinary Institute, Oslo, Norway; Mr T. Laidler, Veterinary Investigation Centre, Penrith, Cumbria, UK; Dr W. D. Paterson, Connaught Laboratories, Willowdale, Canada; Dr B. Souter, Freshwater Institute, Winnipeg, Canada; Mrs B. Spacey, Wellcome Research Laboratory, Beckenham, Kent, UK.

suspensions at -20°C (Wellington & Williams, 1979) and on plates of a semi-defined medium (SDM; Embley *et al.*, 1982) supplemented with ascorbic acid (0.05%, w/v), haemin (0.0002%, w/v), inositol (0.0001%, w/v), sodium bicarbonate (0.04%, w/v), sodium chloride (0.1%, w/v) and thiamin (0.0005%, w/v) at room temperature. These latter stocks were subcultured every two weeks and checked for purity from Gram-stained smears (Hucker & Conn, 1923).

Collection of data. Each strain was examined for 104 unit characters (Table 2) based on an array of biochemical, degradative, enzymic, morphological and tolerance tests. The biochemical, degradative and morphological tests were done in Petri dishes. Inocula from 4 to 8 d modified SDM plates were streaked over the surface of test media (four organisms per plate). The tolerance tests were done in polystyrene Replidishes (Sneath & Stevens, 1967) using a multiloop inoculation procedure (Alderson, 1982). Thus, master plates were prepared by dispensing 1 to 2 ml of each bacterial suspension into the wells of Replidishes and a multiloop inoculator, consisting of an aluminium template (12 × 12 × 0.6 cm) carrying 25 nichrome inoculating loops (Medical Wire and Equipment Co., Potley, Wilts, UK), was used to transfer 5 µl of each suspension to the test media. The bacterial suspensions were prepared in distilled water using 4 to 8 d growth from modified SDM plates; suspensions were diluted to give an OD₆₂₅ of approximately 0.5, i.e. 10⁶ to 10⁸ cells ml⁻¹, unless otherwise stated.

Nearly all of the strains grew well on test media, adjusted to pH 6.8 when necessary, at 15 °C. *Actinomyces viscosus* ATCC 15987 and *Rothia dentocariosa* ATCC 17931 were incubated at 30 °C as they did not grow at the lower temperature. The remaining marker strains were incubated at both 15 and 30 °C for all but the temperature tests in order to determine the effect of temperature on the numerical classifications. Modified SDM agar was used as the basal medium throughout. Tests were repeated when ambiguous or clearly unexpected results were obtained. Tests were read weekly up to 21 d unless stated otherwise. Data for computation were taken from the final test reading unless stated to the contrary.

API tests. API-ZYM kits (API Laboratory Products) were used to determine the ability of the strains to produce the enzymes listed in Table 2. Cupules were inoculated with two drops of dense bacterial suspensions (OD₆₂₅ 2–3) in distilled water. Reproducible results were obtained with the renibacteria after 6 h at 15 °C, and with the marker strains after 12 h at 15 °C and 6 h at 30 °C. Tests were read as instructed by the manufacturer. The kits were examined under a high density lamp in order to eliminate any yellow coloration due to an excess of Fast Blue (ZYM B reagent). Weak positive results were confirmed by comparing treated against control kits containing distilled water. Both weak and strong reactions were scored positive.

Biochemical tests. Catalase (Cowan, 1974) and oxidase production (Kovacs, 1956) were detected on 4 to 8 d cultures. Phosphatase activity (Wilkinson & Jones, 1977) was examined after 14 d.

Colonial properties. Pigmentation was recorded after 7 and 14 d. Motility was examined in wet preparations from 7 d modified SDM broth cultures at ×400 magnification under phase contrast.

