***Marmoricola aurantiacus* gen. nov., sp. nov., a coccoid member of the family *Nocardioideae* isolated from a marble statue**

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A Gram-positive, aerobic bacterium with coccoid cells occurring singly, in pairs and in clusters was isolated from the surface of a marble statue. The peptidoglycan contains LL-diaminopimelic acid as diagnostic diamino acid and a single glycine residue as interpeptide bridge (type A3_γ). The major menaquinone is MK-8(H₄). The cellular fatty acid pattern consists of straight chain saturated and monounsaturated components and 10-methyl octadecanoic (tuberculostearic) acid as the only branched chain fatty acid. Phosphatidylinositol, phosphatidylglycerol and diphosphatidylglycerol occur as characteristic polar lipids. The DNA G+C composition is 72 mol%. According to its phylogenetic position and 16S rDNA signature nucleotides, the organism is a member of the family *Nocardioideae*. The combination of chemotaxonomic characteristics is unique within this family and supports the description of a new genus and new species, *Marmoricola aurantiacus*. The type strain is strain BC 361^T (= DSM 12652^T).

Keywords: *Marmoricola aurantiacus*, marble, *Nocardioideae*, chemotaxonomy, phenotypic characteristics

INTRODUCTION

Actinobacteria with LL-diaminopimelic acid (LL-A₂pm) cluster into five families belonging to four suborders on the basis of characteristic signature nucleotides of 16S rDNA: (i) *Nocardioideae* (suborder *Propionibacterineae*), (ii) *Propionibacteriaceae* (suborder *Propionibacterineae*), (iii) *Intrasporangiaceae* (suborder *Micrococcineae*), (iv) *Sporychthiaceae* (suborder *Frankineae*) and (v) *Streptomycetaceae* (suborder *Streptomycineae*) (Stackebrandt *et al.*, 1997). The genera of these families can readily be distinguished by their chemotaxonomic characteristics (Schumann *et al.*, 1997; Prauser *et al.*, 1997; Busse & Schumann, 1999). The family *Nocardioideae* (Stackebrandt *et al.*, 1997) comprises the genera *Nocardioideae* and *Aeromicrobium* which differ in their major isoprenoid quinone, cellular fatty acid profile and polar lipid pattern.

The genus *Nocardioideae* includes at present six species,

namely *Nocardioideae albus* (Prauser, 1976), *Nocardioideae simplex* (O'Donnell *et al.*, 1982), *Nocardioideae jensenii* (Suzuki & Komagata, 1983; Collins *et al.*, 1989), *Nocardioideae luteus* (Prauser, 1984), *Nocardioideae plantarum* (Collins *et al.*, 1994) and *Nocardioideae pyridinolyticus* (Yoon *et al.*, 1997). *Nocardioideae fastidiosa* proposed by Collins & Stackebrandt (1989) has been transferred to the genus *Aeromicrobium* as *Aeromicrobium fastidiosum* by Tamura & Yokota (1994) on the basis of chemotaxonomic and phylogenetic data. Though *Nocardioideae* species are heterogeneous with respect to morphology, embracing strains that form mycelium which may fragment into irregular rod- and coccus-like cells as well as true rods and cocci, they are coherent in chemotaxonomic properties. The peptidoglycan of all representatives of the genus *Nocardioideae* contains LL-A₂pm and is of the A3_γ type (Schleifer & Kandler, 1972) with a single glycine residue as interpeptide bridge. The genus lacks mycolic acids and has a tetrahydrogenated menaquinone with eight isoprene units [MK-8(H₄)] as predominant isoprenoid quinone (O'Donnell *et al.*, 1982). *Nocardioideae* strains show complex cellular fatty acids including iso- and anteiso-branched, straight-chain saturated and unsaturated components and 10-methyl octadecanoic

Abbreviations: LL-A₂pm, LL-diaminopimelic acid; TBSA, 10-methyl octadecanoic acid (tuberculostearic acid).

The GenBank/EMBL/DBJ accession number for the 16S rDNA sequence of strain BC 361^T is Y18629.

acid (tuberculostearic acid; TBSA). The predominance of iso-C_{16:0} is characteristic for the genus (Yoon *et al.*, 1997; Park *et al.*, 1998). The polar lipid profiles include phosphatidylglycerol, diphosphatidylglycerol and two α -glycol positive phospholipids (O'Donnell *et al.*, 1982).

