Marmoricola korecus sp. nov.

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A novel actinomycete, designated strain Sco-A36^T, was isolated from volcanic ash. Cells were aerobic, Gram-stain-positive, oxidase-negative, catalase-positive, non-motile cocci; colonies were yellow-coloured, smooth, entire and convex. Phylogenetic analysis showed that the organism formed a distinct phyletic line within the radiation of the genus *Marmoricola*. Its closest phylogenetic neighbours were *Marmoricola aurantiacus* DSM 12652^T (97.9% 16S rRNA gene sequence similarity), *Marmoricola scoriae* Sco-D01^T (97.9%), *Marmoricola aequoreus* SST-45^T (97.4%) and *Marmoricola bigeumensis* MSL-05^T (96.3%). The diagnostic diamino acid in the cell walls was LL-diaminopimelic acid. The predominant menaquinone was MK-8(H₄). The polar lipids contained phosphatidylinositol, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine and an unknown phospholipid. The predominant fatty acids were C_{16:0}, C_{17:1ω8c}, C_{18:1ω9c} and C_{16:1ω7c} and/or iso-C_{15:0} 2-OH. The DNA G + C content of strain Sco-A36^T was 71.0 mol%. Levels of DNA–DNA relatedness between the new isolate and the type strains of recognized species of the genus *Marmoricola* were 4.9–29.2%. The phenotypic and DNA–DNA hybridization data presented here strongly suggest that strain Sco-A36^T represents a novel species of the genus *Marmoricola*, for which the name *Marmoricola korecus* sp. nov. is proposed. The type strain is Sco-A36^T (=KCTC 19596^T=DSM 22128^T).

The genus *Marmoricola* was proposed by Urzi *et al.* (2000), with the description of *Marmoricola aurantiacus* as the type and only species. Subsequently, *Marmoricola aequoreus* (Lee, 2007), *Marmoricola bigeumensis* (Dastager *et al.*, 2008) and *Marmoricola scoriae* (Lee & Lee, 2010) have been described. These bacteria form a phylogenetically coherent cluster within the family *Nocardioidaceae* and are well separated from other genera of the family (Lee & Lee, 2010). Members of the genus have been isolated from marble, beach sediment, agricultural soil and volcanic ash. In the present study, we describe the identification and classification of a *Marmoricola* strain isolated from scoria, designated Sco-A36^T, based on a polyphasic taxonomic approach.

Strain Sco-A36^T was isolated from a red layer of scoria (volcanic ash) collected in Jeju, Republic of Korea. Treatment of the volcanic ash sample and preparation of media for bacterial isolation were performed as described

by Lee & Lee (2010). The pure culture was maintained in a 20% (v/v) glycerol solution at –20 and –80 °C. For DNA–DNA hybridization experiments, *M. aequoreus* SST-45^T, *M. aurantiacus* DSM 12652^T and *M. scoriae* Sco-D01^T were used as reference strains.

Cell biomass for DNA extraction and chemotaxonomic characterization was obtained from cultures grown in International *Streptomyces* Project medium 2 (ISP 2) broth [0.4% glucose, 1.0% malt extract and 0.4% yeast extract, pH 7.2 (Shirling & Gottlieb, 1966)] at 30 °C. Chromosomal DNA was extracted and purified by using a Wizard Genomic DNA Purification kit (Promega) according to the manufacturer's instructions. Amplification and sequencing of the 16S rRNA gene and phylogenetic analyses were performed as described by Lee & Lee (2008).

A partial 16S rRNA gene sequence (1426 nt) of strain Sco-A36^T was determined in this study and was aligned with those of members of the genus *Marmoricola* and related taxa. A neighbour-joining tree (Fig. 1) based on 16S rRNA gene sequence analyses revealed that strain Sco-A36^T belonged to the genus *Marmoricola* and formed a distinct phyletic line between a cluster comprising *M. scoriae*–*M. aurantiacus* and *M. aequoreus*. This relationship was supported by a bootstrap value of 97% and was also found in trees generated by the maximum-parsimony and

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Sco-A36^T is FN386723.

A supplementary figure and table are available with the online version of this paper.

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences showing the position of strain Sco-A36^T within the radiation occupied by the genus *Marmoricola*. The tree was constructed by using the model of Jukes & Cantor (1969) and the neighbour-joining method (Saitou & Nei, 1987). *Aeromicrobium erythreum* NRRL B-3381^T was used as an outgroup. Asterisks indicate branches that were also found in the maximum-likelihood (Fitch, 1971) and maximum-parsimony (Felsenstein, 1981) trees. Bootstrap values are shown at branch points based on 1000 resamplings. Bar, 0.01 substitutions per nucleotide position.

maximum-likelihood methods. Levels of 16S rRNA gene sequence similarity between strain Sco-A36^T and *M. aurantiacus* DSM 12652^T, *M. scoriae* Sco-D01^T, *M. aequoreus* SST-45^T and *M. bigeumensis* MSL-05^T were 97.9, 97.9, 97.4 and 96.3 %, respectively. Strain Sco-A36^T shared low levels of 16S rRNA gene sequence similarity (<95 %) with the type strains of related species of the genus *Nocardioideae*.

