**Micromonospora maritima** sp. nov., isolated from mangrove soil

Apakorn Songsumanus,1 Somboon Tanasupawat,1 Yasuhiro Igarashi2 and Takuji Kudo3

1Department of Biochemistry and Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand
2Biotechnology Research Center, Toyama Prefectural University, 5180 Kurokawa, Kosugi, Toyama 939 0398, Japan
3Japan Collection of Microorganisms, RIKEN BioResource Center, Wako, Saitama 351-0198, Japan

Strain D10-9-5^T was isolated from mangrove soil in Samut Sakhon province, Thailand. A polyphasic approach was used to determine the taxonomic position of the strain. The strain presented single rough spores on substrate mycelium and no aerial mycelium. Chemotaxonomic data supported the assignment of strain D10-9-5^T to the genus *Micromonospora* based on the presence of meso-diaminopimelic acid and glycolyl muramic acid in the peptidoglycan, ribose, mannose, galactose, xylose and glucose as whole-cell sugars, MK-10(H4) (14.8 %), MK-10(H6) (46.7 %) and MK-10(H8) (27.5 %) as the predominant isoprenoid quinones, iso-C15 : 0 (17.9 %), anteiso-C17 : 0 (14.6 %), iso-C17 : 0 (9.6 %), C17 : 0 (8.0 %), iso-C16 : 0 (7.7 %) and C17 : 1\(^\omega 8c\) (7.0 %) as the major cellular fatty acids, and diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides and phosphatidylethanolamine as the predominant phospholipids in the cell wall. The 16S rRNA gene sequence and phylogenetic analysis showed that strain D10-9-5 was closely related to *Micromonospora marina* JCM 12870^T (99.6 %), *Micromonospora coxensis* JCM 13248^T (99.4 %), *Micromonospora aurantiaca* JCM 10878^T (99.3 %), *Micromonospora humi* JCM15292^T (99.3 %), *Micromonospora halophytica* JCM 3125^T (99.1%) and *Micromonospora chalcea* JCM 3031^T (99.1 %). Strain D10-9-5^T could be clearly distinguished from related members of the genus *Micromonospora* by its physiological and biochemical characteristics as well as its phylogenetic position and level of DNA–DNA relatedness. Therefore, the strain represents a novel species for which the name *Micromonospora maritima* sp. nov. is proposed; the type strain is D10-9-5^T (=JCM 17013^T=NBRC 108767^T=PCU 322^T=TISTR 2000^T).

The genus *Micromonospora* belonging to the family *Micromonosporaceae* was first described by Ørskov (1923) for actinomycete strains on the basis of morphological properties, i.e. the absence of true aerial mycelium and production of spores borne singly on the substrate mycelium. Species of the genus *Micromonospora* were classified on the basis of chemotaxonomic characteristics, gyrB and 16S rRNA gene sequence analyses including DNA–DNA relatedness (Kroppenstedt, 1985; Kawamoto, 1989; Koch et al., 1996; Kasai et al., 2000). Members of the genus have been isolated in increasing numbers and the genus currently encompasses 47 species with validly published names (Euzéby, 2012). Strains of the genus *Micromonospora* have distinct morphological characteristics; they produce single spores on the substrate mycelium and lack aerial mycelium. There are some species of the genus *Micromonospora* that have been isolated from mangrove soil, e.g. *Micromonospora rifamycinica* (Huang et al., 2008), *Micromonospora rhizosphaerae* (Wang et al., 2011) and *Micromonospora patallongensis* (Thawai et al., 2008).

Many antibiotics from species of the genus *Micromonospora* have been reported and they have been found continuously, e.g. a new actinomycin complex (Wagman et al., 1976), sibanomicin (Itoh et al., 1988), mycinamicins I and II (Kinoshiba et al., 1992), arisostatins A and B (Igarashi et al., 2000) and kosinostatin (Furumai et al., 2002). In this study, the compounds SEK34 and SEK34b were isolated from strain D10-9-5^T, purified and described.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain D10-9-5^T is HQ704071.