The genus *Aeromicrobium* embraces the two species *Aeromicrobium erythreum* and *A. fastidiosum*. Representatives of the genus *Aeromicrobium* form rod-like or coccoid cells. Members of this genus display the same peptidoglycan type A3 γ as *Nocardioides* species but differ from them in that they contain the tetrahydrogenated menaquinone with nine isoprenoid units MK-9(H₄), exclusively straight-chain saturated and monounsaturated fatty acids and phosphatidylethanolamine (Miller *et al.*, 1991; Tamura & Yokota, 1994).

This paper describes the morphological, physiological and chemotaxonomic characterization of a coccoid LL-A₂pm containing strain isolated from a marble statue of the Nordfriedhof cemetery in Munich (Germany) and the determination of its phylogenetic position on the basis of its 16S rDNA sequence. Because of the unique combination of its characteristics among members of the family *Nocardioidaceae*, a new genus and species, *Marmoricola aurantiacus*, is proposed.

METHODS

Bacterial strain. Strain BC 361^T was isolated from a marble statue (Wagmüller's monument), in the old Nordfriedhof cemetery in Munich, Germany. The organism was isolated by dilution plating on Bunt and Rovira medium (Bunt & Rovira, 1955), modified by addition of 0.5% glucose, 0.5% NaCl and 0.03% Na₂CO₃, pH 8.6 (BRII). For morphological and chemotaxonomic studies, strain BC 361^T was cultivated in Luedemann medium (Luedemann, 1968).

Staining procedures. Gram staining and acid-fast staining were done as described previously (Lanyi, 1987) and Luedemann staining was performed as described by Luedemann (1968).

Morphological characteristics. Cell and aggregate morphology were determined by bright-field microscopy and by phase-contrast microscopy (DML, Leica; oil immersion objective, magnification $\times 100$) and scanning electron microscopy (SEM) (Leo Electron Microscopy), from material obtained from surface growth on agar plates.

For SEM observations, a 14 d old strain BC 361^T culture from Luedemann medium was suspended in PBS (0.13 M NaCl in 0.1 M KH₂PO₄/NaOH buffer, pH 7.4). The cells were fixed with 0.5% glutaraldehyde (pH 7.4), washed several times and dehydrated in a series of ethanol concentrations. After the cells were sputter-coated with gold-palladium, they were observed under a scanning electron microscope (Groth *et al.*, 1997).

Physiological and biochemical characterization. All physiological tests were performed at 28 °C. Oxidase activity was determined by monitoring the oxidation of *N,N,N',N'*-tetramethyl-*p*-phenylenediamine on filter paper. Acidification of carbohydrates was verified by a colour change from blue to yellow in a medium containing 0.5% tryptone

(Oxoid), 0.4% bacto casamino acids (Difco), 0.07% (NH₄)₂HPO₄, 0.5% NaCl, 0.003% bromocresol purple, and the substrates at a final concentration of 1% (Schumann *et al.*, 1997). Catalase production was shown by the production of bubbles after a suspension of the cells was mixed with a drop of a 3% hydrogen peroxide solution on a slide. DNA hydrolysis was indicated by clear zones in spot-inoculated plates containing DNase agar (Oxoid). The hydrolysis of casein and the decomposition of tyrosine, xanthine and hypoxanthine were determined as described previously (Gordon *et al.*, 1974).

Urease activity, nitrate reduction, aesculin hydrolysis, hydrolysis of starch, hydrolysis of Tween (20, 40, 60, 80), phosphatase production and hydrolysis of gelatin were studied by using previously described methods (Lanyi, 1987). Strain BC 361^T was examined for its ability to grow in Czapek Dox modified agar (CZ; Oxoid), potato dextrose agar (PDA; Oxoid), brain-heart infusion agar (BHIA; Oxoid), 1.5% agar-H₂O, YMS-Glc agar (0.5% yeast extract, 1.5% malt extract, 1% starch, 1% glucose, 1.5% agar), YCS-Glc agar (0.5% yeast extract, 1.5% casein hydrolysate, 1% starch, 1% glucose, 1.5% agar), Y-Glyc agar (0.5% yeast extract, 2% glycerol, 1.5% agar), YG-Glyc agar (0.5% yeast extract, 1% glucose, 10% glycerol, 1.5% agar) (Luedemann & Fonseca, 1989) and in medium M53 *Corynebacterium* agar (Collins & Stackebrandt, 1989).

