Kineococcus aurantiacus gen. nov., sp. nov., a New Aerobic, Gram-Positive, Motile Coccus with *meso*-Diaminopimelic Acid and Arabinogalactan in the Cell Wall

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A new aerobic, gram-positive, motile coccus isolated from soil is described. Strain RA 333^T (T = type strain) has the following characteristics: menaquinone MK-9(H₂); G+C content of DNA of 73.9 mol%; *meso*-diaminopimelic acid, glutamic acid, and alanine in a molar ratio of ca. 1:1:2 (type A1 γ); and arabinose and galactose in the cell wall. The two sugars are contained in the cell wall as an arabinogalactan polymer. Mycolic acids are not present. The taxonomic characteristics of this organism are different from those of previously described aerobic, gram-positive cocci. The name *Kineococcus aurantiacus* gen. nov., sp. nov., is proposed. The type strain is strain RA 333, which has been deposited in the Institute for Fermentation, Osaka, as strain IFO 15268.

The following six genera of aerobic, gram-positive cocci have been described previously: *Micrococcus* (11), *Deinococcus* (2), *Planococcus* (11), *Marinococcus* (9), *Sporosarcina* (3), and *Salinicoccus* (23). Among the members of these genera, *Planococcus*, *Marinococcus*, and *Sporosarcina* species and some strains of *Micrococcus agilis* (12) are known to be motile.

During the course of our taxonomic study of motile actinomycetes isolated from natural sources, we found that a gram-positive coccus with a high DNA guanine-plus-cytosine (G+C) content and *meso*-diaminopimelic acid (DAP) in its cell wall, which was isolated from soil in India, is motile and has taxonomic characteristics distinct from those of previously described aerobic, gram-positive cocci. In this paper, we describe the morphological, physiological, and chemotaxonomic characteristics of this isolate, strain RA 333^T, (T = type strain), and propose a new genus and species for this strain.

MATERIALS AND METHODS

Bacterial strains and culture conditions. Microorganisms exhibiting motility were screened by the dilution plate method on a medium containing 1.0% soluble starch, 0.1% casein, 0.05% K₂HPO₄, and 1.5% agar (pH 7.0 to 7.5) supplemented with 25 μ g of nalidixic acid per ml, 12.5 μ g of kanamycin per ml, 5.0 μ g of cefsulodin per ml, and 6.25 μ g of kabicidin per ml. Strain RA 333^T was isolated from soil obtained from the Indore region of India. For chemotaxonomic studies this strain was grown in shake cultures (10 g of yeast extract and 10 g of glucose in 1 liter of water; pH 7.0) at 28°C, harvested in the stationary phase, washed twice with water, and then freeze-dried. *M. agilis* IFO 15260^T was used as a reference strain for the polar lipid study.

Morphological and cultural characteristics. Cell morphology was examined by using cells grown on yeast extract-malt extract agar (4 g of yeast extract, 10 g of malt extract, 4 g of D-glucose, and 15 g of agar; pH 7.3). The sample used for scanning electron microscopy with a model JSM 5400 scanning electron microscope (JEOL, Ltd., Tokyo, Japan) was prepared by dehydrating cells through a graded ethanol

series and then in a Hitachi model HCP-2 critical point drying apparatus. Motility was observed under a light microscope by using cells in the logarithmic growth phase grown on yeast extract-malt extract agar or cells in the stationary phase after incubation at 28°C for 1 h in 0.01 M phosphate buffer (pH 7.0) containing 10% soil extract. Flagellation was observed with a model JEM-1200EX transmission electron microscope (JEOL) after shadowing with platinum-palladium.

