Marmoricola aequoreus sp. nov., a novel actinobacterium isolated from marine sediment

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A yellow-coloured, marine actinobacterium, designated SST-45^T, was isolated from sandy sediment under the surface of a beach and taxonomically characterized by physiological, chemotaxonomic and phylogenetic methods. The cells of the isolate were Gram-positive, aerobic, non-sporulating, non-motile, spherical cells that occurred singly, in pairs, in clusters or as short chains. The isolate grew at 10–37 °C, an initial pH 5.1–12.1 and in the presence of 5 % (w/v) NaCl. The organism possessed LL-2,6-diaminopimelic acid as the diagnostic diamino acid in the cell wall, MK-8(H₄) as the major menaquinone, a polar lipid profile including diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol and an unknown phospholipid, C_{18:1} and C_{16:0} as the major fatty acids, and a DNA G + C content of 72.4 mol%. A phylogenetic analysis based on 16S rRNA gene sequences showed that the organism was related to members of the genera *Marmoricola* and *Nocardioide*s. The closest neighbours were *Marmoricola aurantiacus* DSM 12652^T (97.0 % sequence similarity) and *Nocardioide*s *jensenii* KCTC 9134^T (96.7 %). The combination of morphological and chemotaxonomic characters supported the assignment of the isolate to the genus *Marmoricola*. However, the organism is clearly distinguished phenotypically from the single described species of this genus, *Marmoricola aurantiacus*. Based on the data obtained, the organism has been assigned as a novel species, for which the name *Marmoricola aequoreus* sp. nov. is proposed. The type strain is strain SST-45^T (= JCM 13812^T = NRRL B-24464^T).

The genus *Marmoricola* was proposed by Urzì *et al.* (2000) for a Gram-positive, aerobic, non-motile, non-sporulating, spherical bacterium without a rod–coccus life cycle, and currently contains only the type strain of the type species, *Marmoricola aurantiacus* DSM 12652^T, which was isolated from the surface of a marble statue. The genus was chemotaxonomically characterized as having LL-2,6-diaminopimelic acid in the cell wall, MK-8(H₄) as the major menaquinone, the characteristic phospholipids phosphatidylinositol, phosphatidylglycerol and diphosphatidylglycerol, and a cellular fatty acid pattern consisting of straight-chain saturated and monounsaturated components, with tuberculostearic acid as the only branched-chain fatty acid. This genus, together with the phylogenetically related genera *Nocardioide*s (Prauser, 1976) and *Aeromicrobium* (Miller *et al.*, 1991), belongs to the family *Nocardioideaceae* Nesterenko *et al.* 1985 emend. Rainey *et al.* 1997 (Nesterenko *et al.*, 1985; Stackebrandt *et al.*, 1997).

Strain SST-45^T studied in this work was isolated from a sandy sediment sample (1 m below the surface) taken from Samyang beach in Jeju, Republic of Korea. The isolation agar (SC-SW) and procedure have been reported previously (Lee, 2006). A colony was subcultured on yeast extract/malt extract agar (Shirling & Gottlieb, 1966) prepared in a 60 : 40 mixture of natural sea water and distilled water (YE-SW agar). Pure cultures were maintained in 20 % (v/v) glycerol suspensions containing 20 % (v/v) distilled water and 60 % (v/v) natural sea water at –20 and –80 °C.

Chromosomal DNA was extracted and purified by using the method of Hopwood *et al.* (1985). The G + C content of DNA was determined by HPLC as described by Mesbah *et al.* (1989). The DNA G + C content of strain SST-45^T was 72.4 mol%. The chromosomal 16S rRNA gene was amplified by PCR as described previously (Lee *et al.*, 2000). The resultant PCR product was directly sequenced using an ABI PRISM BigDye Terminator cycle sequencing kit (Applied Biosystems) and an automatic DNA sequencer (Model 3730xl; Applied Biosystems). A partial 16S rRNA gene sequence for strain SST-45^T (1398 nt) was determined in this study. The result of a preliminary BLAST search showed that the organism was related to members of the family *Nocardioideaceae*. The sequence was aligned with those

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SST-45^T is AM295338.