Cell wall analysis. Purified cell wall preparations were obtained by the method of Schleifer (1985). The amino acids and peptides in cell wall hydrolysates were analysed by two-dimensional ascending TLC on cellulose plates using the described solvent systems (Schleifer, 1985). Whole-cell sugars were determined as alditol acetates by GC as described (Groth *et al.*, 1996). The molar ratios of amino acids were determined by GC and GC-MS of *N*-heptafluorobutyl amino acid isobutyl esters (MacKenzie, 1987). The glycolate content of bacterial cell walls was determined by the colorimetric method of Uchida & Aida (1984).

Lipid analysis. Cellular fatty acid methyl esters obtained from cells grown in bacto tryptic soy broth at 28 °C by the method described by Stead *et al.* (1992) were separated by GC (Groth *et al.*, 1996) and identified as described previously (Schumann *et al.*, 1997). Menaquinones were extracted as described by Collins *et al.* (1977) and were analysed by HPLC (Groth *et al.*, 1996). Polar lipids extracted by the method of Minnikin *et al.* (1979) were identified by two-dimensional TLC and spraying with specific reagents (Collins & Jones, 1980). The absence of mycolic acids was demonstrated by TLC (Minnikin *et al.*, 1975).

DNA base composition. DNA was isolated and its G+C content was determined by HPLC of deoxyribonucleosides as described previously (Groth *et al.*, 1996).

16S rDNA sequence determination and analysis. Extracted genomic DNA was used for PCR-mediated amplification of 16S rDNA (Rainey *et al.*, 1996). The purified PCR product was directly sequenced by using previously described protocols (Rainey *et al.*, 1996), and the sequence reaction mixtures were electrophoresed with a model 373A automatic DNA sequencer.

The 16S rDNA sequences were manually aligned with the sequences of members of the order *Actinomycetales* by using the ae2 editor (Maidak *et al.*, 1994). Evolutionary distances for strain BC 361^T and for a selection of actinobacterial strains (data not shown) were calculated by a previously described method (Jukes & Cantor, 1969). Phylogenetic

Fig. 1. Phylogenetic dendrogram based on the results of a 16S rDNA sequence comparison. Bar, 2 nucleotide substitutions per 100 nucleotides.

dendrograms were reconstructed by using the treeing algorithm of DeSoete (1983) and the neighbour-joining algorithm contained in the PHYLIP package (Felsenstein, 1993). Bootstrap analyses were used to evaluate the tree topologies of the neighbour-joining data by performing 500 resamplings (Felsenstein, 1993). Strain numbers and rDNA accession numbers of reference organisms are included in Fig. 1.

RESULTS

Cultural and morphological characteristics

Growth occurred after 2 weeks in Luedemann medium at 28 °C. The colonies were circular (diameter 2–5 mm), with a smooth surface, shiny and orange coloured (Fig. 2). In old cultures (2–3 months incubation), colonies had the tendency to become shaped like craters and were sometimes rough. Cells were spherical (0.5–0.7 µm in diameter) and occurred singly,

Fig. 2. Single BC 361^T colony (after 30 d incubation on Luedemann medium); diameter 4.5 mm. Bar, 1 mm.

in pairs or in tetrads (Fig. 3) and in some cases in clusters (old cultures). The temperature range for growth on Luedemann medium was 18–28 °C, no growth occurred at 6 and 37 °C. Growth occurred within a range of pH between 5.1 and 8.7. Addition of 2% NaCl to Luedemann medium resulted in a restricted growth, whereas no growth occurred in the presence of 4% NaCl.

