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Proposal of six new species in the genus *Microbacterium* and transfer of *Flavobacterium marinotypicum* ZoBell and Upham to the genus *Microbacterium* as *Microbacterium maritypicum* comb. nov.

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Reference strains, including two mis-named organisms, '*Chromobacterium chocolatum*' and *Flavobacterium marinotypicum*, isolates from soil and clinical specimens, all previously recognized as *Aureobacterium* or *Microbacterium*, were characterized taxonomically. On the basis of morphological, physiological and chemotaxonomic characteristics, as well as DNA–DNA hybridization data, six new species and one new combination are proposed in the genus *Microbacterium*: *Microbacterium ketosireducens* sp. nov. (type strain IFO 14548^T), *Microbacterium chocolatum* sp. nov. (type strain IFO 3758^T), *Microbacterium aurantiacum* sp. nov. (type strain IFO 15234^T), *Microbacterium hominis* sp. nov. (type strain IFO 15708^T), *Microbacterium thalassium* sp. nov. (type strain IFO 16060^T), *Microbacterium halophilum* sp. nov. (type strain IFO 16062^T) and *Microbacterium maritypicum* comb. nov. (type strain IFO 15779^T).

Keywords: *Microbacterium ketosireducens* sp. nov., *Microbacterium chocolatum* sp. nov., *Microbacterium aurantiacum* sp. nov., *Microbacterium hominis* sp. nov., *Microbacterium thalassium* sp. nov., *Microbacterium halophilum* sp. nov., *Microbacterium maritypicum* comb. nov.

INTRODUCTION

As a result of the unification of the genera *Microbacterium* and *Aureobacterium*, 19 species are accommodated in the genus *Microbacterium* (Takeuchi & Hatano, 1998). The taxonomic study that led to the unification revealed that 11 reference strains including two mis-named organisms, '*Chromobacterium chocolatum*' and *Flavobacterium marinotypicum*, isolates from soil and clinical specimens, should be accommodated in the redefined genus *Microbacterium*, although their taxonomic positions in the genus were not certain. All strains had all of the characteristic chemotaxonomic markers of the genus *Microbacterium*, the presence of D-diamino acid or L-diamino acid (D-ornithine or L-lysine), glycine and *N*-glycolyl residues in the cell walls, isoprenoid quinones MK-11, 12, and/or 13, and G+C contents of 67·2–71·5 mol%. Furthermore, the polyphasic assignment of these strains to the genus *Microbacterium* was confirmed by the results of 16S rDNA sequence comparisons and phylogenetic analyses which included all previously validly described *Microbacterium* species (Takeuchi & Hatano, 1998).

This paper describes the characterization of these 11 strains. On the basis of morphological and chemotaxonomic characteristics, DNA–DNA hybridization data and previously obtained results of the 16S rDNA sequence analysis (Takeuchi & Hatano, 1998), we concluded that 10 out of the 11 strains represent six new *Microbacterium* species and one new combination in the genus *Microbacterium*, while the remaining strain was identified as *Microbacterium laevaniformans*.

METHODS

Bacterial strains and culture conditions. Strains used in this study are listed in Table 1. *Microbacterium ketosireducens* IFO 14548^T and IFO 14549, which reduce 2-5-diketo-D-gluconic acid (2,7-anhydro-β-arabino-2-5-hepto-diulopyranose) to 2-keto-gulonic acid (2,7-anhydro-β-D-ido-heptulopyranose), were isolated from soil in 1983, and

Table 1. Bacterial strains used

Brackets indicate misidentifed name. T, Type strain. ATCC, American Type Culture Collection, Rockville, USA; BUCSAV, Biologicky Ustav. Czech Akademie Ved, Prague, Czech Republic; CDC, Centers for Disease Control and Prevention, Atlanta, USA; LCDC, Laboratory Center for Disease Control Tunney's Pasture, Ottawa, Canada; NCIB (= NCIMB, NCMB), National Collection of Industrial and Marine Bacteria, Aberdeen, UK; NCFB, National Collection of Food Bacteria, Reading Laboratory, Reading, UK.

Proposed reclassification	Species	Strain (IFO no.)	Other designation	Source
Microbacterium ketosireducens	'*Aureobacterium ketoreductum*'	14548^T		Soil
Microbacterium ketosireducens	'*Aureobacterium ketoreductum*'	14549		Soil
Microbacterium chocolatum	'*Chromobacterium chocolatum*'	3758^T	BUCSAV 207^T, NCIB 8181^T	Culture contamination
Microbacterium maritypicum	[*Flavobacterium marinotypicum*]	15779^T	ATCC 19260^T, NCMB 1050^T	Sea water and marine mud
Microbacterium aurantiacum	[*Microbacterium laevaniformans*]	15234^T	NCFB 2288^T, ATCC 49090^T	Sewage
Microbacterium aurantiacum	[*Microbacterium laevaniformans*]	15235	NCFB 2289, ATCC 49091	Sewage
Microbacterium hominis	*Microbacterium* sp. (CDC group A-4)	15708^T	LCDC 84-209^T	Lung aspirate
Microbacterium laevaniformans	*Microbacterium* sp. (CDC group A-5)	15709	LCDC 91-039^T	Blood
Microbacterium thalassium	Strain No. 10	16060^T		Mangrove rhizosphere
Microbacterium thalassium	Strain No. 71	16061		Mangrove rhizosphere
Microbacterium halophilum	Strain No. 76	16062^T		Mangrove rhizosphere

