**Nocardiopsis coralliicola** sp. nov., isolated from the gorgonian coral, *Menella praelonga*

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An actinobacterial strain, SCSIO 10427T, was isolated from a gorgonian coral sample collected from Weizhou Island, Guangxi province, China, and its taxonomic position was investigated using a polyphasic approach. The organism was found to have a range of chemical and morphological properties consistent with its classification in the genus *Nocardiopsis*. Phylogenetic analysis indicated that 16S rRNA gene sequence similarity between strain SCSIO 10427T and type strains of other recognized members of the genus *Nocardiopsis* was lower than 98.4%. Furthermore, phenotypic characteristics revealed that the strain differed from the currently recognized species of the genus *Nocardiopsis*. Therefore, strain SCSIO 10427T represents a novel species of the genus *Nocardiopsis*, for which the name *Nocardiopsis coralliicola* sp. nov. is proposed. The type strain is SCSIO 10427T (=CCTCC AA 2011010T=DSM 45611T).

The genus *Nocardiopsis* was proposed by Meyer (1976) on the basis of chemotaxonomic and morphological characteristics. At the time of writing, this taxon comprised 35 recognized species. Numerous studies have shown that *Nocardiopsis* strains are ubiquitously distributed in the natural environment (Kroppenstedt & Evtushenko, 2002). The natural habitat of most described strains is soil (Kroppenstedt & Evtushenko, 2002), but they have also been isolated from marine environments, including seashore sediments (Sabry et al., 2004) and a marine animal (Chen et al., 2009), plant rhizosphere soil (Hamedi et al., 2010), plant tissue (Qin et al., 2009), animal guts (Vasanthi & Hoti, 1992), indoor environments (Peltola et al., 2001), the atmosphere of a composting facility (Kämper et al., 2002), clinical material (Bernatchez & Lebreux, 1991; Yassin et al., 1997) and saline soils (Li et al., 2003, 2004, 2006; Chen et al., 2008). Many of these species prefer moderately alkaline conditions (pH 8.5) (Kroppenstedt, 1992), and some grow better on media supplemented with sodium chloride (Hamedi et al., 2011). Members of the genus *Nocardiopsis* are known to produce bioactive metabolites such as griseusin D, apoptolidin, methylpen-dolymycin, thiopeloteptide (designated TP-1161), naphthospir-onone A and a lipopeptide biosurfactant (Sun et al., 1991; Kim et al., 1997; Li et al., 2007a; Gandhimathi et al., 2009; Engelhardt et al., 2010; Ding et al., 2010). Therefore, the isolation of members of this genus from different environments should provide access to new bioactive products and contribute to an understanding of their ecological roles.

Strain SCSIO 10427T was isolated from the gorgonian coral *Menella praelonga*, collected from a depth of 6.5 m in the south-western coastal waters of Weizhou Island, Guangxi province, China. The coral sample was washed with 75 % (v/v) ethanol and sterilized distilled water, processed in a sterile commercial blender, and 0.2 ml volumes were plated on trehalose-proline isolation medium (trehalose 1 g, proline 0.5 g, MgCl2.6H2O 0.2 g, KNO3 0.5 g, agar 12 g, 1 l distilled water, pH 7.0) and incubated at 28 °C. The purified strain was routinely cultured on nutrient agar medium supplemented with 3% (w/v) NaCl at 28 °C and stored as glycerol suspensions (20%, v/v) at −70 °C.

Aerial spore-mass colour, substrate mycelium pigmentation and coloration of the diffusible pigments of strain SCSIO...
10427T were recorded on International Streptomyces Project (ISP) media 2, 3, 4 and 5, Czapek’s agar, potato-glucose agar and nutrient agar prepared as described by Dong & Cai (2001). Colours were determined by using colour chips from the ISCC–NBS colour charts (standard samples, no. 2106) (Kelly, 1964). Morphological properties were examined using a light microscope (Eclipse E600; Nikon) and a scanning electron microscope (S-3400N; Hitachi) after 7–14 days incubation on nutrient agar medium supplemented with 3 % (w/v) NaCl at 28 °C. Oxidase activity was determined from the oxidation of tetramethyl-p-phenylenediamine. Catalase activity was detected by the production of bubbles following addition of a drop of 3 % (v/v) H2O2. Hydrolysis of starch, gelatin and Tweens 20, 40 and 80 was determined as described by Smibert & Krieg (1994). Carbon source utilization was determined according to the method described by Smibert & Krieg (1994). Nitrogen source utilization was assessed according to Williams et al. (1989). The tolerance to different NaCl concentrations (0, 1, 3, 5, 7, 10, 12, 15, 17, 18, 19 and 20 %, w/v) was tested on nutrient agar medium as the basal medium by incubating the cultures for 28 days at 28 °C. Growth at different temperatures (10, 15, 20, 25, 28, 37, 40, 45, 50 and 60 °C) was tested on nutrient agar medium supplemented with 3 % (w/v) NaCl over 28 days. The pH range for growth [pH 4.0–11.0 (at intervals of 1.0 pH unit), using the buffer system described by Xu et al. (2005)] was tested at 28 °C for 28 days by culturing the strains in tryptic soy broth (TSB).

