Marmoricola scoriae sp. nov., isolated from volcanic ash

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A novel Gram-stain-positive, coccoid actinobacterium, designated strain Sco-D01^T, was isolated from volcanic ash collected from Oreum (a parasitic volcanic cone) on Jeju Island, Republic of Korea. Cells were aerobic, oxidase-negative and catalase-positive. Colonies were vivid yellow, circular, smooth and convex. The diagnostic diamino acid in the cell wall was L-diaminopimelic acid. The predominant menaquinone was MK-8(H₄). The polar lipids were phosphatidylinositol, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine and an unknown phospholipid. The fatty acid profile was represented by large amounts of saturated, unsaturated, 10-methyl and hydroxyl components. The DNA G+C content of strain Sco-D01^T was 72.0 mol%. 16S rRNA gene sequence analysis revealed that strain Sco-D01^T belonged to the family *Nocardioideae* and formed a distinct sublineage within the radiation of the genus *Marmoricola*. The level of DNA–DNA relatedness between strain Sco-D01^T and its closest phylogenetic relative, *Marmoricola aurantiacus* DSM 12652^T, was 30.2 % (35.4 % in duplicate measurements). On the basis of phenotypic and DNA–DNA hybridization data, strain Sco-D01^T is considered to represent a novel species of the genus *Marmoricola*, for which the name *Marmoricola scoriae* sp. nov. is proposed. The type strain is Sco-D01^T (=KCTC 19597^T=DSM 22127^T).

The genus *Marmoricola* was proposed by Urzì *et al.* (2000) to accommodate an aerobic, Gram-positive, coccoid actinobacterium isolated from marble, *Marmoricola aurantiacus*, which was assigned to the family *Nocardioideae* on the basis of comparative 16S rRNA gene sequence analysis. Two other species in the genus, *Marmoricola aequoreus* (Lee, 2007) and *Marmoricola bigeumensis* (Dastager *et al.*, 2008), were subsequently described, members of which were isolated from various environments such as marble, beach sediment and agricultural soil. The aim of the present study was to characterize a novel Gram-stain-positive, coccoid actinobacterium, designated strain Sco-D01^T, by using a polyphasic taxonomic approach. An emended description of the genus *Marmoricola* is also provided.

Strain Sco-D01^T was isolated from volcanic ash collected from Oreum (a parasitic volcanic cone) on Jeju Island, Republic of Korea. Volcanic ash samples (1 g) were ground into powder with a pestle and suspended in 10 ml distilled water. Aliquots of serial dilutions were spread onto starch-casein agar (1 % soluble starch, 0.03 % casein, 0.2 % KNO₃, 0.2 % NaCl, 0.2 % KH₂PO₄, 0.002 % CaCO₃, 0.005 %

MgSO₄·7H₂O, 0.001 % FeSO₄·7H₂O and 1.8 % agar; pH 7.2), and the plates were incubated at 30 °C for 2 weeks. A single colony was selected and further streaked on ISP (International *Streptomyces* Project) medium 2 (Shirling & Gottlieb, 1966) at least three times. For phenotypic and genotypic comparisons, *M. aurantiacus* DSM 12652^T, *M. aequoreus* SST-45^T and *M. bigeumensis* KCTC 19287^T were grown on R2A agar (Difco) for 5 days at 30 °C.

Unless specified otherwise, the following morphological and physiological characteristics were investigated by using ISP 2 as the basal medium. The temperature and pH ranges for growth were determined at 4, 10, 20, 30, 37 and 42 °C and at initial pH values of 4.1–12.1 (at intervals of 1.0 pH units). NaCl tolerance for growth was tested on ISP 2 supplemented with 1–9 % (w/v) NaCl. Gram staining, oxidase and catalase activities, degradation ability and utilization of carbohydrates were determined according to the methods described by Lee & Lee (2008). Other physiological and biochemical properties were tested by using the API 20E and API ZYM kits (bioMérieux) according to the manufacturer's recommendations. Colony morphology and cell pigmentation were observed and recorded after incubation on ISP 2 for 5 days at 30 °C. Cell morphology and motility were observed by using phase-contrast and transmission electron microscopy. For the latter, cells were stained with 1 % phosphotungstic acid and observed with a model JEM-1200EX II (JEOL) transmission electron microscope.

Abbreviation: DAP, diaminopimelic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Sco-D01^T is FN386750.