maintained at the Institute for Fermentation, Osaka, as '*Aureobacterium ketoreductum*'. *Microbacterium chocolatum* was isolated from culture contamination by M. H. Knutsen in 1944 (Clise, 1948). *Microbacterium maritypicum* appeared on the Approved List of Bacterial Names (Skerman *et al*., 1980) as [*Flavobacterium marinotypicum*] (Breed, 1957), but it is excluded from the genus *Flavobacterium* because it is Gram-positive and motile (Holmes *et al*., 1984). *Microbacterium aurantiacum* IFO 15234^T and IFO 15235 were isolated from sewage and identified as *Microbacterium laevaniformans*. But they differ from the type strain of *M. laevaniformans* IFO 14471^T in menaquinone composition and amino acid composition of the peptidoglycan. On the basis of the amino acids in their cell walls, the organisms should be excluded from the genus *Microbacterium* and probably be assigned to the genus *Aureobacterium* (Yokota *et al*., 1993b). *Microbacterium hominis* IFO 15708^T and *Microbacterium laevaniformans* IFO 15709 were isolated from clinical specimens (Funke *et al*., 1995). *Microbacterium thalassium* IFO 16060^T and IFO 16061, and *Microbacterium halophilum* IFO 16062^T were isolated from soil in mangrove rhizospheres in Okinawa prefecture. For chemotaxonomic studies, each strain was cultured at 28 °C with aerobic shaking in peptone-yeast extract medium (PY medium), which contained 1% peptone, 0·2% yeast extract, 0·2% NaCl and 0·2% D-glucose (pH 7·2), and then harvested by centrifugation during the stationary phase, washed with water and lyophilized.

Morphological, physiological and biochemical characterization. Cell morphology was determined by phase-contrast microscopy following cell growth on PY agar. Motility was determined by the hanging drop method. Unless otherwise indicated, biochemical tests were performed at 28 °C. Catalase activity was determined by the presence of bubbles in a 3% hydrogen peroxide solution. Acid production from carbohydrates was studied in a medium containing 1% peptone, 0·5% NaCl, 0·003% bromothymol blue and 0·5% carbohydrate (pH 7·2). Assimilation of carbohydrates and organic acids was studied in a medium containing 0·1% NH_4NO_3, 0·1% K_2HPO_4, 0·05% $MgSO_4.7H_2O$, 0·02% KCl and 0·5% of carbohydrates or organic acids (pH 7·0). Nitrate reduction and hydrolysis of starch, aesculin, gelatin and Tweens 20, 40, 60 and 80 were tested by the methods described by Cowan (1974). API 20 NE tests were also used to determine physiological and biochemical characteristics.

Cell wall sugar analysis. Cell walls were prepared as described by Schleifer & Kandler (1972). They were hydrolysed with 2 M HCl at 100 °C for 2 h, dried *in vacuo*, then analysed as described by Mikami & Ishida (1983), using a model LC-5A HPLC apparatus (Shimadzu) equipped with a Shim-pack ISA 07/S2504 column (250 × 4 mm) and a Shimadzu model RF 530 spectrofluorometer.

Analysis of cellular fatty acids. Fatty acids were extracted from dried cells, purified, and analysed by GLC-MS with a GCMS-QP5000 spectrometer (Shimadzu) combined with a CLASS-5000 MS Workstation computer system as described previously (Takeuchi & Hatano, 1998).

DNA–DNA hybridization. DNA–DNA hybridization was performed fluorometrically in microdilution wells by using biotinylated DNA (Ezaki *et al*., 1989).

RESULTS AND DISCUSSION

Morphological, physiological and biochemical characteristics

All 11 strains were Gram-positive, irregular short rods ranging from 0·2 to 0·5 by 0·6 to 2·0 μm. Many cells were arranged at angles, forming V shapes, but primary branching was not noted. In older cultures, rods were shorter, but a marked rod–coccus growth cycle did not occur. Colonies on solid media were circular, low convex with entire margins, opaque and moist. The pigments of the colonies of *M. aurantiacum* IFO 15234^T, IFO 15235 and *M. chocolatum* IFO 3758^T were orange, and those of the other eight strains were light yellow, yellowish-white or yellow. *M. maritypicum* IFO 15779^T was motile, but the other strains were non-motile. Two strains of *M. thalassium*, IFO 16060^T and IFO 16061, and two strains of *M. ketosireducens*, IFO 14548^T and IFO 14549, did not grow at 37 °C, but the other seven strains grew well. All strains except for *M. chocolatum* IFO 3758^T grew

Table 2. Phenotypic characteristics of *Microbacterium* species

Strains: 1, *M. ketosireducens* IFO 14548^T, IFO 14549; 2, *M. chocolatum* IFO 3758^T; 3, *M. maritypicum* IFO 15779^T; 4, *M. aurantiacum* IFO 15234^T, IFO 15235; 5, *M. hominis* IFO 15708^T; 6, *M. thalassium* IFO 16060^T, IFO 16061; 7, *M. halophilum* IFO 16062^T; 8, *M. laevaniformans* IFO15709; 9, *M. laevaniformans* IFO14471^T. Abbreviations: +, all strains positive; +w, weakly positive; −, all strains negative; ND, not determined; Y, yellow; YW, yellowish white; LY, light yellow; O, orange.