For chemotaxonomic analyses, cell mass was obtained from TSB medium after cultivation for 3 days at 28 °C and 200 r.p.m. The isomer of diaminopimelic acid in whole-cell hydrolysates was determined using TLC as described by Stanec & Roberts (1974). The whole-cell sugars were detected by HPLC (1100; Agilent) after precolumn derivatization with 1-phenyl-3-methyl-5-pyrazolone (PMP) according to the method described by Tang et al. (2009). Phospholipids were extracted, examined by two-dimensional TLC and identified using previously described procedures (Minnikin et al., 1979; Collins & Jones, 1980). Menaquinones were extracted according to Collins et al. (1977) and separated by HPLC (Tamaoka et al., 1983). Cellular fatty acids were extracted, methylated and analysed by using the Sherlock Microbial Identification System (MIDI) according to the manufacturer’s instructions. The fatty acid methyl esters were then identified by using the Microbial Identification software package (Sherlock Version 6.1; MIDI database: TSBA6). The DNA G+C content was determined by using the HPLC method (Mesbah et al., 1989).

Extraction of genomic DNA, PCR amplification and sequencing of the 16S rRNA gene were performed as described by Li et al. (2007b). The sequence obtained was compared with available 16S rRNA gene sequences from GenBank using the BLAST program and the EzTaxon server (http://www.eztaxon.org, Chun et al., 2007) to determine an approximate phylogenetic affiliation. Gene sequences were aligned with those of closely related species by CLUSTAL_X (Thompson et al., 1997). The phylogenetic trees were constructed by the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) tree-making algorithms by using the MEGA version 5.05 software package (Tamura et al., 2011). The topologies of the phylogenetic trees were evaluated by using the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

Strain SCSIO 10427T grew well on yeast extract-malt extract agar (ISP 2), nutrient agar and potato-glucose agar; grew moderately on oatmeal agar (ISP 3), glycerol-asparagine agar (ISP 5) and Czapek’s agar; and grew poorly on inorganic salts-starch agar (ISP 4). White aerial mycelia formed slowly on ISP 3 and Czapek’s agar, but not on the other media tested. The substrate mycelia were yellow–white on ISP 3, ISP 4, ISP 5 and Czapek’s agar, pale yellow on ISP 2, and pale grey–yellow on nutrient agar and potato-glucose agar. A pale yellow soluble pigment was produced on nutrient agar. Vegetative hyphae were long, well-developed and fragmented. Long spore chains were borne on the aerial mycelium and the spores were smooth and non-motile (Fig. S1, available in IJSEM online). Strain SCSIO 10427T grew at 20–45 °C (optimum, 28–37 °C), pH 7.0–10.0 (optimum, pH 8.0–9.0) and at NaCl concentrations up to 18 % (w/v). Detailed physiological results are given in Table 1 and in the species description.

Strain SCSIO 10427T contained meso-diaminopimelic acid as the diagnostic diamino acid in the cell-wall peptidoglycan. Whole-cell hydrolysates contained ribose, glucose and mannose. The major phospholipids were diphosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidyl-ethanolamine, phosphatidyglycerol, phosphatidylcholine and three unknown phospholipids (Fig. S2). The phospholipid pattern was of type III according to Lechevalier et al. (1977). The menaquinone profile was composed of two major components MK-10(H8) (42 %) and MK-10(H6) (43 %), with minor amounts of MK-9(H4) (8 %) and MK-9(H6) (7 %). Major fatty acids (>10 %) of strain SCSIO 10427T were iso-C16 : 0 (21.73 %), anteiso-C17 : 0 (14.42 %), C17 : 1o8c (11.33 %) and C18 : 1o9c (10.38 %), and the minor components were anteiso-C15 : 0 (7.61 %), 10-methyl C17 : 0 (6.36 %), 10-methyl C18 : 0 (tuberculostearic acid, 6.09 %), C17 : 0 (5.37 %), C16 : 0 (4.14 %), C18 : 0 (2.84 %), C16 : 1o7c (1.87 %), iso-C18 : 0 (1.63 %), iso-C14 : 0 (1.32 %) and iso-C17 : 0 (1.17 %). The DNA G+C content of strain SCSIO 10427T was 69.5 mol%.

