Red-Pigmented Micrococci: a Basis for Taxonomy

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Fifty strains of red-pigmented, gram-positive, nonfermentative micrococci were studied, including organisms from diverse collections identified as *Micrococcus roseus*, *M. agilis*, "*Sarcina erythromyxa*," "*M. radiodurans*," "*M. radiophilus*," and "*M. radioproteolyticus*" and miscellaneous unidentified strains usually labelled *M. roseus* (names in quotation marks are not on the Approved Lists of Bacterial Names, Int. J. Syst. Bacteriol. 30:225-420, 1980). Although similar in physiological attributes (negative characters predominated), the cell wall structural profiles separated *M. roseus* and *M. agilis* (simple homogeneous profile) from "*M. radiodurans*" and the radiation-resistant group (complex, multilayered profile). Simple reactions (growth in 5% NaCl broth, growth at 37°C, and nitrate reduction) distinguished *M. roseus* from *M. agilis*, and acid production from glucose and other sugars distinguished "*M. radioproteolyticus*" from the rest. The members of the "*M. radiodurans*" group could be typified by physiological reactions but not with great reliability. Gas-liquid chromatography of extracted lipids showed that veritable *M. roseus* and *M. agilis* strains had at least 50% of fatty acids in the form of 15:0 branched chains. "*M. radiodurans*" and the rest had straight chains with a 16:1 component which formed at least 25% of total fatty acids and which was not possessed by *M. roseus* or *M. agilis*. Further studies were based on representative strains of clusters derived from the above-mentioned tests. Zymograms for nonspecific esterases and chromatograms of extracted pigments showed no identical patterns for any 2 of 10 strains. Absorption spectra for pigments had maxima at 450 to 510 nm. The guanine plus cytosine contents of the deoxyribonucleic acids ranged from 62 to 74 mol%; the *M. roseus*-*M. agilis* cluster was >69 mol%, and the radiation-resistant cluster was <71 mol%. There was little deoxyribonucleic acid homology between *M. roseus* and *M. agilis* (8%) or among any of the rest (<21%). The named radiation-resistant species showed <18% homology to each other, but alignments were detected in the miscellaneous group. Ribosomal ribonucleic acid (16S) cataloguing showed that "*M. radiodurans*," "*M. radiophilus*," and one of the miscellaneous radiation-resistant strains were related ($S_{AB} = 0.51$ to 0.63) but that they had as little oligonucleotide similarity to *M. luteus* and *M. roseus* ($S_{AB} = 0.23$) as they all did to *Escherichia coli*. It is concluded that "*M. radiodurans*" and its relatives are not species of *Micrococcus* and that they represent clones that separated from the main stem early in procaryotic evolution. There are at least five such species: "*M. radiodurans*," "*M. radiophilus*," "*M. radioproteolyticus*," "*M. erythromyxa*" (*Sarcina erythromyxa*), and one other unnamed.

The genus *Micrococcus* Cohn 1872, as outlined by Baird-Parker (3), consists of three species: *Micrococcus luteus* (Schroeter) Cohn 1872, *M. varians* Migula 1900; and *M. roseus* Flügge 1886. This represents not only a reduction in the number of species from 16 in the seventh edition

of *Bergey's Manual* (1957) but also the inclusion of red-pigmented strains previously in the genus *Sarcina* Goodsir 1842 (e.g., *Sarcina rosea*).

The red-pigmented *Micrococcus* strains are of particular interest because a number of them are known to be extremely radiation resistant, notably "*M. radiodurans*" (1). (Names in quotation marks are not on the Approved Lists of Bacterial Names [43].) Due to their limited action on substrates, these organisms were assumed by Hill (15) to form a natural group. This

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was a hazardous assumption because the similarity between strains was based upon negative characters. Some of the strains possess distinctive features indicative of inhomogeneity. One strain, previously identified as a member of *M. agilis* Ali-Cohen 1889, was originally described as having swimming motility. Recent studies of radiation-resistant strains have shown that the cell wall of these organisms has extraordinary structural features (25, 44, 50, 55); other radiation-resistant organisms seem to have unusually thick walls (38). Baird-Parker (3) included the radiation-resistant micrococci in one *species incertae sedis*, "*M. radiodurans*" (35), and thus maintained the alliance with the genus *Micrococcus*. Stackebrandt and Woese (47) included "*M. radiodurans*" in their survey of ribonucleic acid (RNA) sequences in the *Micrococcaceae* and concluded that it was distinct from other species in the genus and bore no detectable specific relationship to gram-positive bacteria in general.

Interest in resolving the problems concerning the classification of the red-pigmented micrococci was stimulated by the isolation of other radiation-resistant, red-pigmented micrococci (10, 23, 27). Reports in the literature (14, 27, 44, 45, 50, 55) suggest that these organisms are not closely related to the typical gram-positive micrococci and that they should be reclassified because of their unusual cell wall structure and composition. There is insufficient information for a thorough comparison. Some of the isolates have been described as new species: "*M. radiophilus*" (27) and "*M. radioproteolyticus*" (23).

We have studied 50 strains of red-pigmented, gram-positive, catalase-positive cocci from worldwide sources with the objective of identifying and assessing characters that would allow clustering and a more effective approach to classification and taxonomy.

(This paper is derived in part from an M.Sc. thesis by B. W. Brooks, University of Western Ontario, Ontario, Canada, 1978.)

MATERIALS AND METHODS

Bacterial strains. The strains used in this study and their sources are given in Table 1. Stock cultures were maintained on the tryptone-glucose-yeast extract-methionine (TGYM) medium of Work and Griffiths (55) with the addition of 1.5% (wt/vol) agar; incubation was at 30°C.

Growth of bacteria in batch cultures. The inoculum for batch cultures was obtained from 20 ml of TGYM broth in a 125-ml Erlenmeyer flask inoculated from the appropriate stock culture and shaken (120 rpm) at 30°C for 18 to 48 h in a reciprocating water bath shaker (New Brunswick Scientific Co., New Brunswick, N.J.). Some strains grew slowly and re-

quired more than 18 h to attain noticeable turbidity. Baffled culture flasks (2 liter, Bellco Glass, Inc., Vineland, N.J.) containing 1 liter of TGYM were inoculated with 5 ml of the above-mentioned culture and were shaken (Gyrotary shaker, New Brunswick Scientific Co.) at 30°C and 120 rpm. Growth was monitored by measuring turbidity with a Klett-Summerson photoelectric colorimeter (Klett Manufacturing Co., Inc., New York, N.Y.).