Phenotypic characterization. Unless indicated otherwise, all tests were carried out at 28°C. Catalase activity was determined by bubbling in a 3% hydrogen peroxide solution. Oxidase activity was determined by the oxidation of 1% tetramethyl-*p*-phenylenediamine on filter paper. Acid production from carbohydrates was studied in media containing 0.5% peptone, 0.25% NaCl, 0.003% bromocresol purple, and each carbohydrate at a concentration of 0.5% (pH 7.2). Nitrate reduction and hydrolysis of starch, gelatin, casein, and esculin were tested by the methods described by Cowan and Steel (6). Susceptibility to antibiotics and antimicrobial agents was examined by placing antibiotic disks (diameter, 8 mm; Showa Yakuhin Kako Co., Ltd., Tokyo, Japan) on yeast extract-malt extract agar plates seeded with a suspension of the strain. Plates were incubated at 28°C for 3 days, and the diameters of inhibition zones were measured.

Peptidoglycan analysis. Cell walls were prepared from ca. 500 mg of dry cells by mechanical disruption with an ultrasonic oscillator and were purified as described by Schleifer and Kandler (19). The amino acid composition of complete wall hydrolysates was determined with a model LC-6AD high-performance liquid chromatography (HPLC) apparatus (Shimadzu Co., Ltd., Kyoto, Japan) equipped with a Wakopak WS-PTC column (Wako Pure Chemical Industries, Ltd., Osaka, Japan) as phenyl thiocarbamoyl (PTC) derivatives according to the manufacturer's instructions (24). The amino acid composition and isomers of DAP were determined by developing preparations on cellulose thin-layer chromatography plates (Tokyo Kasei Co., Ltd., Tokyo, Japan), using two-dimensional descending chromatography as described by Harper and Davis (10).

Cell wall sugar analysis. Cell walls were hydrolyzed with 2 N HCl at 100°C for 2 h, dried in vacuo, and then analyzed by the method of Mikami and Ishida (14), using a model LC-5A

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FIG. 1. Scanning electron micrograph of strain RA 333^T cells grown on yeast extract-malt extract agar.

HPLC apparatus (Shimadzu) equipped with a Shim-pack ISA 07/S2504 column (250 by 4 mm) and a Shimadzu model RE-530 spectrofluorometer.

Isolation of cell wall polysaccharide. Cell wall polysaccha-

ride was prepared by the method described previously (21). Briefly, a cell wall preparation was suspended in 0.5 N NaOH and heated at 90°C for 16 h. The suspension was neutralized with acetic acid and centrifuged, and the po-

FIG. 2. Transmission electron micrograph of a motile cell of strain RA 333^T.

TABLE 1. Differential characteristics of strain RA 333^T, aerobic gram-positive cocci, and related taxa^a

Taxon	Cell morphology	Motility	Endospores	G+C content (mol%)	Diamino acid	Murein type ^b	Mycolic acid	Glycolate test	Cell wall sugar	Menaquinone system	Cellular fatty acid(s)
Isolate RA 333 ^T	Coccus	+	—	73.9	<i>meso</i> -DAP	A1γ	—	Ace	Ara, Gal	MK-9(H ₂)	anteiso-15:0
<i>Micrococcus</i>	Coccus	—/+ ^c	—	65–75	L-Lys	A4α	—	Ace	NC	MK-8(H ₂), MK-9(H ₂), MK-7(H ₂), MK-8, MK-7	anteiso-15:0
						A3α					
<i>Deinococcus</i>	Coccus	—	—	62–71	L-Orn	A3β	—	ND	NC	MK-8	16:0
					L-Lys	A3α					16:1
<i>Planococcus</i>	Coccus	+	—	39–52	L-Lys	A3α	—	ND	NC	MK-8, MK-7	anteiso-15:0
											anteiso-17:0
<i>Sporosarcina</i>	Coccus	+	+	40–42	L-Lys	A4α	—	ND	NC	MK-7	ND
					L-Orn	A4β					
<i>Marinococcus</i>	Coccus	+	—	44–47	<i>meso</i> -DAP	A1γ	—	ND	NC	MK-7, MK-6	anteiso-15:0
											anteiso-17:0
<i>Salinicoccus</i>	Coccus	—	—	51.2	L-Lys	A4α	—	ND	ND	None	anteiso-15:0, iso-15:0
<i>Corynebacterium</i>	Rod	—	—	51–65	<i>meso</i> -DAP	A1γ	+	Ace	Ara, Gal	MK-8(H ₂), MK-9(H ₂)	16:0, 18:1
<i>C. amycolata</i>	Rod	—	—	61	<i>meso</i> -DAP	A1γ	—	Ace	Ara, Gal	MK-8(H ₂), MK-9(H ₂)	16:0, 18:1
<i>Rhodococcus</i>	Rod	—	—	60–69	<i>meso</i> -DAP	A1γ	+	Gly	Ara, Gal	MK-8(H ₂)	16:0, 18:1
<i>Tsukamurella</i>	Rod	—	—	66–73	<i>meso</i> -DAP	A1γ	+		Ara, Gal	MK-9	16:0, 18:1
<i>Arthrobacter</i>	Rod	—	—	59–66	L-Lys	A3α	—	Ace	NC	MK-9(H ₂)	anteiso-15:0, anteiso-17:0
						A4α				MK-8, MK-9	

