**Sciscionella marina gen. nov., sp. nov., a marine actinomycete isolated from a sediment in the northern South China Sea**

Xin-Peng Tian,1,2† Xiao-Yang Zhi,2† Yun-Qi Qiu,1 Yu-Qin Zhang,2 Shu-Kun Tang,2 Li-Hua Xu,2 Si Zhang1,3 and Wen-Jun Li1,2

1Guangdong Key Laboratory of Marine Materia Medica, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou, 510301, PR China
2The Key Laboratory for Microbial Resources of the Ministry of Education, PR China, and Laboratory for Conservation and Utilization of Bio-resources, Yunnan Institute of Microbiology, Yunnan University, Kunming, Yunnan, 650091, PR China
3Hainan Key Lab of Tropical Marine Biotechnology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Sanya, 572000, PR China

The taxonomic position of an actinomycete, designated SCSIO 00231T, isolated from a sediment sample collected from the northern South China Sea, was determined by using a polyphasic approach. The organism formed fragmented substrate hyphae and sparse aerial mycelium on modified ISP 2 medium. Phylogenetic analysis based on the 16S rRNA gene sequence showed that strain SCSIO 00231T fell into the family *Pseudonocardiaceae*, in which it formed a distinct lineage and was loosely associated with *Thermocrispum municipale* DSM 44069T, with 93 % similarity. The other closest phylogenetic neighbours were *Saccharopolyspora erythraea* NRRL 2338T (92.6 % similarity) and *Amycolatopsis sacchari* DSM 44468T (93.1 % similarity). The isolate had cell-wall type IV (*meso*-diaminopimelic acid and whole-cell sugars arabinose, galactose and glucose) and phospholipid type III. The predominant menaquinone was MK-9(H4). The G+C content of the genomic DNA was 69 mol%. Based on these data, strain SCSIO 00231T can be readily distinguished from previously described organisms and represents a new genus within the family *Pseudonocardiaceae*. The name *Sciscionella* gen. nov. is proposed, with the novel species *Sciscionella marina* sp. nov. The type strain of *Sciscionella marina* is SCSIO 00231T (=KCTC 19433T =CCTCC AA208009T).

More than 70 % of our planet’s surface is covered by oceans, which play a crucial role in the global ecological system. Unexplored marine environments are now a popular research area due to the potentially huge resources present within them. Recently, marine actinomycete research has received more attention, especially after the establishment of the new genus *Salinispora* and the discovery that it is an excellent source of secondary metabolites (Laatsch, 2006; Lam, 2006). Many novel bioactive secondary metabolites isolated from marine actinomycetes have been reported (Lam, 2006), and they may be a source of novel compounds with pharmaceutical potential (Fiedler et al., 2005; Jensen et al., 2005a; Fenical & Jensen, 2006). Culture-independent studies have shown that marine sediment environments contain a wide diversity of actinomycetes, and many unique taxa are very different from their terrestrial counterparts (Stach et al., 2003; Gontang et al., 2007). In addition, culture-dependent studies have also shown that marine actinomycetes are ubiquitous in marine sediment environments (Maldonado et al., 2005; Jensen et al., 2005b; Gontang et al., 2007). During an investigation of the diversity of cultivable marine actinomycetes, strain SCSIO 00231T was isolated from a grey sand sediment sample. Based on phylogenetic analysis, morphological and physiological data and chemotaxonomic markers, strain SCSIO 00231T can be readily distinguished from described genera and represents a new member of the family *Pseudonocardiaceae*. Here, we report the taxonomic description of this strain.

Samples were collected in September 2006 from the northern South China Sea (20° 36’ N 116° 21’ E; depth 516 m). The surface layer of the sediment, about 40 cm in

†These authors contributed equally to this work.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SCSIO 00231T is EU503139.

Scanning electron micrographs of mycelium of strain SCSIO 00231T are available as supplementary material with the online version of this paper.