The result of phylogenetic analyses indicated that strain SCSIO 10427T clustered with members of the genus Nocardiopsis (Figs 1 and S3). The neighbour-joining phylogenetic tree revealed that it formed a distinct clade with Nocardiopsis chromatogenes YIM 90109T (98.4 % 16S rRNA gene sequence similarity), Nocardiopsis halophila DSM 44494T (98.0 %) and Nocardiopsis baikchengensis YIM 90130T (98.0 %); this cluster was also recovered with the other algorithms tested (Figs 1 and S3). It has been shown that species of the genus Nocardiopsis show high 16S rRNA gene sequence similarities (>99 %) and have low DNA–DNA relatedness values (Peltola et al., 2001;
Table 1. Phenotypic characteristics that differentiate strain SCSIO 10427<sup>T</sup> from its closest phylogenetic neighbours

Taxa: 1, SCSIO 10427<sup>T</sup>; 2, *N. chromatogenes* YIM 90109<sup>T</sup>; 3, *N. halophila* DSM 44494<sup>T</sup>; 4, *N. baichengensis* YIM 90130<sup>T</sup>; 5, *N. composta* KS9<sup>T</sup>; 6, *N. potens* IMMIB L-21<sup>T</sup>. Data for all strains were determined in this study except where indicated. +, Positive; −, negative; w, weak; ND, no data available.

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<td>Major menaquinones</td>
<td>MK-10(H&lt;sub&gt;3&lt;/sub&gt;), MK-10(H&lt;sub&gt;4&lt;/sub&gt;)</td>
<td>MK-10, MK-10(H&lt;sub&gt;3&lt;/sub&gt;), MK-10(H&lt;sub&gt;4&lt;/sub&gt;)</td>
<td>MK-10(H&lt;sub&gt;3&lt;/sub&gt;), MK-10(H&lt;sub&gt;4&lt;/sub&gt;)</td>
<td>MK-9(H&lt;sub&gt;4&lt;/sub&gt;), MK-10(H&lt;sub&gt;3&lt;/sub&gt;), MK-10(H&lt;sub&gt;4&lt;/sub&gt;)</td>
<td>MK-10(H&lt;sub&gt;3&lt;/sub&gt;), MK-11(H&lt;sub&gt;3&lt;/sub&gt;), MK-10(H&lt;sub&gt;4&lt;/sub&gt;)</td>
<td>MK-11(H&lt;sub&gt;2,4,6,8&lt;/sub&gt;), MK-10(H&lt;sub&gt;2,4,6,8&lt;/sub&gt;)</td>
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<td>Major fatty acids (&gt;10%)†</td>
<td>iso-C&lt;sub&gt;16:0&lt;/sub&gt; (21.73 %), anteiso-C&lt;sub&gt;17:0&lt;/sub&gt; (14.42 %), C&lt;sub&gt;18:1&lt;/sub&gt;ω9c (11.33 %), C&lt;sub&gt;18:1&lt;/sub&gt;ω9c (10.38 %)</td>
<td>iso-C&lt;sub&gt;16:0&lt;/sub&gt; (26.02 %), anteiso-C&lt;sub&gt;17:0&lt;/sub&gt; (10.07 %), TBSA (29.38 %)</td>
<td>ND</td>
<td>iso-C&lt;sub&gt;16:0&lt;/sub&gt; (24.17 %), anteiso-C&lt;sub&gt;17:0&lt;/sub&gt; (13.64 %), TBSA (33.19 %)</td>
<td>iso-C&lt;sub&gt;16:0&lt;/sub&gt; (16.0 %), anteiso-C&lt;sub&gt;15:0&lt;/sub&gt; (18.9 %), anteiso-C&lt;sub&gt;17:0&lt;/sub&gt; (12.8 %)</td>
<td>anteiso-C&lt;sub&gt;15:0&lt;/sub&gt; (18.82 %), anteiso-C&lt;sub&gt;17:0&lt;/sub&gt; (11.16 %)</td>
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<td>DNA G+C content (mol%)</td>
<td>69.5</td>
<td>71.8</td>
<td>71.5</td>
<td>73.2</td>
<td>74.7</td>
<td>ND</td>
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* indicates data from previous studies for taxa 2–6 (Al-Tai & Ruan, 1994; Kämpfer et al., 2002; Li et al., 2006; Yassin et al., 2009).
† TBSA, tuberculostearic acid.
Schippers et al., 2002; Li et al., 2006; Yang et al., 2008; Chen et al., 2009; Fang et al., 2011; Hamedi et al., 2011). Stackebrandt & Ebers (2006) also demonstrated that strains showing less than 98.7% 16S rRNA gene sequence similarity always have DNA–DNA reassociation values lower than 70%. Considering the 16S rRNA gene sequence similarity values between strain SCSIO 10427T and N. chromatogenes YIM 90109T, N. halophila DSM 44494T, N. baichengensis YIM 90130T, Nocardiopsis composta KS9 and Nocardiopsis potens IMMIB L-21T were lower than 98.5%, and phenotypic and chemotaxonomic traits distinguished strain SCSIO 10427T from its closest phylogenetic neighbours, DNA–DNA relatedness studies were not performed.

