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

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The taxonomic position of an actinomycete, designated SCSIO 00231^T, 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 00231^T fell into the family *Pseudonocardiaceae*, in which it formed a distinct lineage and was loosely associated with *Thermocrispum municipale* DSM 44069^T, with 93 % similarity. The other closest phylogenetic neighbours were *Saccharopolyspora erythraea* NRRL 2338^T (92.6 % similarity) and *Amycolatopsis sacchari* DSM 44468^T (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(H_4). The G+C content of the genomic DNA was 69 mol%. Based on these data, strain SCSIO 00231^T 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 00231^T (=KCTC 19433^T =CCTCC AA208009^T).

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 00231^T was isolated from a grey sand sediment sample. Based on phylogenetic analysis, morphological and physiological data and chemotaxonomic markers, strain SCSIO 00231^T 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

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

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

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 00231^T 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 00231^T grew well on media ISP 2, ISP 4 and ISP 5 (Shirling & Gottlieb, 1966), Czapek 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 (JSM5600LV; 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 H_2S 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 00231^T 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.

Analysis of whole-cell sugars was done according to procedures described by Staneck & Roberts (1974). Amino acids and peptides in cell-wall hydrolysates 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 hydrolysates contained *meso*-diaminopimelic acid and the whole-cell sugars were galactose, arabinose and glucose. Phospholipids were type III, including diphosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol and unknown phosphoglycolipids. The predominant menaquinone was MK-9(H_4) (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-$C_{16:0}$ (43.9 %), unknown fatty acid (peak name 16.048) (15.52 %), i-$C_{16:0}$ 2-OH (12.70 %), ai-$C_{17:0}$ (9.84 %), ai-$C_{17:0}$ 2-OH (3.02 %), 10-methyl $C_{16:0}$ (2.81 %), i-$C_{15:0}$ (2.48 %), i-$C_{17:0}$ (2.21 %), $C_{16:0}$ (1.87 %), $C_{14:0}$ (1.82 %), ai-$C_{15:0}$ (1.08 %), $C_{18:0}$ 3-OH (0.89 %), i-$C_{15:0}$ 2-OH/$C_{16:1}$ *t*9 (0.73 %), $C_{16:1}$ 2-OH (0.56 %) and i-$C_{14:0}$ (0.55 %). Chemotaxonomic characteristics of strain SCSIO 00231^T 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_{nuc} 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 replicates.

BLAST search results using the 16S rRNA gene sequence of strain SCSIO 00231^T showed that the new isolate had the highest similarities to members of the family *Pseudonocardiaceae*, such as *Saccharopolyspora erythraea* NRRL 2338^T (92.6 % similarity), *Thermocrispum municipale* DSM 44069^T (93 %) and *Amycolatopsis sacchari* DSM 44468^T (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 00231^T, 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^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.

Strain	Cell-wall type	Whole-cell sugars*	Phospholipid type	Phospholipids†	Predominant menaquinone	Major fatty acid(s) (>10 %)
Strain SCSIO 00231^T	IV	Ara, Gal, Glc	P III	DPG, PC, PE, PI, PL, PME	9(H_4)	i-$C_{16:0}$, i-$C_{16:0}$ 2-OH
Thermocrispum municipale DSM 44069^T	III	Ara, Man, Glc	P II	PE, PE-OH, PI	9(H_4)	i-$C_{16:0}$
Amycolatopsis orientalis NBRC 12806^T	IV	Ara, Gal	P II	DPG, PE, PG, PI, PME	9(H_4)	i-$C_{15:0}$, i-$C_{16:0}$, $C_{16:1}\omega6c$/$C_{16:1}\omega7c$, $C_{17:1}\omega8c$
Amycolatopsis sacchari DSM 44468^T	IV	Ara, Gal	P II	DPG, PE, PE-OH, PG, PI	9(H_4)	i-$C_{16:0}$, ai-$C_{17:0}$
Saccharopolyspora hirsuta DSM 43463^T	IV	Ara, Gal	P III	DPG, PC, PE, lyso-PE, PE-OH, PI, PME	9(H_4)	i-$C_{15:0}$, i-$C_{16:0}$, i-$C_{17:0}$, ai-$C_{17:0}$
Saccharopolyspora erythraea NRRL 2338^T	IV	Ara, Gal	P III	DPG, PC, PE, lyso-PE, PI	9(H_4)	i-$C_{15:0}$, i-$C_{16:0}$, i-$C_{17:0}$, ai-$C_{17:0}$, $C_{17:0}$
Prauserella rugosa DSM 43194^T	IV	Ara, Gal	P II	DPG, PE, PG, PI, PME	9($H_{2,4}$)	i-$C_{16:0}$, i-$C_{16:1}$ H, $C_{16:1}$, $C_{17:1}$ C
Saccharomonospora halophila DSM 44411^T	IV	Ara, Gal	P II	DPG, PI, PE PE-OH, lyso-PE	9(H_4)	i-$C_{16:0}$, $C_{16:0}$, $C_{16:1}$, i-$C_{16:0}$ 2-OH
Pseudonocardia spinosispora LM 141^T	IV	Ara, Gal	P III	DPG, PC, PE, PE-OH, PG, PIM, PL, PME	8(H_4)	i-$C_{15:0}$, i-$C_{16:0}$, i-$C_{17:0}$
Kibdelosporangium aridum subsp. *largum* DSM 44150^T	IV	Ara, Gal, Glc, Rha	P II	PE, PG, PI, PME	9(H_4)	i-$C_{16:0}$, $C_{16:0}$, ai-$C_{17:0}$, $C_{17:0}$
Actinomycetospora chiangmaiensis YIM 0006^T	IV	Ara, Gal	P III	PC, PG, PI	9(H_4)	$C_{16:1}\omega7c$/i-$C_{15:0}$ 2-OH, i-$C_{16:0}$, $C_{16:0}$