Two supplementary figures and four supplementary tables are available with the online version of this paper.
Strain D10-9-5\textsuperscript{T} was isolated from mangrove soil from Samut Sakhon Province in Thailand by using starch casein nitrate agar (Tanasupawat et al., 2010) supplemented with nystatin (50 mg l\textsuperscript{-1}) and nalidixic acid (20 mg l\textsuperscript{-1}) and incubated at 30 \textdegree C for 14 days. The single colony was transferred onto yeast extract–malt extract agar [International Streptomyces Project (ISP) medium number 2] as described by Shirling & Gottlieb (1966). The pure isolate was maintained on ISP 2 slants at 4–10 \textdegree C. Strain D10-9-5\textsuperscript{T} was incubated for 14 days on ISP 2 and observed using light and scanning electron microscopes (Itoh et al., 1989). The Hucker–Conn method was used for Gram staining (Hucker & Conn, 1923). Phenotypic properties were examined by using standard methods (Arai, 1975; Williams & Cross, 1971; Gordon et al., 1974). For determining the cultural characteristics, the strain was grown at 30 \textdegree C for 14 days on various agar media (Shirling & Gottlieb, 1966; Asano & Kawamoto, 1986) after which the colony colours were determined using the National Bureau of Standards/Inter Society Color Council (NBS/IBCC) Colour System. The growth of strain D10-9-5\textsuperscript{T} was tested at various temperatures on ISP 2 for 14 days as well as at various pH and NaCl concentrations on ISP 2 at 30 \textdegree C. The carbon utilization of the strain was determined by using carbon utilization medium (ISP 9) supplemented with 1 % sole carbon source (Shirling & Gottlieb, 1966). Melanin and H\textsubscript{2}S production were examined on tyrosine agar (ISP 7) and peptone iron agar (ISP 6), respectively (Shirling & Gottlieb, 1966).

For chemotaxonomy, freeze-dried cells were collected from 4-day-old cultures grown in ISP 2 broth on a rotary shaker at 30 \textdegree C. The cell wall peptidoglycan was prepared by the method of Kawamoto et al. (1981). The isomer of diaminopimelic acid was determined by the TLC method of Staneck & Roberts (1974). The N-acyl group of the muramic acid in the peptidoglycan was analysed by spectrophotometry using the method of Uchida & Aida (1984). Isopenoid quinones were extracted by the method of Collins et al. (1977) and then analysed by HPLC equipped with a Cosmosil 5C18 column (4.6 × 150 mm; Nacalai Tesque). Whole-cell sugars were analysed according to Mikami & Ishida (1983). Methyl esters of cellular fatty acids were prepared from cells grown on ISP 2 and identified by GC according to the instructions of the Microbial Identification System (MIDI) Sherlock version 6.0 (Sasser, 1990; Kämpfer & Kropfenstedt, 1996) with the RXTSA6 MIDI database. Phospholipids were extracted and analysed by the two-dimensional TLC method of Minnikin et al. (1984).

Chromosomal DNA was extracted from cells grown in ISP 2 broth supplemented with 0.1 % (w/v) glycine (Tamaoka, 1994; Yamada & Komagata, 1970). The G+C content of the DNA was determined by HPLC (Tamaoka & Komagata, 1984). DNA–DNA relatedness was determined according to Ezaki et al. (1989). The 16S rRNA gene was amplified and sequenced by the procedures described previously (Nakajima et al., 1999). The 16S rRNA gene sequence was multialigned with selected sequences obtained from the GenBank/EMBL/DDBJ databases by the CLUSTAL\textsubscript{X} version 1.83 program (Thompson et al., 1997). The aligned sequences were manually edited before constructing the phylogenetic tree by the neighbour-joining (Saitou & Nei, 1987) and the maximum-parsimony (Kluge & Farris, 1969) methods in the MEGA4 software (Tamura et al., 2007). The branch confidence values of the phylogenetic tree were examined by bootstrap analysis (Felsenstein, 1985) based on 1000 resamplings. Gaps and ambiguous nucleotides were deleted manually before analysing the similarity values by CLUSTAL\textsubscript{X} (Thompson et al., 1997).

