Marinactinospora thermotolerans gen. nov., sp. nov., a marine actinomycete isolated from a sediment in the northern South China Sea

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A novel marine actinomycete, designated SCSIO 00652T, was isolated from a marine sediment collected from the northern South China Sea at a depth of 3865 m. The strain formed branched substrate mycelia and no fragmentation was found. Abundant aerial mycelia differentiated into long spore chains and the spores had a wrinkled surface. Growth occurred on ISP medium 2 with 0–5 % (w/v) NaCl and at 10–55 °C. The whole-cell hydrolysate contained meso-diaminopimelic acid and glucose as the whole-cell sugar. BLAST search results based on an almost-complete 16S rRNA gene sequence showed the novel strain had the highest similarity (96.5 %) with Nocardiopsis trehalosi VKM Ac-942T. The phylogenetic tree of the family Nocardiopsaceae indicated that strain SCSIO 00652T formed a distinct lineage at the deepest branch with a high bootstrap value. Additionally, the profiles of menaquinones, phospholipids and fatty acids showed there were marked differences between strain SCSIO 00652T and the recognized genera of the family Nocardiopsaceae. Based on the polyphasic data, a new genus, Marinactinospora gen. nov., is proposed within the family Nocardiopsaceae with the type species Marinactinospora thermotolerans sp. nov. The type strain of the type species is SCSIO 00652T (=DSM 45154T =CCTCC AA 208041T).

The family Nocardiopsaceae was created, with Nocardiopsis as the type genus, by Rainey and co-workers in 1996 based on polyphasic data (Rainey et al., 1996). At present it contains four genera, namely, Nocardiopsis (Meyer, 1976), Thermobifida (Zhang et al., 1998), Streptomonospora (Cui et al., 2001) and Haloactinospora (Tang et al., 2008). Members of this family can form long or short spore chains or sporangia with abundant spores, which increase the survival rate of these organisms in many extreme environments. Members of the genus Thermobifida and some species of the genus Nocardiopsis are thermophilic actinomycetes, which can grow at high temperatures (40–65 °C) (Kroppenstedt & Evtushenko, 2006). Two genera, Streptomonospora and Haloactinospora, are strictly halo-philic micro-organisms. Many Nocardiopsis strains have been isolated from hypersaline environments (Tang et al., 2008), one Nocardiopsis strain has been isolated from an Antarctic glacier and a few species have been found in marine sediment (Dixit & Pant, 2000; Evtushenko et al., 2000; Kroppenstedt & Evtushenko, 2006). Extreme environments are the general habitat of members of the family Nocardiopsaceae.

In this study, an abundant filamentous actinomycete strain was isolated on raffinose–histidine agar (Vickers et al., 1984) from a deep-sea sediment sample collected from site E410 (17° 58.742′ N 116° 00.228′ E; black soft mud at 3865 m depth) in the northern South China Sea. Based on phylogenetic analysis, strain SCSIO 00652T could be readily distinguished from the previously described genera of the family Nocardiopsaceae and should be recognized as representing a new genus and novel species.

The samples were collected aseptically with a grab-bucket collection sampler in September 2006 from the northern...
South China Sea. After collection, the surface layer of sediment, from 0–10 cm depth, was obtained as a subsample. Wet samples were first air-dried aseptically by being placed into a laminar flow hood and then a 2 g air-dried sample was suspended in 18 ml sterile seawater before 0.1 ml was spread on isolation media plates. After being incubated at 28 °C for 3 weeks, colonies were selected, purified and maintained on ISP 2 agar medium modified with seawater instead of distilled water. The purified strains were suspended in 20 % (w/v) glycerol at −20 °C.

Strain SCSIO 00652T grew well on ISP 2, ISP 3, ISP 4 and ISP 5 agars (Shirling & Gottlieb, 1966), Czapek solution agar (Waksman, 1961), nutrient agar (Difco) and potato agar (Waksman, 1961). During incubation at 28 °C for 7 days, white aerial mycelia developed well on agar plates of the above media (except ISP 2) and yellow–white substrate mycelia grew well on all of the media. After 14 days incubation, yellow–white aerial mycelia were observed on all media. By comparing the cultures with the most suitable colour chips from the ISCC-NBS colour charts (Kelly, 1964), it was evident that the novel strain did not produce diffusible pigments on any of the media. Micromorphology was observed by light microscopy (BH 2; Olympus) and electron microscopy (JEM-1010; JEOL) using cells incubated for 7, 14, 21 and 28 days. After 14 days, the white aerial mycelia were observed to be differentiated into long spore chains and the spores had a wrinkled surface (Fig. 1).