^a Data from references 2 through 5, 8, 9, 11, 18, and 23. Abbreviations: L-Lys, L-lysine; L-Orn, L-ornithine; Ace, acetyl residues; Gly, glycolyl residues; Ara, arabinose; Gal, galactose; ND, not determined; NC, not characteristic.

^b Designations for murein types are the designations of Schleifer and Kandler (19).

^c —/+, absent except for one species (*M. agilis*).

lysaccharide in the supernatant was purified by chromatography performed with a DEAE-Toyopearl column. Sugar composition was determined by HPLC as described above.

Glycolyl analysis. Glycolyl testing was carried out by using the method of Uchida and Aida (22).

Analysis of cellular fatty acids. Fatty acids were extracted from dry cells (50 mg) by acid methanolysis and were examined by using a model GC-9A gas-liquid chromatography apparatus (Shimadzu) equipped with a glass column (5 m by 0.28 cm) containing 10% diethyleneglycol succinate on Chromosorb W at 180°C (20).

Analysis of polar lipids. Free lipids were extracted from dry cells (100 mg), purified by the method of Minnikin et al. (16), and examined by two-dimensional thin-layer chromatography, using Kiesegel 60 F₂₅₄ plates. Lipids were visualized by spraying the plates with 10% molybdophosphoric acid in ethanol, followed by heating at 140°C for 10 min. Specific spray reagents for lipid phosphate, α-naphthol (for sugar), and ninhydrin (for amino groups) were also used.

Analysis of mycolic acids. Mycolic acids were analyzed by using the method of Minnikin et al. (15).

Analysis of isoprenoid quinones. Menaquinones were extracted from dry cells (200 mg) with chloroform-methanol (2:1, vol/vol) and were purified by thin-layer chromatography, using benzene as the solvent. The menaquinones were extracted with diethyl ether, dried by using a nitrogen stream, and then analyzed by HPLC, using a Shimadzu model LC-5A apparatus equipped with a Zorbax octyldecyl silane column (4.6 by 150 mm).

DNA base composition. DNA was obtained by the method of Saito and Miura (17). The G+C content of the DNA was determined by the method of Mesbah et al. (13) after treatment with P1 nuclease and alkaline phosphatase and by

HPLC, using a model LC-6AD apparatus (Shimadzu) equipped with a Comosil 5C₁₈-AR column (4.6 by 150 mm; Nacalai Tesque, Inc., Tokyo, Japan).

