**Nocardiopsis litoralis** sp. nov., a halophilic marine actinomycete isolated from a sea anemone

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A Gram-positive, moderately halophilic, alkalitolerant, filamentous, aerobic actinomycete, designated strain JSM 073097T, was isolated from a sea anemone collected from a tidal flat in the South China Sea. Phylogenetic analyses based on 16S rRNA gene sequences indicated that the new isolate was a member of the genus *Nocardiopsis* and was most closely related to *Nocardiopsis kunsanensis* HA-9T, *Nocardiopsis xinjiangensis* YIM 90004T and *Nocardiopsis salina* YIM 90010T (99.6, 98.5 and 98.1 % similarity, respectively). Phenotypic characteristics and chemotaxonomic data also indicated that strain JSM 073097T was a member of the genus *Nocardiopsis*. The strain grew well on most of the media tested, producing white to yellow–white substrate mycelium and white aerial mycelium and straight to flexuous hyphae. The substrate mycelium was well developed and fragmented with age; the aerial mycelium produced long, straight to flexuous spore chains with non-motile, smooth-surfaced, rod-shaped spores. The strain grew in the presence of 1–15 % (w/v) total salts and at pH 6.0–10.5 and 20–35 °C; optimum growth occurred in the presence of 5–7 % (w/v) total salts and at pH 8.5 and 25 °C. Whole-cell hydrolysates of strain JSM 073097T contained meso-diaminopimelic acid and no diagnostic sugars. The predominant menaquinones were MK-10(H4), MK-10(H6) and MK-10(H8). The major cellular fatty acids were iso-C15 : 0, iso-C16 : 0, anteiso-C16 : 0 and 10-methyl C18 : 0. Polar lipids comprised diphosphatidylglycerol, phosphatidylcholine and phosphatidylglycerol. The DNA G+C content of strain JSM 073097T was 70.4 mol%. The combination of phylogenetic analysis, DNA–DNA relatedness data, phenotypic characteristics and chemotaxonomic data supported the suggestion that strain JSM 073097T represents a novel species of the genus *Nocardiopsis*, for which the name *Nocardiopsis litoralis* sp. nov. is proposed. The type strain is JSM 073097T (=DSM 45168T =KCTC 19473T).

The genus *Nocardiopsis* was first described by Meyer (1976) and, at the time or writing, comprises 28 recognized species (Meyer, 1976; Grund & Kroppenstedt, 1990; Yassin et al., 1993, 1997; Al-Tai & Ruan, 1994; Chun et al., 2000; Evtushenko et al., 2000; Peltola et al., 2001; Al-Zarban et al., 2002; Kämpfer et al., 2002; Schippers et al., 2002; Li et al., 2003, 2004, 2006; Hozzein et al., 2004; Sabry et al., 2004; Chen et al., 2008; Hozzein & Goodfellow, 2008; Yang et al., 2008; Zhang et al., 2008). During an investigation of the diversity of the microbial population of invertebrates inhabiting the South China Sea (Chen et al., 2009a, b; Xiao et al., 2009), a moderately halophilic, alkalitolerant,
Strain JSM 073097<sup>T</sup> was isolated from homogenates of a sea anemone collected from a tidal flat near Naozhou Island, southern China. Based on the results of the present taxonomic study, this strain is considered to represent a novel species of the genus *Nocardiopsis*.