Characteristic	1	2	3	4	5	6	7	8	9
Colour of colonies	Y	O	LY	O	YW	Y	YW	YW	Y
Motility	−	−	+	−	−	−	−	−	−
Growth at 37 °C	−	+	+	+	+	−	+	+	+
Growth in 2% NaCl	+	+w	+	+	+	+	+	+	+
Growth in 5% NaCl	+w	−	+	+	+w	+	+	+	ND
Growth in 6·5% NaCl	−	−	+w	+w	+w	+w	+	−	ND
Hydrolysis of:									
Gelatin	+	−	+	−	−	+w/−	+w	−	−
Starch	+	+w	−	+	−	+	+	+	+
H_2S formation	+	+	−	+	+	−	−	+	+
MR test	+	−	−	−	+	+	+	+	+
VP test	−	−	−	−	+	−	−	+	+
Assimilation of:									
Arabinose	+	−	−	−/+w	+	−	−	−	−
Maltose	+	+	+	+	+	+	+	+	+
Mannose	+	+w	+	+	+	+	+	+	+
Mannitol	+	+w	+	+	+	+	+	+	+
N-Acetylglucosamine	−	−	+	−	+	−	−	−	−
Acetate	+w	−	−	−	+	−	−	+	+
Adipate	−	−	−	−	−	−	−	−	−
Caprate	−	−	−	−	−	−	−	−	−
Citrate	+/−	−	+	−	+	−	−	−	+
Fumarate	+w	−	−	+	+	−	−	+	+
Gluconate	+	+	+	+	+	+	+	+	+
Lactate	+w	−	−	−/+w	+	−	−	+	+
Malate	−	−	+	−/+w	+	−	−	+	+
Phenyl acetate	−	−	+	−	−	−	−	−	−
Propionate	−	−	−	+	+	−	−	+	+
Acid produced from:									
L-Arabinose	+	−	−	−	+	−	−	−	−
Galactose	+/−	−	−	−	+	−	−	+	+
Glucose	+	−	+	+w/−	+	+	+	+	+
Inulin	−/+w	−	−	−	−	−	−	−	−
Mannose	+	+	+	+	+	+	+	+	+
Melezitose	−	−	−	−	+	−	−	−	−
Raffinose	−	−	−	−	−	−	−	−	+
Rhamnose	−	−	−	−	−	−	+	−	+
Ribose	−	−	−	−	−	−	−	−	ND
Sucrose	+	+	+w	+	+	−	−	−	+
Trehalose	−	−	−	−	+	−	−	−	+
Xylose	+	+	−	−/+	−	+	−	−	−

weakly in broth media containing 5% NaCl. *M. halophilum* IFO 16062^T grew well in broth media containing 6·5% NaCl, but other strains grew weakly or not at all. The other physiological properties of the 11 strains and *M. laevaniformans* IFO 14471^T, which was added as a reference strain, are shown in Table 2.

Two strains of *M. aurantiacum* IFO 15234^T and IFO 15235, two strains of *M. ketosireducens* IFO 14548^T and IFO 14549, and two strains of *M. thalassium* IFO 16060^T and IFO 16061, showed identical phenotypic characteristics, respectively, and *Microbacterium* sp. IFO 15709 was similar to *M. laevaniformans* IFO

Table 3. Cellular fatty acids of *Microbacterium* species

Species	Strain (IFO no.)	Cellular fatty acid composition (%)							
		i14:0	i15:0	ai15:0	i16:0	16:0	i17:0	ai17:0	18:0
M. ketosireducens	14548^T	–	11·3	34·9	19·0	3·2	6·7	22·0	2·9
M. ketosireducens	14549	–	11·9	34·4	18·1	3·6	6·8	20·4	4·8
M. chocolatum	3758^T	–	6·3	34·7	10·9	4·6	4·7	38·8	–
M. maritypicum	15779^T	1·3	5·8	63·6	21·3	1·3	–	6·7	–
M. aurantiacum	15234^T	–	13·1	34·2	12·2	5·8	7·9	24·3	2·5
M. aurantiacum	15235	–	13·0	30·0	12·9	8·0	7·8	21·7	6·6
M. hominis	15708^T	–	5·7	40·9	21·0	6·7	2·3	23·5	–
M. laevaniformans	15709	–	4·0	36·1	20·6	7·5	–	26·8	5·0
M. thalassium	16060^T	–	10·1	30·5	25·7	2·6	6·4	24·7	–
M. thalassium	16061	–	21·4	25·8	28·0	2·6	9·2	13·0	–
M. halophilum	16062^T	0·9	22·3	24·0	23·5	3·9	11·3	12·1	2·0

Table 4. Chemotaxonomic characteristics of *Microbacterium* species

Data from this study and previous studies (Takeuchi & Hatano, 1998). Parentheses indicate that a trace amount is present. Abbreviations: ai, anteiso-branched acid; i, iso-branched acid; Hsr, homoserine; Orn, ornithine; Lys, lysine; Rha, rhamnose; Gal, galactose; Glc, glucose; 6dT, 6-deoxytalose; Man, mannose; Fuc, fucose; Xyl, xylose.

Species	Strain (IFO no.)	G+C content (mol%)	Menaquinone (MK)	Cellular fatty acid	Cell wall composition		
					Amino acid		Sugar
					Position 3	Linkage	
M. ketosireducens	14548^T	69·8	13	ai15, ai17, i16	Hsr	Orn	ND
M. ketosireducens	14549	69·7	13	ai15, ai17, i16	Hsr	Orn	ND
M. chocolatum	3758^T	69·5	12	ai15, ai17, i16	Hsr	Orn	Rha, Gal, (Man), (Xyl)
M. maritypicum	15779^T	71·6	12	ai15, (ai17), i16	Hsr	Orn	Gal
M. aurantiacum	15234^T	70·3	12	ai15, ai17, i16	Hsr	Orn	Rha, Gal, Fuc
M. aurantiacum	15235	70·1	12	ai15, ai17, i16	Hsr	Orn	Rha, Gal, Fuc
M. hominis	15708^T	71·2	11, 12	ai15, ai17, i16	Lys	Lys	Rha, 6dT, Gal, Man
M. laevaniformans	15709	68·8	11, 12	ai15, ai17, i16	Lys	Lys	Rha, Gal, (Glc), (Man)
M. thalassium	16060^T	69·7	11, 12	ai15, ai17, i16	Hsr	Orn	Gal, Glc
M. thalassium	16061	69·1	11, 12	ai15, ai17, i15, i16	Hsr	Orn	Rha, Man, Gal, Glc
M. halophilum	16062^T	67·2	11, 12, 13	ai15, ai17, i15, i16	Hsr	Orn	Man, Gal, Glc

14474^T, but differed in such features as assimilation of citrate and acid production from some carbohydrates.

Chemical characteristics

As shown in Table 3, cellular fatty acids of all strains except *M. maritypicum* IFO 15779^T and two strains isolated from mangrove rhizosphere, *M. thalassium* IFO 16061 and *M. halophilum* IFO 16062^T, were composed mainly of anteiso-C15:0, anteiso-C17:0 and iso-C16:0. In *M. maritypicum* IFO 15779^T, anteiso-C17:0 was detected as a minor component. In *M. thalassium* IFO 16061 and *M. halophilum* IFO 16062^T, iso-C15:0 was detected as a major component. Chemotaxonomic characteristics of the 11 strains are summarized in Table 4. The cell wall sugars rhamnose, galactose, fucose, mannose, 6-deoxytalose, mannose and glucose were detected in various combinations.