The isomer of diaminopimelic acid in the cell-wall peptidoglycan (Staneck & Roberts, 1974), major menaquinones (Kroppenstedt, 1985), polar lipids (Minnikin *et al.*, 1977), G + C content of the DNA (Mesbah *et al.*, 1989) and cellular fatty acids were analysed as described by Lee & Lee (2010). For fatty acid analysis, strain Sco-A36^T was grown on R2A agar for 5 days at 30 °C.

The diagnostic diamino acid in the cell-wall peptidoglycan of strain Sco-A36^T was LL-diaminopimelic acid. The predominant menaquinone was MK-8(H₄). The polar lipids contained phosphatidylinositol, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine and an unknown phospholipid. Phosphatidylcholine is also found in *M. scoriae*, but not in *M. aequoreus*, *M. aurantiacus* or *M. bigeumensis*. The fatty acid profile of strain Sco-A36^T contained saturated, unsaturated, 10-methyl and hydroxyl components, with the major fatty acids being C_{16:0}, C_{17:1}ω8c, C_{18:1}ω9c and C_{16:1}ω7c and/or iso-C_{15:0} 2-OH. The detailed cellular fatty acid profiles of strain Sco-A36^T and the type strains of recognized species of the genus *Marmoricola* are given in Supplementary Table S1 in IJSEM Online. The DNA G + C content of strain Sco-A36^T was 71.0 mol%.

Tests for morphological, cultural, physiological and biochemical properties were performed as described by Lee & Lee (2010). Cells of strain Sco-A36^T were aerobic, Gram-positive, oxidase-negative, catalase-positive, non-motile cocci (1.1–1.2 µm in diameter; see Supplementary Fig. S1). Colonies were yellow, circular and convex with entire margins. Data for other physiological and biochemical features are given in Table 1 and in the species description below.

Morphological and cultural characteristics of strain Sco-A36^T were similar to those of the type strain of *M. scoriae* (Lee & Lee, 2010), although the two were isolated from different sources of scoria; strain Sco-A36^T was isolated from a red layer of a volcanic ash, whereas *M. scoriae* Sco-D01^T was isolated from a yellow–brown layer. The two strains could be differentiated from each other based on temperature and pH ranges for growth and, in particular, based on utilization of carbohydrates. Differential characteristics between strain Sco-A36^T and the type strains of recognized species of the genus *Marmoricola* are given in Table 1.

DNA–DNA hybridization was carried out by using the method of Ezaki *et al.* (1989); levels of DNA–DNA relatedness were determined fluorometrically as described by Yoon *et al.* (2007). Levels of DNA–DNA relatedness between Sco-A36^T and the type strains of recognized species of the genus *Marmoricola* were 4.9–29.2 % (SD, 12.2 %, five replications), showing that strain Sco-A36^T represents a distinct genomic species (Wayne *et al.*, 1987).

On the basis of the data presented here, we suggest that strain Sco-A36^T represents a novel species of the genus *Marmoricola*, for which the name *Marmoricola korecus* sp. nov. is proposed.

Description of *Marmoricola korecus* sp. nov.

Marmoricola korecus (ko.re'cus. N.L. masc. adj. *korecus* pertaining to Korea, from where the type strain was isolated).

Cells are aerobic, Gram-positive, oxidase-negative, catalase-positive, non-motile cocci. Colonies are yellow, circular and convex with entire margins. Growth occurs at pH 5.1–12.1 (optimum, pH 6.1–10.1), at 4–37 °C (optimum, 30 °C) and in the presence of up to 2 % (w/v) NaCl. Aesculin is hydrolysed, but CM-cellulose, hypoxanthine, DL-tyrosine and xanthine are not. Nitrate is not reduced to nitrite. Gelatin liquefaction is observed. Positive in tests for alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine

Table 1. Differential characteristics between strain Sco-A36^T and the type strains of recognized species of the genus *Marmoricola*