Degradation tests. The degradation of adenine, chitin, hypoxanthine, tyrosine, xanthine (all 0.5%, w/v), casein (1%, w/v; skimmed milk), elastin (0.3%, w/v), guanine and testosterone (0.1%, w/v) was detected as clearing of the insoluble compounds from in and around areas of growth. Plates of modified SDM medium supplemented with Tweens 20, 40, 60 and 80 (1%, v/v) were examined for opacity (Sierra, 1957), and lecithin (1%, v/v) and tributyrin (1%, v/v) plates for clearing. Starch degradation was observed after 21 d by flooding plates with iodine solution (Cowan, 1974) and scoring zones of clearing as positive. Hyaluronidase and chondroitin sulphatase (400 µg ml⁻¹; Schofield & Schaal, 1981) activities were detected when 21 d plates were flooded with 2 M-acetic acid, left for 15 min and examined for clear zones around bacterial growth. Degradation of DNA and RNA (0.2%, w/v) was observed when 21 d plates were flooded with 1 M-HCl; clearance zones were recorded as positive. Blackening of test media indicated a positive result for aesculin and arbutin degradation (0.5%, w/v; Broom & Sneath, 1981).

Growth tests. Growth at 45 °C was recorded after 2 weeks, and that at 4, 15 and 30 °C after 4 weeks. Resistance to bile salts, dyes and inorganic chemical inhibitors at various concentrations (Table 2) was scored weekly for 3 weeks by comparing growth in the presence of the inhibitory compound against that in the negative control; any sign of inhibition was scored negative. The first readable result was taken for computation. The same procedure was followed with the antibiotics but in this case Replidishes were read after 7, 10 and 14 d at 15 °C, and after 3 and 7 d at 30 °C. Growth at pH 5.8 and pH 7.8 was examined in buffered (0.5 M-potassium phosphate) media for 4 weeks.

Utilization of 4-methylumbelliferone (4MU) derivatives. Samples (50 µl) of dense bacterial suspensions (OD₆₂₅ 2–3) in buffer were added to equal volumes of 4-methylumbelliferyl fluorogenic substrates (Koch-Light) in microtitre plate wells which were then covered with plastic lids (Grange & Clarke, 1977; Grange, 1978). The substrates were dissolved in 2 ml dimethyl sulphoxide (Sigma) to give 0.02 M solutions of acyl ester and 0.04 M solutions of glycoside and sulphate substrates (Grange, 1978); the stock solutions were stored at –20 °C. Working solutions were prepared by adding 0.2 ml stock solution to 9.8 ml sterile buffer. Glycoside substrates were prepared in phosphate buffer at pH 5.4, and acyl ester and sulphate substrates in Dulbecco A buffer at pH 7.4 as recommended by Grange (1978). Glycosidase activity was read after 18 h, the hydrolysis of acyl esters after 6, 12 and 18 h, and sulphatase activity after 12 and 18 h. After incubation, a drop of 1 M-NaOH was added to each well containing glycoside substrates as this caused any 4MU released by enzyme activity to fluoresce intensively under long wave (366 nm) UV light. The remaining tests were read without modifying the pH of the reaction mixture as the 4MU-conjugates of these substrates are known to hydrolyse spontaneously at high pH giving false positive results (Grange, 1978).

Coding of data. Many of the characters existed in one of two mutually exclusive states and were scored plus (1) or

minus (0). The character states for pigmentation were mutually exclusive and were scored plus (1) for the state exhibited and minus (0) for the remaining states (Sneath & Sokal, 1973). Quantitative multistate characters, such as tolerance to chemical inhibitors, were coded using the additive method of Sneath & Sokal (1973). Eighteen tests were deleted from the original data matrix because they were constant in the matrix. The final data matrix, therefore, contained data for 56 strains incubated at 15 °C and 86 unit characters (Table 2). An enlarged matrix containing the information obtained with the 12 marker strains incubated at 30 °C was also prepared.

Computer analysis. Data were examined using the Clustan IC program (Wishart, 1978) on an IBM 370/180 computer using the simple matching coefficient (S_{SM} ; Sokal & Michener, 1958), which includes both positive and negative matches, and the Jaccard coefficient (S_J ; Sneath, 1957), which includes positive matches only. Clustering on each of the coefficients was achieved using the unweighted pair group method with the arithmetic averages (UPGMA) algorithm (Sneath & Sokal, 1973); the results are presented as dendrograms. Cophenetic discrepancies, that is the lack of correlation between respective similarity values in a matrix and its corresponding dendrogram, were assessed for each coefficient using the correlation coefficient (r ; see Sneath, 1978).