DNA–DNA hybridization

High DNA–DNA relatedness values (> 70%) were obtained between two strains of *M. aurantiacum* IFO 15234^T and IFO 15235, two strains of *M. ketosireducens* IFO 14548^T and IFO 14549, two strains of *M. thalassium* IFO 16060^T and IFO 16061, and *Microbacterium* sp. IFO 15709 and *M. laevaniformans* IFO 14471^T (Table 5), but none of these pairs showed high homology values with each other or with previously described species of the genus *Microbacterium*. These results indicate that each of these strains constitutes a separate taxon.

The conclusions described above are supported by phylogenetic analysis (Takeuchi & Hatano, 1998). We previously determined almost complete 16S rDNA sequences of these 11 strains, and compared them with published sequences of species included in the genus

Table 5. Levels of DNA–DNA similarity among strains of the genus *Microbacterium*

Species	Strain (IFO No.)	15234^T	15235	3758^T	16062^T	14548^T	14549	14471^T	15709	15779^T	15708^T	16060^T	16061
M. arabinogalactanolyticum	14344^T	11	2	17	19	12	13	15	ND	10	19	10	11
M. arborescens	3750^T	17	ND	6	15	36	ND	10	4	17	11	14	10
M. aurantiacum	15234^T	**100**	83	38	ND	29	16	18	ND	11	13	ND	ND
M. aurantiacum	15235	86	**100**	44	ND	23	20	23	ND	13	23	ND	ND
M. aurum	15204^T	38	ND	10	8	36	ND	19	13	7	16	7	17
M. barkeri	15036^T	5	1	4	36	11	9	10	ND	15	3	15	8
M. chocolatum	3758^T	51	46	**100**	ND	30	21	13	ND	21	19	ND	ND
M. dextranolyticum	14592^T	16	ND	11	11	10	ND	10	14	14	13	12	9
M. esteraromaticum	3751^T	6	1	6	37	12	10	12	ND	11	13	7	11
M. flavescens	15039^T	29	10	23	31	30	56	7	ND	13	18	16	12
M. halophilum	16062^T	ND	ND	ND	**100**	ND	ND	ND	ND	ND	ND	21	15
M. imperiale	12610^T	28	ND	11	14	13	ND	14	13	6	25	7	21
M. keratanolyticum	13309^T	10	2	7	49	11	17	10	ND	9	34	9	7
M. ketosireducens	14548^T	16	12	18	ND	**100**	95	13	ND	9	15	ND	ND
M. ketosireducens	14549	15	8	13	35	76	**100**	19	ND	11	25	ND	ND
M. lacticum	14135^T	19	ND	15	31	35	ND	14	37	28	25	16	7
M. laevaniformans	14471^T	17	ND	16	16	31	ND	**100**	90	17	25	17	12
M. laevaniformans	15709	23	ND	23	ND	26	ND	81	**100**	ND	17	ND	ND
M. liquefaciens	15037^T	6	3	5	14	11	14	5	ND	13	6	12	10
M. luteolum	15074^T	25	13	36	11	40	36	20	ND	27	10	9	7
M. maritypicum	15779^T	9	1	20	ND	9	8	22	ND	**100**	39	ND	ND
M. hominis	15708^T	21	ND	11	ND	21	ND	18	7	39	**100**	ND	ND
M. saperdae	15038^T	10	7	6	13	14	23	18	ND	19	7	12	8
M. schleiferi	15075^T	20	13	14	21	21	21	19	ND	9	12	14	7
M. terrae	15300^T	4	8	5	13	47	41	28	ND	10	30	15	8
M. terregens	12961^T	9	7	11	6	31	32	25	ND	13	9	17	7
M. testaceum	12675^T	20	2	9	41	15	9	23	ND	15	14	14	9
M. thalassium	16060^T	ND	ND	ND	42	ND	ND	ND	ND	ND	ND	**100**	76
M. thalassium	16061	ND	ND	ND	41	ND	ND	ND	ND	ND	ND	79	**100**
M. trichothecenolyticum	15077^T	9	5	13	24	15	18	5	ND	13	7	13	12

ND, Not determined.

Microbacterium and other members of the family *Microbacteriaceae* (Takeuchi & Hatano, 1998). As shown in Fig. 1, the 16S rDNA sequences of these strains represent seven distinct lines of descent within the genus *Microbacterium*. These correspond to *M. maritypicum* IFO 15779^T, *M. ketosireducens* IFO 14548^T, *M. thalassium* IFO 16060^T, *M. aurantiacum* IFO 15234^T, *M. chocolatum* IFO 3758^T, *M. hominis* IFO 15708^T and *M. halophilum* IFO 16062^T, respectively.

On the basis of morphological, physiological and chemotaxonomic characteristics, together with DNA–DNA hybridization and 16S rDNA sequence comparison results, we conclude that these organisms should be classified as new species in the genus *Microbacterium*, and propose six new species and one new combination in the genus *Microbacterium*: *Microbacterium ketosireducens* sp. nov. (type strain IFO 14548^T), *Microbacterium chocolatum* sp. nov. (type strain IFO 3758^T), *Microbacterium aurantiacum* sp. nov. (type strain IFO 15234^T), *Microbacterium hominis* sp. nov. (type strain IFO 15708^T), *Microbacterium thalassium* sp. nov. (type strain IFO 16060^T), *Microbacterium halophilum* sp. nov. (type strain IFO 16062^T) and *Microbacterium maritypicum* comb. nov. (type strain IFO 15779^T).