Strains: 1, Sco-A36^T; 2, *M. aequoreus* SST-45^T (data from Lee, 2007); 3, *M. aurantiacus* DSM 12652^T (Urzi *et al.*, 2000; Lee & Lee, 2010); 4, *M. bigeumensis* MSL-05^T (Dastager *et al.*, 2008; Lee & Lee, 2010); 5, *M. scoriae* Sco-D01^T (Lee & Lee, 2010). All of the strains were aerobic, Gram-positive, oxidase-negative, catalase-positive and positive for aesculin hydrolysis. All were positive for alkaline phosphatase, esterase lipase (C8), leucine arylamidase, α -glucosidase, β -glucosidase and the Voges–Proskauer reaction but negative for lipase (C14), α -galactosidase, β -glucuronidase, *N*-acetyl- β -glucosaminidase, α -mannosidase, α -fucosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, tryptophan deaminase and indole production (API ZYM and API 20E strips). All of the strains utilized glucose, D-mannitol and methyl α -D-glucoside as sole carbon source but not formate, *meso*-inositol, methyl α -D-mannoside or L-sorbose. +, Positive; –, negative; w, weakly positive; ND, not determined.

Characteristic	1	2	3	4	5
Colony colour	Yellow	Yellow	Orange	Lemon yellow	Vivid yellow
Cell motility	–	–	–	+	–
Temperature range for growth (°C)	4–37	10–37	18–28	20–37	10–37
pH range for growth	5.1–12.1	5.1–12.1	5.1–8.7	6.0–12.0	6.1–12.1
NaCl concentration for growth (%)	0–2.0	0–7.0	0.5–2.0	0–7.0	0–3.0
Nitrate reduction	–	+	–	+	–
β -Galactosidase	–	+	w	+	+
Degradation of:					
Casein	+	+	–	–	+
DNA	–	ND	–	–	+
Gelatin	+	+	–	–	+
Starch	–	–	–	+	–
Enzyme activities					
Acid phosphatase	+	–	+	+	+
α -Chymotrypsin	–	–	–	+	–
Cystine arylamidase	w	w	–	+	w
Esterase (C4)	–	–	w	+	–
Naphthol-AS-BI-phosphohydrolase	+	–	+	+	+
Trypsin	w	–	–	+	w
Valine arylamidase	+	+	w	–	+
Utilization of:					
Acetate	–	+	+	+	+
Adonitol	+	–	+	–	–
D-Arabinose	–	–	+	–	+
Cellobiose	+	+	+	+	–
Citrate	–	+	+	–	+
Dextran	–	+	+	+	–
Dulcitol	+	–	–	–	–
D-Fructose	–	+	+	–	+
D-Galactose	–	+	+	–	+
Glycerol	–	+	+	–	–
Lactose	–	+	+	+	–
DL-Malate	–	+	+	–	+
Maltose	–	+	+	+	+
D-Mannose	+	+	+	+	–
Melezitose	–	+	+	–	+
<i>meso</i> -Erythritol	+	–	–	–	–
Raffinose	–	–	+	–	+
L-Rhamnose	–	–	+	+	+
L-Ribose	–	+	+	–	+
Salicin	–	–	+	–	+
D-Sorbitol	–	+	+	+	–
Succinate	+	+	+	–	–
Sucrose	–	+	+	–	+
DL-Tartrate	–	–	+	–	–
Trehalose	–	+	+	–	+

Table 1. cont.

Characteristic	1	2	3	4	5
D-Xylitol	+	—	+	+	—
D-Xylose	+	+	+	+	—
Phosphatidylcholine	+	—	—	—	+
Major fatty acids (>10 %)*	C _{16:0} , C _{17:1} ω8c, C _{18:1} ω9c, SF4	C _{16:0} , C _{18:1} ω9c	C _{16:0} , C _{18:1} ω9c, C _{16:1} †	iso-C _{16:0}	C _{16:0} , C _{18:1} ω9c, TBSA
DNA G + C content (mol%)	71.0	72.4	72.0	72.9	72.0

*SF4, C_{16:1}ω7c and/or iso-C_{15:0} 2-OH; TBSA, tuberculostearic acid.

†Present as a main component in a previous report (Urzi *et al.*, 2000), but not detected in our study (Lee & Lee, 2010).

arylamidase (weak response), trypsin (weak response), acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, β-glucosidase (API ZYM) and the Voges–Proskauer reaction (API 20E). Utilizes adonitol, cellobiose, meso-erythritol, D-glucose, D-mannitol, D-mannose, methyl α-D-glucoside, succinate, D-xylitol and D-xylose as sole carbon and energy sources. Data for other physiological properties are given in Table 1. The polar lipids include phosphatidylinositol, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine and an unknown phospholipid. The major fatty acids are C_{16:0}, C_{17:1}ω8c, C_{18:1}ω9c and C_{16:1}ω7c and/or iso-C_{15:0} 2-OH.

The type strain, Sco-A36^T (=KCTC 19596^T=DSM 22128^T), was isolated from scoria (volcanic ash) in Jeju, Republic of Korea. The G + C content of the DNA of the type strain is 71.0 mol%.

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