For fermentation and extraction, pure colonies were transferred to the Bn-2 agar slant consisting of (w/v) 0.5 % glucose, 0.5 % soluble starch, 0.1 % meat extract, 0.1 % yeast extract, 0.2 % NZ-case, 0.2 % NaCl, 0.1 % CaCO\textsubscript{3} and 1.5 % agar (pH 7.0). A loopful of a mature slant culture was inoculated into 100 ml of the seed medium (V-22) consisting of soluble starch 1 %, glucose 0.5 %, NZ-case 0.3 %, yeast extract 0.2 %, tryptone 0.5 %, K\textsubscript{2}HPO\textsubscript{4} 0.1 %, MgSO\textsubscript{4} .7H\textsubscript{2}O 0.05 % and CaCO\textsubscript{3} 0.3 % (pH 7.0) and incubated at 30 \textdegree C for 3–4 days on a rotary shaker (200 r.p.m.). Aliquots (3 ml) of the seed was transferred into 100 ml production medium consisting of 0.2 % glucose, 2.5 % soluble starch, 0.5 % yeast extract, 0.5 % polypeptide, 0.5 % NZ-amine, 0.3 % CaCO\textsubscript{3}, 1.0 % Dianon HP-20 (Mitsubishi Chemical); pH was adjusted to 7.0 and cultures were incubated for 5–6 days at 30 \textdegree C on a rotary shaker (200 r.p.m.). The culture broth was extracted with 100 ml butanol and the extract was purified by silica gel and octadecylsilane (ODS) column chromatographies and HPLC separation. The isolated secondary metabolites were identified by NMR and MS analysis.

Strain D10-9-5\textsuperscript{T} had morphological, cultural and chemotaxonomic properties consistent with its classification in the genus Micromonospora. The strain formed well-developed and branched substrate hyphae. No aerial mycelium was produced. Spores at maturity were spherical to oval, appeared to be rough and were non-motile (Fig. 1). The phenotypic characteristics are presented in the species description and in Table 1 and Table S1 (available in IJSEM Online). The strain contained meso-diaminopimelic acid in the cell wall. The N-acyl type of muramic acid in the peptidoglycan was determined to be the glycolyl type. Ribose, mannose, galactose, xylose, glucose and trace amount of arabinose were detected as the whole-cell sugars [pattern D of Lechevalier & Lechevalier (1970)]. The polar lipid profile was characterized by the presence of diphostatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides, phosphatidylethanolamine, an unknown phosphoglycolipid and unknown phospholipids [type PII pattern of Lechevalier et al. (1977)] as shown in Fig. S1. Significant fatty acids of strain D10-9-5\textsuperscript{T} were iso-C\textsubscript{15 : 0} (17.9 %), anteiso-C\textsubscript{17 : 0} (14.6 %), iso-C\textsubscript{17 : 0} (9.6 %), C\textsubscript{17 : 0} (8.0 %), iso-C\textsubscript{16 : 0} (7.7 %) and C\textsubscript{17 : i0}8c (7.0 %) (Table S2). This pattern corresponds to fatty acid type 3b as proposed by
Kroppenstedt (1985). The predominant menaquinones were MK-10(H₄) (14.8 %), MK-10(H₆) (46.7 %) and MK-10(H₈) (27.5 %). The DNA G+C content was 73.6 mol%.