Nineteen species have hitherto been accommodated in the genus *Microbacterium*. From the results of this study, seven species have been added to the genus *Microbacterium*, and 26 species are accommodated in this genus. Differential characteristics for these species and the 19 previously described species of the genus *Microbacterium* are summarized in Table 6. These results show that species in the genus *Microbacterium* can be separated from each other by their morphological, physiological and chemotaxonomic characteristics.

Microbacterium species were widely distributed in various environments such as soil [*Microbacterium arabinogalactanolyticum*, *Microbacterium keratanolyticum*, *Microbacterium luteolum*, *Microbacterium schleiferi*, *Microbacterium terrae*, *Microbacterium trichothecenolyticum* (Yokota *et al*., 1993a), *Microbacterium flavescens*, *Microbacterium terregens*, *Microbacterium testaceum* (Collins *et al*., 1983) and *Microbacterium dextranolyticum* (Yokota *et al*., 1993b)], sewage [*Microbacterium barkeri* (Collins *et al*., 1983) and *Microbacterium laevaniformans* (Collins *et al*., 1986)], lake water [*Microbacterium arborescens* (Breed, 1957)], steep liquor [*Microbacterium aurum* (Yokota *et al*., 1993b)], milk products or cheese [*Microbacterium lacticum* (Collins *et al*., 1986) and *Microbacterium liquefaciens* (Collins *et al*., 1983)]. In addition to these organisms, some *Microbacterium* species have been isolated from different sources; *Microbacterium esteraromaticum* was isolated from accidental contamination in culture (Breed, 1957; Yokota *et al*., 1993a). *Microbacterium imperiale* was isolated from the alimentary tract of the imperial moth *Eacles imperialis*

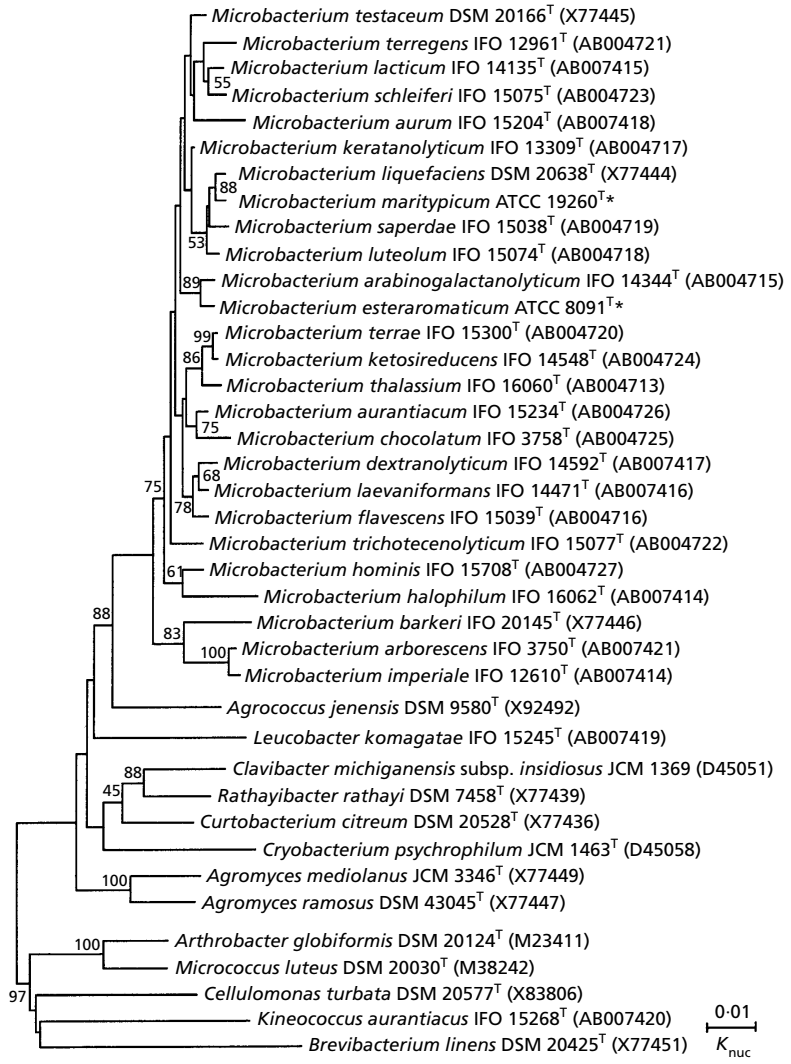

Fig. 1. Phylogenetic dendrogram based on a comparison of the 16S rDNA sequences of *Microbacterium* species and some close relatives (Takeuchi & Hatano, 1998). The tree was created by using the neighbour-joining method and K_{nuc} values. The numbers on the tree indicate the percentages of bootstrap samplings, derived from 1000 samples, supporting the internal branches (Felsenstein, 1985). Asterisks indicate that sequence data were obtained from the Ribosomal Database Project (RDP) (Larsen *et al.*, 1993).

Dru (Collins *et al.*, 1986), and *Microbacterium saperdae* was isolated from dead larvae (Collins *et al.*, 1983). Recently, *Microbacterium* spp. and other actinobacteria were isolated from the surface of cheese (Kollöffel *et al.*, 1997), and *Aureobacterium* spp. were isolated from clinical specimens (Saweljew *et al.*, 1996).

As shown in Table 1, each species which was identified as a new species or a new combination in the genus *Microbacterium*, was isolated from soil (*M. ketosireducens*, *M. halophilum* and *M. thalassium*), culture contamination (*M. chocolatum*), sea water or marine mud [*M. maritypicum* (Breed, 1957)], sewage (*M. aurantiacum*) or clinical specimens (*M. hominis* and *M. laevaniformans*). Among the species isolated from soil, *M. thalassium* and *M. halophilum* were isolated from soil in mangrove rhizosphere in Okinawa prefecture, Japan, where complex environments have formed under the influence of tidal ebb and flow, the influx of fresh water, and high temperature and humidity in these subtropical and tropical climates. The soils in such environments are muddy, and are reported to be anoxic, low in nutrients, and to have higher concentrations of heavy metals and higher salinity than terrestrial soils (Wakushima *et al.*, 1994). These results also show the variety of environments in which *Microbacterium* species are distributed.