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depth, was collected by a grab bucket, and the top 10 cm layer was obtained aseptically for sampling; samples were placed in sterile 50 ml conical tubes. All samples were processed for cultivation experiments by using a standard dilution plating method on ship within 2 h, and the remainder was frozen at −20°C. Strain SCSIO 00231T was isolated on Gauze No. 1 medium prepared with seawater instead of distilled water, incubated at 28°C for 3 weeks. The purified strain was maintained on modified ISP medium 2 (prepared with natural seawater) (Shirling & Gottlieb, 1966) and as 20% (w/v) glycerol suspensions at −20°C. Biomass for chemotaxonomic and molecular systematic studies was obtained by cultivation using modified ISP 2 broth (28°C, 1 week, 150 r.p.m.).

Strain SCSIO 00231T grew well on media ISP 2, ISP 4 and ISP 5 (Shirling & Gottlieb, 1966), Caspeck solution agar (Waksman, 1961), nutrient agar (Difco) and potato agar (Waksman, 1961), but not on ISP 3 medium at 28°C. Diffusible pigments were not observed and the colony colour was yellow–white, examined by comparing the cultures with the most suitable colour chips from the ISCC–NBS Color Charts (Kelly, 1964). Micromorphology was examined by light microscopy (model BH 2; Olympus) and electron microscopy (ISM560LV; JEOL) using cells incubated in modified ISP 2 medium for 14 and 28 days. The organism formed branching substrate mycelium and fragmented into rod-shaped elements, 2.5–3.5 μm long. Sparse mycelium was produced on modified ISP 2 medium after incubation for 28 days (Supplementary Fig. S1, available in IJSEM Online).

The growth temperature was tested at 4–55°C and pH range for growth was determined at pH 4.0–12.0, based on the buffer system described by Xu et al. (2005), using modified ISP 2 as the basal medium. Tolerance of NaCl was examined at 0–20% (w/v). Carbon source utilization (0.5%, w/v) was tested as described by Shirling & Gottlieb (1966). Physiological tests including hydrolysis of cellulose, gelatin, starch and Tweens 20, 40, 60 and 80, nitrate reduction, utilization of urea, milk coagulation and peptonization and H₂S and melanin production were performed as described previously (Gonzalez et al., 1978; Smibert & Krieg, 1981). Antibiotic susceptibility was examined as described by Groth et al. (2004) using antibiotic discs on modified ISP 2 medium.

Cells of strain SCSIO 00231T were Gram-positive and aerobic. The strain was susceptible to (μg per disc) penicillin G (10), erythromycin (15), gentamicin (10), novobiocin (30), trimethoprim (1.25), netilmicin (30), amikacin (30), tobramycin (10) and neomycin (10) and resistant to streptomycin (10), tetracycline (30), vancomycin (30), lincomycin (2), rifampicin (5), chloramphenicol (30), ampicillin (10), norfloxacin (10), amoxicillin (10) and ciprofloxacin (5). Detailed physiological properties of the strain are given in the species description.

Amino acids and peptides in cell-wall hydrolysatess were analysed by the methods described by Schleifer (1985) and Schleifer & Kandler (1972) with the modification that TLC on cellulose sheets was applied instead of paper chromatography. Menaquinones were isolated using the methods of Minnikin et al. (1984) and separated by HPLC (Kroppenstedt, 1982; Kroppenstedt et al., 1981). Phospholipids were extracted and examined by using published procedures (Minnikin et al., 1979; Collins & Jones, 1980). 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 isolate had a type IV cell wall; whole-cell hydrolysatess contained meso-diaminopimelic acid and the whole-cell sugars were galactose, arabinose and glucose. Phospholipids were type III, including diphosphatidylglycerol, phosphatidylmethyl ethanolamine, phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol and unknown phosphoglycolipids. The predominant menaquinone was MK-9(H₄) (93%). The G+C content of the genomic DNA was 69 mol%, determined by using the HPLC method (Mesbah et al., 1989). The fatty acid profile contained i-C₁₆:0 (43.9%), unknown fatty acid (peak name 16.048) (15.52%), i-C₁₆:0 2-0H (12.70%), ai-C₁₇:0 (9.84%), ai-C₁₇:0 2-0H (3.02%), 10-methyl C₁₆:0 (2.81%), i-C₁₅:0 (2.48%), i-C₁₇:0 (2.21%), C₁₅:0 (1.87%), C₁₄:0 (1.82%), ai-C₁₅:0 (1.08%), C₁₈:0 3-0H (0.89%), i-C₁₅:0 2-0H/C₁₆:1 9 (0.73%), C₁₆:1 2-0H (0.56%) and i-C₁₄:0 (0.55%). Chemotaxonomic characteristics of strain SCSIO 00231T and its closest phylogenetic neighbours are compared in Table 1.