Determination of fatty acid composition by gas-liquid chromatography. Cells from batch cultures were harvested during the exponential phase of growth by centrifugation at $4,000 \times g$ for 15 min in a Sorvall superspeed centrifuge (Ivan Sorvall, Inc., Norwalk, Conn.), washed twice in 50 ml of 0.9% (wt/vol) sodium chloride, resuspended in 10 ml of 0.9% (wt/vol) sodium chloride, and freeze-dried. The methods used for extraction of the lipids and transmethylation were those of Tadayan et al. (49). Fatty acid methyl esters were analyzed by gas-liquid chromatography on a 10-ft, 1/8-in. (3 m, 3.2 mm) stainless-steel polar column of 10% SP-2330 on 100/200 Chromosorb (Supelco, Inc., Bellefonte, Pa.) in a Beckman GC-65 chromatograph with hydrogen flame detector. The column was operated at 190°C (detector temperature, 260°C) with nitrogen used as the carrier gas. Identification of fatty acids was based on comparison of retention times with those of methyl ester standards (Supelco, Inc., and Applied Science Laboratories, State College, Pa.). Relative percentages of individual fatty acid components were determined from gas-liquid chromatography tracings (6).

Electron microscopy of thin sections. For ultrathin sectioning, log-phase cells, grown in TGYM and incubated at 30°C, were fixed by the procedure of Rytter and Kellenberger (37). Two modifications of this procedure that were used with a few strains involved: (i) prefixation with 10% (wt/vol) saturated picric acid and (ii) postfixation with 10% saturated picric acid added to 1% OsO₄ in Veronal-acetate buffer immediately before use. Dehydration was carried out in increasing concentrations of ethanol. Blocks were embedded in Epon 812 (Ladd Research Industries, Burlington, Vt.) and cured for 72 h at 60°C. Sections were stained by floating grids carrying sections on 2% (wt/vol) uranyl acetate and, in some cases, were further stained with lead citrate by the method of Reynolds (36). Thin sections were examined with a Philips EM-200 electron microscope operating at 60 kV. Micrographs were taken on 35-mm Kodak Fine Grain-positive film.

Metabolic and physiological testing. Catalase production, nitrate reduction, and the presence of β -galactosidase (*o*-nitrophenyl- β -D-galactopyranoside test) were determined by the methods described by MacFaddin (28). The procedure of Sneath (46) was used to determine the ability of an organism to hydrolyze esculin. TGYM agar was used to determine growth at 30 and 37°C. Salt tolerance was observed on TGYM agar with 5% (wt/vol) sodium chloride. Two different basal media were used for fermentation tests: (i) the standard medium adopted by the International Committee on Systematic Bacteriology Subcommittee on the Taxonomy of Staphylococci and

Micrococci (48) and (ii) a peptone-water base which contained proteose peptone 3 (1%, wt/vol; Difco) sodium chloride (0.5%, wt/vol), bromocresol purple (0.004%, wt/vol), and the appropriate carbohydrate (1%, wt/vol). Tubes were inoculated and incubated aerobically at 30°C for up to 3 weeks.

Comparative zone electrophoresis of esterases. *Staphylococcus aureus* PM261 (obtained from W. E. Kloos, Genetics Department, North Carolina State University, Raleigh, NC 27607) was used as a reference strain. The methods of Zimmerman and Kloos (57) were used. Vertical gel electrophoresis was performed on a GE-4 gel electrophoresis apparatus (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.).

Pigment analysis. Cells from batch culture were harvested during the exponential phase of growth by centrifugation at $4,000 \times g$ for 15 min at 4°C, washed twice in 0.9% (wt/vol) sodium chloride, resuspended in 0.9% (wt/vol) sodium chloride, frozen, and stored at -20°C. Cell suspensions were brought to room temperature, and the cells were pelleted by centrifugation at $5,000 \times g$ for 15 min at 4°C. The wet pellet was extracted by the two-phase method of Bligh and Dyer (4). Light was excluded, and access of oxygen was minimized as much as possible throughout the entire procedure by displacement with nitrogen gas. The combined chloroform phases were evaporated to dryness, resuspended in benzene, and stored at -20°C under nitrogen and in the dark. Thin-layer chromatography was carried out with the use of glass plates with 0.25-mm thick layers of silica gel H (Merck AG, Darmstadt, Germany; 1 part silica gel H to 5 parts distilled water). Immediately before use, plates were activated by heating at 120°C for 1 h. The solvent system was a mixture of petroleum ether-acetone-chloroform (25:25:20, vol/vol [9]). β -Carotene (Sigma Chemical Co., St. Louis, Mo.) was included as a standard with each run. Absorption spectra (250 to 600 nm) were determined for extracted segments with a Unicam SP1700 UV spectrophotometer.

Peptidoglycan typing. The peptidoglycan composition of the representative strains was determined by the methods used by Schleifer and Kandler (41) in comparing the structure of this polymer from a wide range of bacteria. The isomeric configurations of amino acids were not determined.

DNA isolation. The cells were suspended in 0.15 NaCl-0.01 M ethylenediaminetetraacetic acid buffer, pH 8.0 (29). Lysozyme (100 μ g/ml) was added, and the suspension was incubated at 37°C for 0.5 to 1.0 h to make the cells susceptible to lysis with 1% sodium dodecyl sulfate. The deoxyribonucleic acid (DNA) from most of the lysates was isolated by a hydroxylapatite procedure (19). High-molecular-weight DNA was used for in vitro labeling. After an initial extraction with chromatography-grade phenol, high-molecular-weight DNA was isolated by the Marmur procedure (29).

G+C content of DNA. The guanine plus cytosine (G+C) contents of DNA preparations were calculated by using the thermal melting points (T_m) of the DNA preparations (20, 30).

Preparation of labeled DNA. High-molecular-weight DNA (200 μ g/ml) was nicked by incubating it

with 0.1 μ g of deoxyribonuclease I (electrophoretically purified) per ml for 1 min. The DNA (10 μ g) was then labeled with [3 H]thymidine triphosphate by a nick translation method (7). The specific activity of the labeled DNA preparations was about 100,000 cpm/ μ g.

DNA homology experiments. A variation of the S1 nuclease procedure described by Crosa et al. (8, 18, 21) was used in DNA homology experiments. The reassociation mixtures contained 10 μ l (0.025 μ g) of labeled DNA, 50 μ l (30 μ g) of unlabeled DNA (either homologous, heterologous bacterial DNA or native fragmented salmon sperm DNA), and 50 μ l of 0.88 M NaCl + 0.001 M HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer (pH 7.0). Just prior to each experiment, the amount of labeled DNA needed for the experiment was denatured by heating the tube in a boiling-water bath for 5 min. Reaction vials containing native salmon sperm DNA were used to measure the amount of self-renaturation of labeled DNA during the incubation period. The reaction vials were incubated for 17 h at 70°C.

After incubation, a 100- μ l sample was removed from each vial and transferred into a tube (13 by 100 mm) for S1 nuclease digestion. In addition to the sample of reassociation mixture, each digestion tube contained 1.0 ml of the 25 μ g of fragmented, denatured salmon sperm DNA and 50 μ l of S1 nuclease (Calbiochem-Behring). The preparation was diluted such that twice the amount of enzyme was added that was needed to digest totally 25 μ g of denatured DNA in 1 hr (21). After incubation for 1 h at 50°C, an equal volume of 10% trichloroacetic acid was added to each tube. After cooling in a refrigerator for at least 1 h, the precipitates were collected on nitrocellulose filters (BA 85, Schleicher & Schuell Co., Keene, N.H.). The membranes were dried, and the radioactivity was measured by use of liquid scintillation counter and a toluene-base scintillation fluid.