Description of *Microbacterium aurantiacum* sp. nov.

Microbacterium aurantiacum (au.ran.ti′a.cum. L. n. *aurantium* specific name of the orange; M.L. neut. adj. *aurantiacum* orange-coloured).

Good growth occurs on solid media in air; colonies are 2–4 mm in diameter, circular, low convex with entire margins, opaque and moist. Orange pigment is produced. In young cultures, small, slender rods are formed. Many cells are arranged at angles, forming V shapes; primary branching is uncommon. In older cultures, rods are shorter, but a marked rod–coccus growth cycle does not occur. Gram-positive non-motile rods. The optimum growth temperature is about 28 °C, and growth occurs at 37 °C. Growth

Table 6. Differential characteristics of *Microbacterium* species

Abbreviations: +, all strains positive; +w, weakly positive; −, all strains negative; d, differs among strains; ND, not determined;Y, yellow; YW, yellowish white; LY, light yellow; O, orange; GEL, gelatin; STA, starch; VP test, Voges–Proskauer test; ADH, arginine dihydrolase; ARA, arabinose; NAc-GlcN, *N*-acetylglucosamine; MLT, malate; CIT, citrate; PAC, phenyl acetate; Glc, glucose.

Species	Colour of colony	Motility	Growth		Hydrolysis of:		H_2S prodn	VP test	ADH	Assimilation of:					Acid from Glc	Cell wall diamino	Major menaquinone acid
			37 °C	2% NaCl	GEL	STA				ARA	NAc-GlcN	MLT	CIT	PAC			
M. arabinogalactanolyticum	YW	−	−	+	+	+	+	−	+	+	+	+	−	+	−	Orn	MK-12,13
M. arborescens	O	+	−	ND	+	−	+	−	−	+	+	+	+w	−	+	Lys	MK-11,12
M. aurantiacum	O	−	+	+	−	+	+	−	−	d	−	d	−	−	d	Orn	MK-12
M. aurum	YW	−	+	ND	+	+	+	−	−	−	+w	−	−	−	+	Lys	MK-11, 12
M. barkeri	W	+	+	+	+	+	+	−	+	+	+	+	+	−	−	Orn	MK-11, 12
M. chocolatum	O	−	+	+w	−	+w	+	−	−	−	+w	−	−	−	−	Orn	MK-12
M. dextranolyticum	W	−	−	ND	−	−	+	+	−	+	−	+	−	−	+	Orn	MK-11, 12
M. esteraromaticum	YW	+	d	−	−	+	+	−	−	+	−	−	−	−	−	Orn	MK-12, 13
M. flavescens	Y	−	−	+	+	+	+	−	−	+	−	+	−	−	+	Orn	MK-13, 14
M. halophilum	Y	−	+	+	+w	+	−	−	−	−	−	−	−	−	+	Orn	MK-11, 12, 13
M. hominis	YW	−	+	+	−	−	+	+	−	+	+	+	+	−	+	Lys	MK-11, 12
M. imperiale	O	+	+	ND	−	+	+	−	−	+	+w	+	−	−	+	Lys	MK-11, 12
M. keratanolyticum	Y	+	−	+	+	−	+	−	+w	+w	+	−	−	−	−	Orn	MK-12, 13
M. ketosireducens	Y	−	−	+	+	+	+	−	−	+	−	−	d	−	+	Orn	MK-13
M. lacticum	Y	−	−	ND	−	+	−	−	−	+w	+	+	−	−	+	Lys	MK-11, 12
M. laevaniformans	Y/YW	−	+	ND	d	+	+	+	d	−	−	+	−	−	+	Lys	MK-11, 12
M. liquefaciens	Y	−	−	+	+	−	+	−	+	−	+	−	+	−	−	Orn	MK-11, 12
M. luteolum	YW	−	−	−	−	−	+	−	−	+	+	+	−	−	−	Orn	MK-12
M. maritypicum	Y	+	+	+	+	−	−	−	−	−	+	+	+	+	+	Orn	MK-12
M. saperdae	YW	+	−	−	−	+	+	−	+	+	+	+	−	−	+	Orn	MK-11, 12
M. schleiferi	YW	−	+w	+w	−	−	−	+	−	+w	+w	+	−	−	+	Orn	MK-11, 12
M. terrae	Y	−	−	+	+	+	+	−	−	+	−	−	−	−	+	Orn	MK-13, 14
M. terregens	Y	−	−	+w	−	−	−	−	−	−	−	+	−	−	+	Orn	MK-12, 13
M. testaceum	O	+	−	−	+	−	+	−	−	+	+	+	+	+w	−	Orn	MK-10, 11
M. thalassium	Y/LY	−	−	+	d	+	−	−	−	−	−	−	−	−	−	Orn	MK-11, 12
M. trichothecenolyticum	Y	−	−	+w	−	+	+	−	−	−	+	+	+	−	+	Orn	MK-12, 13

occurs in the presence of 2 and 5% NaCl, but is very weak in the presence of 6·5% NaCl. Maltose, mannose, mannitol, fumarate, gluconate and propionate are assimilated, but arabinose, *N*-acetylglucosamine, acetate, adipate, caprate, citrate, lactate, malate and phenyl acetate are assimilated weakly or are not assimilated. Starch, Tweens 20, 40, 60 and 80 are hydrolysed. Gelatin is not hydrolysed. H_2S is produced. Methyl red test and Voges–Proskauer test are negative. Arginine dihydrolase is not produced. Acid is produced from glucose, mannose and sucrose, but is not produced or is produced weakly from L-arabinose, galactose, glucose, inulin, melezitose, raffinose, rhamnose, ribose, trehalose and xylose. The cell wall peptidoglycan contains ornithine, homoserine, glutamate plus hydroxyglutamate, glycine and alanine. The cell wall sugars are rhamnose, galactose and fucose. The major cellular fatty acids are anteiso-C15:0, anteiso-C17:0 and iso-C16:0. Unsaturated menaquinones with 12 isoprene units are present. The DNA G+C content of the type strain is 70·1–70·3 mol%. Source is sewage. The type strain is IFO 15234^T.