*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, phosphatidylmethylethanolamine.

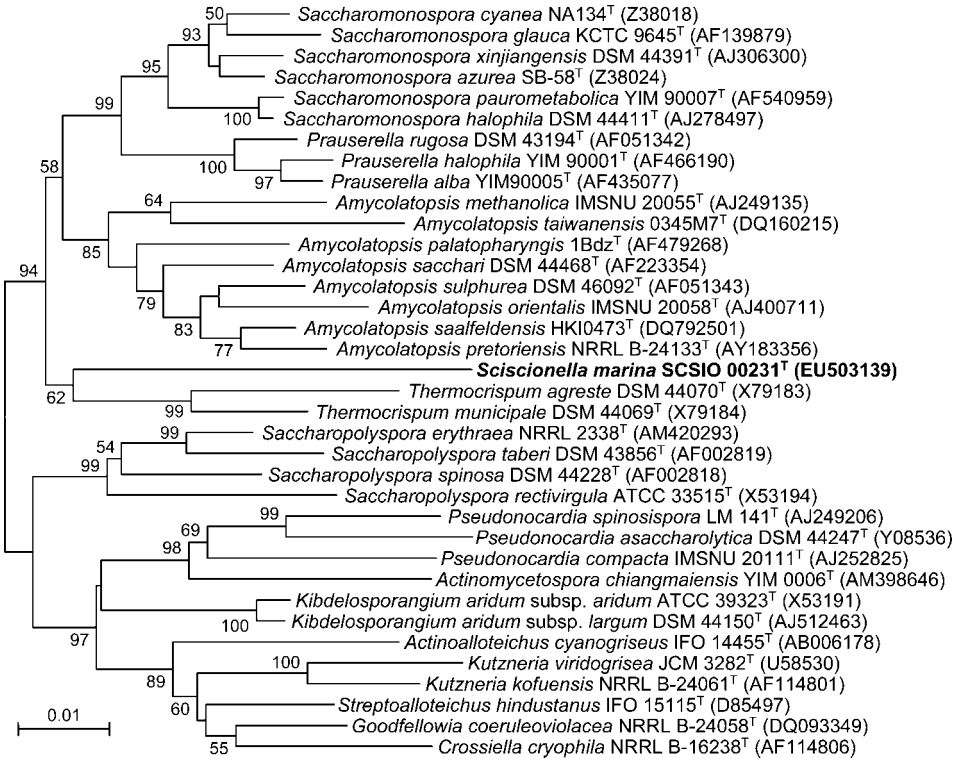

Fig. 1. Phylogenetic dendrogram of strain SCSIO 00231^T and its closest relatives within the family *Pseudonocardiaceae*, reconstructed by using the neighbour-joining method, based on 16S rRNA gene sequences of more than 1300 nt. Numbers at nodes are bootstrap percentages based on 1000 resamplings (only values of 50 % or more are indicated). Bar, 1 % sequence divergence.

In the phylogenetic tree based on the 16S rRNA gene sequences of representatives of all genera in family *Pseudonocardiaceae* (Fig. 1), strain SCSIO 00231^T formed a distinct lineage and was loosely associated with the genus *Thermocrispum*, with the highest similarity of 93 % to *Thermocrispum municipale* DSM 44069^T, which showed the earlier evolutionary divergence between strain SCSIO 00231^T 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 00231^T and other genera of the family *Pseudonocardiaceae* in some signature sites (Table 2). Thus, based on the phylogenetic position and chemotaxonomic data, a novel genus is proposed for strain SCSIO 00231^T, to be named *Sciscionella* gen. nov., with the type species *Sciscionella marina* sp. nov.