Comparative cataloging of 16S ribosomal RNA. A limited set of organisms ("*M. radiodurans*" Sark 298, "*M. radiophilus*" 1055, *M. roseus* 294, and *M. roseus* 1057) were compared by cataloging the T₁ ribonuclease digests of the 16S ribosomal RNA from each strain. This ubiquitous and essential macromolecule provides evidence of sequences showing extreme conservation as well as variable regions appropriate to a semantide useful for estimation of both distant and close relationships (53). The labeling (32 P), extraction, purification, digestion, electrophoretic separation, and analysis of the resulting oligonucleotide "fingerprint" have been systematized and have been published in detail (40, 51, 54). The oligonucleotide catalogs were subjected to a computer-assisted comparison, and an association coefficient, S_{AB} , was generated according to the derivation given by Fox et al. (13); all oligomers of length six and longer, the post-transcriptionally modified oligomers, and the 3' and 5' termini were used as the data base.

RESULTS

Metabolic and physiological characters. All strains were gram positive and catalase positive. The majority of the metabolic and physiological tests, including methyl red, Voges-Proskauer

TABLE 1. Strains used in this study

Acquisition no. in culture collections ^a				Name given	Strain designation	Source ^b
UWO	ATCC	CCM	NCTC			
288	13939	1700		" <i>M. radiodurans</i> "	Anderson R ₁	1
289	144	2257	7511	<i>M. roseus</i>		2, 4
290	177	557	7512	<i>M. roseus</i>		2, 4
291	179	560	7514	<i>M. roseus</i>		2, 4
292	185	365	7515	<i>M. roseus</i>		2
293	19172			<i>M. roseus</i>	David 248	1
294				<i>M. roseus</i>	Davis 253	1
295	516	839	7521	<i>M. roseus</i>		2
296	534	618	7522	<i>M. roseus</i>		2
297	9815	691	7738	<i>M. roseus</i>		2, 4
298				" <i>M. radiodurans</i> "	Sark S	1
395				" <i>M. radiodurans</i> "	Irradiated Sark	1
1036				<i>M. roseus</i>	CN 6713	7
1037	966	2390	7509	<i>M. roseus</i>	CN 6205	7, 4
1038				" <i>M. radiodurans</i> "		7
1039	998	2131	2676	<i>M. roseus</i>		2, 4
1043				<i>M. roseus</i>	LMD 22.58 <i>Sarcina rosea</i>	9
1044				<i>M. roseus</i>	LMD 29.25 <i>M. agilis</i>	9
1045	187	706		<i>M. roseus</i>	LMD 22.44 <i>Sarcina erythromyxa</i>	9
1048				" <i>M. radiodurans</i> "	rec 30	8
1050				" <i>M. radiodurans</i> "	UV-17	8
1051				" <i>M. radiodurans</i> "	UV-38	8
1052				" <i>M. radiodurans</i> "	ts-2	8
1053				" <i>M. radiodurans</i> "	ts-1	8
1054				" <i>M. radiodurans</i> "	ts-3	8
1055	27603	2564	10785	" <i>M. radiophilus</i> "	Lewis	3
1056		2703		" <i>M. radioproteolyticus</i> "	Kobatake	3, 4
1057	186	679	7523	<i>M. roseus</i>	Neotype-ATCC catalog 1974	3, 4
1059	397	562	7528	<i>M. roseus</i>		4
1061			10580	<i>M. roseus</i>		4
1062			3874	<i>M. roseus</i>	(<i>M. agilis</i>)	4
1064	418 (178)		7520 (7513)	<i>M. roseus</i>		4
1071				" <i>M. radiodurans</i> "	T ₁	10
1072				" <i>M. radiodurans</i> "	T ₃	10
1073				" <i>M. radiodurans</i> "	T ₆	10
1074				" <i>M. radiodurans</i> "	T ₆ H	10
1075				" <i>M. radiodurans</i> "	T ₇	10
1076				" <i>M. radiodurans</i> "	T ₈	10
1077				" <i>M. radiodurans</i> "	T ₉	10
1078				" <i>M. radiodurans</i> "	T ₁₁	10

1079	" <i>M. radiodurans</i> "	JB 301	10
1080	" <i>M. radiodurans</i> "	II-100-2, 0-10	10
1081	" <i>M. radiodurans</i> "	II-100-2, 5-5	10
1082	" <i>M. radiodurans</i> "	HV-0, 5-1	10
1083	" <i>M. radiodurans</i> "	JB 302	10
1084	" <i>M. radiodurans</i> "	HI-1, 1	10
1085	" <i>M. radiodurans</i> "	S.11.A	10
1086	<i>Micrococcus</i> sp.	A ₉	5
1087	<i>M. roseus</i>	37251	2
1088	<i>M. roseus</i>	BKM B11 (also labeled <i>M. agilis</i>)	6
1108	<i>Staphylococcus aureus</i>	PM 261	11

^a Abbreviations: UWO, Culture Collection of The University of Western Ontario, London, Ontario, Canada; ATCC, American Type Culture Collection, Rockville, Md.; CCM, Czechoslovak Collection of Microorganisms, Brno, Czechoslovakia; NCTC, National Collection of Type Cultures, Colindale, London, England.

^b 1, Department of Microbiology and Immunology Culture Collection, University of Western Ontario, London, Ontario, Canada; 2, American Type Culture Collection, Rockville, Md.; 3, Czechoslovak Collection of Microorganisms, tr. Obrancu miru, J. E. Purkyne University, Brno, CSSR; 4, National Collection of Type Cultures, Central Public Health Laboratory, London, England; 5, B. Osterberg, Johnson & Johnson Biological Laboratory, Sollentuna, Sweden; 6, W. W. Alferow, The Institute of Microbiology, Academy of Sciences, USSR, Moscow, USSR; 7, J. F. Spilsbury, Wellcome Bacterial Collection, The Wellcome Research Laboratories, Beckenham, Kent, England; 8, M. M. Werner, Biology Branch, Atomic Energy of Canada Ltd., Chalk River Nuclear Laboratories, Chalk River, Ontario, Canada; 9, J. Van der Toorn, Culture Collection, Technische Hogeschool Delft, Laboratorium voor Microbiologie, Julianalaan, Delft, The Netherlands; 10, E. A. Christensen, Statens Seruminstitut, Copenhagen, Denmark; (11) W. E. Kloos, Department of Genetics, North Carolina State University, Raleigh, NC.

kauer, indole, and citrate tests, gave negative results. No acid production was detected for any of the 52 strains using the following carbohydrates as substrates: arabinose, mannitol, sucrose, lactose, maltose, galactose, xylose, or sorbitol. However, some tests distinguished strains, and the results are depicted in Table 2.