Extraction of genomic DNA and PCR amplification of the 16S rRNA gene were done as described by Li et al. (2007). Multiple alignments with sequences of the most closely related taxa and calculations of levels of sequence similarity were carried out using CLUSTAL_X (Thompson et al., 1997). A phylogenetic tree and distance matrix were reconstructed using the neighbour-joining method of Saitou & Nei (1987) from K_{nu} values (Kimura, 1980, 1983) using 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 replications.

BLAST search results using the 16S rRNA gene sequence of strain SCSIO 00231T showed that the new isolate had the highest similarities to members of the family Pseudonocardiaceae, such as Saccharopolyspora erythraea NRRL 2338T (92.6% similarity), Thermocresipum municipal DSM 44069T (93%) and Amycolatopsis sacchari DSM 44468T (93.1%). Additionally, patterns of selected 16S rRNA gene signature nucleotides defined for the family Pseudonocardiaceae (Stackebrandt et al., 1997) were also consistent with nucleotides determined for the 16S rRNA gene sequence of strain SCSIO 00231T, except that G–G and U were determined at positions 183:194 and 747, respectively. All the above data confirmed that the new isolate should be assigned to the family Pseudonocardiaceae.
Table 1. Chemotaxonomic characteristics of strain SCSIO 00231\(^\text{T}\) and its closest relatives in described genera of the family Pseudonocardiaceae