A table of comparative fatty acid data is available as supplementary data with the online version of this paper.

of related organisms retrieved from GenBank by using CLUSTAL_X (Thompson *et al.*, 1997). Phylogenetic analyses and tree construction were carried out using several tree-making algorithms, as described by Lee (2006).

The neighbour-joining tree (Fig. 1) showed that the organism was related to members of the genera *Marmoricola* and *Nocardioideis*. The closest neighbours were *Marmoricola aurantiacus* DSM 12652^T (97.0 % sequence similarity) and *Nocardioideis jensenii* KCTC 9134^T (96.7 %). All tree-making algorithms used in this study supported the phylogenetic relationships of the isolate with *Marmoricola aurantiacus* and *Nocardioideis jensenii*. Levels of 16S rRNA gene sequence similarities of strain SST-45^T to other *Nocardioideis* species and representatives of the genus *Aeromicrobium* were in the ranges of 92.1–95.5 % and 93.2–93.5 %, respectively.

Cell morphology and motility were observed using phase-contrast and transmission electron microscopes. Cells were grown on YE-SW agar at 30 °C for 15, 24 and 72 h. Colony pigmentation was observed visually and recorded after incubation for 5 days at 30 °C on YE-SW agar. Growth was tested on YE-SW agar at temperatures of 4, 10, 20, 30, 37 and 42 °C, and at pH 4.1–12.1. To test the pH for growth, the pH of the basal medium was adjusted at intervals of 1.0, after autoclaving, with 6 M HCl and 10 M NaOH. NaCl tolerance for growth was studied on yeast extract–malt extract agar, with the addition of NaCl at final concentrations of 0–9 % (w/v). The ability to utilize a variety of substrates as sole carbon source was determined using ISP 9 medium (Shirling & Gottlieb, 1966). Each filter-sterilized carbon source was added in the basal medium at a final concentration of 1 % (w/v) for carbohydrates and alcohols, and at 0.1 % (w/v) for organic acids. Strain SST-45^T was grown on YE-SW agar at 30 °C for 3 days and harvested by scraping the cells from the agar surface with a sterile cotton swab. After washing twice with sterile distilled water, cells were resuspended in distilled water before using them as an inoculum. The production of hydrogen sulfide was detected on Trypticase Soy Broth (TSB; Difco) by using lead acetate strips. Catalase activity was determined with a 3 % (v/v) H₂O₂ solution. Urease activity was determined using Bacto urea broth (Difco). Oxidase activity and Gram staining were determined as described by MacFaddin (1980). Decomposition of hypoxanthine, DL-tyrosine and xanthine were examined by the method of Gordon *et al.* (1974). Nitrate reduction, casein hydrolysis and gelatin liquefaction were examined by using the methods of MacFaddin (1980). The ability to hydrolyse starch was determined using starch agar (Difco). The production of acid from carbohydrates and alcohols was determined on Bacto OF basal medium (Difco) supplemented with a filter-sterilized carbon source at a final concentration of 1 % (w/v). Other enzyme activities were tested by using API ZYM strips, according to the instructions of the manufacturer, with cells grown on YE-SW at 30 °C for 2 days.

Cells of strain SST-45^T were aerobic, Gram-positive, oxidase-negative, catalase-positive, non-motile and coccoid (Fig. 2), irrespective of culture age. After incubation on

YE-SW at 30 °C for 5 days, the colonies were bright yellow in colour and circular, smooth and convex with an entire margin. Cells of strain SST-45^T showed an ability to utilize a variety of substrates, namely carbohydrates, alcohols and organic acids as sole carbon and energy sources for growth. The optimum temperature and pH of the isolate were 30 °C and pH 7.1, respectively. Detailed results of the physiological characterization are given in Table 1 and in the species description below.