A supplementary table giving the cellular fatty acid profiles of strain Sco-D01^T and the type strains of recognized *Marmoricola* species is available with the online version of this paper.

Cells of strain Sco-D01^T were aerobic, Gram-stain-positive, non-motile cocci (0.6–1.0 µm in diameter) that were present singly, in pairs or in clusters. Colonies were vivid yellow, circular, convex with entire margins and 0.1–0.2 mm in diameter after incubation on ISP 2 for 5 days at

30 °C. Other physiological and biochemical characteristics are given in Table 1 and in the species description below.

Extraction of genomic DNA and amplification and sequencing of the 16S rRNA gene were performed as

Table 1. Characteristics that differentiate strain Sco-D01^T from the type strains of recognized *Marmoricola* species

Strains: 1, Sco-D01^T; 2, *M. aequoreus* SST-45^T (data from Lee, 2007); 3, *M. aurantiacus* DSM 12652^T (Urzi *et al.*, 2000); 4, *M. bigeumensis* MSL-05^T (Dastager *et al.*, 2008). All were aerobic, Gram-positive, oxidase-negative and catalase-positive and hydrolysed aesculin. All were positive for enzyme activities of alkaline phosphatase, esterase lipase (C8), leucine arylamidase, α-glucosidase and β-glucosidase, but negative for lipase (C14), α-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase (API ZYM). All were positive for β-galactosidase and the Voges–Proskauer reaction, but negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, tryptophan deaminase and indole production (API 20E). All utilized D-mannitol and methyl α-D-glucoside as sole carbon source, but not formate, myo-inositol, methyl α-D-mannoside or L-sorbose. +, Positive; –, negative; w, weakly positive; ND, not determined.

Characteristic	1	2	3*	4*
Colony colour	Vivid yellow	Yellow	Orange	Lemon yellow
Cell arrangement	Single, pairs or clusters	Single, pairs or clusters	Single, pairs or clusters	Single
Motility	–	–	–	+
Temperature range (°C)	10–37	10–37	18–28	20–37
NaCl tolerance range (%)	0–3.0	0–7.0	0.5–2.0	0–7.0
pH range	6.1–12.1	5.1–12.1	5.1–8.7	6.0–12.0
Nitrate reduction	–	+	–	+
Gelatin liquefaction	+	+	–	–
Degradation of:				
Casein	+	+	–	–
DNA	+	ND	–	–
Starch	–	–	–	+
Xanthine	–	–	–	+
Enzyme activity				
Acid phosphatase	+	–	+	+
Cystine arylamidase	w	w	–	+
Esterase (C4)	–	–	w	+
Trypsin	w	–	–	+
Utilization of (on ISP 9):				
Adonitol	–	–	+	–
D-Arabinose	+	–	+	–
Citrate	+	+	+	–
Dextran	–	+	+	+
Glycerol	–	+	+	–
D-Lactose	–	+	+	+
Raffinose	+	–	+	–
L-Rhamnose	+	–	+	+
Salicin	+	–	+	–
D-Sorbitol	–	+	+	+
Succinate	–	+	+	–
DL-Tartrate	–	–	+	–
D-Xylitol	–	–	+	+
Polar lipids†	DPG, PC, PG, PI, PL	DPG, PG, PI, PL	DPG, PG, PI, PL	DPG, PG, PI, PL
Major fatty acids (>10%)	C _{16:0} , C _{18:1ω9c} , 10-methyl C _{18:0}	C _{16:0} , C _{18:1ω9c}	C _{16:0} , C _{18:1ω9c}	iso-C _{16:0}
DNA G + C content (mol%)	72.0	72.4	72.0	72.9
Isolation source	Volcanic ash	Beach sediment	Marble	Agricultural soil

*Data for fatty acids and utilization of carbon sources are from the present study.