Physiological characteristics

The physiological and biochemical characteristics of strain BC 361^T are summarized in Table 1. Strain BC 361^T was negative for oxidase and urease tests. Catalase was produced. Nitrate was not reduced to nitrite. No acid was produced from D-ribose, L-arabinose, D-xylose, L-rhamnose, D-glucose, D-mannose, D-galactose, maltose, lactose, D-cellobiose, D-trehalose, D-raffinose, glycerol, D-mannitol and myo-inositol. Casein and gelatin were not hydrolysed. Xanthine and tyrosine were not decomposed, whereas hypoxanthine was decomposed. Aesculin was hydrolysed, Tween 20, 40, 60 were not hydrolysed, whereas Tween 80 was only weakly hydrolysed. DNA was not hydrolysed. The strain did not grow in the following media: CZ, YMS-Glc, YCS-Glc, YG-Glyc, Y-Glyc, agar-H₂O and M53. Growth occurred in PDA and BHIA medium.

Chemotaxonomic characteristics

The peptidoglycan of strain BC 361^T contained LL-A₂pm, Ala, Gly and Glu in a molar ratio of 1.0:1.0:0.9:0.7. From these results and from the two-dimensional TLC peptide pattern of partial cell-wall hydrolysates (data not shown), it was concluded that strain BC 361^T represented peptidoglycan type A3γ with a single glycine residue as an interpeptide bridge (Schleifer & Kandler, 1972). Glucose and traces of ribose could be detected only as whole-cell sugars. The acyl type was acetyl. The fatty acid profile of strain BC 361^T was characterized by the predominance of hexadecanoic acid (C_{16:0}) and octadecenoic acid (C_{18:1}), and by the presence of C_{16:1}, C_{14:0}, C_{18:0} and TBSA as

Fig. 3. Scanning electron micrograph of coccoid cells from 14 d old culture of strain BC 361^T grown at 28 °C on Luedemann medium. Bar, 1 µm.

minor components (Table 2). The isoprenoid quinones were MK-8(H₄), MK-7(H₄), MK-8(H₂), MK-6(H₄); the peak area ratio was 73:4:1:1. The polar lipid pattern consisted of phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol and one unknown phospholipid. Mycolic acids were absent. The DNA G+C composition was 72 mol% (Table 3).

16S rDNA sequence analysis

The almost complete 16S rDNA sequence of 1470 nt (95.5% of the *Escherichia coli* sequence) was determined for strain BC 361^T and compared to the sequences of the 16S rDNA database of members of the class *Actinobacteria* (Stackebrandt *et al.*, 1997). The highest similarity values were found with representatives of the family *Nocardioidaceae* (> 93 %), whereas they were slightly lower with members of the neighbouring families, i.e. *Microthax*, *Propioniferax*, *Luteococcus*, *Friedmanniella* and *Propionibacterium* (similarity between 89.6 and 92.6 %).

Strain BC 361^T shares the highest binary 16S rDNA similarity with the type strain *N. jensenii* (96.4 %). The two treeing algorithms place these two organisms as a sister lineage to the lineage containing the other species of *Nocardioides* [Fig. 1 showing the phylogenetic tree according to the algorithm of DeSoete (1983)], whereas the *Aeromicrobium* line of descent branches slightly deeper. The inclusion of the 16S rDNA sequence into the database does not change the branching point of *N. jensenii* which has been demonstrated in previous phylogenetic studies to represent the deepest point within the genus *Nocardioides* (Tamura & Yokota, 1994; Schumann *et al.*, 1997; Yoon *et al.*, 1998a).

Although strain BC 361^T clusters within the radiation of *Nocardioides* species and shares with them the

Table 1. Morphological and biochemical characteristics of strain BC 361^T

–, Negative; +, positive; w, weakly positive.

Characteristic	Strain BC 361 ^T
Colour of colony	Orange
Cell morphology	Coccus, 0.5–0.6 µm, packet, clusters
Gram staining	+
Acid-fast staining	–
Spore formation	–
KOH	–
Catalase	+
Oxidase	–
Growth in:	–
CZ	–
PDA	+
BHIA	+
YMS-Glc	–
YCS-Glc	–
YG-Glyc	–
Y-Glyc	–
Agar-H ₂ O	–
M53	–
Growth at:	
6 °C	–
18 °C	+
22 °C	+
25 °C	+
28 °C	+
37 °C	–
Growth at pH:	
5.1	+
6	+
7.2	+
8.7	+
Growth in the presence of NaCl:	
0.5 %	+
2 %	+
4 %	–
6 %	–
10 %	–
Phosphatase	–
Nitrate reduction	–
Degradation tests:	
Aesculin	+
Urea	–
Xanthine	–
Casein	–
Hypoxanthine	+
Gelatin	–
Starch	–
Tween 20	–
Tween 40	–
Tween 60	–
Tween 80	w
Tyrosine	–
DNA	–