RESULTS AND DISCUSSION

Morphological, physiological, and biochemical characteristics. Isolate RA 333^T is a gram-positive coccus. The cells are 1.0 to 1.5 μm in diameter and occur in clusters, as well as in pairs, tetrads, and octads (Fig. 1). Rod-shaped or filamentous cells were not observed at any stage of growth or when the organism was cultured under different growth conditions. On most solid media the strain formed rough, round, convex, orange colonies. Cells in the early phase grown on yeast extract-malt extract agar were motile at a low frequency. Cells in the stationary phase formed clusters and were not motile. However, after incubation at 28°C for few hours in 0.01 M phosphate buffer (pH 7.0) containing 10% soil extract, almost all of the cells were actively motile. Motile cells had tufts of flagella, as shown in Fig. 2. Cells grown under all conditions were surrounded by a slime- or capsular polysaccharide-like substance (Fig. 1), which seemed to prohibit motility of the cells. However, when the cells were incubated under the conditions described above, almost all of the cells were motile. The flagella shown in Fig. 2 appeared to be thick lines, which might have been due to the deposit of polysaccharide-like substance on the surface.

The biochemical characteristics of strain RA 333^T are summarized in Table 2. This strain had catalase and urease activities but not oxidase activity. It did not hydrolyze esculin and weakly utilized propionate; it was strictly aerobic. The temperature range for growth was 9 to 36°C, and the optimum temperature was 28°C. The pH range for growth

TABLE 2. Phenotypic characteristics of strain RA 333^T

Characteristic	Strain RA 333 ^T
Color	Orange
Morphology	Cocci (diameter, 1.0–1.5 μ m), in clusters
Gram staining	+
Motility	+
Spore formation	–
Oxidase	–
Catalase	+
Urease	+
Nitrate reduction	–
Hydrolysis of:	
Gelatin	–
Casein	–
Esculin	–
Starch	–
Utilization of propionate	+
Acid production from:	
Glycerol	–
D-Xylose	+
D-Ribose	–
L-Arabinose	+
D-Arabinose	–
D-Glucose	+
D-Mannose	–
D-Galactose	–
D-Fructose	+
L-Rhamnose	–
Mannitol	–
Inositol	–
Raffinose	–
Maltose	–
Sucrose	+
Trehalose	–
OF test ^a	Oxidation
Temp range for growth (°C)	9–37
Optimum temp (°C)	28
Growth in 2% NaCl	+
Growth in 5% NaCl	+
Growth in 7% NaCl	–
Anaerobic growth	–

^a OF, Oxidation-fermentation.

was pH 6.0 to 9.0. The strain was resistant to sulbenicillin, cefsulodin, colistin, polymyxin B, and fosfomycin but was susceptible to cefem and tetracycline antibiotics.

Chemotaxonomic characteristics. The chemotaxonomic characteristics of strain RA 333^T are summarized in Table 3. The quinone was hydrogenated menaquinone MK-9(H₂). The amino acids in the cell wall were *meso*-DAP, alanine, and glutamic acid (molar ratio, ca. 1:2:1); this corresponds to murein type A1 γ of Schleifer and Kandler (19). The only cell wall sugars that were detected were arabinose and galactose. An examination of the polysaccharide isolated from the cell wall indicated that these two sugars were present as an arabinogalactan polymer in the cell wall (molar ratio of galactose to arabinose, ca. 1.00:1.10). Mycolic acids were not present. The glycan moiety of the murein contained only acetyl residues. The major cellular fatty acid of RA 333^T was 12-methyltetradecanoic (anteiso-C_{15:0}) acid (88.7%). The strain had the polar lipid pattern shown in Fig. 3A. Diphosphatidylglycerol and phosphatidylglycerol were identified by their chromatographic behavior and staining characteristics. In addition, unknown glycolipids were present. Thus, the polar lipid pattern of strain RA 333^T distinguishes this organism from *Micrococcus* species (Fig. 3B).