†DPG, Diphosphatidylglycerol; PC, phosphatidylcholine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PL, unknown phospholipid.

described by Lee & Lee (2008). Multiple alignments of sequences were performed by using CLUSTAL X (Thompson *et al.*, 1997). A phylogenetic tree was constructed by using the neighbour-joining method (Saitou & Nei, 1987) and the model of Jukes & Cantor (1969). The topology of the tree was assessed by performing bootstrap analysis of 1000 replicated neighbour-joining datasets (Felsenstein, 1985). An almost-complete 16S rRNA gene sequence (1425 nt) of strain Sco-D01^T determined in this study was compared with those of representatives of the family *Nocardioidaceae*. The phylogenetic tree (Fig. 1) revealed that strain Sco-D01^T belonged to the genus *Marmoricola* and formed a tight cluster with the type strain of *M. aurantiacus* (98.6 % 16S rRNA gene sequence similarity) that was supported by a

93 % bootstrap value. This topology was also recovered in trees generated with the maximum-likelihood (Fitch, 1971) and maximum-parsimony (Felsenstein, 1981) methods. The next closest phylogenetic neighbours of strain Sco-D01^T were the type strains of *M. aequoreus* and *M. bigeumensis* (97.6 and 95.9 % 16S rRNA gene sequence similarity, respectively).

Chemotaxonomic characteristics were investigated as described by Lee & Lee (2008), with cell biomass obtained from a culture grown in ISP 2 broth for 5 days at 30 °C. The following chemotaxonomic characteristics were determined by using standard methods: isomer of diaminopimelic acid (DAP) in the cell wall (Staneck & Roberts,

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences, showing the phylogenetic position of strain Sco-D01^T within the radiation of the family *Nocardioidaceae*. The sequence of *Streptomyces griseus* KCTC 9080^T (GenBank accession no. M76388) served as the outgroup (not shown). Asterisks indicate branches that were also recovered in the maximum-likelihood and maximum-parsimony trees. Bootstrap values (expressed as percentages of 1000 replications) of ≥ 50 % are shown at nodes. Bar, 0.01 substitutions per nucleotide position.

1974), respiratory quinones (Collins, 1985; Kroppenstedt, 1985), polar lipids (Minnikin *et al.*, 1975) and DNA G+C content (Mesbah *et al.*, 1989). For fatty acid analysis, strain Sco-D01^T and the type strains of the three recognized *Marmoricola* species were grown on R2A agar for 5 days at 30 °C. Cellular fatty acid methyl esters were prepared and analysed according to the standard protocol of the Microbial Identification System (version 6; MIDI).

Strain Sco-D01^T contained LL-DAP as the diagnostic diamino acid in the cell-wall peptidoglycan. The predominant menaquinone was MK-8(H₄). The polar lipids comprised phosphatidylinositol, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine and an unknown phospholipid. The presence of phosphatidylcholine distinguishes strain Sco-D01^T from the type strains of recognized *Marmoricola* species, all of which lack this component in their polar lipid profiles. The DNA G+C content of strain Sco-D01^T was 72.0 mol%. The fatty acid profile of strain Sco-D01^T was characterized by large amounts of saturated and unsaturated components, together with moderate amounts of 10-methyl and hydroxyl fatty acids. The predominant fatty acids (>10% of the total) were C_{16:0} (27.7%), C_{18:1ω9c} (25.9%) and 10-methyl C_{18:0} (10.2%). Strain Sco-D01^T could be differentiated from the type strains of *M. aequareus* and *M. aurantiacus* based on the presence/absence of iso-C_{16:0}, C_{17:1ω6c}, C_{17:0} 2-OH and anteiso-C_{19:0}, and could be distinguished easily from the type strain of *M. bigeumensis* based on the relative amounts of the major components (C_{16:0}, iso-C_{16:0}, C_{18:1ω9c} and 10-methyl C_{18:0}). The cellular fatty acid profiles of strain Sco-D01^T and the type strains of recognized *Marmoricola* species are given in Supplementary Table S1, available in IJSEM Online.

DNA–DNA hybridization experiments were performed by using the method of De Ley *et al.* (1970) with the modifications described by Huß *et al.* (1983). The level of DNA–DNA relatedness between strain Sco-D01^T and *M. aurantiacus* DSM 12652^T was 30.2% (35.4% in duplicate measurements). Differential characteristics between strain Sco-D01^T and the type strains of *M. aurantiacus*, *M. aequareus* and *M. bigeumensis* are detailed in Table 1.

On the basis of phenotypic features and DNA–DNA hybridization data, strain Sco-D01^T is considered to represent a novel species of the genus *Marmoricola*, for which the name *Marmoricola scoriae* sp. nov. is proposed.