Chemotaxonomic analyses were performed as described by Lee (2006). The isomer of diaminopimelic acid in the cell wall, isoprenoid quinones, polar lipids and mycolic acids were determined according to the methods of Stanek & Roberts (1974), Kroppenstedt (1985), Minnikin *et al.* (1977) and Minnikin *et al.* (1980), respectively. To determine cellular fatty acid composition, strain SST-45^T was grown on TSB supplemented with Bacto agar (Difco) at 30 °C for 3 days. Cellular fatty acid methyl esters were prepared and analysed using the Sherlock Microbial Identification System (version 6.0; MIDI), according to the instructions of the manufacturer. The determined chemotaxonomic markers (given in the species description) were consistent with those of *Marmoricola aurantiaca*. The cellular fatty acids of strain SST-45^T were represented by straight-chain saturated and unsaturated acids and consisted predominantly of C_{18:1} (40.1 %) and C_{16:0} (35.0 %). Minor components were C_{14:0} (1.4 %), C_{17:0} (2.9 %), C_{18:0} (7.6 %), C_{16:1} (6.6 %) and C_{17:1} (2.0 %). The fatty acid profile of strain SST-45^T differed from that of *Marmoricola aurantiaca* in the absence/presence of 10-Me C_{18:0} and some other minor acids (see supplementary Table S1 with the online version of this paper). This difference, however, may be due to different growth and analysis conditions as demonstrated for some actinobacterial species (Park *et al.*, 1999; Sohn *et al.*, 2003; Trujillo *et al.*, 2006).

The isolate also differed from *Marmoricola aurantiaca* in the yellow-pigmented colonies, growth at 37 °C and in the presence of 5 % (w/v) NaCl, the ability to reduce nitrate and to produce acid from maltose, and some degradation activities (Table 1). On the other hand, the other phylogenetic neighbour, *Nocardioideis jensenii*, as well as other members of that genus, showed a striking dissimilarity to the isolate by the presence of iso- and anteiso-branched fatty acids, and in their rod-shaped cells or fragmenting mycelium (Collins *et al.*, 1989; Miller *et al.*, 1991; Prauser, 1976; Schumann *et al.*, 1997; Suzuki & Komagata, 1983; Tamura & Yokota, 1994).

On the basis of the phenotypic and phylogenetic data, it was shown that the isolate merits classification as a novel species of the genus *Marmoricola* for which the name *Marmoricola aequoreus* sp. nov. is proposed. The type strain is SST-45^T (= JCM 13812^T = NRRL B-24464^T).

Description of *Marmoricola aequoreus* sp. nov.

Marmoricola aequoreus (ae.qu.o.re'us. L. masc. adj. *aequoreus* belonging to the sea, referring to the isolation of the type strain from the sea).

Fig. 1. A phylogenetic tree showing the relationship of strain SST-45^T with members of related genera in the family Nocardioidaceae. The tree was constructed using the neighbour-joining method (Saitou & Nei, 1987) and the method of Jukes & Cantor (1969). *Streptomyces griseus* was used as an outgroup. Asterisks indicate branches of the tree that were recovered using both maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods. Bootstrap values of 1000 analyses are shown at the top of the branching points for neighbour-joining analysis (only values greater than 50 % are indicated). Bar, 1 substitution per 100 nt positions.

Table 1. Phenotypic characteristics that differentiate strain SST-45^T from the type strain of *Marmoricola aurantiacus*

Data for strain DSM 12652^T are taken from Urzì *et al.* (2000). Results were recorded as: +, positive; –, negative.

Characteristic	SST-45 ^T	DSM 12652 ^T
Colour of colony	Yellow	Orange
Cell morphology	Coccus, 0.5–0.7 µm	Coccus, 0.5–0.6 µm
Growth at 37 °C	+	–
Growth on 5 % NaCl	+	–
Nitrate reduction	+	–
Hydrolysis of:		
Casein	+	–
Gelatin	+	–
Hypoxanthine	–	+
Acid production from maltose	+	–
DNA G + C content (mol%)	72.4	72.0
Origin of isolation	Beach sediment	Marble