Description of *Sciscionella* gen. nov.

Sciscionella (Sci.sci.o.nel′la. N.L. fem. dim. n. *Sciscionella* arbitrary name formed from the acronym of the South China Sea Institute of Oceanology, SCISCIO, 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, phosphatidylmethylethanolamine, phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol and unknown phospholipids. Mycolic acids are absent. The predominant menaquinone is MK-9(H_4). Major fatty acids are i-$C_{16:0}$, ai-$C_{17:0}$ and i-$C_{16:0}$ 2-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.

Sciscionella marina (ma.ri′na. L. fem. adj. *marina* of the sea).

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

Table 2. Patterns of selected 16S rRNA gene signature nucleotides detected among the various genera of the family *Pseudonocardiaceae*

Taxa: 1, SCSIO 00231^T; 2, *Thermocrispum*; 3, *Amycolatopsis*; 4, *Saccharopolyspora*; 5, *Saccharomonospora*; 6, *Prauserella*; 7, *Pseudonocardia*; 8, *Kutzneria*; 9, *Actinoalloteichus*; 10, *Crossiella*; 11, *Kibdelosporangium*; 12, *Streptoalloteichus*; 13, *Goodfellowia*; 14, *Actinostreptospora*; 15, *Actinomycetospora*. All 16S rRNA gene sequences of type strains belong to the family *Pseudonocardiaceae* were included in this analysis. The signatures given below for each group were chosen for their presence in more than 95 % of the members of the respective genera. −, Residue absent; *, no signature residue.

Position(s)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
42 : 400	U–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C
43 : 399	U–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G
51	U	A	A/C	A	A	A	A	A	A	A	A	A	A	A	A
52 : 359	A–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G
70 : 98	G–G	U–A	U–A	U–A	U–A	U–A	U–A	*–A	U–A	U–A	U–A	U–A	U–A	U–A	U–A
72	C	A	−	−	A	A	−	A	A	A	A	A	A	A	A
183 : 194	G–G	G–C	*–G	G–G	G–C	G–C	G–G	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C
208	G	G	U	U	U	G	U	G	G	G	G	G	G	G	G
210	A	A	G	G	G	A	G	A	A	A	A	A	A	A	A
213	G	G	A	A	A	C	A	G	U	U	U	U	U	U	U
248 : 276	U–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G
252 : 274	A–U	U–A	U–A	U–A	U–A	U–A	U–A	U–A	U–A	U–A	U–A	U–A	U–A	U–A	U–A
280	U	U	C	C	C	U	C/U	C	C	C	C	C	C	C	C
289 : 311	G–U	U–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C
502 : 543	A–U	A–U	A–U	A–U	A–U	A–U	A–U	A–U	A–U	A–U	A–U	G–C	A–U	A–U	A–U
591 : 648	U–U	U–A	U–A	U–A	U–A	U–A	U–A	U–A	U–A	U–A	U–A	U–A	U–A	U–A	U–A
658	G	G/U	U	U	U	U	U	U	U	U	U	U	U	U	U
659 : 746	U–A	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G
747	U	A/C	A	A	A	A	A/G	A	A	A	A	A	A	A	A
811	U	C	U	C	C	U	C	C	C	C	C	C	C	C	C
1004	G	G	A	A	A/G	G	A	A	A	A	A	A	A	A	A
1138	G	U	U	U	C/U	U	U	U	U	U	*	U	U	U	U
1159	U	C	U	U	C/U	U	U	U	U	U	U	U	U	U	U
1189	U	C	U	U	C/U	U	U	U	U	U	U	U	U	U	U
1192	G	C	C	C	C	C	C	C	G	C	C	C	C	C	C
986 : 1219	U–A	U–U	U–A	U–A	U–A	U–A	U–A	U–A	U–A	U–A	U–A	U–A	U–A	U–A	U–A
1257	A	U	U	*	G/U	G	G/U	U	U	U	U	U	U	U	U
1285	A	U	U	U	A/U	U	U	A	U	U	A/U	U	U	G	G
1311 : 1326	A–U	A–U	A–U	G–C	*–*	A–U	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C
1362	C	A	A	A	A/G	A	A	A	A	A	A	A	A	A	A

produced. Gelatin liquefaction, catalase and hydrolysis of Tweens 20, 40, 60 and 80 are positive. Hydrolysis of starch and cellulose, H_2S 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.

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