Emended description of *Marmoricola Urzì et al.* 2000 emend Dastager *et al.* 2008

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

The description is as given by Urzì *et al.* (2000) and Dastager *et al.* (2008), with the following addition. Oxidase-negative and catalase-positive. Tuberculoheptanoic acid (10-methyl C_{18:0}) and other branched fatty acids may

be present. The presence of phosphatidylcholine is variable depending on species.

Description of *Marmoricola scoriae* sp. nov.

Marmoricola scoriae [sco.ri'ae. L. gen. n. *scoriae* of scoria (volcanic ash), referring to the site from which the type strain was isolated].

Cells are aerobic, Gram-stain-positive, oxidase-negative, catalase-positive, non-motile cocci (0.6–1.0 µm in diameter) that are present singly, in pairs or in clusters. Colonies are vivid yellow, circular, convex with entire margins and 0.1–0.2 mm in diameter after incubation on ISP 2 for 5 days at 30 °C. Growth is observed at pH 6.1–12.1 (optimum at pH 8.1–11.1), at 10–37 °C (optimum at 30 °C) and in the presence of 0–3% (w/v) NaCl (optimum at 0% NaCl). CM-cellulose, hypoxanthine, tyrosine and xanthine are not hydrolysed. H₂S production is not observed. Positive for enzyme activities of naphthol-AS-BI-phosphohydrolase and valine arylamidase, but negative for activity of α-chymotrypsin (API ZYM). Acetate, L-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, DL-malate, maltose, D-mannose, melezitose, L-ribose, sucrose, trehalose and D-xylose are utilized as sole carbon and energy sources, but benzoate, dulcitol, *meso*-erythritol and melibiose are not. Data for other physiological and biochemical properties are given in Table 1. LL-DAP is the diagnostic diamino acid in the cell-wall peptidoglycan. Polar lipids comprise phosphatidylinositol, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine and an unknown phospholipid. The predominant menaquinone is MK-8(H₄). The major fatty acids are C_{16:0}, C_{18:1ω9c} and 10-methyl C_{18:0}. The DNA G+C content of the type strain is 72.0 mol%.

The type strain, Sco-D01^T (=KCTC 19597^T=DSM 22127^T), was isolated from volcanic ash of Oreum (a parasitic volcanic cone) in Jeju, Republic of Korea.

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References

- Collins, M. D. (1985). Analysis of isoprenoid quinones. *Methods Microbiol* **18**, 329–366.
- Dastager, S. G., Lee, J.-C., Ju, Y.-J., Park, D.-J. & Kim, C.-J. (2008). *Marmoricola bigeumensis* sp. nov., a member of the family Nocardioidaceae. *Int J Syst Evol Microbiol* **58**, 1060–1063.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.

- Felsenstein, J. (1981).** Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.
- Felsenstein, J. (1985).** Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Fitch, W. M. (1971).** Towards defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.
- Huß, V. A. R., Festl, H. & Schleifer, K. H. (1983).** Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.
- Jukes, T. H. & Cantor, C. R. (1969).** Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- Kroppenstedt, R. M. (1985).** Fatty acid and menaquinone analysis of actinomycetes and related organisms. In *Chemical Methods in Bacterial Systematics*, pp. 173–199. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.
- Lee, S. D. (2007).** *Marmoricola aequoreus* sp. nov., a novel actinobacterium isolated from marine sediment. *Int J Syst Evol Microbiol* **57**, 1391–1395.
- Lee, D. W. & Lee, S. D. (2008).** *Aeromicrobium ponti* sp. nov., isolated from seawater. *Int J Syst Evol Microbiol* **58**, 987–991.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989).** Precise measurement of the G + C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Minnikin, D. E., Alshamaony, L. & Goodfellow, M. (1975).** Differentiation of *Mycobacterium*, *Nocardia*, and related taxa by thin layer chromatographic analysis of whole-cell methanolysates. *J Gen Microbiol* **88**, 200–204.
- Saitou, N. & Nei, M. (1987).** The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Shirling, E. B. & Gottlieb, D. (1966).** Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* **16**, 313–340.
- Staneck, J. L. & Roberts, G. D. (1974).** Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* **28**, 226–231.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997).** The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Urzi, C., Salamone, P., Schumann, P. & Stackebrandt, E. (2000).** *Marmoricola aurantiacus* gen. nov., sp. nov., a coccoid member of the family *Nocardioideaceae* isolated from a marble statue. *Int J Syst Evol Microbiol* **50**, 529–536.