The analysis of an almost complete 16S rRNA gene sequence (1495 nt) of strain D10-9-5T indicated that it was placed in a monophyletic clade with the closely related species, *Micromonospora marina* JCM 12870T (99.6 %), *Micromonospora cocoensis* JCM 13248T (99.4 %), *Micromonospora aurantiaca* JCM 10878T (99.3 %), *Micromonospora humi* JCM 3136T (99.3 %), *Micromonospora halophytica* JCM 3125T (99.1 %) and *Micromonospora chalcea* JCM 3031T (99.1 %), based on the results from both the neighbour-joining and maximum-parsimony methods (Fig. 2). Comparison with the descriptions of these previously characterized species of *Micromonospora* showed that our isolate could be distinguished from them by a combination of biochemical and physiological properties, in particular, the decomposition of biochemical and physiological properties, in particular, the decomposition of tyrosine, growth at pH 5, tolerance to 5 % (w/v) NaCl and utilization of L-arabinose, cellobiose, D-fructose, D-galactose, glycerol, D-mannitol, inositol, lactose, melibiose, raffinose, L-rhamnose and salicin (Table 1).

The level of DNA–DNA relatedness between strain D10-9-5T and closely related species was less than 55.8 % (Table S3). These values were obtained from the means of four determinations and are below the threshold value of 70 % for distinguishing genomic species (Wayne et al., 1987). It is evident from the genotypic and phenotypic data that strain D10-9-5T occupies a new taxonomic position in the genus *Micromonospora*.

Strain D10-9-5T was found to produce two aromatic polyketides SEK34 and SEK34b (Fig. S2) on the basis of spectroscopic analysis including NMR and MS (Table S4). These compounds were previously isolated as an ‘unnatural’ natural product from a transformant of *Streptomyces coelicolor* harbouring recombinant biosynthetic genes for actinorhodin. (McDaniel et al., 1994). This is the first report on the isolation of SEK34 and SEK34b as a natural product, suggesting that the new actinomycete species are the important source of unknown small molecules.

Based on the genotypic and phenotypic data, it is proposed that strain D10-9-5T represents a novel species, *Micromonospora maritima* sp. nov.

### Description of *Micromonospora maritima* sp. nov.

*Micromonospora maritima* sp. nov. (ma.ri.ta.ma. L. fem. adj. maritima of or belonging to the sea, maritime, referring to coastal soil from which the type strain was isolated).

Aerobic, Gram-positive, mesophilic actinomycete that forms a well-developed and extensively branched substrate mycelium. No aerial mycelium is produced. The colour of the vegetative mycelium on ISP 2 is dark greyish brown. Spores are spherical to oval, appear to be rough and are non-motile. Starch hydrolysis, gelatin liquefaction and milk peptonization are positive. Nitrate reduction and L-tyrosine decomposition are negative. Utilizes L-arabinose, inositol, melibiose and D-xylene but not D-mannitol, L-rhamnose, raffinose, lactose, D-ribose, glycerol, D-galactose or cellobiose as sole carbon sources for energy. Grows at 20–40 °C, at pH 6–8 and in a maximum of 5 % (w/v) NaCl. Grows optimally at 30 °C, at pH 7.3–8.0 and in the presence less than 5 % (w/v) NaCl. The cell wall peptidoglycan contains meso-diaminopimelic acid. The acyl type of the peptidoglycan is the glycolyl type. The predominant menaquinones are MK-10(H₄), MK-10(H₆) and MK-10(H₈). Major cellular fatty acids are iso-C₁₅:0, anteiso-C₁₇:0, iso-C₁₇:0, C₁₇:0 and C₁₆:0 and C₁₇:0ω8c.

The type strain is D10-9-5T (= JCM 17013T =NBRC 108767T =PCU 322T=TISTR 2000T) isolated from mangrove soil
Fig. 2. Neighbour-joining tree based on almost-complete 16S rRNA gene sequences showing relationships among strain D10-9-5\(^T\), *Micromonospora* species with validly published names and representatives of the family *Micromonosporaceae*. *Streptomyces ambofaciens* ATCC 23877\(^T\) was used as an outgroup. Asterisks indicate branches of the tree that were also found using the maximum-parsimony method. The numbers on the branches indicate the percentage bootstrap values for 1000 replicates; only values \(\geq 50\%\) are indicated. Bar, 0.01 substitutions per nucleotide position.
collected in Samut Sakhon Province, Thailand. The DNA G+C content of the type strain is 73.6 mol%.

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References