Most strains of *M. roseus* listed in Table 2 grew at 37°C, grew in the presence of 5% sodium chloride, and reduced nitrate to nitrite. Strains included in this study and formerly recognized as members of *M. agilis* (*M. roseus* UWO 1037, 1039, 1044, and 1062) were distinct in that they all failed to grow at 37°C, failed to grow in the presence of 5% sodium chloride, and were unable to reduce nitrate. Similar results were obtained by Kocur and Schleifer (24) with five strains of *M. agilis*.

Cell wall profiles. Sections of the type strain (UWO 1057) of *M. roseus* showed a single, homogeneous, thick component (40 to 45 nm) external to the plasma membrane (Fig. 1) consistent with the wall profiles of gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus licheniformis* (31). This type of profile was consistent for all strains identifiable with *M. roseus* Flüge and *M. agilis* Ali-Cohen. All others in the series had a multilayered profile (Fig. 2-7), including the organism formerly named "*S. erythromyxa*" (Fig. 2).

The "*M. radiodurans*" strains showed the most complex profile (25, 55), including, from inside to outside: a compartmentalized layer, a delicate unit membrane, and an outermost hexagonal array of macromolecules. The inner dense layers (peptidoglycan containing, 10 to 20 nm thick) of "*M. radiodurans*" UWO 288, 298, 1083, and 1085 and of *M. roseus* UWO 293 and 294 showed regular fenestrations, described in earlier papers (see 25) as the "holey layer." These fenestrations were not seen in "*S. erythromyxa*," "*M. radiophilus*," or "*M. radioproteolyticus*." The complexity of the profile and a fenestrated or nonfenestrated peptidoglycan layer provided the most useful characters.

Fatty acid composition. The fatty acid composition of the organisms included in this study was determined by gas-liquid chromatography. The strains were then sorted (Tables 3, 4, and 5) on the basis of predominant fatty acid components.

The major fatty acid component of the strains with similar patterns assembled in Table 3, clustered around the type strain of *M. roseus*, was a methyl-branched C15 saturated acid. The retention time of this peak corresponded exactly with that of a C15:0 anteiso standard and indicated

TABLE 2. Metabolic and physiological characters of red micrococci^a

Original identification	Strain designation (UWO)	Growth on 5% NaCl	Growth at 37°C	Nitrate reduction	Esculin hydrolysis	Glucose ^b	Glucose ^c	Fructose ^b	Fructose ^c	ONPG ^d
<i>M. roseus</i> group										
<i>M. roseus</i>	289	+	+	+	+	-	-	-	-	-
<i>M. roseus</i>	290	+	+	+	+	-	-	-	-	-
<i>M. roseus</i>	291	+	+	+	+	-	-	-	-	-
<i>M. roseus</i>	292	+	+	+	+	-	-	-	-	-
<i>M. roseus</i>	295	+	+	+	+	-	-	-	-	-
<i>M. roseus</i>	296	+	+	+	+	-	-	-	-	-
<i>M. roseus</i>	297	+	+	+	-	-	-	-	-	-
<i>M. roseus</i>	1036	+	+	+	+	-	-	w	-	-
<i>M. roseus</i>	1043	+	+	+	-	-	-	-	-	-
<i>M. roseus</i>	1057	+	+	+	+	-	-	-	-	-
<i>M. roseus</i>	1059	+	+	+	-	-	-	-	-	-
<i>M. roseus</i>	1061	+	+	+	+	-	-	-	-	-
<i>M. roseus</i>	1064	+	+	+	-	-	-	-	-	-
<i>M. roseus</i>	1087	+	+	+	+	-	-	-	-	-
<i>M. agilis</i> group										
<i>M. roseus</i> (<i>M. agilis</i> *)	1037	-	-	-	-	-	-	-	-	-
<i>M. roseus</i>	1039	-	-	-	-	-	-	-	-	-
<i>M. roseus</i>	1044	-	-	-	-	-	-	-	-	-
<i>M. roseus</i>	1062	-	-	-	-	-	-	-	-	-
" <i>M. radiodurans</i> " group										
" <i>M. radiodurans</i> "	288	-	+	-	-	-	-	-	-	-
" <i>M. radiodurans</i> "	298	-	+	-	-	-	-	-	-	-
" <i>M. radiodurans</i> "	395	-	+	+	-	-	-	-	-	-
" <i>M. radiodurans</i> "	1038	-	+	-	-	-	-	-	-	-
" <i>M. radiodurans</i> "	1048	-	+	-	-	-	-	-	-	-
" <i>M. radiodurans</i> "	1050	-	+	-	-	-	-	-	-	-
" <i>M. radiodurans</i> "	1051	+	+	+	+	-	w	-	w	-
" <i>M. radiodurans</i> "	1052	-	+	-	-	-	-	-	-	-
" <i>M. radiodurans</i> "	1053	-	+	-	-	-	-	-	-	-
" <i>M. radiodurans</i> "	1054	-	+	-	-	-	-	-	-	-
" <i>M. radiodurans</i> "	1071	-	+	-	-	-	-	-	-	-
" <i>M. radiodurans</i> "	1072	-	+	-	-	-	-	-	-	-
" <i>M. radiodurans</i> "	1073	+	+	+	+	-	-	-	-	-
" <i>M. radiodurans</i> "	1074	-	+	-	-	-	-	-	-	-
" <i>M. radiodurans</i> "	1075	+	+	-	-	-	-	-	-	-
" <i>M. radiodurans</i> "	1076	-	+	-	-	-	-	-	-	-
" <i>M. radiodurans</i> "	1077	-	+	-	-	-	-	-	-	-
" <i>M. radiodurans</i> "	1078	-	+	-	-	-	-	-	-	-
" <i>M. radiodurans</i> "	1079	-	-	+	-	-	-	-	-	-

FIG. 1. Section of *M. roseus* 1057 (fixed with OsO_4), showing a single homogeneous wall profile. Bars represent $0.1 \mu\text{m}$ on Fig. 1-7.

FIG. 2. Section of *M. roseus* 1045 (formerly *Sarcina erythromyxa*) fixed as in Fig. 1, showing an outer layer in addition to the thick, homogeneous, peptidoglycan-containing layer.

FIG. 3. Section of "*M. radioproteolyticus*" 1056, fixed as in Fig. 1, showing a substantial set of components (at least two) outside of the peptidoglycan-containing layer.

FIG. 4. Section of "*M. radiophilus*" 1055, fixed as in Fig. 1, showing a complex profile like that of "*M. radioproteolyticus*" but with a thinner peptidoglycan-containing layer.

FIG. 5. Section of "*M. radiodurans*" 298, prefixed with 10% picric acid before treatment with OsO_4 fixative, showing the cell wall profile and at least three layers in addition to the peptidoglycan layer. The latter is fenestrated in other appropriate preparations. The prefixation step provided for a more coherent wall and better adherence of the most superficial protein array.

FIG. 6. Section of *M. roseus* 294, one of the radiation-resistant fish strains, which was fixed in OsO_4 . There are at least two superficial layers and, in appropriate preparations, the peptidoglycan layer is fenestrated.

