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

Jie Li,1 Jian Yang,1 Wen-Yong Zhu,2 Jie He,2 Xin-Peng Tian,1 Qiong Xie,3 Si Zhang1 and Wen-Jun Li2

1Key Laboratory of Marine Bio-resources Sustainable Utilization CAS, RNAM Center for Marine Microbiology, Guangdong Key Laboratory of Marine Materia Medica, South China Sea Institute of Oceanology, Chinese Academy of Sciences, 164 West Xingang Road, Guangzhou 510301, PR China
2Key Laboratory of Microbial Diversity in Southwest China, Ministry of Education and Laboratory for Conservation and Utilization of Bio-Resources, Yunnan Institute of Microbiology, Yunnan University, Kunming 650091, PR China
3State Key Lab of Space Medicine Fundamentals and Application, China Astronaut Research and Training Center, Beijing 100094, PR China

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 (Kämpfer 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-dolomycin, thiopelote (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...
Strain SCSIO 10427^T 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 10427^T 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 10427^T 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, phosphatidymethylethanolamine, phosphatidylethanolamine, phosphatidylglycerol, 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(H6) (42 %) and MK-10(H8) (43 %), with minor amounts of MK-9(H6) (8 %) and MK-9(H8) (7 %). Major fatty acids (>10 %) of strain SCSIO 10427^T were iso-C16 : 0 (21.73 %), anteiso-C17 : 0 (14.42 %), C17 : 1(9c) (11.33 %) and C18 : 1(9c) (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 : 1(9c) (1.87 %), iso-C18 : 0 (1.63 %), iso-C14 : 0 (1.32 %) and C17 : 0 (1.17 %). The DNA G+C content of strain SCSIO 10427^T was 69.5 mol%.

The result of phylogenetic analyses indicated that strain SCSIO 10427^T 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 90109^T (98.4 % 16S rRNA gene sequence similarity), Nocardiopsis halophila DSM 44494^T (98.0 %) and Nocardiopsis baichengensis YIM 90130^T (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

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>−</td>
<td>ND</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Milk coagulation</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Milk peptonization</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gelatin</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Starch</td>
<td>−</td>
<td>+</td>
<td>ND</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Temperature for growth (°C):</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>20–45</td>
<td>20–60</td>
<td>15–37</td>
<td>20–50</td>
<td>15–50</td>
<td>20–37</td>
</tr>
<tr>
<td>Optimum</td>
<td>28–37</td>
<td>37–40</td>
<td>28</td>
<td>37–40</td>
<td>37</td>
<td>28</td>
</tr>
<tr>
<td>NaCl concentration for growth (% w/v):</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0–18</td>
<td>0–18</td>
<td>3–20</td>
<td>0–18</td>
<td>0–15</td>
<td>0–12</td>
</tr>
<tr>
<td>Optimum</td>
<td>3–7</td>
<td>5–8</td>
<td>5–15</td>
<td>5–8</td>
<td>10</td>
<td>ND</td>
</tr>
<tr>
<td>Carbon source utilization:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellobiose</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycerol</td>
<td>−</td>
<td>+</td>
<td>ND</td>
<td>−</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Inositol</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Lactose</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Maltose</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Raffinose</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>1-&lt;wbr/&gt;Rhamnose</td>
<td>−</td>
<td>+</td>
<td>W</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Sodium acetate</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Sucrose</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>D-Xylose</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Nitrogen source utilization:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Glutamic acid</td>
<td>+</td>
<td>−</td>
<td>ND</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Chemotaxonomic characteristic:*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Major menaquinones</td>
<td>MK-10(H&lt;sub&gt;4&lt;/sub&gt;), MK-10(H&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>MK-10, MK-10(H&lt;sub&gt;2&lt;/sub&gt;), MK-10(H&lt;sub&gt;4&lt;/sub&gt;)</td>
<td>MK-10(H&lt;sub&gt;4&lt;/sub&gt;), MK-10(H&lt;sub&gt;2&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;2&lt;/sub&gt;), MK-10(H&lt;sub&gt;4&lt;/sub&gt;)</td>
<td>MK-10(H&lt;sub&gt;4&lt;/sub&gt;), MK-10(H&lt;sub&gt;2&lt;/sub&gt;), MK-10(H&lt;sub&gt;4&lt;/sub&gt;)</td>
<td>MK-10(H&lt;sub&gt;4&lt;/sub&gt;), MK-11(H&lt;sub&gt;3&lt;/sub&gt;)</td>
</tr>
<tr>
<td>Major fatty acids (&gt;10%):&lt;sup&gt;†&lt;/sup&gt;</td>
<td>iso-C&lt;sub&gt;16&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt; (21.73%), anteiso-C&lt;sub&gt;17&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt; (14.42%), C&lt;sub&gt;17&lt;/sub&gt;:&lt;sub&gt;1&lt;/sub&gt;iso8c (11.33%), C&lt;sub&gt;18&lt;/sub&gt;:&lt;sub&gt;1&lt;/sub&gt;iso9c (10.38%)</td>
<td>iso-C&lt;sub&gt;16&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt; (26.02%), anteiso-C&lt;sub&gt;17&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt; (10.07%), TBSA (29.38%)</td>
<td>ND</td>
<td>iso-C&lt;sub&gt;16&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt; (24.17%), anteiso-C&lt;sub&gt;17&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt; (13.64%), TBSA (33.19%)</td>
<td>ND</td>
<td>iso-C&lt;sub&gt;16&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt; (16.0%), anteiso-C&lt;sub&gt;15&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt; (18.9%), anteiso-C&lt;sub&gt;17&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt; (12.8%)</td>
</tr>
<tr>
<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>
</tr>
</tbody>
</table>

* 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 IMMIB L-21T 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.cola. 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 mycelia are long, well-developed and fragment-
ted. 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
coaulation and peptization. Hydrolysates Tween 20 and
40, gelatin and hypoxanthine, but not Tween 80, urea,
cellulose, starch or adenine. 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, glyceral, inositol, lactose, maltose, raffinose, L-
rhamnose, sodium pyruvate, D-sorbitol, sucrose, D-xylene
or xyitol. 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 diphosphatidylgly-
cerol, phosphatidylethanolamine, phosphatidylletha-
nolamine, phosphatidylglycerol, phosphatidylcholine and
three unknown phospholipids. The predominant mena-
quiones are MK-10(H₁₀) and MK-10(H₁₂). Major fatty
acids (>10 %) are iso-C₁₆:0 anteiso-C₁₇:0 C₁₇:0 10 mol% and
C₁₈:1 96 mol%.

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. 2010CB83801), the National Natural Science Foundation of China (No. 41106139), and the Academic Frontier Project for young researchers (No. SQ201013).

**References**


Microbiol 425. 57

Microbiol description of Nocardiopsis Environ Microbiol

N. exhalans species, method for reconstructing phylogenetic trees.

and emended description of the genus novel actinobacterium isolated from forest soil in Yunnan (China), Nocardiopsis the genus

International Journal of Systematic and Evolutionary Microbiology