Test reproducibility. The test results for the nine duplicate cultures and their partners (Table 3) were compared for the 86 unit characters and an estimate of individual test variance (s_i^2) was calculated (formula 15; Sneath & Johnson, 1972). The average test variance was determined to obtain a pooled variance (s^2) which was used to calculate the average probability (p) of an erroneous test result (formula 4; Sneath & Johnson, 1972). The similarity between duplicates was estimated by computation of the S_{SM} coefficient.

RESULTS

Criteria of goodness

Inclusion of duplicate strains in the analysis enabled experimental test error to be calculated. The average probability (p) of an erroneous test result was 0.72% calculated from the pooled variance ($s^2 = 0.007$) of all the unit characters scored for the duplicate strains grown at 15 °C. The nine pairs of duplicate strains showed a mean observed similarity of around 98.5% S_{SM} . Values of s_i^2 ranged from zero for the majority of the tests, through 0.055 for alkaline phosphatase, catalase, oxidase and esterase (C_4) production, casein and Tween 40 degradation, and growth at pH 5.8 and 7.8 and in the presence of methylene blue (0.0001%, w/v), to 0.11 for growth in the presence of Nile blue sulphate (0.001%, w/v).

The correlation coefficients (r) between the similarity matrix and corresponding dendrograms were 0.98 for the S_{SM} and 0.99 for the S_J coefficients. Both of these values are very high and well within acceptable limits for a study using UPGMA.

Clustering of strains using the S_{SM} coefficient and the UPGMA algorithm

The classification based on the S_{SM} , UPGMA analysis is described in detail for, in general, it gave the most compact clusters and aggregate clusters. Thus, all but one of the 56 strains were recovered in three aggregate clusters defined at the 66% similarity (S) level or above (Fig. 1). The remaining strain, *Planococcus citreus* NCMB 1493, formed a single member aggregate cluster which showed its closest similarity to aggregate cluster 3.

The first aggregate cluster, defined at the 72% S -level, contained 44 strains received as *Corynebacterium* sp. KD, *Renibacterium salmoninarum*, kidney disease or Dee disease micro-organisms, and *Actinomyces viscosus* ATCC 15987, *Micrococcus luteus* NCIB 9278 and *Rothia dentocariosa* ATCC 17931. All of the fish pathogens were designated *Renibacterium salmoninarum* as they formed a tight cluster defined at the 93% S -level. The three single member clusters shared only a loose relationship to one another and to the *R. salmoninarum* strains.

The second aggregate cluster, delineated at the 76% S -level, contained 3 lactobacilli including *Lactobacillus brevis* NCDO 1749 and *Lactobacillus casei* NCDO 161 which joined at the 85% S -level. The remaining strain, *Lactobacillus curvatus* NCIB 9710, was united with the other two at the 69% S -level. The final aggregate cluster, cut-off at the 69% S -level, contained 5 single member clusters, *Arthrobacter aureus* NCIB 8912, *Arthrobacter globiformis* NCIB 8907, *Arthrobacter nicotianae* NCIB 9458, *Listeria denitrificans* ATCC 14870 and *Microbacterium lacticum* NCIB 8450.

Fig. 1. A simplified dendrogram showing the relationships between clusters recovered in the S_{SM} -UPGMA analysis.

Clustering of strains using the S_j coefficient and the UPGMA algorithm

The compositions of both the *R. salmoninarum* cluster and the three aggregate clusters were only marginally affected when the data were analysed using the S_j coefficient and the UPGMA algorithm (Fig. 2). However, aggregate cluster 1, defined at the 36% S -level, contained 46 as opposed to 47 strains as *M. luteus* NCIB 9278 was recovered on the periphery of aggregate cluster 3. The renibacteria formed a compact cluster at the 87% S -level apart from *R. salmoninarum* K42 which joined the cluster at the 76% S -level. *Actinomyces viscosus* ATCC 15987 and *Rothia dentocariosa* ATCC 17931 were again sharply separated from one another and from the *R. salmoninarum* cluster. Aggregate cluster 2 was again recognized but now showed a close similarity to aggregate cluster 3. Within the latter the three *Arthrobacter* strains formed a cluster at the 57% S -level.