Table 2. Cellular fatty acid composition of strain BC 361^T and of type strains of *Aeromicrobium* and *Nocardioide* species

Fatty acid: 1, i-C_{14:0}; 2, C_{14:0}; 3, i-C_{15:0}; 4, ai-C_{15:0}; 5, C_{15:0}; 6, i-C_{16:0}; 7, i-C_{16:1}; 8, C_{16:0}; 9, C_{16:1}; 10, i-C_{17:0}; 11, ai-C_{17:0}; 12, ai-C_{17:1}; 13, C_{17:0}; 14, C_{17:1}; 15, i-C_{18:0}; 16, C_{18:0}; 17, C_{18:1}; 18, TBSA (10-methyl octadecanoic acid). i-C_{15:1}, C_{15:1}, i-C_{17:1} and C_{18:2} were not observed. Values less than 1% are not shown; i, iso; ai, anteiso.

Taxon	Fatty acid composition (%)*																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
BC 361 ^{T†}		3.4						41.4	14.9							2.4	33.7	2.8
<i>N. jensenii</i>	1.7		16.7		1.3	28.6	2.8	4.3	2.3	9.6	1.0		3.9	6.5	1.5	1.6	10.3	2.4
<i>N. albus</i>	2.3		1.7		1.5	70.8		3.0	1.9		2.1		2.1	6.0	2.1		2.6	1.9
<i>N. simplex</i>	4.6		2.6			41.8	1.1	3.8	5.0	1.9				5.2			13.1	5.2
<i>N. plantarum</i>			3.4		1.0	26.0		2.9	1.0	5.4	16.8		6.1	4.0	1.8		15.7	1.9
<i>N. pyridinolyticus‡</i>			5.1	1.4		47.3	4.2	1.2		1.7	14.2	2.1	1.2	1.7		1.0	2.0	3.4
<i>A. erythreum§</i>		3.1			3.1			47.0	5.2				2.4	2.1		3.0	18.9	5.0
<i>A. fastidiosum¶</i>		1.1			1.4			20.6	5.4							2.7	20.2	15.8

* Data (except for strain BC 361^T and *N. pyridinolyticus*) from Schumann *et al.* (1997).

† Strain contained additionally 1.4% 2-hydroxy hexadecanoic acid.

‡ Data from Yoon *et al.* (1997). Strain contained additionally 5.5% 10-methyl heptadecanoic acid and 5.0% 10-methyl hexadecanoic acid.

§ Strain contained additionally 4.6% 2-hydroxy hexadecanoic acid.

¶ Strain contained additionally 20.3% 2-hydroxy hexadecanoic acid.

Table 3. Differential characteristics of strain BC 361^T and members of the family *Nocardioideaceae*

Abbreviations: DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PG-OH, PG containing 2-hydroxy fatty acids; PI, phosphatidylinositol; PE, phosphatidylethanolamine; PL, unknown phospholipid(s); menaquinones e.g. MK-9(H₄), menaquinone with two of nine isoprene units hydrogenated.

Taxon	Cell morphology	Major menaquinone	Polar lipids	G + C (mol %)	Reference
BC 361 ^T	Cocci in packets	MK-8(H ₄)	PI, PG, DPG, PL	72	
<i>Aeromicrobium</i>	Rods	MK-9(H ₄)	PE, PG	71–73	Miller <i>et al.</i> (1991); Tamura & Yokota (1994)
<i>N. albus</i>	Hyphae	MK-8(H ₄)	PG, DPG, PL, PG-OH	66.5–68.6	Prauser (1986); Collins <i>et al.</i> (1989); O'Donnell <i>et al.</i> (1982)
<i>N. simplex</i>	Rods/cocci	MK-8(H ₄)	PG, DPG, PL, PG-OH	71.7	Collins <i>et al.</i> (1989); O'Donnell <i>et al.</i> (1982)

family-specific 16S rDNA signature nucleotides, strain BC 361^T exhibits several secondary-structure-forming nucleotides that are not present in the 16S rDNA sequences of any other member of the family. These nucleotides are found at positions (numbered according to *E. coli*) 183–194 (G–C), 987–1218 (A–U), 1002–1138 (A–U), 1012–1017 (G–C), 1089–1216 (G–C), 1422–1478 (U–G) and 1423–1479 (U–A).

