Streptomyces aomiensis sp. nov., isolated from a soil sample using the membrane-filter method

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A Gram-positive actinobacterium, designated M24DS4T, was isolated from a soil sample collected from Aomi, Tokyo, Japan, using the membrane-filter method. Strain M24DS4T exhibited low 16S rRNA gene sequence similarity (96.1 %) with Streptomyces scabrisporus NBRC 100760T. Cell hydrolysates contained the L-isomer of diaminopimelic acid and the predominant quinones were MK-9(H4) and MK-9(H6). The genomic DNA G+C content was 75 mol%.

Comparison of the characteristics of strain M24DS4T and related members of the genus Streptomyces with validly published names showed that the strain represents a novel species of the genus, for which the name Streptomyces aomiensis sp. nov. is proposed. The type strain is M24DS4T (=NBRC 106164T =KACC 14925T).

Actinomycetes are known to produce pharmaceutically important compounds and have been extensively studied (Bull et al., 2000; McVeigh et al., 1994; Newman & Hill, 2006). Soil has served as the primary isolation source of these actinomycetes. For the isolation of novel strains from a soil sample, the membrane-filter method described by Hirsch & Christensen (1983) is used. This method employs a membrane filter (0.22–0.45 μm pore size) to selectively eliminate non-actinomycete bacteria on the basis of their inability to form mycelia. Briefly, the agar plate for isolation is overlaid with a cellulose ester membrane filter and the filter surface is inoculated with a soil suspension. During incubation, the branched mycelia of actinomycetes penetrate the filter through the pores and grow on the underlying medium; bacteria that cannot penetrate the filter fail to grow. The membrane is removed after a few days of incubation and the plate is reincubated to allow the development of actinomycete colonies.

During our studies on the isolation of actinobacteria using the membrane-filter method from a soil sample, strain M24DS4T was isolated from a soil sample collected from Aomi, Tokyo, Japan. A soil sample (1 g) was suspended in PBS (10 ml) and the solution was serially diluted in sterile water. Aliquots (100 μl) of appropriate dilutions were plated on the surface of 0.3 μm-pore cellulose ester membrane filters (Advantec) on humic acid-vitamin agar (Hayakawa & Nonomura, 1987) without antibiotics. The membrane filters were removed after 4 days of incubation at 28 °C and the plates were reincubated at 28 °C for 2–3 weeks. Actinomycete colonies appearing on the plates were transferred to International Streptomyces Project (ISP) 2 agar (Shirling & Gottlieb, 1966) for further purification. For long-term preservation, the isolate was stored at −80 °C in 15% glycerol (v/v).

For analysis of the 16S rRNA gene sequence, genomic DNA was prepared using the Prepman Ultra system (Applied Biosystems). The 16S rRNA gene was amplified using a universal primer set (9f and 1492r; Brosius et al., 1978) and sequenced directly using a BigDye Terminator v3.1 Cycle Sequencing kit and an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). In BLAST searches (Altschul et al., 1990) comparing the almost-complete 16S rRNA gene sequence (1486 bp) of strain M24DS4T against sequences available in the EzTaxon server (Chun et al., 2007), strain M24DS4T showed highest 16S rRNA gene sequence similarity (96.13 %, 59 bp difference) with Streptomyces scabrisporus NBRC 100760T. 16S rRNA gene sequences related to strain M24DS4T were downloaded from DDBJ and a multiple alignment (1430 bp) was created using CLUSTAL X (Thompson et al., 1997). A phylogenetic tree was
reconstructed using the neighbour-joining algorithm (Saitou & Nei, 1987) and the robustness of the tree topology was evaluated by bootstrap analysis (Felsenstein, 1985) using 1000 resamplings of the sequences. Strain M24DS4T clustered with S. scabrisporus NBRC 100760T (Fig. 1) as a monophyletic clade supported by a high bootstrap value of 98%. This cluster was also obtained with maximum-parsimony analysis (Swofford, 2000).