Physiological characteristics, including temperature and pH ranges for growth and tolerance to sodium chloride, were tested using ISP 2 as the basal medium. Carbon-source utilization for growth was carried out as described by Shirling & Gottlieb (1966). Tests for hydrolysis of cellulose, gelatin, starch and Tweens 20, 40, 60 and 80, nitrate reduction, utilization of urea, milk coagulation and peptonization and production of H2S and melanin were performed as described by Gonzalez et al. (1978). Antibiotic susceptibility was examined as described by Groth et al. (2004) using antibiotic discs on modified ISP 2 agar. The detailed physiological properties of the strain are given in the species description.

Biomass for chemotaxonomic studies was obtained by cultivation using modified ISP 2 broth at 28 °C for 1 week and centrifugation at 150 r.p.m. Analysis of whole-cell sugars was performed according to the procedure described by Staneck & Roberts (1974). Amino acids and peptides in the cell-wall hydrolysate were analysed by the methods described by Hasegawa et al. (1983). Menaquinones were isolated using the methods of Minnikin et al. (1984) and separated by HPLC (Kroppenstedt et al., 1981; Kroppenstedt, 1982). Phospholipids were extracted and examined by using published procedures (Collins & Jones, 1980; Minnikin et al., 1979). Fatty acid analysis was performed by using standard methods (Sasser, 1990) and the results were compared with the database of fatty acids in the Microbial Identification System.

The whole-cell hydrolysate of the novel isolate contained meso-diaminopimelic acid as the diagnostic diamino acid and glucose. The menaquinones consisted of MK-11(H8) (43.8 %), MK-11(H10) (18.7 %), MK-10(H8) (16.5 %), MK-12(H8) (8.8 %), MK-10(H10) (7.9 %) and MK-11(H8) (4.4 %). Phospholipids comprised diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, phosphatidylinositol mannosides, phosphatidylinositol and unknown phosphoglycolipids. The G+C content of the genomic DNA was 72 mol%, as determined by using the HPLC method (Mesbah et al., 1989). The fatty acid profile mainly contained 10-methyl-C18:0 (24.5 %), i-C16:0 (24.5 %), i-C16:1G (10.9 %) and ai-C17:0 (9.5 %).

Genomic DNA extraction and PCR amplification of the 16S rRNA gene were conducted as described by Li et al. (2007). Multiple alignments with sequences of closely related taxa were carried out using CLUSTAL_X (Thompson et al., 1997). A phylogenetic tree was reconstructed using the neighbour-joining method of Saitou & Nei (1987) from K_nuc values (Kimura, 1980, 1983) and MEGA version 4.0 (Tamura et al., 2007). The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.
BLAST results for the 16S rRNA gene sequence of strain SCSIO 00652\(^T\) showed that its closest relatives were members of the genus *Nocardiopsis* in the family *Nocardiopsaceae*, with the highest gene sequence similarity of 96.5\% with *Nocardiopsis trehalosi* VKM Ac-942\(^T\). The phylogenetic tree (not shown) of the family *Nocardiopsaceae* based on 16S rRNA gene sequences showed that the novel isolate formed a distinct but unstable lineage among members of the family *Nocardiopsaceae*, with a bootstrap value of <30\%. However, in the reconstructed phylogenetic tree (Fig. 2) based on the 16S rRNA gene sequences of type strains of species of the family *Nocardiopsaceae*, strain SCSIO 00652\(^T\) formed a distinct and stable lineage at the deepest branch with a bootstrap value of 100\%. The chemotaxonomic characteristics of the novel strain could be readily distinguished from those of the recognized genera in the family *Nocardiopsaceae* (Table 1). Additionally, analysis of the nucleotide signatures also showed differences in some signature sites between SCSIO 00652\(^T\) and the other genera in the family *Nocardiopsaceae* (see Supplementary Table S1, available in IJSEM Online). Based on these polyphasic data, strain SCSIO 00652\(^T\) represents a novel genus for which the name *Marinactinospora* gen. nov. is proposed. The type species of the genus is *Marinactinospora thermotolerans* sp. nov.