Characterization of *Renibacterium salmoninarum*

The properties of the renibacteria and marker strains are shown in Table 2, together with characters of potential diagnostic value. It is particularly interesting that the majority of the renibacteria gave a characteristic profile of positive reactions with the API-ZYM tests, with only *R. salmoninarum* K25 and K42 giving negative responses for alkaline phosphatase and 2-D-mannosidase, respectively. Indeed, the results of the API-ZYM tests seem to be sufficiently diagnostic to distinguish renibacteria from all of the other test organisms.

[illegible]

Kanamycin (16-0)	- (0)	+	-	-	-	+	+	-	-	+	+	-	-	+	+	-
(32-0)	- (0)	+	-	-	-	-	+	-	-	-	+	+	-	+	+	+
Oxolinic acid (4-0)	- (4)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(16-0)	+	+	-	-	-	-	-	-	-	+	+	-	-	+	+	-
Oxytetracycline (32-0)	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
Utilization of 4-methyl-umbelliferone (4MU) derivatives:																
α -L-Arabinopyranoside*	- (0)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
β -D-Cellobiopyranoside monohydrate	- (0)	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Elaidate	- (0)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
β -D-Fucopyranoside	- (0)	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-
2-Acetamido-2-deoxy- β -D-galactopyranoside	- (0)	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
2-Acetamido-2-deoxy- β -D-glucopyranoside	- (0)	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Stearate	- (0)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
β -D-Xylopyranoside*	- (0)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-

+, Positive; -, negative; v, variable.

(+) or (-), Results observed at 30 °C when they differed from those obtained at 15 °C.

* Characters of presumptive diagnostic value.

† Number in brackets is number of strains of *R. salmoninarum* giving positive results.

All strains produced leucine arylamidase and phosphoamidase, were sensitive to novobiocin (16 µg ml⁻¹) and oxytetracycline (32 µg ml⁻¹), and utilized 4MU-acetate, 4MU-butyrate, 4MU-heptanoate, 4MU-laurate, 4MU-nonanoate, 4MU-oleate and 4MU-propionate, but none degraded hyaluronic acid, produced lipase (C₁₄: myristate) or grew on methylene blue (0.01%, w/v), phenol (0.1 and 0.15%, w/v), potassium thiocyanate (3.75%, w/v) and thallos acetate (0.025%, w/v).

Fig. 2. A simplified dendrogram showing the relationships between clusters recovered in the S_J UPGMA analysis.

Table 3. Overall similarity values between duplicate sets of marker strains

Strains were incubated at 15 °C and 30 °C for tests included in the numerical phenetic analyses based on the S_J and S_{SM} coefficients and UPGMA clustering.

Duplicate cultures	Coefficient...	Percentage similarities	
		S_J	S_{SM}
<i>Arthrobacter aureescens</i> NCIB 8912		84	88
<i>Arthrobacter globiformis</i> NCIB 8907		85	92
<i>Arthrobacter nicotianae</i> NCIB 9458		88	93
<i>Lactobacillus brevis</i> NCDO 1749		91	97
<i>Lactobacillus casei</i> NCDO 161		65	86
<i>Lactobacillus curvatus</i> NCIB 9710		79	94
<i>Listeria denitrificans</i> ATCC 14870		84	92
<i>Microbacterium lacticum</i> NCIB 8450		77	90
<i>Micrococcus luteus</i> NCIB 9278		83	93
<i>Planococcus citreus</i> NCMB 1493		87	92

Effect of temperature on the numerical classification of marker cultures

The results obtained when 10 of the marker cultures were examined at 30 °C were in most instances similar to those obtained at 15 °C (Table 2). In the S_{SM} analysis, the duplicate strains generally showed similarity values of over 90%, slightly lower values being recorded for the duplicate cultures of *A. aureescens* and *L. casei* (Table 3). The relationships between the marker cultures were only marginally altered when the data obtained at 30 °C were added to the data

matrix though *L. casei* NCDO 161 joined with *L. brevis* NCDO 1749 at the 88% *S*-level, and thereby showed a greater similarity to the latter than to its own duplicate incubated at 15 °C.