Several physiological and biochemical characteristics support the distinctiveness of strain SCSIO 10427T from its closest relatives, including nitrate reduction, milk coagulation, milk peptonization, hydrolysis of urea, gelatin and starch, the temperature range for growth, and carbon and nitrogen source utilization. Strain SCSIO 10427T showed positive results in the tests for nitrate reduction and milk coagulation and peptonization, while strains N. chromatogenes YIM 90109T and N. baichengensis YIM 90130T showed negative results. The ability of strain SCSIO 10427T to hydrolyse gelatin differed from N. chromatogenes YIM 90109T, N. halophila DSM 44494T and N. potens IMMIB L-21T. The temperature range and optimum NaCl concentration for growth were different between strain SCSIO 10427T and its closest relatives. Strain SCSIO 10427T and N. halophila DSM 44494T possessed the same predominant menaquinones, which were different from the components of the other four related strains. Differences were also observed in fatty acid profiles and in DNA G+C contents (Table 1). Based on the phenotypic and genotypic results obtained in this study, strain SCSIO 10427T represents a novel species, for which the name Nocardiopsis coralliicola sp. nov. is proposed.

**Description of Nocardiopsis coralliicola sp. nov.**

Nocardiopsis coralliicola [co.ral.li.i’co.la. L. n. corallium coral; L. suff. –cola (from L. n. incola), inhabitant; N.L. n. coralliicola inhabitant of corals].

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences (1312 bp) showing the phylogenetic relationships between strain SCSIO 10427T and species of the genera Nocardiopsis and Streptomonospora. Bootstrap values (expressed as percentages of 1000 replications) ≥50% are given at the nodes. Asterisks indicate the clades that were also conserved when the maximum-likelihood and maximum-parsimony methods were used to construct phylogenetic trees. Bar, 1 nt substitution per 200 nt.
Vegetative hyphae are long, well-developed and fragmented. Aerial mycelia differentiate into long spore chains, and spores are smooth-surfaced and non-motile. Substrate mycelia are yellow–white to pale grey–yellow. A pale yellow soluble pigment is produced on nutrient agar. Growth occurs at 20–45 °C, at pH 7.0–10.0, and in the presence of up to 18 % (w/v) NaCl. Catalase is produced. Negative result in tests for the oxidase reaction and production of H₂S, but positive results for nitrate reduction, milk coagulation and peptization. Hydrolyses Trehwens 20 and 40, gelatin and hypoxanthine, but not Trehwen 80, urea, cellulosum, starch or adonine. Utilizes D-arabinose, D-fructose, D-galactose, D-glucose, D-mannose, D-mannitol, D-ribose and sodium acetate as sole carbon sources, but not cellulbiose, glycerc, inositol, lactose, maltose, raffinose, L-rhamnose, sodium pyruvate, D-sorbitol, sucrose, D-xyllose or xylitol. L-alanine, L-arginine, L-asparagine, L-glutamic acid, glycine, L-histidine, L-lysine, L-proline, L-serine, L-threonine and L-valine can be used as sole nitrogen sources, but not L-cysteine. The diagnostic diamino acid in the cell-wall peptidoglycan is meso-diaminopimelic acid, and ribose, glucose and mannose are present in whole-cell hydrolysates. Major phospholipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylcholine and three unknown phospholipids. The predominant menaquinones are MK-10(H₄) and MK-10(H₈). Major fatty acids (>10 %) are iso-C₁₆ : 0, anteiso-C₁₇ : 0, C₁₇ : 0ω8c and C₁₈ : 1ω9c.

The type strain, SCSIO 10427T (=CCTCC AA 2011010T =DSM 45611T), was isolated from the gorgonian coral *Menella praelonga*, collected from Weizhou Island, Guangxi province, China. The DNA G+C content is 69.5 mol%.

**Acknowledgements**

We would like to gratefully acknowledge the help of Miss Ling-Ling Yang for fatty acid and menaquinone analyses, and furthermore, the help of Dr Lu-Ping Zhang and Chao Long for the scanning electron microscopic analysis. This research was supported by the National Basic Research Program of China (No. 2010CB833801), the National Natural Science Foundation of China (No. 41106221), and the Academic Frontier Project for young researchers (No. SQ201013).

**References**