Strain JSM 073097<sup>T</sup> was isolated from plate 1:10 serial dilutions of the sample on marine agar 2216 (MA; Difco) supplemented with 10 % (w/v) NaCl and cultivating at 25 °C for 2 weeks. After primary isolation and purification, the isolate was preserved both on slants of MA supplemented with 3 % (w/v) NaCl (containing approximately 5 % NaCl and 6.4 % total salts, pH 8.5; hereafter referred to as MA3) at 4 °C and in marine broth 2216 (MB; Difco) supplemented with 20 % (v/v) glycerol at −80 °C. *Nocardiopsis kunsanensis* DSM 44524<sup>T</sup>, *Nocardiopsis xinjiangensis* YIM 90004<sup>T</sup> and *Nocardiopsis salina* YIM 9010<sup>T</sup> were obtained from the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany) and the YIM (Yunnan Institute of Microbiology, Kunming, China). Morphological characteristics were observed by using light microscopy (BH 2; Olympus) and scanning electron microscopy (JSM5600LV; JEOL) after 2 and 4 weeks growth on MA3. Cultural characteristics were determined after 2–4 weeks by using the methods applied in the International *Streptomyces* Project (Shirling & Gottlieb, 1966). The colours of both substrate and aerial mycelia and any soluble pigments produced were determined by comparison with chips from the colour charts of the Inter-Society Colour Council – National Bureau of Standards (Kelly, 1964). Growth was tested at various temperatures (5–45 °C, at increments of 5 °C) and pH (5.0–11.0, at pH increments of 0.5) in MB supplemented with 3 % (w/v) NaCl and on MA3. Turbidity was monitored at 610 nm after the MB tubes had been incubated for 5 days in a reciprocal water-bath shaker. Growth at different total salt contents (0, 0.1 and 0.5 %, w/v, and 1–20 %, w/v, at increments of 1 %) was determined on MA prepared according to the formula of Atlas (1993). The concentration of total salts contained in MA was changed (proportions maintained) as an integer in the salt-response experiment. Growth under anaerobic conditions and catalase and oxidase activities were detected as described previously (Chen et al., 2007). The media and procedures used to determine physiological and biochemical features, as well as utilization of carbon and nitrogen sources, were those described by Shirling & Gottlieb (1966) and Kroppenstedt & Evtushenko (2006). Unless indicated otherwise, all tests were carried out with 6 % (w/v) NaCl at pH 8.5 with incubation at 25 °C.

Strain JSM 073097<sup>T</sup> showed macroscopic and microscopic characteristics typical of most species of the genus *Nocardiopsis* (Meyer, 1976, 1994; Kroppenstedt & Evtushenko, 2006). The novel strain was Gram-positive, obligately aerobic and catalase-positive. It formed circular colonies that had white mycelia on MA3. Good growth was also observed on yeast extract-malt extract, oatmeal, peptone-yeast extract-iron, Czapek’s, nutrient and potato extract agar media supplemented with 6 % (w/v) NaCl, but poor growth occurred on inorganic salts-starch and glycerol-asparagine agar media supplemented with 6 % (w/v) NaCl. Substrate mycelia were well developed and fragmented with age. Aerial mycelia produced long, straight to flexuous spore chains with non-motile, smooth-surfaced, rod-shaped spores (see Supplementary Fig. S1 in IJSME Online). No diffusible pigments were produced on any of the media tested. Strain JSM 073097<sup>T</sup> was able to grow at 20–35 °C (optimum 25 °C) and at pH 6.0–10.5 (optimum pH 8.5) in the presence of 1–15 % (w/v) total salts (optimum 5–7 %). The results of other phenotypic tests are summarized in the species description below and in Table 1.

DNA was isolated according to the method of Hopwood et al. (1985) and the G+C content was determined by using the HPLC method (Mesbah et al., 1989). The 16S rRNA gene was amplified and sequenced as described by Cui et al. (2001). The resulting 16S rRNA gene sequence was compared with sequences obtained from public databases (GenBank/EMBL/DDBJ) to find the most closely related species. Multiple alignments with sequences of the most closely related strains were carried out by using CLUSTAL_X (Thompson et al., 1997). Distances were calculated by using distance options according to Kimura’s two-parameter model (Kimura, 1980). A neighbour-joining (Saitou & Nei, 1987) tree was constructed by using the software package MEGA version 4.1 (Tamura et al., 2007). Maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) trees were constructed using the treeing algorithms contained in the PHYLIB package (Felsenstein, 2002). Confidence values for the branches of phylogenetic trees were determined by using bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings. DNA–DNA hybridization experiments were carried out by using photobiotin-labelled probes in microplate wells as described by Ezaki et al. (1989).