The following four genera of aerobic, gram-positive, motile cocci have been described previously: *Planococcus*, *Marinococcus*, *Micrococcus*, and *Sporosarcina*. Chemotaxonomy has proved to be very useful in providing characteristics that distinguish these taxa from neighboring taxa. Strain RA 333^T can be distinguished readily from all of these taxa on the basis of chemical characteristics (Table 1). The menaquinone and cellular fatty acid compositions, the G+C content of the DNA, and the presence of motility in strain RA 333^T most closely resemble the characteristics of *M. agilis* (12). However, these organisms are different, as *M. agilis* contains type A4 α peptidoglycan, has no characteristic sugars in its cell wall, and produces a different polar lipid pattern (Fig. 3B). Thus, the combination of the presence of *meso*-DAP and arabinogalactan in the cell wall, a G+C content of 73.9 mol%, and the presence of MK-9(H₂) readily

FIG. 3. Two-dimensional thin-layer chromatograms of the polar lipids of strain RA 333^T (A) and *M. agilis* IFO 15260^T (B). Chloroform-methanol-water (65:25:4, vol/vol/vol) was used in the first dimension, and chloroform-methanol-acetic acid-water (80:12:15:4, vol/vol/vol/vol) was used in the second dimension. Lipids were visualized by spraying the plates with 10% molybdophosphoric acid in ethanol, followed by heating at 150°C for 10 min. Abbreviations: DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PGL, unidentified phosphoglycolipid; GL, unidentified glycolipid.

TABLE 3. Chemotaxonomic characteristics of strain RA 333^T

Characteristic	Strain RA 333 ^T
Quinone system	MK-9(H ₂)
Cellular fatty acids	anteiso-C _{15:0} (88.7), anteiso-C _{17:0} (1.7) ^a
Peptidoglycan amino acids (molar ratio)	
<i>meso</i> -DAP	0.56
Alanine	2.00
Glutamic acid	0.92
Cell wall sugars (molar ratio)	
Arabinose	1.10
Galactose	1.00
Mycolic acid	Absent
G+C content of DNA (mol%)	73.9

^a The values in parentheses are the percentages of the total fatty acids.

distinguishes strain RA 333^T from all previously described aerobic, gram-positive cocci (Table 3). The presence of MK-9(H₂), acetyl residues in the murein, and *meso*-DAP and arabinogalactan in the cell wall is consistent with organisms classified in the mycolic acid-containing taxa, such as the genera *Corynebacterium*, *Mycobacterium*, *Tsukamurella*, and *Rhodococcus*, but the absence of mycolic acids and the occurrence of branched fatty acids, together with cell morphology, clearly distinguish our isolate from all of these taxa. The mycolic acid-lacking coryneform bacterium *Corynebacterium amycolata* (4) has chemotaxonomic characteristics similar to those of our strain, but the two organisms can be differentiated on the basis of cellular fatty acid composition, cell morphology, and motility.

Thus, on the basis of biochemical and chemical criteria, our new isolate can be distinguished readily from all previously described aerobic, gram-positive cocci and in our opinion warrants a new taxon. Therefore, we formally propose that strain RA 333^T should be classified in a new genus, *Kineococcus*, as *Kineococcus aurantiacus* gen. nov., sp. nov.

Much additional work, including DNA-rRNA hybridization studies and comparative analysis of the 16S rRNA sequences of *Kineococcus* strains and other gram-positive, aerobic cocci and rods, will be necessary to determine the relationship of this new genus to previously described gram-positive bacteria. Meanwhile, the genus *Kineococcus* should be considered one of the several genera belonging to the recently proposed family *Pseudonocardiaceae* (1, 7).

Descriptions for the new genus and new species are given below.