Cultural and physiological characteristics of strain M24DS4T and S. scabrisporus NBRC 100760T were determined as described by Shirling & Gottlieb (1966). Growth at 4, 10, 15, 20, 25, 30, and 35°C was assessed on ISP 2 agar for 6 days. Growth at pH 5 and 6 (adjusted with HCl) and pH 8, 9, and 10 (adjusted with NaOH) was examined on ISP 2 agar at 28°C for 9 days. The growth characteristics on ISP 1 and ISP 3–9 were also determined (Shirling & Gottlieb, 1966). The API ZYM and API Coryne systems (bioMérieux) were used, according to the manufacturer’s instructions, for biochemical characterization. Strain M24DS4T could be differentiated phenotypically from S. scabrisporus by a number of characteristics (Table 1), namely spore morphology, aerial mycelium on ISP 2, colony base colour, temperature range for growth, utilization of carbon sources, activities of alkaline phosphatase, α-chymotrypsin and urease, and hydrolysis of gelatin.

For strain M24DS4T, the hyphal arrangement, spore chain morphology and spore surface ornamentation were observed by light microscopy (CX41LF; Olympus) and scanning electron microscopy (JSM-6060; JEOL) after growth on ISP 2 agar at 28°C for 15–20 days. A micrograph showing the spore chain morphology of strain M24DS4T is given in Supplementary Fig. S1 (available in IJSEM Online). Genomic DNA was extracted from cells grown to late exponential growth phase using the protocol of Minamisawa (1990) and the G+C content of the genomic DNA was determined using the method described by Mesbah et al. (1989). Menaquinones, cellular fatty acids and the diaminopimelic acid isomer in whole-cell hydrolysates were determined as described by Tamura et al. (1994). The culture used to determine the fatty acid content was prepared on ISP 2 agar at 28°C for 10 days. The fatty acid content of strain M24DS4T is given in Supplementary Table S1. Other characteristics are given in the species description.

On the basis of significant differences shown in the 16S rRNA gene sequence analysis and the phenotypic differences between strain M24DS4T and its closest phylogenetic neighbour (Stackebrandt & Ebers, 2006), we propose that strain M24DS4T should be classified in a novel species of the genus Streptomyces, for which the name Streptomyces aomiensis sp. nov. is proposed.

**Description of Streptomyces aomiensis sp. nov.**

Streptomyces aomiensis (ao.mi.en’sis. N.L. masc. adj. aomiensis pertaining to Aomi, the place from where the strain was isolated).

Aerobic and Gram-positive actinomycete. Spore chains are rectifiables and the spore surface is smooth. Spores and aerial mycelium are grey. The diaminopimelic acid isomer in the cell hydrolysate is the ll-type. Growth occurs at 15–37°C (optimum 25–28°C). Optimum pH is pH 6.0–8.0 (weak growth at pH 9.0 and 10.0). Growth is good on yeast extract-malt extract agar (ISP 2) and peptone-yeast extract iron agar (ISP 6), but is average-to-poor on tryptone yeast extract agar (ISP 1), oatmeal agar (ISP 3), inorganic salts-starch agar (ISP 4), glycerol-asparagine agar (ISP 5) and tyrosine agar (ISP 7). Melanin pigments are not produced on ISP 6 or ISP 7. With
API ZYM and API Coryne, positive for acid phosphatase, catalase, β-glucosidase, leucine arylamidase, N-acetyl-β-glucosaminidase, naphthol-AS-BI-phosphohydrolase, pyrazinamidase and nitrate reduction and weakly positive for cystine arylamidase, pyrrolidonyl arylamidase and valine arylamidase, but negative for alkaline phosphatase, esterase, esterase lipase, α-chymotrypsin, α-fucosidase, and β-galactosidase, β-glucuronidase, α-glucosidase, α-mannosidase, lipase, trypsin, urease and gelatin hydrolysis. As sole carbon sources, L-rhamnose, D-glucose, lactose and D-xylose are utilized and L-arabinose, D-fructose, sorbitol and sucrose are weakly utilized, but myo-inositol, D-mannitol and raffinose are not utilized. The predominant fatty acids are iso-C₁₆:₀, anteiso-C₁₅:₀, iso-C₁₅:₀ and C₁₆:₀. The major respiratory quinones are MK-9(H₈) and MK-9(H₆); a small amount of MK-9(H₄) is also present. The DNA G+C content of the type strain is 75 mol%.

The type strain, M24DS₄ᵀ (=NBRC 106164ᵀ = KACC 14925ᵀ), was isolated from a soil sample using the membrane-filter method.

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