FIG. 7. Section of "*M. radiodurans*" 1085, one of the miscellaneous strains from Risø, which was fixed in OsO_4 . As in Fig. 6., there are at least two superficial layers, and the peptidoglycan layer proved to be fenestrated.

gels; as is evident, there was great diversity. Banding patterns and staining intensities of non-specific esterase in independent protein extracts of the same strain were reproducible. Some bands were always sharply defined whereas others were diffuse.

Pigment analysis of representative strains. Absorption spectra of the total pigment

fraction (in distilled chloroform) extracted from representative strains were determined. In the visible range, the absorption maximum for each strain was as follows: *M. roseus* UWO 1057, 485 to 490 nm; *M. roseus* UWO 1039, 510 to 520 nm; "*M. radiodurans*" UWO 288, 495 to 500 nm; "*M. radiodurans*" UWO 298, 485 to 495 nm; "*M. radiodurans*" 1085, 490 to 510 nm; "*M. radi-*

TABLE 4. Fatty acid composition of various *M. radiodurans* strains^a

Strain designation	UWO no.	Fatty acid															19:1	W	X	Y	Z
		14:0i	14:0	14:1	15:0 Br	15:1	16:0i	16:1	17:0a	17:1	18:0	18:1	18:2	18:3	18:4	18:5					
" <i>M. radiodurans</i> "	288	0.6	0.6	T	1.0	2.6	13.8	70.8	4.6	4.8	1.7										
" <i>M. radiodurans</i> "	298	0.6	0.6	0.7	4.7	14.8	8.4	57.8	4.7	7.7	0.6										
" <i>M. radiodurans</i> "	395	0.7	0.9	0.9	4.9	13.0	9.0	56.5	5.3	8.9	0.8										
" <i>M. radiodurans</i> "	1038	T	0.6	0.6	1.2	3.4	19.8	60.1	7.5	4.7	2.3										
" <i>M. radiodurans</i> "†	1048	T	0.6	0.6	0.4	1.7	14.5	71.5	5.7	2.3	2.9										
" <i>M. radiodurans</i> "†	1050	0.6	0.5	0.5	1.9	2.6	16.6	69.0	3.9	3.8	1.1										
" <i>M. radiodurans</i> "†	1051	T	T	—	1.4	1.4	13.0	71.4	6.2	4.3	2.2										
" <i>M. radiodurans</i> "†	1052	0.7	0.7	—	1.1	3.2	16.4	61.8	5.7	6.9	4.2										
" <i>M. radiodurans</i> "†	1053	T	T	T	1.5	2.5	12.4	74.1	2.5	5.1	1.3										
" <i>M. radiodurans</i> "†	1054	0.7	0.6	0.6	1.7	2.4	12.3	71.6	4.3	4.8	1.6										
" <i>M. radiodurans</i> "*	1071	1.0	1.0	1.4	2.7	9.8	10.6	65.8	2.8	5.2	0.7										
" <i>M. radiodurans</i> "*	1072	0.6	0.6	0.8	1.9	4.7	10.6	67.9	3.9	8.4	1.3										
" <i>M. radiodurans</i> "*	1073	0.6	0.6	0.8	1.9	7.3	8.4	66.7	5.2	7.8	1.4										
" <i>M. radiodurans</i> "*	1074	0.6	0.6	0.7	2.9	6.0	12.8	60.9	5.2	9.9	1.0										
" <i>M. radiodurans</i> "*	1075	T	T	0.6	1.5	7.7	7.3	66.3	4.1	10.8	1.3										
" <i>M. radiodurans</i> "*	1076	T	T	T	1.1	4.6	9.1	72.4	6.3	5.1	1.1										
" <i>M. radiodurans</i> "*	1077	0.8	0.8	1.1	4.0	13.6	9.6	60.0	2.9	7.0	1.1										
" <i>M. radiodurans</i> "*	1078	0.9	0.9	2.1	1.7	12.3	6.8	63.5	8.3	4.0	0.6										
" <i>M. radiodurans</i> "*	1079	1.4	1.4	2.7	6.5	12.7	15.8	48.7	7.0	4.8	0.5										
" <i>M. radiodurans</i> "*	1080	0.7	0.5	0.5	0.7	3.3	16.6	72.4	3.9	1.8	1.1										
" <i>M. radiodurans</i> "*	1081	T	T	0.5	1.1	4.1	10.5	72.2	3.6	5.9	1.9										
" <i>M. radiodurans</i> "*	1082	T	T	T	1.5	12.1	6.7	62.1	4.4	11.3	1.3										
" <i>M. radiodurans</i> "*	1084	0.9	0.9	1.1	1.2	2.6	10.4	71.6	5.2	4.6	2.4										
<i>Micrococcus</i> sp.	1086	T	T	T	2.3	11.0	7.2	57.4	4.1	16.3	1.0										

^a Conventions as in Table 3. Strains derived from UWO 288 are marked with a dagger, and isolates independent of UWO 288 and 298 are marked with an asterisk.

TABLE 5. Fatty acid composition of miscellaneous strains^a

Strain designation	UWO no.	Fatty acid														
		14:0i	14:0	14:1	15:0 Br	15:1	16:0i	16:0	16:1	17:0a	17:1	18:0	18:1	19:1	W	Z
" <i>M. radiodurans</i> "**	1083		T	1.5	9.4	22.5	9.4	25.2	9.5	13.6	2.2	3.2	3.5			
" <i>M. radiodurans</i> "**	1085		0.6	2.8	12.2	23.8	10.4	24.2	12.5	13.4	T	T	T			
<i>M. roseus</i>	293		1.0		15.4	6.4	12.0	26.9	27.4	2.6		T				
<i>M. roseus</i>	294		0.9		13.0	9.4	11.5	25.8	24.8	4.1		T				
<i>M. roseus</i>	1045		1.8	2.6	3.8		33.2	9.3	9.1	0.7	30.8	0.7	8.1			
<i>M. roseus</i>	1088		1.5	0.5	1.9		32.0	11.5	5.7	7.6	32.2	0.9	7.5			
" <i>M. radiophilus</i> "	1055		0.5	T	0.5	9.5	4.0	51.1	8.1	18.1	1.2			3.7	2.8	
" <i>M. radioproteolyticus</i> "	1056		T	T	T	0.9	3.7	73.0	4.8	6.9	7.8	2.4				

^a Conventions as in Tables 3 and 4.

ophilus" UWO 1055, 495 to 500 nm; *M. roseus* UWO 293, 490 to 500 nm; and *M. roseus* UWO 1045, 450 to 465 nm.