In the corresponding *S_J* analysis, the *S*-levels obtained between the two sets of duplicate cultures were 77% or above apart from the cultures of *L. casei*, which united at the 65% *S*-level. Once again the detailed relationships between the marker cultures were only slightly altered when the data obtained at 30 °C were considered. However, *L. casei* NCDO 161 again showed a greater similarity with *L. brevis* NCDO 1749 at 30 °C than with its own duplicate incubated at 15 °C.

DISCUSSION

The numerical phenetic classification of the test strains was only marginally affected by the coefficient of association (*S_J* or *S_{SM}*), or by the test error of 0.36%. It was also encouraging that the clustering of the marker strains was only slightly influenced by the incubation temperature employed. The renibacteria, received from diverse sources under a variety of labels, formed a homogeneous cluster that was sharply separated from representative strains of *Actinomyces*, *Arthrobacter*, *Lactobacillus*, *Microbacterium*, *Micrococcus*, *Planococcus*, *Rothia* and *Listeria denitrificans*, all of which have DNA relatively rich in G + C, and lysine as the diamino acid of the wall peptidoglycan (Goodfellow & Cross, 1984). It is evident that *Renibacterium salmoninarum* is a good species, a finding that is in excellent agreement with chemotaxonomic data (Embley *et al.*, 1983; Kusser & Fiedler, 1983). It was also significant, given the problems experienced in the diagnosis of bacterial kidney disease (Fryer & Sanders, 1981), that the renibacteria could be distinguished from the other test strains by their API-ZYM profiles and by their reactions to a few simple biochemical tests. These diagnostic properties have already been used to identify renibacteria growing on selective isolation plates (Austin *et al.*, 1983), and also serve to distinguish these organisms from *Actinomyces pyogenes* and *Arcanobacterium haemolyticum* (Schofield & Schaal, 1981).

The *R. salmoninarum* cluster was loosely associated within an aggregate cluster with the type strains of *Actinomyces viscosus*, *Arthrobacter nicotianae*, *Micrococcus luteus* and *Rothia dentocariosa*. The low similarity found between *A. viscosus* and *R. dentocariosa* was in good agreement with the results of earlier numerical phenetic surveys (Holmberg & Hallander, 1973; Schofield & Schaal, 1981). However, *R. salmoninarum* can also be distinguished from other members of aggregate cluster 1 by chemical criteria (Embley *et al.*, 1983). Thus, *Actinomyces viscosus* ATCC 15987 and *Arthrobacter nicotianae* NCIB 8907 have the peptidoglycan type A4 α (Schleifer & Kandler, 1972; Weiss *et al.*, 1981) and *Micrococcus luteus* NCIB 9278 and *Rothia dentocariosa* ATCC 17931 have peptidoglycan types A2 α and A3 α , respectively (Schleifer & Kandler, 1972), whereas *Renibacterium salmoninarum* ATCC 33209 has a unique variation of the A3 α type (Kusser & Fiedler, 1983).

The possibility of close relationships between *Lactobacillus* and *Renibacterium* (Vladík *et al.*, 1974), and between the latter and *Listeria denitrificans* (Collins *et al.*, 1983) is not supported by the numerical phenetic data. It is also interesting that *R. salmoninarum* and *L. denitrificans* have markedly different peptidoglycan types (Fiedler & Seger, 1983; Kusser & Fiedler, 1983; Fiedler *et al.*, 1984). The sharp separation of the lactobacilli from the actinobacteria included in the present study is in good agreement with the results of previous numerical taxonomic studies (Davis *et al.*, 1969; Jones, 1975; Wilkinson & Jones, 1977). The type strain of *Paracoccus citreus* also showed little overall similarity to either *Renibacterium* or the actinobacteria. These findings are not surprising as 16S rRNA cataloguing data show that both lactobacilli and paracocci are not related to the actinomycetes but belong to the *Bacillus-Clostridium* phyletic line (Stackebrandt & Woese, 1979, 1981). The relatively close relationship found between *Arthrobacter aurescens* NCIB 8912, *Arthrobacter globiformis* NCIB 8907 and *Arthrobacter nicotianae* NCIB 9458 in the *S_J* and *S_{SM}*, UPGMA analyses is also in line with current trends in the taxonomy of these organisms (Stackebrandt *et al.*, 1983).