The DNA G+C content of strain JSM 073097<sup>T</sup> was 70.4 mol%. An almost-complete 16S rRNA gene sequence (1404 bp) of this organism was determined. Phylogenetic analyses based on 16S rRNA gene sequences revealed that strain JSM 073097<sup>T</sup> belonged to the genus *Nocardiopsis*, and was most closely related to the type strain of *N. kunsanensis* (99.6 % similarity; Chun et al., 2000), followed by those of *N. xinjiangensis* (98.5 %; Li et al., 2003) and *N. salina* (98.1 %; Li et al., 2004). The four strains formed a robust lineage supported by a significant bootstrap resampling value (99 %) in the neighbour-joining tree (Fig. 1). The topology was similar to those of the phylogenetic trees constructed by using the maximum-likelihood and maximum-parsimony methods (see Supplementary Fig. S2). Levels of DNA–DNA relatedness between strain JSM 073097<sup>T</sup> and the type strains of *N. kunsanensis*, *N. xinjiangensis* and *N. salina* were 30.7 ± 2.8, 20.6 ± 2.3 and 22.1 ± 2.9 % (mean ± SD of 3 determinations),
respectively. These values are far below the threshold of 70% recommended by Wayne et al. (1987) for assignment of strains to the same species. The other closest phylogenetic neighbours of strain JSM 073097T (showing 16S rRNA gene sequence similarity 97%) were the type strains of Nocardiopsis aegyptia (97.7%), Nocardiopsis lucentensis (97.5%), Nocardiopsis alba (97.4%), Nocardiopsis dassonvillei subsp. dassonvillei (97.4%) and Nocardiopsis dassonvillei subsp. albirubida (97.2%). These levels of sequence similarity are lower than those found for some other individual pairs of recognized Nocardiopsis species, as discussed previously (Al-Zarban et al., 2002; Schippers et al., 2002; Hozzein et al., 2004; Sabry et al., 2004; Yang et al., 2008). Furthermore, Stackebrandt & Ebers (2006) suggested an increase from 97 to 98.7–99% in the 16S rRNA gene sequence similarity threshold used for the delineation of separate species, provided that these data are supported by clear phenotypic differences. On this basis, together with DNA–DNA hybridization data, the present phylogenetic analyses strongly suggested that strain JSM 073097T represents a previously unknown species of the genus Nocardiopsis.

Biomass for chemical studies was obtained from cultures grown in MB (pH 8.5) supplemented with 3% (w/v) NaCl on a rotary shaker (about 200 r.p.m.) at 25°C for 3 days. Cells were harvested by centrifugation and were washed twice with distilled water. Amino acids and sugars of whole-cell hydrolysates were analysed as described by Staneck & Roberts (1974). Polar lipids were extracted, examined by using two-dimensional TLC and identified by using standard procedures (Minnikin et al., 1984). Menaquinones were isolated by using the methods of Minnikin et al. (1984) and were then separated by HPLC (Kroppenstedt, 1982, 1985). Fatty acids were determined as described by Sasser (1990) by using the Microbial Table 1. Differential characteristics between strain JSM 073097T and phylogenetically related species of the genus Nocardiopsis