Data were taken from our study and references Al-Zarban et al. (2002), Goodfellow et al. (2001), Jiang et al. (2008), Korn-Wendisch et al. (1989, 1995), Lee et al. (2002), Majumdar et al. (2006), Mertz & Yao (1993) and Tomita et al. (1993). Cell walls of all taxa contain meso-diaminopimelic acid.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Cell-wall type</th>
<th>Whole-cell sugars*</th>
<th>Phospholipid type</th>
<th>Phospholipids†</th>
<th>Predominant menaquinone</th>
<th>Major fatty acid(s) (&gt;10 %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain SCSIO 00231(^\text{T})</td>
<td>IV</td>
<td>Ara, Gal, Glc</td>
<td>P III</td>
<td>DPG, PC, PE, PI, PL, PME</td>
<td>9(\text{H}_4)</td>
<td>i-C(<em>{16:0}), i-C(</em>{16:0}) 2-OH</td>
</tr>
<tr>
<td>Thermocrispum municipale DSM 44069(^\text{T})</td>
<td>III</td>
<td>Ara, Man, Glc</td>
<td>P II</td>
<td>PE, PE-OH, PI</td>
<td>9(\text{H}_4)</td>
<td>i-C(_{16:0})</td>
</tr>
<tr>
<td>Amycolatopsis orientalis NBRC 12806(^\text{T})</td>
<td>IV</td>
<td>Ara, Gal</td>
<td>P II</td>
<td>DPG, PE, PG, PI, PME</td>
<td>9(\text{H}_4)</td>
<td>i-C(<em>{15:0}), i-C(</em>{16:0}), C(<em>{16:1}) (\text{cis}6), C(</em>{16:1}) (\text{cis}7), C(_{17:0}) (\text{cis}8)</td>
</tr>
<tr>
<td>Amycolatopsis sacchari DSM 44468(^\text{T})</td>
<td>IV</td>
<td>Ara, Gal</td>
<td>P II</td>
<td>DPG, PE, PE-OH, PG, PI</td>
<td>9(\text{H}_4)</td>
<td>i-C(<em>{16:0}), ai-C(</em>{17:0})</td>
</tr>
<tr>
<td>Saccharopolyspora hirsuta DSM 43463(^\text{T})</td>
<td>IV</td>
<td>Ara, Gal</td>
<td>P III</td>
<td>DPG, PC, PE, lyso-PE, PE-OH, PI, PME</td>
<td>9(\text{H}_4)</td>
<td>i-C(<em>{15:0}), i-C(</em>{16:0}), i-C(<em>{17:0}), ai-C(</em>{17:0})</td>
</tr>
<tr>
<td>Saccharopolyspora erythraea NRRL 2338(^\text{T})</td>
<td>IV</td>
<td>Ara, Gal</td>
<td>P III</td>
<td>DPG, PC, PE, lyso-PE, PI</td>
<td>9(\text{H}_4)</td>
<td>i-C(<em>{15:0}), i-C(</em>{16:0}), i-C(<em>{17:0}), ai-C(</em>{17:0}), C(_{17:0})</td>
</tr>
<tr>
<td>Prauserella rugosa DSM 43194(^\text{T})</td>
<td>IV</td>
<td>Ara, Gal</td>
<td>P II</td>
<td>DPG, PE, PG, PI, lyso-PE</td>
<td>9(\text{H}_{2,4})</td>
<td>i-C(<em>{16:0}), i-C(</em>{16:1}) H, C(<em>{16:1}), C(</em>{17:1}) C</td>
</tr>
<tr>
<td>Saccharomonospora halophila DSM 44411(^\text{T})</td>
<td>IV</td>
<td>Ara, Gal</td>
<td>P II</td>
<td>DPG, PI, PE PE-OH, lyso-PE</td>
<td>9(\text{H}_4)</td>
<td>i-C(<em>{16:0}), C(</em>{16:0}), C(<em>{16:1}), i-C(</em>{16:2}) 2-OH</td>
</tr>
<tr>
<td>Pseudonocardia spinosiora LM 141(^\text{T})</td>
<td>IV</td>
<td>Ara, Gal</td>
<td>P III</td>
<td>DPG, PC, PE, PE-OH, PG, PIM, PL, PME</td>
<td>8(\text{H}_4)</td>
<td>i-C(<em>{15:0}), i-C(</em>{16:0}), i-C(_{17:0})</td>
</tr>
<tr>
<td>Kibdelosporangium aridum subsp. largum DSM 44150(^\text{T})</td>
<td>IV</td>
<td>Ara, Gal, Glc, Rha</td>
<td>P II</td>
<td>PE, PG, PI, PME</td>
<td>9(\text{H}_4)</td>
<td>i-C(<em>{16:0}), C(</em>{16:0}), ai-C(<em>{17:0}), C(</em>{17:0})</td>
</tr>
<tr>
<td>Actinomycetospora chiangmaiensis YIM 0006(^\text{T})</td>
<td>IV</td>
<td>Ara, Gal</td>
<td>P III</td>
<td>PC, PG, PI</td>
<td>9(\text{H}_4)</td>
<td>C(<em>{16:1}) (\text{cis}6), (\text{cis}7) i-C(</em>{15:0}) 2-OH, i-C(<em>{16:0}), C(</em>{16:0})</td>
</tr>
</tbody>
</table>