Cells are aerobic, Gram-positive, non-spore-forming and non-motile cocci (0.5–0.7 µm in diameter) that occur singly, in pairs, as clusters or as short chains. Colonies are circular, smooth, convex and bright yellow in colour. Chemoheterotrophic. Growth is good at 10–37 °C, but poor at 4 °C, and does not occur at 42 °C. Growth occurs at an initial pH of 5.1–12.1, with an optimum at pH 7.1. Good growth is observed at 0–5 % (w/v) NaCl, but poor at 6–7 % (w/v) NaCl. Nitrate is reduced to nitrite. Gelatin is liquefied. Aesculin and casein are hydrolysed. H₂S production is observed. Hypoxanthine, DL-tyrosine or xanthine are not degraded. Urease activity is not detected. Acid is produced from dextran, maltose and salicin. No acid is produced from L-arabinose, D-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, inulin, D-lactose, D-mannose, D-melezitose, methyl- α -D-glucoside, methyl- α -D-mannoside, D-raffinose, L-rhamnose, L-ribose, L-sorbose, sucrose, D-trehalose, D-xylose, adonitol, 2,3-butanediol, D-dulcitol, *meso*-erythritol, glycerol, *meso*-inositol, D-mannitol, 1,2-propanediol, D-sorbitol or D-xylitol. In the API ZYM system, alkaline phosphatase, leucine arylamidase, valine arylamidase and α -glucosidase are positive. The following tests are weakly positive: esterase lipase (C8), cystine arylamidase, β -galactosidase and β -glucosidase. Esterase (C4), lipase (C14), trypsin, α -chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -galactosidase, β -glucuronidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase are negative. The following compounds are utilized as sole carbon and energy sources: D-cellobiose, dextran, D-fructose, D-galactose, D-glucose, inulin, D-lactose, maltose, D-mannose, D-melezitose, methyl- α -D-glucoside, L-ribose, sucrose, D-trehalose, D-xylose, glycerol, D-mannitol, 1,2-propanediol and D-sorbitol. Assimilation of acetate, citrate, malate and succinate is detected. The following carbon sources are not utilized: L-arabinose, D-arabinose, methyl- α -D-mannoside, D-raffinose, L-rhamnose, salicin, L-sorbose,

Fig. 2. Electron microscopy of strain SST-45^T grown on YE-SW agar for 72 h at 30 °C. (a) Cocci as aggregates; (b) cocci in a short chain. Bars, 1 µm.

adonitol, 2,3-butanediol, D-dulcitol, *meso*-erythritol, *meso*-inositol and D-xylitol. Tests for benzoate, formate and tartrate assimilation are negative. LL-2,6-Diaminopimelic acid is the diagnostic diamino acid in the cell wall. The predominant menaquinone is MK-8(H₄). The polar lipids contain phosphatidylinositol, diphosphatidylglycerol, phosphatidylglycerol and an unknown phospholipid. The predominant cellular fatty acids are C_{18:1} and C_{16:0}. The G + C content of the DNA is 72.4 mol%.

The type strain is strain SST-45^T (=JCM 13812^T = NRRL B-24464^T), isolated from sandy sediment of Samyang beach in Jeju Island, Republic of Korea.