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<tr>
<td>Fragmentation of substrate mycelia</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td>NaCl range (optimum) (%) w/v)</td>
<td>1-15 (5-7)</td>
<td>3-20 (10)</td>
<td>3-20 (10)</td>
<td>3-20 (10)</td>
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<tr>
<td>Temperature range (optimum) (°C)</td>
<td>20-35 (25)</td>
<td>20-50 (37)</td>
<td>20-40 (28)</td>
<td>20-40 (28)</td>
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<tr>
<td>pH range (optimum)</td>
<td>6.0-10.5 (8.5)</td>
<td>7.0-11.0 (9.0)</td>
<td>6.0-10.0 (7.2)</td>
<td>6.0-9.0 (7.2)</td>
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<td>Urease activity</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<td>–</td>
<td>–</td>
<td>+</td>
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<td>Nitrate reduction</td>
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<td>Casein</td>
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<td>+</td>
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<td>+</td>
<td>W</td>
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<td>Starch</td>
<td>–</td>
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<td>Utilization of:</td>
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<td>d-Cellobiose</td>
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<td>+</td>
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<td>d-Fructose</td>
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<td>L-Proline</td>
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<td>L-D-Xyllose</td>
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<td>Diagnostic sugars</td>
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<tr>
<td>Major phospholipids‡</td>
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<td>None</td>
<td>Xylose, arabinose, galactose</td>
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<tr>
<td>Major fatty acids</td>
<td>iso-C15:0, iso-C16:0, anteiso-C16:0, 10-methyl C18:0</td>
<td>iso-C15:0, anteiso-C16:0, 10-methyl C18:0</td>
<td>iso-C14:0, anteiso-C15:0, iso-C16:0, C18:0, 10-methyl C18:0</td>
<td>iso-C16:0, C18:109c, 10-methyl C18:0</td>
</tr>
<tr>
<td>Major menaquinone(s)</td>
<td>MK-10(H8, H9, H8)</td>
<td>MK-10(H8)</td>
<td>MK-10(H8, H9, H4)</td>
<td>MK-9(H8), MK-10(H8)</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>70.4</td>
<td>71</td>
<td>74.3</td>
<td>73.1</td>
</tr>
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</table>

*Data for strain JSM 073097T were for total salts.
†Data obtained from Li et al. (2004).§DPG, diphasphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PME, phosphatidylmethylethanolamine.

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Table 1. Differential characteristics between strain JSM 073097T and phylogenetically related species of the genus Nocardiopsis

Taxa: 1, strain JSM 073097T (data from the present study); 2, N. kunsanensis HA-9T (Chun et al., 2000); 3, N. xinjiangensis YIM 90004T (Li et al., 2003); 4, N. salina YIM 90010T (Li et al., 2006). +, Positive; –, negative; W, weak reaction; ND, no data available.

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Identification System (MIDI; Microbial ID). Chemotaxonomic data for strain JSM 073097T were consistent with its assignment to the genus Nocardiopsis (Meyer, 1976, 1994; Kroppenstedt & Evtyushenko, 2006). Whole-cell hydrolysates contained meso-diaminopimelic acid as the diagnostic diamino acid and no diagnostic sugars. Polar lipids of the novel strain comprised diphosphatidylglycerol, phosphatidylcholine and phosphatidylglycerol. The predominant menaquinones were MK-9(H6) (5.8 %), MK-9(H8) (2.7 %) and MK-10(H8) (36.8 %) and MK-10(H6) (32.3 %), with MK-9(H6) (5.8 %), MK-9(H8) (2.7 %) and MK-10(H8) (36.8 %) present as minor components. The fatty acid profile of strain JSM 073097T was similar to those of related Nocardiopsis species (Table 1). The type strain, JSM 073097T, was isolated from homogenates of a sea anemone collected from a tidal flat on Naozhou Island in the South China Sea, near Zhanjiang City, southern China. The DNA

Nocardiopsis litoralis sp. nov.

Nocardiopsis litoralis (li.to.ra’lis. L. adj. litoralis of the shore).