DISCUSSION

Rock and monument surfaces support a high variety of microflora that often have been only poorly studied. In the last few years, however, it has become evident

that a great variety of *Actinobacteria* could be considered as the major colonizer of stone surfaces exposed to the outdoor environment. Among them, strains of *Micrococcus*, *Geodermatophilus* and *Micromonospora* (Urzi & Realini, 1998) were very often isolated and representatives of novel taxa might be expected (unpublished data). Tests based upon morphological as well as biochemical and physiological characteristics turned out to be insufficient to identify all isolated strains at the genus level. In the case of strain BC 361^T, the first attempt carried out using classical identification methods misplaced it in the genus *Micrococcus*. Later on, LL-A₂pm was found to be the characteristic diamino acid of the cell wall. This

finding was in conflict with the affiliation of strain BC 361^T to the genus *Micrococcus* or related genera (Stackebrandt *et al.*, 1995) and initiated a study of chemotaxonomic characteristics and phylogenetic relatedness of the strain in question.

The combination of the chemotaxonomic characteristics, peptidoglycan structure, menaquinone profiles and fatty acid composition is very helpful to differentiate genera of the order *Actinomycetales* and especially to distinguish strains containing LL-A₂pm in their cell wall (Schumann *et al.*, 1997; Prauser *et al.*, 1997). Strain BC 361^T showed the peptidoglycan type A3 γ with a single glycine residue as interpeptide bridge, MK-8(H₄) as predominant menaquinone and only straight-chain saturated and unsaturated fatty acids and TBSA (Tables 2 and 3). Peptidoglycan structure and the major menaquinone of strain BC 361^T are consistent with the genus description of the genus *Nocardioides*, but the complete absence of iso- and anteiso-branched chain fatty acids and the presence of phosphatidylinositol clearly separates strain BC 361^T from representatives of this genus. Exclusively straight-chain saturated and unsaturated fatty acids and TBSA in combination with peptidoglycan type A3 γ with a single glycine residue as interpeptide bridge are in agreement with the genus description of *Aeromicrobium*, but strain BC 361^T differs from all strains of this genus in containing MK-8(H₄) as major menaquinone and by the absence of phosphatidylethanolamine. In regard to its chemotaxonomic characteristics, strain BC 361^T seemed to occupy an 'intermediate position' between the genera *Nocardioides* and *Aeromicrobium*.

The phylogenetic analysis based on 16S rDNA sequence comparison placed strain BC 361^T between the genera *Nocardioides* and *Aeromicrobium* and revealed *N. jensenii* (96.4% similarity) as its closest phylogenetic neighbour (Fig. 1). As published by Yoon *et al.* (1998a, b), *N. jensenii* shows the highest 16S rDNA similarity (95.0%) and the highest 16S–23S internally transcribed spacer sequence similarity (73.1%) of all validly published *Nocardioides* species to *Aeromicrobium erythreum*, the type species of the genus *Aeromicrobium*. Despite its phylogenetic proximity to the genus *Aeromicrobium*, the fatty acid profile and all other chemotaxonomic features characterize *N. jensenii* as a true member of the genus *Nocardioides* (Schumann *et al.*, 1997; Suzuki & Komagata, 1983; Collins *et al.*, 1989). Strain BC 361^T differs from its closest phylogenetic neighbour *N. jensenii* not only by chemotaxonomic characteristics but also by sequences of the PCR primer designed for rapid identification of *N. jensenii* by multiplex PCR (Park *et al.*, 1998) and of 16S rDNA of strain BC 361^T. The nucleotide pair G-G at position 445 and 446 in *N. jensenii* is replaced by the pair C-A in the sequence of strain BC 361^T. In addition, strain BC 361^T exhibits several secondary-structure-forming nucleotides that are not present in the 16S rDNA sequences of any other member of the family. Thus, strain BC 361^T possesses a unique combination

of chemotaxonomic and phylogenetic characteristics. Since it could readily be distinguished from representatives of all phylogenetically related genera, it is concluded that the organism should be assigned to a new genus and species for which the name *Marmoricola aurantiacus* is proposed.