Thin-layer chromatography was used to separate the total pigment fraction into components, not necessarily single pigments, for comparative purposes. Relative mobilities were calculated relative to the fast-migrating β -carotene front, which was assigned a value of 1. The number of components detected in each strain and their relative mobilities were as follows: *M. roseus* UWO 1057, four components, R_f values 0.17, 0.42, 0.66, and 0.80; *M. roseus* UWO 1039, one component, R_f value 0.87; "*M. radiodurans*" UWO 288, five components, R_f values 0.17, 0.36, 0.55, 0.81, and 0.89; "*M. radiodurans*" UWO 298, six components, R_f values 0.07, 0.44, 0.58, 0.82, 0.89, and 0.99; "*M. radiodurans*" UWO 1083, four components, R_f values 0.38, 0.60, 0.84, and 0.91; "*M. radiodurans*" UWO 1085, five components, R_f values 0.17, 0.42, 0.60, 0.83, and 0.90; "*M. radiophilus*" UWO 1055, one component, R_f value 0.90; *M. roseus* UWO 293, six components, R_f values 0.17, 0.36, 0.57, 0.81, 0.89, and 0.95; *M. roseus* UWO 294, six components, R_f values 0.18, 0.36, 0.57, 0.80, 0.89, 0.95; and *M. roseus* UWO 1045, one component, R_f value 0.89.

Peptidoglycan typing of representative strains. There was a clear difference in the diamino acids of the radiation-sensitive and -resistant representative strains. *M. roseus* exhibited the Lys-Ala₃₋₄ type, and *M. agilis*, the Lys-Thr-Ala₃ type. All the radiation-resistant strains were of the Orn-Gly₂ or ₂₋₃ type. One exception was found by an incidental testing of one strain outside of the group, *M. roseus* 1088, which proved to have diaminopimelic acid rather than ornithine.

The molar ratios of the amino acids were consistent with the primary structure proposed for "*M. radiodurans*" by Schleifer and Kandler (41), as follows:

FIG. 8. Schematic zymogram showing relative mobilities of nonspecific esterases of representative strains calculated on the basis of the mobility of the fast-migrating esterase band of *Staphylococcus aureus* PM 261, which was assigned a value of 1.0.

DNA base composition and DNA/DNA homology of representative strains. The results of the DNA base composition and DNA/DNA homology studies are shown in Table 6. The range of G+C ratios for veritable *M. roseus* and *M. agilis* strains was 69 to 74 mol%, whereas groups with 16:1 fatty acids and ornithine in the peptidoglycan showed 62 to 71 mol%. The distinction was more clearly made by the lack of homology (< 21%) between *M. roseus* 1057 and all the other strains. *M. agilis* 1039 showed insignificant homology to either veritable *M. roseus*, "*M. radiodurans*," or "*M. radioproteolyticus*" strains or to one of the miscellaneous group, *M. roseus* 294. There were only two areas of significant homology between strains representing the radiation-resistant grouping as shown by 33% homology for the Anderson and

Sark strains of "*M. radiodurans*," by the seeming identity of the fish strains (*M. roseus* 293 and 294), and by the 44% homology of these two strains with the two strains isolated at Risø ("*M. radiodurans*" 1083 and 1085). The only exception to the internal consistency of results was the 29% homology observed between "*M. radiodurans*" 1085 and "*M. radioproteolyticus*" 1056 when the parallel result with "*M. radiodurans*" 1083 gave 5%. The possibility of a mixed culture was proved by showing that about one-third of 50 colonies of strain 1085 as maintained in the laboratory had the phenotype of strain 1056, an object lesson which reflects on the figure in question and the G+C molar ratio for strain 1085.

Comparative cataloging of the 16S ribosomal RNA polynucleotides of selected

TABLE 6. Mole percent G+C and DNA homology values among red-pigmented *Micrococcus* strains

Cluster and UWO no.	G+C (mol %)	% Homology with DNA from strain:			
		1057	298	1056	294
<i>M. roseus</i> 1057	74	(100)	1	3	3
<i>M. agilis</i> 1037	69	2	2	0	6
<i>M. agilis</i> 1039	70	8	0	1	1
" <i>M. radiodurans</i> " 298	67	17	(100)	8	17
" <i>M. radiodurans</i> " 288	67	11	33	6	17
" <i>M. radioproteolyticus</i> " 1056	65	17	10	(100)	8
" <i>M. radiophilus</i> " 1055	62	5	4	18	4
<i>S. erythromyxa</i> 1045	71	21	1	3	3
<i>S. erythromyxa</i> 1088(?)	70	9	6	5	5
Miscellaneous (fish) 294	69	17	13	6	(100)
Miscellaneous (fish) 293	68	13	12	8	100
Miscellaneous (Risø) 1083	70	5	10	5	44
Miscellaneous (Risø) 1085	66 ^a	5	11	29 ^a	44

^a These figures are in error; see text.

strains. Oligonucleotide catalogs (Table 7) were developed. From the data base of 6-mers and longer, plus post-transcriptionally modified oligomers and termini, the S_{AB} values were calculated and are shown in Table 8. The S_{AB} values, expressed as a dendrogram (Fig. 9), showed that the three red micrococci formed a cluster quite separate (S_{AB} 0.17 to 0.29) from the other bacteria, including *M. roseus* and *M. luteus*, which showed an S_{AB} of 0.73. For comparative purposes we include data for *Escherichia coli* (51), *Bacillus brevis* (54), *M. luteus* (47), and *Corynebacterium diphtheriae* (B. J. Lewis, Ph.D. thesis, University of Illinois, Urbana, 1977). Any arrangement of the three red micrococci with respect to one another in the dendrogram would be arbitrary and so is expressed as somewhere between the S_{AB} values of 0.51 and 0.63. The catalogs and dendrogram here provided can be compared to those provided by Stackebrandt and Woese (47) for other *Micrococcaceae*.

DISCUSSION

Despite a remarkable superficial phenotypic similarity, it has been possible to determine that "*M. radiodurans*" and organisms like it are distinct from the equally red micrococci, *M. roseus* and *M. agilis*. The distinction is so fundamental, involving genomic and ribosomal sequences as well as the structure of polymeric constituents, that we have to consider these micrococci as being phylogenetically far removed from the *Micrococcaceae*. However, the tests are esoteric, and practical determinative requirements sug-

gest that, even if a new genus were required (B. Brooks and R. G. E. Murray, unpublished data), it would still have to be appended to the *Micrococcaceae* in Part 14 of *Bergey's Manual* (3) as a genus of unknown affiliation. Feltham (12) recently published a taxonomic survey of *Micrococcaceae* (277 strains; 61 characters determined) and concluded, as we have, that *M. roseus*, *M. agilis*, and "*M. radiodurans*" (including the equivalents of our strains UWO 288, 1055, and 1056) form three distinct clusters deserving of species distinction.