Description of *Kineococcus* gen. nov. *Kineococcus* (Ki.ne.o.coc'cus. Gr. n. *kinesis*, motion; Gr. n. *coccus*, a grain or berry; M.L. fem. n. *Kineococcus*, motile coccus) cells are spherical and 1.0 to 1.5 µm in diameter and occur in pairs, in tetrads, or in clusters. Motile. The motile cells have tufts of flagella. Endospores are not formed. Gram positive. Colonies are circular and rough and may be cream colored to orange. Strictly aerobic. Catalase and urease positive. Oxidase negative. The cells do not reduce nitrate to nitrite. Acid is produced from glucose and some other sugars. Esculin is hydrolyzed. Starch, gelatin, and casein are not hydrolyzed. The optimum growth temperature is 27°C.

The cell wall peptidoglycan contains *meso*-DAP, alanine, and glutamic acid. The major menaquinone is MK-9(H₂). Mycolic acid is not present. The major cellular fatty acid is anteiso-C_{15:0}. Diphosphatidylglycerol, phosphatidylglycerol, and unidentified glycolipids are present as polar lipids.

The type species is *Kineococcus aurantiacus*.

Description of *Kineococcus aurantiacus* sp. nov. *Kineococcus aurantiacus* (au.ran.ti'a.cus. M. L. n. *aurantium*, generic name of orange; M. L. adj. *aurantiacus*, orange colored) cells are spherical and 1.0 to 1.5 µm in diameter and occur in pairs, in tetrads, or in clusters. Motile. The motile cells have tufts of flagella. Endospores are not formed. Gram positive. Colonies are circular and rough and may be cream colored to orange. Strictly aerobic. Catalase and urease positive. Oxidase negative. The cells do not reduce nitrate to nitrite. Acid is produced from D-glucose, D-xylose, D-fructose, sucrose, and L-arabinose. Acid is not produced from glycerol, D-ribose, D-arabinose, raffinose, maltose, D-galactose, D-mannose, inositol, L-rhamnose, mannitol, or trehalose. Esculin, starch, gelatin, and casein are not hydrolyzed. The optimum growth temperature is 28°C.

Chemical characteristics, such as amino acid composition of the cell wall peptidoglycan, menaquinone system, mycolic acids, cellular fatty acids, and polar lipids, are the same as the chemical characteristics described above for the genus. The DNA base composition of the type strain is 73.9 mol%, as determined by HPLC. Distribution: soil.

The type strain is strain RA 333 which has been deposited in the Institute for Fermentation, Osaka, as strain IFO 15268.

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REFERENCES

1. Bowen, T., E. Stackebrandt, M. Dorsch, and T. M. Embley. 1989. The phylogeny of *Amycolata autotrophica*, *Kibdelosporangium aridum* and *Saccharothrix australiensis*. J. Gen. Microbiol. 135:2529-2536.
2. Brooks, B. W., and R. G. E. Murray. 1981. Nomenclature for "*Micrococcus radiodurans*" and other radiation-resistant cocci: *Deinococcaceae* fam. nov. and *Deinococcus* gen. nov., including five species. Int. J. Syst. Bacteriol. 31:353-360.
3. Claus, D., and F. Fahmy. 1986. Genus *Sporosarcina* Kluyver and van Niel 1936, 401^{AL}, p. 1202-1206. In P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), *Bergey's manual of systematic bacteriology*, vol. 2. The Williams & Wilkins Co., Baltimore.
4. Collins, M. D., R. A. Burton, and D. Jones. 1988. *Corynebacterium amycolatum* sp. nov., a new mycolic acid-less *Corynebacterium* species from human skin. FEMS Microbiol. Lett. 49: 349-352.
5. Collins, M. D., J. Smida, M. Dorsch, and E. Stackebrandt. 1988. *Tsukamurella* gen. nov. harboring *Corynebacterium paurometabolum* and *Rhodococcus aurantiacus*. Int. J. Syst. Bacteriol. 38:385-391.
6. Cowan, S. T., and K. J. Steel. 1965. Manual for the identification of medical bacteria. Cambridge University Press, London.
7. Embley, M. T., J. Smida, and E. Stackebrandt. 1988. The phylogeny of mycolate-less wall chemotype IV actinomycetes and description of *Pseudonocardiaceae* fam. nov. Syst. Appl. Microbiol. 11:44-52.
8. Goodfellow, M. 1986. Genus *Rhodococcus* Zopf 1891, 28^{AL}, p. 1472-1481. In P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), *Bergey's manual of systematic bacteriology*, vol. 2. The Williams & Wilkins Co., Baltimore.
9. Hao, M. V., M. Kocur, and K. Komagata. 1984. *Marinococcus* gen. nov., a new genus for motile cocci with *meso*-diaminopimelic acid in the cell wall; and *Marinococcus albus* sp. nov. and *Marinococcus halophilus* (Novitsky and Kushner) comb.