Description of *Microbacterium chocolatum* sp. nov.

Microbacterium chocolatum (choc.o.lat.um. M.L. neut. adj. *chocolatum* chocolate-coloured, derived from the Mexican *chocolatl*, chocolate).

Good growth occurs on solid media in air; colonies are 2–4 mm in diameter, circular, low convex with entire margins, opaque and moist. Orange or dull orange pigment is produced. In young cultures, small, slender rods are formed. Many cells are arranged at angles, forming V shapes; primary branching is uncommon. In older cultures, rods are shorter, but a marked rod–coccus growth cycle does not occur. Gram-positive non-motile rods. The optimum temperature for growth is about 28 °C, and growth occurs at 37 °C. Growth is weak in the presence of 2% NaCl, but does not occur in the presence of 5 and 6·5% NaCl. Maltose and gluconate are assimilated, but arabinose, mannose, mannitol, *N*-acetylglucosamine, acetate, adipate, caprate, citrate, fumarate, lactate, malate, phenyl acetate and propionate are assimilated weakly or are not assimilated. Starch, Tweens 20, 40, 60 and 80 are hydrolysed. Gelatin is not hydrolysed. H_2S is produced. Methyl red test and Voges–Proskauer test are negative. Arginine dihydrolase is not produced. Acid is produced from mannose, sucrose and xylose, but is not produced from L-arabinose, galactose, glucose, inulin, melezitose, raffinose, rhamnose, ribose and trehalose. The cell wall peptidoglycan contains ornithine, homoserine, glutamate plus hydroxyglutamate, glycine and alanine. The cell wall sugars are rhamnose and galactose as major components, and mannose and xylose as minor components. The major cellular fatty acids are anteiso-C15:0, anteiso-C17:0

and iso-C16:0. Unsaturated menaquinones with 12 isoprene units are present. The DNA G+C content of the type strain is 69·5 mol%. Source is culture contamination. The type strain is IFO 3758^T.

Description of *Microbacterium halophilum* sp. nov.

Microbacterium halophilum (ha.lo′phi.lum. Gr. n. *hals* salt; Gr. adj. *philos* loving; M.L. neut. adj. *halophilum* salt-loving).

Good growth occurs on solid media in air; colonies are 2–4 mm in diameter, circular, low convex with entire margins, opaque and moist. Yellowish white pigment is produced. In young cultures, small, slender rods are formed. Many cells are arranged at angles, forming V shapes; primary branching is uncommon. In older cultures rods are shorter, but a marked rod–coccus growth cycle does not occur. Gram-positive non-motile rods. The optimum temperature for growth is about 28 °C, and growth occurs at 37 °C. Good growth occurs in the presence of 2, 5 and 6·5% NaCl. Maltose, mannose, mannitol and gluconate are assimilated, but arabinose, *N*-acetylglucosamine, acetate, adipate, caprate, citrate, fumarate, lactate, malate, phenyl acetate and propionate are not assimilated. Starch, Tweens 40, 60 and 80 are hydrolysed, and gelatin and Tween 20 are hydrolysed weakly. H_2S is not produced. Methyl red test is positive and Voges–Proskauer test is negative. Arginine dihydrolase is not produced. Acid is produced from glucose, mannose and rhamnose, but is not produced from L-arabinose, galactose, inulin, melezitose, raffinose, ribose, sucrose, trehalose and xylose. The cell wall peptidoglycan contains ornithine, homoserine, glutamate plus hydroxyglutamate, glycine and alanine. The cell wall sugars are mannose, galactose and glucose. The major cellular fatty acids are anteiso-C15:0, anteiso-C17:0, iso-C15:0 and iso-C16:0. Unsaturated menaquinones with 11, 12 and 13 isoprene units are present. The DNA G+C content of the type strain is 67·2 mol%. Source is soil in mangrove rhizosphere. The type strain is IFO 16062^T (= strain No. 76^T).

Description of *Microbacterium hominis* sp. nov.

Microbacterium hominis (ho′mi.nis. L. masc. n. *homo* man; L. gen. masc. n. *hominis* of man).

Good growth occurs on solid media in air; colonies are 2–4 mm in diameter, circular, low convex with entire margins, opaque and moist. Yellowish white pigment is produced. In young cultures, small, slender rods are formed. Many cells are arranged at angles, forming V shapes; primary branching is uncommon. In older cultures, rods are shorter, but a marked rod–coccus growth cycle does not occur. Gram-positive non-motile rods. The optimum temperature for growth is about 28 °C, and growth occurs at 37 °C. Growth occurs in the presence of 2% NaCl, but is weak in the presence of 5 and 6·5% NaCl. Arabinose, maltose, mannose, mannitol, *N*-acetylglucosamine, acetate, citrate, fumarate, gluconate, lactate, malate and propionate are assimilated, but adipate, caprate and phenyl acetate are not assimilated. Tweens 20, 40, 60 and 80 are hydrolysed, but gelatin and starch are not hydrolysed. H_2S is produced. Methyl red test and Voges–Proskauer test are positive. Arginine dihydrolase is not produced. Acid is produced from L-arabinose, galactose, glucose, mannose, melezitose, sucrose and trehalose, but is not produced from inulin, raffinose, rhamnose, ribose and xylose. The cell wall peptidoglycan contains lysine, glutamate plus hydroxyglutamate, glycine and alanine. The cell wall sugars are rhamnose, 6-deoxy-L-talose, galactose and mannose. The major cellular fatty acids are anteiso-C15:0, anteiso-C17:0 and iso-C16:0. Unsaturated menaquinones with 11 and 12 isoprene units are present. The DNA G+C content of the type strain is 71·2 mol%. Source is lung aspirate. The type strain is IFO 15708^T.