We can justify the alignment of *M. roseus* Flüge 1886 and *M. agilis* (Ali-Cohen 1889) Kocur and Schleifer 1975 (24) with the genus *Micrococcus* assembled by Baird-Parker (3). They exhibit appropriate wall profiles, peptidoglycan types, branched-chain 15:0 fatty acids, and an appropriate range of G+C molar ratios. *M. roseus* shows a 0.73 association coefficient in 16S ribosomal RNA catalogs to the type species, *M. luteus* (47). Despite this very clear structural and molecular support for the distinction of *M. agilis* and *M. roseus* made by Kocur and Schleifer (24), we anticipate problems in identifying these micrococci using biochemical determinative tests without further standardization of each procedure. For example, our results for these two organisms differ from those of Kocur and Schleifer (24) for glucose oxidation, esculin hydrolysis, and β -galactosidase (*o*-nitrophenyl- β -D-galactopyranoside test) activity. Similarly, Feltham (12) found that nine strains of *M. roseus* produced acid from xylose according to the Analytab Products, Inc., (Plainview, N.Y.) identi-

TABLE 7. *Oligonucleotide catalogs for three red micrococci*^a

Sequences	Organisms	Sequences	Organisms
5-mers		6-mers—Continued	
CCCAG	3	UCUUCG	1
CACCG	1-3	CUUCUG	1
CAACG	1-3; 1, 2	UUCAUG	2
AACCG	3	UUACUG	1
ACACG	1-3		
CAAAG	3	UUUUCG	1-3
AAAAG	3	UUCUUG	2
		UUUAUG	1, 3
UCCCG	1-3	7-mers	
CUCAG	1-3	CAAACCG	1, 2
CACUG	1-3	AACACCG	2
UACCG	1-3; 3	CCAAAAG	2
ACCUG	2; 2		
CUAAG	1, 3	CCUCACG	1
UAACG	3	CCUACAG	2
AACUG	2, 3	CAACICG	1-3
ACAUG	1-3; 1	CAUACCG	1-3
AAUCG	1-3	AUACCCG	1-3
AUCAG	2	AUCACCG	2
UAAAG	1-3; 1-3; 2	UACAACG	2
		UAACACG	1, 3
UCCUG	1	AACCUAG	1
CCUUG	1-3	AUACCAG	1-3
UUCAG	1-3	CUAAAAG	3
UCUAG	1-3	AUAAACG	1-3
CUAUG	2	CCCUUCG	1-3
UCAUG	1	CUUCCAG	1
UAUCG	3	UUACCCG	1-3
UUAAG	1-3; 3	CACUCUG	1-3
AAUUG	1-3	ACCUCUG	1-3
AUUAG	1-3	CAUUCAG	1, 2
UUCUG	3	UAAUACG	1-3
UCUUG	1, 2; 1	UACAAUG	3
CUUUG	2, 3	AUAACUG	2
AUUUG	1, 3	CCUUUAG	3
		UUUACCG	3
6-mers		UAAAUG	2
CCACAG	1	8-mers	
(CCA, CA)G	3	CCCCAAG	2
CACAAG	1-3		
ACAACG	1	CUCAACCG	2
ACACAG	1	CCAACUCG	2
AAACCG	1, 3	AACCCUG	2
CCUCAG	3	CAAACAUG	1
ACUCCG	1, 2		
CUAACG	1-3	CAUCCUG	2
UCACAG	2	CUCAACUG	3
UACCAG	1, 2	CACUCUAG	3
AAUCAG	3	ACUCCAUG	3
AAUACG	1-3	CUAAUACG	1, 2
		CUACAAUG	1-3
UUCCCG	1-3	+CAAACUUG	3
UCCUCG	2	AAUCACUG	1-3
CCUUCG	1	AAUAACUG	1
CCUAUG	2		
CUACUG	3	CCCUUAUG	3
CACUUG	3	UCCUUACG	1-3
ACCUUG	3	CAUCUUCG	2
AUCCUG	1-3	AAUCCUG	1, 2
UAAACUG	1-3	UCUUAAAG	3
UAAUCG	1, 2; 1	CAUUUAAG	3
AAUCUG	1-3	CCUUUAUG	3
AUACUG	1, 2	AAUUUCUG	3
UAAUAG	1, 3; 3	AUUUAUCG	1
	1-3	UUUUAAAG	1, 2

TABLE 7—Continued

Sequences	Organisms	Sequences	Organisms
9-mers		11-mers	
CCCACCAAG	2	AACCUUACCAG	1-3
CCUACCAAG	1	12-mers	
CUCAAACCG	2	None	
CUACACACG	1-3	13-mers	
UACACACG	1-3	+CUACAAUCAAAG	1
+CCUACCAUG	3	UCCCACUCCUACG	1-3
CUAACUCCG	1-3	14-mers	
CAACCUUG	1, 3	+AAACCUAUCCCCAG	1
CUAAUACAG	3	AAUCUCCCCAAUG	2
AAUAACCUG	3	AAUCUCCACAAUG	1, 3
UUCCAUCAG	1-3	15-mers	
AUAUUUAAG	1	+UCCACACCCUAAACG	1-3
UUUAAUUCG	1-3	16-mers	
10-mers		+CAACCCCUACCUUCAG	2
CCAUCCCCAG	2	Post-transcriptionally	
ACCUACCCCG	3	modified oligomers	
UCACACCAG	2	AAG	1-3
ACCUACCCAG	1	AAG	1-3
ACAUCCACAG	3	GGGC	1-3
+AACACCAAUG	1, 3	CCCCG	1-3
AAACUCAAG	1-3	CAACG	1-3
CUCAACCUUG	1	NAAUCG	1-3
UCACACCAUG	1, 3	UAACAAG	1-3
ACAUCCCAUG	2	Termini	
UAAACCUCUG	1-3	5' end:	
		pUUUUAUG	1, 2
		(not determined in 3)	
		3' end:	
		AUCACCUCCUUX _{OH}	1, 2
		AUCACCUCCUUCU _{OH}	3

"Organisms are designated by numbers as follows: 1, "*M. radiodurans*" UWO-298; 2, *M. roseus* UWO-294; 3, "*M. radiophilus*" UWO-1055. Numbers before the semicolon or numbers that stand alone indicate the organisms in which the sequence is present; numbers after the semicolon indicate organisms in which it occurs a second time. Symbols: *, base is modified but precise nature of modification has not been determined; (+), not fully sequenced, other orders possible; N, underlying base is of undetermined character; X, unidentified nucleotide.

TABLE 8. Number of coincident nucleotides and the S_{AB} values in comparing red micrococci with various reference strains^a

Representative strain	1	2	3	4	5	6	7	8
1. " <i>Micrococcus radiophilus</i> " 1055	—	359	285	97	111	110	168	140
2. " <i>M. radiodurans</i> " 298	0.62	—	366	117	132	139	174	138
3. <i>M. roseus</i> 294	0.51	0.63	—	106	112	122	169	116
4. <i>M. roseus</i> 1057	0.17	0.20	0.19	—	411	274	165	154
5. <i>M. luteus</i> ATCC 23262	0.20	0.23	0.20	0.73	—	309	178	148
6. <i>Corynebacterium diphtheriae</i> ATCC 11913	0.20	0.24	0.22	0.49	0.55	—	189	159
7. <i>Bacillus brevis</i> ATCC 8185	0.29	0.29	0.29	0.29	0.31	0.33	—	165
8. <i>Escherichia coli</i> B-236	0.24	0.23	0.20	0.27	0.26	0.28	0.28	—

^a Data in upper triangle are the total number of nucleotides presented in the coincident oligomers; data in the lower triangle are S_{AB} values.