Description of *Marmoricola* gen. nov.

Marmoricola. (Mar.mo.ri'co.la. L. neutr. n. *marmor* marble; L. masc. suffix *-cola* inhabitant of; *Marmoricola* inhabitant of marble).

Cells are Gram-positive, not acid-fast, spherical, occurring singly, in pairs, tetrads and small clusters. Non-motile and non-sporulating. No rod/coccus life cycle. Colonies are circular, convex, entire, orange-pigmented, shiny and smooth. They can become rough and shaped like craters in old cultures. Aerobic. Catalase-positive, oxidase-negative. The cell wall peptidoglycan contains LL-A₂pm as characteristic diamino acid and a single glycine residue as interpeptide bridge (type A3 γ). The acyl type is acetyl. Phospholipids are phosphatidylinositol, phosphatidylglycerol and diphosphatidylglycerol. Mycolic acids are absent. The cellular fatty acid profile consists of straight-chain saturated and monounsaturated components and TBSA as the only branched chain fatty acid. The major menaquinone is MK-8(H₄). The DNA G+C content is 72 mol%. Phylogenetically, this genus is a member of the family *Nocardioideaceae* (Stackebrandt *et al.*, 1997). The type species is *Marmoricola aurantiacus*.

Description of *Marmoricola aurantiacus* sp. nov.

Marmoricola aurantiacus (au.ran.ti'a.cus. M.L. adj. *aurantiacus* orange-coloured).

Cells are spherical occurring singly, in pairs, tetrads and small clusters (0.5–0.7 μ m in diameter). Non-motile and non-sporulating. No rod/coccus life cycle. Colonies are circular, convex, entire, orange-pigmented, shiny and smooth (2–5 mm in diameter). They can become rough and shaped like craters in old cultures. Aerobic. Catalase-positive, oxidase-negative. Nitrate is not reduced to nitrite. No acid production is observed from D-ribose, L-arabinose, D-xylose, L-rhamnose, D-glucose, D-mannose, D-galactose, maltose, lactose, D-cellobiose, D-trehalose, D-raffinose, glycerol, D-mannitol or *myo*-inositol. Casein and gelatin are not hydrolysed. Xanthine and tyrosine are not decomposed; hypoxanthine is decomposed. Aesculin is hydrolysed, Tween 80 is only weakly hydrolysed. DNA is not hydrolysed. The strain does not grow in the following media: CZ, YMS-Glc, YCS-Glc, YG-Glyc, Y-Glyc, agar-H₂O and M53. Growth occurs in Luedemann, PDA and BHIA media. The cell wall peptidoglycan contains LL-A₂pm as characteristic diamino acid and a single glycine residue as interpeptide bridge (type A3 γ). The acyl type is acetyl. Phospholipids are phosphatidylinositol, phosphatidyl-

glycerol and diphosphatidylglycerol. Mycolic acids are absent. The cellular fatty acid profile consists of straight-chain saturated and monounsaturated components and TBSA as the only branched chain fatty acid. The major menaquinone is MK-8(H₄). The DNA G + C content is 72 mol %. Phylogenetically, it is a member of the family *Nocardioidaceae*. The only known isolation site is a Carrara marble statue (Wagmüller's monument) located in the Nordfriedhof Cemetery in Munich, Germany. The type strain is strain BC 361^T (bacterial collection of the Institute of Microbiology, Messina). It has been deposited in the DSMZ – German Collection of Microorganisms and Cell Cultures GmbH as strain DSM 12652^T.

ACKNOWLEDGEMENTS

This study was supported by the financial contribution of CNR (PF 97.00720. PF36) and EC (project ENV4-CT98-0707). P.S. acknowledges the grant received by EC in the frame of project no. ENV4-CT98-0704. The authors would like to thank Dr Helmut Prauser for the stimulating discussion and Professor Hans G. Trüper for his valuable help in assigning the generic and specific name to the strain BC 361^T.

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