It is timely to redescribe *R. salmoninarum* in the light of data derived from this and some recent studies (Sanders & Fryer, 1980; Embley *et al.*, 1982, 1983; Embley, 1983).

Emendation of *Renibacterium salmoninarum* Sanders and Fryer 1980

(*Re.ni.bac.te'rium* L. n. *renes* the kidneys; Gr. neut. n. *bakterion* small rod; M.L. neut. n. *Renibacterium* kidney bacterium. *sal.mo.ni.na'rum* M.L. *Salmoninae* subfamily of the *Salmonidae*; M.L. gen. n. *salmoninarum*, of the *Salmoninae*.)

Aerobic, strongly Gram-positive, asporogenous, non-acid alcohol fast, non-encapsulated, non-motile short rods, 0.3 to 1.0 by 1.0 to 1.5 μm , often occurring in pairs. White to creamy yellow, circular, convex colonies of varying sizes are formed on cysteine serum agar. With Dorset egg medium, growth appears as a raised, smooth, shiny yellow layer and on Loeffler coagulated serum a creamy growth with a matt surface is produced. In cysteine serum broth, creamy yellow growth occurs only at the surface of the medium but uniformly turbid growth is formed in Mueller–Hinton broth. Growth in all of these media is slow, often requiring several weeks of incubation. Good growth can be obtained with a semi-defined medium (SDM) within 15 d. On SDM agar, raised, smooth, shiny, creamy yellow colonies are found. Cysteine is required for growth. Growth is enhanced by albumin, blood, charcoal, serum and Tween 60.

Growth is very slow at 5 °C and 22 °C and absent at 30 °C. Optimal growth occurs between 15 and 18 °C and pH 5.8 to 7.8. Catalase positive (actively inhibited by 0.01 M-sodium azide), cytochrome oxidase negative. There is no apparent acid production from sugars. Does not liquefy gelatin. Proteolysis without any pH change is produced in litmus milk. Additional biochemical, enzymic and physiological properties are shown in Table 2.

The cell wall peptidoglycan contains alanine, glutamic acid, glycine and lysine as the diamino acid and is the unique A3 α type (Kusser & Fielder, 1983). The principal cell wall sugar is glucose; arabinose, mannose and rhamnose are also present.

Produces unsaturated menaquinones with nine isoprene units as the predominant isoprenologue and has a very characteristic polar lipid pattern consisting of diphosphatidylglycerol, two major and six or seven uncharacterized glycolipids, and two unclassified minor phospholipids (Embley *et al.*, 1983). The fatty acid profile contains major amounts of methyl-branched acids with 12-methyltetradecanoic, 13-methyltetradecanoic and 14-methylhexadecanoic predominating (Embley *et al.*, 1983). Mycolic acids are absent.

The mol% G + C of the DNA is 53 to 54.

Causes a slowly developing chronic infection of salmonid fish, characterized by grey-white, enlarged necrotic abscesses in the kidney. The organism has only been isolated from members of the *Salmoninae*, the char, salmon and trout, of the family *Salmonidae*.

Type strain: ATCC 33209.

The present study has gone some way towards clarifying the taxonomy of *R. salmoninarum* and has highlighted a number of properties that may prove to be of value in the identification of unknown strains. However, representative renibacteria need to be included in comparative taxonomic studies based on techniques such as 16S rRNA cataloguing if the suprageneric affinities of *Renibacterium* are to be established.

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