FIG. 9. Dendrogram to show the relationship, in terms of 16S ribosomal RNA association coefficients (S_{AB}), of "*M. radiodurans*" and like organisms to veritable *Micrococcus* species and diverse other bacteria. The strains used for comparison were *E. coli* B236 (51), *Bacillus brevis* ATCC 8185 (54), *M. luteus* ATCC 23262 (47), and *Corynebacterium diphtheriae* ATCC 11913. The relative positions of the three radiation-resistant strains to each other is uncertain and (see Table 8) is in the range of S_{AB} 0.5 to 0.6, so the range is shown by a dotted line.

fication system (5 days at 30°C), which was not the case for our 14 strains. Media and conditions are likely to differ. We record strain variability (summary in Table 9), but differences of this degree are perplexing. Perhaps as simple an addition as 0.5 to 3% NaCl (52) might alter results.

We conclude that identifications based on the biochemical tests are similarly hazardous in the "*M. radiodurans*" grouping because four of the seven predominantly negative characters that might be useful for phenotypic distinction from others (Table 2 and summary in Table 9) are not totally reliable. New, positive characters must be sought to aid in identification; some possible approaches such as nonspecific esterases and the carotenoids were not helpful.

Lipid composition provides some useful data for the purposes of bacterial classification, as is apparent in the reviews by Kates (22), Shaw (42), and Lechevalier (26). Although exceptions do exist, these authors indicate that gram-positive bacteria generally have a high proportion of odd-numbered branched-chain (iso and anteiso) saturated or unsaturated fatty acids. Gram-neg-

ative bacteria have a high proportion of even-numbered, straight-chain saturated and unsaturated acids and, occasionally, odd-numbered cyclopropane acids. *M. roseus* and its allies in the genus certainly have the gram-positive selection of fatty acids and the group aligned with "*M. radiodurans*" has the selection usually attributed to gram-negative bacteria despite being definitely gram-positive. It is improbable that these organisms have any close relationship to gram-negative bacteria because the *Limulus* test (56) for endotoxic lipopolysaccharide was negative for the reference strains (S. W. Watson, Woods Hole Oceanographic Institution, Woods Hole, Mass., personal communication).

The lipid peculiarities of this organism are extended by our recent observation (B. G. Thompson, R. Anderson, and R. G. E. Murray, unpublished data) that a form of lipid-containing "outer membrane" lies external to the peptidoglycan of "*M. radiodurans*." Furthermore, the polar lipids are peculiar in character, and there is no trace of the usual phospholipids (phosphatidylcholine, phosphatidylserine, or phosphatidylethanolamine) in an appropriate extract of

TABLE 9. Phenotypes among 50 strains of red-pigmented micrococci^a

No. of strains	Cluster and representative strain	UWO no.	Growth in 5% NaCl	Growth at 37°C	Nitrate ^b	Esculin hydrolysis	Glucose ^c	ONPG ^d
14	<i>M. roseus</i>	1057	+	+	+	+10/14	—	—
4	<i>M. agilis</i>	1039	—	—	—	—	—	—
24	" <i>M. radiodurans</i> "	288	—18/24	+23/24	—18/24	—	—23/24	—
1	" <i>M. radiophilus</i> "	1055	+	+	—	—	—	—
1	" <i>M. radioproteolyticus</i> "	1056	—	+	—	+	A	—
2	<i>S. erythromyxa</i>	1045	+	+	+	—	—	—
2	"Haddock" strains	293	—	+	+	—	—	+
2	"Riso" strains	1083	—	—	+	—	—	+

^a All are gram-positive, catalase-positive, red-pigmented, tetrad-forming micrococci (2 to 3 µm). The reactions given are for the representative strains; the fraction of the number of strains giving the reaction is shown when results are inconsistent.

^b Nitrate reduced to nitrite.

^c In peptone-water basal medium.

^d *o*-Nitrophenyl-β-D-galactopyranoside test for β-galactosidase.

whole cells as well as outer membrane. These facts also argue for a unique niche for these organisms in a phylogenetic taxonomy.

Further to the observations of Stackebrandt and Woese (47), the micrococci aligned with "*M. radiodurans*" are characterized by complex wall profiles, Orn-Gly peptidoglycan types, predominant 16:1 straight-chain fatty acids, and 16S ribosomal RNA catalogs dissimilar (S_{AB} 0.23) to those of veritable *Micrococcus* species and a wide range of bacteria. If they separated from mainstream procaryotes at an early stage, one might expect a wide range of these organisms in nature. The diversity of the miscellaneous strains, the low order of intragroup DNA/DNA homology, and the 51 to 63% association coefficient for the 16S ribosomal RNAs of the three members tested suggest that other distinct clones should exist.

The G+C molar ratios, DNA homology data, and the S_{AB} values give support to the individuality of "*M. radiodurans*," "*M. radiophilus*," and "*M. radioproteolyticus*." The high S_{AB} values for these species and a representative of the miscellaneous group provide an argument in favor of association within a single genus in the face of very low DNA hybridization levels. The latter technique, despite an incomplete matrix, provides supportive data for maintaining that strains 288 and 298 are members of the species "*M. radiodurans*" and that strains 293, 294, 1083, and 1985 should be associated in some as yet unnamed species. The remaining miscellaneous strains may as well be put together with what used to be called *S. erythromyxa* (UWO 1045). The status of one strain, UWO 1088, remains uncertain because, despite many appropriate characteristics, the peptidoglycan contains diaminopimelic acid and no ornithine. Further studies are needed to settle the matter.

The strains of these extraordinary micrococci are very resistant to radiation (1, 5, 16, 23, 27), and this has usually, but not always, been used

as a selective factor for isolation. Moseley (32) has shown that radiation resistance is mutable, and, therefore, some relatives may be expected to be found in nature that are not radiation resistant. There is also the observation that "*M. radiodurans*" is unusually resistant to desiccation (11), and this seems to apply to other radiation-resistant bacteria and provides a means for selection that does not involve radiation (39).

It seems premature to attempt some new alignment in the scheme of bacterial classification. Baird-Parker (2) suggested, on more tenuous grounds than ours, that these organisms should be reclassified. At this moment they stand alone, and this isolation is emphasized by the sequence data from ribosomal nucleic acid. It is the opinion of the group concerned with the phylogenetic applications of RNA sequence catalogs (G. E. Fox et al., *Science*, in press) that "*M. radiodurans*" and its allies form one of eight groups of true bacteria that diverged from some common stem at an early time and that the order of branching is hard to determine. The associations within these groups, based on oligonucleotide sequence, can be surprising; in the case of the *Micrococcaceae*, for instance, *M. luteus* and *M. roseus* 1057 have strong associations (S_{AB} 0.78) with *Arthrobacter globiformis* (47).

Other molecular peculiarities, such as the seemingly unique assortment of polar lipids (Thompson et al., unpublished data), may contribute in the future to defining the level of divergence and possible alignments.

There is one inescapable set of conclusions: "*M. radiodurans*," "*M. radiophilus*," "*M. radioproteolyticus*," and at least two sets of strains in the miscellaneous group (*Sarcina erythromyxa* and the set clustering around *M. roseus* UWO 294) could be given the status of species. Altogether, they share characters that would allow the circumscription and naming of a new genus.

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