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References

- Collins, M. D., Dorsch, M. & Stackebrandt, E. (1989). Transfer of *Pimelobacter tumescens* to *Terrabacter* gen. nov. as *Terrabacter tumescens* comb. nov. and of *Pimelobacter jensenii* to *Nocardioidea* as *Nocardioidea jensenii* comb. nov. *Int J Syst Bacteriol* **39**, 1–6.
- Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.
- Fitch, W. M. (1971). Towards defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.
- Gordon, R. E., Barnett, D. A., Handershan, J. E. & Pang, C. H.-N. (1974). *Nocardia coeliaca*, *Nocardia autotrophica*, and the nocardin strain. *Int J Syst Bacteriol* **24**, 54–63.
- Hopwood, D. A., Bibb, M. J., Chater, K. F., Kieser, T., Bruton, C. J., Kieser, H. M., Lydiate, D. J., Smith, C. P., Ward, J. M. & Schrempf, H. (1985). *Genetic Manipulation of Streptomyces: a Laboratory Manual*. Norwich: John Innes Foundation.
- 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. (2006). *Kineococcus marinus* sp. nov., isolated from marine sediment of the coast of Jeju, Korea. *Int J Syst Evol Microbiol* **56**, 1279–1283.
- Lee, S. D., Kang, S.-O. & Hah, Y. C. (2000). *Hongia* gen. nov., a new genus of the order Actinomycetales. *Int J Syst Evol Microbiol* **50**, 191–199.
- MacFaddin, J. F. (1980). *Biochemical Tests for Identification of Medical Bacteria*, 2nd edn. Baltimore: Williams & Wilkins.
- 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.
- Miller, E. S., Woese, C. R. & Brenner, S. (1991). Description of the erythromycin-producing bacterium *Arthrobacter* sp. strain NRRL B-3381 as *Aeromicrobium erythreum* gen. nov., sp. nov. *Int J Syst Bacteriol* **41**, 363–368.
- Minnikin, D. E., Patel, P. V., Alshamaony, L. & Goodfellow, M. (1977). Polar lipid composition in the classification of *Nocardia* and related bacteria. *Int J Syst Bacteriol* **27**, 104–117.
- Minnikin, D. E., Hutchinson, I. G., Caldicott, A. B. & Goodfellow, M. (1980). Thin layer chromatography of methanolysates of mycolic acid-containing bacteria. *J Chromatogr* **188**, 221–233.
- Nesterenko, O., Kvasnikov, E. I. & Nogina, T. M. (1985). *Nocardioidea* fam. nov., a new family of the order Actinomycetales Buchanan 1917. *Mikrobiol Zh* **47**, 3–12.
- Park, Y. H., Yoon, J. H., Shin, Y. K., Suzuki, K., Kudo, T., Seino, A., Kim, H. J., Lee, J. S. & Lee, S. T. (1999). Classification of ‘*Nocardioidea fulvus*’ IFO 14399 and *Nocardioidea* sp. ATCC 39419 in *Kribbella* gen. nov., as *Kribbella flavida* sp. nov. and *Kribbella sandramycinii* sp. nov. *Int J Syst Evol Microbiol* **49**, 743–752.
- Prauser, H. (1976). *Nocardioidea*, a new genus of the order Actinomycetales. *Int J Syst Bacteriol* **26**, 58–65.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Schumann, P., Prauser, H., Rainey, F. A., Stackebrandt, E. & Hirsch, P. (1997). *Friedmanniella antarctica* gen. nov., sp. nov., an LL-diaminopimelic acid-containing actinomycete from Antarctic sandstone. *Int J Syst Bacteriol* **47**, 278–283.
- Shirling, E. B. & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* **16**, 313–340.
- Sohn, K., Hong, S. G., Bae, K. S. & Chun, J. (2003). Transfer of *Hongia koreensis* Lee et al. 2000 to the genus *Kribbella* Park et al. 1999 as *Kribbella koreensis* comb. nov. *Int J Syst Evol Microbiol* **53**, 1005–1007.
- Stackebrandt, E., Rainey, F. A. & Ward-Rainey, N. L. (1997). Proposal for a new hierarchic classification system, *Actinobacteria* classis nov. *Int J Syst Bacteriol* **47**, 479–491.
- Staneck, J. L. & Roberts, G. D. (1974). Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* **28**, 226–231.
- Suzuki, K. & Komagata, K. (1983). *Pimelobacter* gen. nov., a new genus of coryneform bacteria with LL-diaminopimelic acid in the cell wall. *J Gen Appl Microbiol* **29**, 59–71.
- Tamura, T. & Yokota, A. (1994). Transfer of *Nocardioidea fastidiosa* Collins and Stackebrandt 1989 to the genus *Aeromicrobium* as *Aeromicrobium fastidiosum* comb. nov. *Int J Syst Bacteriol* **44**, 608–611.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **24**, 4876–4882.
- Trujillo, M. E., Kroppenstedt, R. M., Schumann, P. & Martínez-Molina, E. (2006). *Kribbella lupini* sp. nov., isolated from the roots of *Lupinus angustifolius*. *Int J Syst Evol Microbiol* **56**, 407–411.
- Urzi, C., Salamone, P., Schumann, P. & Stackebrandt, E. (2000). *Marmoricola aurantiacus* gen. nov., sp. nov., a coccoid member of the family *Nocardioidea* isolated from a marble statue. *Int J Syst Evol Microbiol* **50**, 529–536.