- nov. J. Gen. Appl. Microbiol. **30**:449–459.
10. Harper, J. J., and G. H. G. Davis. 1979. Two-dimensional thin-layer chromatography for amino acid analysis of bacterial cell walls. Int. J. Syst. Bacteriol. **29**:56–58.
 11. Jones, D., and M. D. Collins. 1986. Section 15. Irregular, non-sporing Gram-positive rods, p. 1261–1434. In P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 2. The Williams & Wilkins Co., Baltimore.
 12. Kocur, M., and K. H. Schleifer. 1975. Taxonomic status of *Micrococcus agilis* Ali-Cohen 1889. Int. J. Syst. Bacteriol. **25**:294–297.
 13. Mesbah, M., U. Premachandran, and W. B. Whitman. 1989. Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. Int. J. Syst. Bacteriol. **39**:159–167.
 14. Mikami, H., and Y. Ishida. 1983. Post-column fluorometric detection of reducing sugars in high-performance liquid chromatography using arginine. Bunseki Kagaku **32**:E207–E210.
 15. Minnikin, D. E., L. Alshamaony, and M. Goodfellow. 1975. Differentiation of *Mycobacterium*, *Nocardia* and related taxa by thin-layer chromatographic analysis of whole-organism methanolysates. J. Gen. Microbiol. **88**:200–206.
 16. Minnikin, D. E., M. D. Collins, and M. Goodfellow. 1979. Fatty acid and polar lipid composition in the classification of *Cellulomonas*, *Oerskovia* and related taxa. J. Appl. Bacteriol. **47**:87–95.
 17. Saito, H., and K. Miura. 1963. Preparation of transforming deoxyribonucleic acid by phenol treatment. Biochim. Biophys. Acta **72**:619–629.
 18. Schleifer, K. H. 1986. Family I. *Micrococcaceae* Prevot 1961, 31^{AL} 1986, p. 1003–1035. In P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 2. The Williams & Wilkins Co., Baltimore.
 19. Schleifer, K. H., and O. Kandler. 1972. Peptidoglycan types of bacterial cell walls and their taxonomic implications. Bacteriol. Rev. **36**:407–477.
 20. Suzuki, K., and K. Komagata. 1983. Taxonomic significance of cellular fatty acid composition in some coryneform bacteria. Int. J. Syst. Bacteriol. **33**:188–193.
 21. Takeuchi, M., and A. Yokota. 1989. Cell-wall polysaccharides in coryneform bacteria. J. Gen. Appl. Microbiol. **35**:233–252.
 22. Uchida, K., and K. Aida. 1977. Acyl type of bacterial cell wall: its simple identification by colorimetric method. J. Gen. Appl. Microbiol. **23**:249–260.
 23. Ventosa, A., M. C. Marquez, F. Ruiz-Berraquero, and M. Kocur. 1990. *Salinicoccus roseus* gen. nov., sp. nov., a new moderately halophilic Gram-positive coccus. Syst. Appl. Microbiol. **13**:29–33.
 24. Wako Pure Chemical Industries, Ltd. 1989. Technical note for the system of PTC-amino acid analysis. Wako Pure Chemical Industries, Ltd., Osaka, Japan. (In Japanese.)