Gram-positive, moderately halophilic, alkalitolerant, non-motile, filamentous, aerobic actinomyces which forms white aerial mycelium and white to yellow-white substrate mycelium and straight to flexuous hyphae. Good growth occurs on most of the media tested. Diffusible pigments are not produced. Substrate hyphae are well developed and fragment with age. Long, straight to flexuous spore chains are borne on aerial hyphae, which fragment into elongated non-motile spores (0.5–0.7 × 1.5–1.8 µm in size) with smooth surfaces. Grows optimally at 25 °C and pH 8.5 and in the presence of 5–7 % (w/v) total salts. Growth is not observed in the absence of salts. Positive for catalase but negative for oxidase. Unable to reduce nitrate to nitrite. H₂S and melanin are not produced. Degrades adenine, gelatin, hypoxanthine, tyrosine and xanthine, but not ascin, casein, cellulose, chitin, DNA, starch, Tween (20, 40, 60, 80) or urea. The following compounds are utilized as sole source of carbon and energy or sole source of carbon, nitrogen and energy: D-glucose, sucrose, D-xylene and L-alanine. The following substances are not utilized: L-arabinose, cellobiose, dextrin, D-fructose, D-galactose, maltose, D-mannose, melezitose, melibiose, raffinose, L-rhamnose, D-ribose, D-salicin, trehalose, adonitol, D-arabitol, glycerol, myo-inositol, D-mannitol, D-sorbitol, acetate, citrate, gluconate, L-arginine, L-asparagine, L-glutamic acid, glycine, L-histidine, hydroxy-L-proline, L-isoleucine, L-leucine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine and L-valine. Whole-cell hydrolysates contain meso-diaminopimelic acid and no diagnostic sugars. The predominant menaquinones are MK-9(H6) (32.3 %), MK-9(H8) (36.8 %) and MK-10(H8) (36.8 %), with MK-9(H6) (5.8 %), MK-9(H8) (2.7 %) and MK-10(H8) (36.8 %) present as minor components. The fatty acid profile of strain JSM 073097T was similar to those of related Nocardiopsis species (Table 1). The major fatty acids of the novel strain were iso-C₁₅:0 (10.1 % of the total), iso-C₁₆:0 (28.2 %), anteiso-C₁₆:0 (11.8 %) and 10-methyl C₁₈:0 (11.0 %), with iso-C₁₄:0 (4.4 %), anteiso-C₁₅:0 (5.7 %), C₁₆:0 10c (2.4 %), C₁₆:0 (3.7 %), iso-C₁₇:0 (1.1 %), anteiso-C₁₇:0 (5.1 %), C₁₇:0 (1.2 %), 10-methyl C₁₇:0 (1.5 %), C₁₈:1ω9c (6.8 %) and C₁₈:0 (4.5 %) present as minor components: iso-C₁₃:0 (5.0 %), C₁₄:0 (7.9 %) and C₁₇:1ω8c and iso-C₁₈:0 were detected at a level of <1 %.

The results of phylogenetic analyses and of morphological and chemotaxonomic investigations supported the view that strain JSM 073097T should be assigned to the genus Nocardiopsis (Meyer, 1976, 1994; Kroppenstedt & Evtyushenko, 2006). However, the comparatively low salinities [1–15 % (w/v) total salts (optimum 5–7 %)] and temperatures [20–35 °C (optimum 25 °C)] for growth, as well as its ability to utilize D-xylene as sole carbon source, markedly differentiated the new isolate from the phylogenetically related Nocardiopsis species (Table 1). Strain JSM 073097T could also be distinguished from its closest phylogenetic neighbour, N. kunsanensis, based on other physiological, biochemical, nutritional and chemotaxonomic differences (see Table 1). Data from the present polyphasic taxonomic study, together with DNA–DNA hybridization data, indicated that strain JSM 073097T represents a novel species of the genus Nocardiopsis, for which we propose the name Nocardiopsis litoralis sp. nov.

Description of Nocardiopsis litoralis sp. nov.

Nocardiopsis litoralis (li.to.ra’lis. L. adj. litoralis of the shore).
G+C content of the type strain is 70.4 mol% (HPLC method).

Acknowledgements

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References