**Description of *Marinactinospora* gen. nov.**

*Marinactinospora* (Ma.rin.ac.ti.no’sp.a.ra. L. adj. marinus of the sea; Gr. n. actis actinos a ray; Gr. n. spora a seed; N.L. fem. n. *Marinactinospora* marine and spored ray, referring to marine spore-forming actinomycete).

Gram-positive-staining, aerobic, moderately thermostolerant, filamentous actinomycetes. Forms are branched but non-fragmented substrate mycelia and yellow–white aerial mycelia that differentiate into long spore chains composed of spores with a wrinkled surface. No diffusible pigments are produced. Whole-cell hydrolysates contain meso-diaminopimelic acid with no diagnostic sugar. The predominant menaquinones are MK-10(H8), MK-11(H8) and MK-11(H10). The phospholipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, phosphatidylinositol mannosides, phosphatidylinositol and unknown phosphoglycolipids. The major fatty acids are 10-methyl-C\(_{18:0}\), i-C\(_{16:0}\), i-C\(_{16:1}\) G and ai-C\(_{17:0}\). The G+C content of the genomic DNA is 72 mol\%. The type species is *Marinactinospora thermotolerans*.

**Description of *Marinactinospora thermotolerans* sp. nov.**

*Marinactinospora thermotolerans* (ther.mo.ta.ler.ans. Gr. n. therme heat; L. pres. part. tolerans tolerating; N.L. part. adj. thermotolerans able to tolerate a high temperature).

Morphological, chemotaxonomic and general characteristics are as given above for the genus. Aerial mycelia are yellow–white on most media after 14 days incubation and no diffusible pigment is produced. Grows at pH 6.0–9.0 and 10–55 °C with 0–5\% (w/v) NaCl, with optimum growth at pH 7.0–8.0 and 28 °C with 0–1\% (w/v) NaCl. Utilizes cellobiose, D-fructose, D-galactose, D-glucose, maltose, D-mannitol, D-mannose, L-rhamnose, D-ribose, D-sorbitol, sucrose and D-xylose as sole carbon sources, but

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**Fig. 2.** Phylogenetic tree, based on 16S rRNA gene sequences of 1440 nt, of strain SCSIO 00652\(^T\) and related strains in the family *Nocardiopsaceae*, reconstructed by using the neighbour-joining method. Numbers at nodes are bootstrap values based on 1000 resamplings (only values >50\% are indicated). Bar, 1\% sequence divergence.
Table 1. Differentiating chemotaxonomic characteristics of strain SCSIO 00652T and recognized genera of the family Nocardiopsaceae

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnostic sugars</td>
<td>None</td>
<td>None</td>
<td>Gal, Rib</td>
<td>Gal, Rib</td>
<td>Gal, Rib</td>
</tr>
<tr>
<td>Predominant menaquinones</td>
<td>MK-11(H8, H16)</td>
<td>MK-10(H8)</td>
<td>MK-10(H8), or MK-9 (H8, H4)</td>
<td>MK-10(H4)</td>
<td></td>
</tr>
<tr>
<td>Diagnostic phospholipids</td>
<td>DPG, PC, PG, PIM, PL</td>
<td>DPG, PME, PC, PL</td>
<td>PC, PME</td>
<td>DPG, PG, PC, PIM</td>
<td>PE, PG, PL</td>
</tr>
<tr>
<td>Major fatty acids (&gt;10%)</td>
<td>i-C16:0, i-C16:1G, 10-methyl-C18:0</td>
<td>i-C16:0, ai-C17:0</td>
<td>i-C16:0, ai-C17:0, 10-methyl-C18:0</td>
<td>i-C16:0, ai-C17:0</td>
<td>i-C15:0, i-C16:0, i-C17:0</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>72</td>
<td>66–72</td>
<td>64–76</td>
<td>68</td>
<td>72–75</td>
</tr>
</tbody>
</table>

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