*Ara, Arabinose; Gal, galactose; Glc, glucose; Man, mannose; Rha, rhamnose.
†DPG, Diphosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PE-OH, hydroxy-PE; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, PI mannosides; PL, unknown phospholipids; PME, phosphatidymethylethanolamine.
In the phylogenetic tree based on the 16S rRNA gene sequences of representatives of all genera in family *Pseudonocardiaceae* (Fig. 1), strain SCSIO 00231T formed a distinct lineage and was loosely associated with the genus *Thermocrispum*, with the highest similarity of 93% to *Thermocrispum municipale* DSM 44069T, which showed the earlier evolutionary divergence between strain SCSIO 00231T and the other previously described genera in this family. Chemotaxonomic characteristics could be distinguished readily from related taxa in the family *Pseudonocardiaceae* (Table 1). We also compared the nucleotide signatures, which also indicate many distinctions between strain SCSIO 00231T and the other previously described genera in this family.从而

### Description of *Sciscionella* gen. nov.

*Sciscionella* (Sci.sci.o.nel: N.L. fem. dim. n. *Sciscionella* arbitrary name formed from the acronym of the South China Sea Institute of Oceanology, SCISIO, where taxonomic studies on this taxon were performed), Gram-positive, aerobic organisms that produce fragmented substrate mycelium and sparse aerial mycelium on modified ISP 2 medium. Characterized by cell-wall chemotype IV, containing meso-diaminopimelic acid and whole-cell sugars arabinose, galactose and glucose, and phospholipid pattern type III sensu *Lechevalier et al.* (1977), comprising diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol and unknown phospholipids. Mycolic acids are absent. The predominant menaquinone is MK-9(H4). Major fatty acids are i-C16 : 0, ai-C17 : 0 and i-C16 : 02-OH. The G+C content of the genomic DNA is about 69 mol%. The type species is *Sciscionella marina*.

### Description of *Sciscionella marina* sp. nov.


Morphological, chemotaxonomic and general characteristics are as given above for the genus. Colonies are yellow–white on most tested media. Good growth occurs at 28 °C on media ISP 2, ISP 4, ISP 5, Czapek solution agar, nutrient agar and potato agar. No diffusible pigments are present.

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**Fig. 1.** Phylogenetic dendrogram of strain SCSIO 00231T and its closest relatives within the family *Pseudonocardiaceae*, reconstructed by using the neighbour-joining method, based on 16S rRNA gene sequences of more than 1300 nt. Numbers at nodes are bootstrap percentages based on 1000 resamplings (only values of 50% or more are indicated). Bar, 1% sequence divergence.
produced. Gelatin liquefaction, catalase and hydrolysis of Tweens 20, 40, 60 and 80 are positive. Hydrolysis of starch and cellulose, H2S and melanin production, utilization of urea, milk coagulation, milk peptonization, nitrate reduction and oxidase are negative. The pH, NaCl concentration and temperature ranges for growth are pH 6.0–8.0, 0–13 % and 10–37 °C, with optimum growth at pH 7.0, 3–5 % (w/v) and 28 °C. Can utilize cellobiose, D-fructose, D-galactose, D-glucose, D-lactose, D-mannitol, D-mannose, D-ribose and trehalose as carbon sources, but not acetate, D-arabinose, citrate, dulcitol, inositol, maltose, raffinose, L-rhamnose, D-sorbitol, sucrose, xylitol or D-xylose.

The type strain is SCSIO 00231 T (=KCTC 19433 T =CCTCC AA208009 T), isolated from a marine sand sediment at a depth about 500 m.

Acknowledgements

The authors are very grateful to Dr J. P. Euzéby for his advice on nomenclature. This research was supported by the National Basic Research Program of China (no. 2004CB719601), the National Natural Science Foundation of China (no. 30600001), the Knowledge Innovation Program of the Chinese Academy of Sciences (KZCX2-YW-216), the China National Key Program for Base Research (2005CCA04800) and the Key Project of Chinese Ministry of Education (no. 206139). W.-J. L. was supported by the Program for New Century Excellent Talents in University.

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