Description of *Microbacterium ketosireducens* sp. nov.

Microbacterium ketosireducens [ke.to.si.redu′cens. M.L. n. *ketosum* keto-sugar; L. part. adj. *reducens* reducing (deoxidizing); M.L. part. adj. *ketosireducens* ketose-deoxidizing].

Good growth occurs on solid media in air; colonies are 2–4 mm in diameter, circular, low convex with entire margins, opaque and moist. Yellow pigment is produced. In young cultures, small, slender rods are formed. Many cells are arranged at angles, forming V shapes; primary branching is uncommon. In older cultures, rods are shorter, but a marked rod–coccus growth cycle does not occur. Gram-positive non-motile rods. The optimum temperature for growth is about 28 °C, but growth does not occur at 37 °C. Growth occurs in the presence of 2% NaCl, but not in the presence of 6·5% NaCl. Weak growth occurs in the presence of 5% NaCl. Arabinose, maltose, mannose, mannitol and gluconate are assimilated, but *N*-acetylglucosamine, acetate, adipate, caprate, citrate, fumarate, lactate, malate, phenyl acetate and propionate are assimilated weakly or are not assimilated. Gelatin, starch, Tweens 20, 40, 60 and 80 are hydrolysed. H_2S is produced. Methyl red test is positive and Voges–Proskauer test is negative. Nitrate is reduced to nitrite. Arginine dihydrolase is not produced. Acid is produced from L-arabinose, glucose, mannose, sucrose and xylose, but is not produced or is produced weakly from galactose, inulin, melezitose, raffinose, rhamnose, ribose and trehalose. The cell wall peptidoglycan contains ornithine, homoserine, glutamate plus hydroxyglutamate, glycine and alanine. The major cellular fatty acids are anteiso-C15:0, anteiso-C17:0 and iso-C16:0. Unsaturated menaquinones with 13 isoprene units are present. The DNA G+C content of the type strain is 69·7–69·8 mol%. Source is soil. The type strain is IFO 14548^T.

Description of *Microbacterium maritypicum* comb. nov.

Microbacterium maritypicum (ma.ri.ty′pi.cum. L. neut. n. *mare* the sea; M.L. neut. adj. *typicum* typical; M.L. neut. adj. *maritypicum* typical of the sea).

Good growth occurs on solid media in air; colonies are 2–4 mm in diameter, circular, low convex with entire margins, opaque and moist. Light yellow pigment is produced. In young cultures, small, slender rods are formed. Many cells are arranged at angles, forming V shapes; primary branching is uncommon. In older cultures, rods are shorter, but a marked rod–coccus growth cycle does not occur. Gram-positive and motile rods with peritrichous flagella. The optimum temperature for growth is about 28 °C, and growth occurs at 37 °C. Growth occurs in the presence of 2 and 5% NaCl, but is weak in the presence of 6·5% NaCl. Maltose, mannose, mannitol, *N*-acetylglucosamine, citrate, gluconate, malate, phenyl acetate are assimilated, but arabinose, acetate, adipate, caprate, fumarate, lactate and propionate are not assimilated. Gelatin, Tweens 20, 40, 60 and 80 are hydrolysed, but starch is not hydrolysed. H_2S is not produced. Methyl red test and Voges–Proskauer test are negative. Arginine dihydrolase is not produced. Acid is produced from glucose, mannose, but is not produced or is produced weakly from L-arabinose, galactose, inulin, melezitose, raffinose, rhamnose, ribose, sucrose, trehalose and xylose. The cell wall peptidoglycan contains ornithine, homoserine, glutamate plus hydroxyglutamate, glycine and alanine. The cell wall sugar is galactose. The major cellular fatty acids are anteiso-C15:0 and iso-C16:0. Unsaturated menaquinones with 12 isoprene units are present. The DNA G+C content of the type strain is 71·6 mol%. Source is sea water and marine mud. The type strain is IFO 15779^T. *Flavobacterium marinotypicum* (Breed, 1957) is the basonym of this species.

Description of *Microbacterium thalassium* sp. nov.

Microbacterium thalassium (tha.las′si.um. M.L. neut adj. *thalassium* based on Gr. adj. *thalassios* pertaining to the sea).

Good growth occurs on solid media in air; colonies are 2–4 mm in diameter, circular, low convex with entire margins, opaque and moist. Yellow or yellowish white pigment is produced. In young cultures, small, slender rods are formed. Many cells are arranged at angles, forming V shapes; primary branching is uncommon. In older cultures, rods are shorter, but a marked rod–coccus growth cycle does not occur. Gram-positive non-motile rods. The optimum temperature for growth is about 28 °C, but growth does not occur at 37 °C. Growth occurs in the presence of 2 and 5% NaCl, but is weak in the presence of 6·5% NaCl. Maltose, mannose, mannitol and gluconate are assimilated, but arabinose, *N*-acetylglucosamine, acetate, adipate, caprate, citrate, fumarate, lactate, malate, phenyl acetate and propionate are not assimilated. Starch, Tweens 40, 60 and 80 are hydrolysed. Gelatin and Tween 20 are hydrolysed weakly. H_2S is not produced. Methyl red test is positive and Voges–Proskauer test is negative. Arginine dihydrolase is not produced. Acid is produced from glucose, mannose and xylose, but is not produced from L-arabinose, galactose, inulin, melezitose, raffinose, rhamnose, ribose, sucrose and trehalose. The cell wall peptidoglycan contains ornithine, homoserine, glutamate plus hydroxyglutamate, glycine and alanine. The cell wall sugars of strain IFO 16060^T are galactose and glucose, and those of strain IFO 16061 are rhamnose, mannose, galactose and glucose. The major cellular fatty acids are anteiso-C15:0, anteiso-C17:0, iso-C16:0 and/or iso-C15:0. Unsaturated mena-quinones with 11 and 12 isoprene units are present. The DNA G+C content of the type strain is 69·1–69·7 mol%. Source is soil in mangrove rhizosphere. The type strain is IFO 16060^T.

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