Nocardiopsis flavescens sp. nov., an actinomycete isolated from marine sediment

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A Gram-positive, aerobic bacterium, strain SA6T, was isolated from marine sediment taken at a depth of 20 cm on the seashore of Lianyungang, Jiangsu Province, China. Strain SA6T contained meso-diaminopimelic acid, no diagnostic sugars, type PIII phospholipids, and MK-10(H2) and MK-10(H4) as the predominant menaquinones. The organism showed a range of chemical and morphological properties consistent with its classification in the genus Nocardiopsis. The almost-complete 16S rRNA gene sequence of strain SA6T was aligned with corresponding sequences of representatives of the genus Nocardiopsis and related taxa by using two tree-making algorithms. Strain SA6T formed a distinct phyletic line within the evolutionary radiation occupied by the genus Nocardiopsis and was related most closely to the type strain of Nocardiopsis lucentensis. Strain SA6T could be distinguished from its nearest phylogenetic relatives in the genus Nocardiopsis based on DNA–DNA relatedness data and a combination of phenotypic properties. Strain SA6T should therefore be assigned to the genus Nocardiopsis as a representative of a novel species, for which the name Nocardiopsis flavescens sp. nov. is proposed. The type strain is SA6T (=CGMCC 4.5723T =JCM 17424T).

The genus Nocardiopsis was proposed by Meyer (1976) and, at the time of writing, comprises 33 recognized species. Nocardiopsis strains are distributed ubiquitously in the environment (Kämpfer et al., 2002; Kroppenstedt & Evtushenko, 2002; Hozzein et al., 2004). They are frequently isolated from habitats with moderate to high salt concentrations such as saline soil or marine sediments (Al-Zarban et al., 2002; Al-Tai & Ruan, 1994; Evtushenko et al., 2000) and salterns (Chun et al., 2000). The genus Nocardiopsis comprises actinomycetes that possess meso-diaminopimelic acid as the cell-wall diamino acid, have no characteristic sugar in whole-cell hydrolysates and no mycolic acids in whole-cell methanolysates (cell-wall chemotype III), have phosphatidylcholine as a characteristic phospholipid (phospholipid type III), have fatty acid profiles which comprise saturated, unsaturated, iso-branched, anteiso-branched and tuberculostearic acids, contain menaquinones with 10 isoprene units with a high degree of hydrogenation as major components, and have DNA G+C contents of 64–71 mol% (Grund & Kroppenstedt, 1990). The aim of this study was to classify a novel actinomycete (designated strain SA6T) isolated from marine sediment by using morphological, physiological, chemotaxonomic and molecular biological methods.

Strain SA6T was isolated on glucose-yeast extract-malt extract [GYM; International Streptomyces Project (ISP) 2 medium; Shirling & Gottlieb, 1966] agar plates, which had been seeded with a sea mud suspension and incubated at 35 °C for 2 weeks. The marine sediment was taken at a depth of 20 cm on the seashore of Lianyungang, Jiangsu Province, China. Strain SA6T was maintained on GYM agar at 4 °C and as suspensions of mycelial fragments in 20 % (v/v) glycerol at −20 °C. Biomass for chemotaxonomic and molecular systematic studies was prepared as described by Zhang et al. (2002).

Extraction of genomic DNA, PCR-mediated amplification of the 16S rRNA gene and purification of the PCR product from isolate SA6T were carried out as described by Rainey et al. (1996). The PCR product was sequenced directly following the procedure described by Lu et al. (2001). Sequence gel electrophoresis was performed and the nucleotide sequences were obtained automatically by using an Applied Biosystems DNA sequencer (model 377) and software provided by the manufacturer. Identification of
phylogenetic neighbours and calculation of levels of pairwise 16S rRNA gene sequence similarity were achieved by using MEGA, version 4.1 (Tamura et al., 2007). The resultant 16S rRNA gene sequence of strain SA6\(^T\) was aligned manually with corresponding sequences of representatives of genera classified in the suborder Streptosporangiineae (Zhi et al., 2009) retrieved from the DDBJ/EMBL/GenBank databases via CLUSTAL X 1.8 software (Thompson et al., 1997). Evolutionary distances were calculated by using distance options according to Kimura’s two-parameter model (Kimura, 1980). Phylogenetic trees were reconstructed with the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Kluge & Farris, 1969) algorithms in MEGA, version 4.1 (Tamura et al., 2007). Topologies of the resultant unrooted trees were evaluated by bootstrap analyses (Felsenstein, 1985) of the neighbour-joining dataset, based on 1000 resamplings. The almost-complete 16S rRNA gene sequence of strain SA6\(^T\) was determined (1401 nt). It is apparent from the neighbour-joining tree shown in Fig. 1 that strain SA6\(^T\) forms a monophyletic clade with the type strain of *Nocardiopsis lucentensis*. Sequence similarity calculations after neighbour-joining analysis indicated that the closest relatives of strain SA6\(^T\) were *N. lucentensis* DSM 44048\(^T\) (99.0 % 16S rRNA gene sequence similarity, Yassin et al., 1993), *Nocardiopsis aegyptia* DSM 44442\(^T\) (98.6 %, Sabry et al., 2004), *Nocardiopsis synnemataformans* IMMIB D-1215\(^T\) (98.6 %, Yassin et al., 1997) and *Nocardiopsis dassonvillei* subsp. *alibirubida* DSM 40465\(^T\) (98.6 %, Evtushenko et al., 2000). These similarity values are lower than those found for some other individual pairs of species of the genus *Nocardiopsis*, for example between *Nocardiopsis exhalans* DSM 44407\(^T\) and *Nocardiopsis prasina* DSM 43845\(^T\) (99.4 %, Peltola et al., 2001), between *Nocardiopsis metallicus* DSM 44598\(^T\) and *N. exhalans* DSM 44407\(^T\) (99.4 %), between *N. metallicus* DSM 44598\(^T\) and *N. prasina* DSM 43845\(^T\) (99.3 %, Schippers et al., 2002), between *Nocardiopsis litoralis* DSM 45168\(^T\) and *Nocardiopsis kunas-nensis* DSM 44524\(^T\) (99.6 %, Chen et al., 2009), or between *Nocardiopsis valliformis* DSM 45023\(^T\) and *N. exhalans* DSM 44407\(^T\) (99.93 %, Yang et al., 2008). It is also significant that the 16S rRNA gene sequence of strain SA6\(^T\) includes signature nucleotides that are characteristic of members of the genus *Nocardiopsis* (Meyer, 1994).

The new isolate was examined for a range of chemotaxonomic markers to determine whether its chemical profile was typical of members of the genus *Nocardiopsis* (Meyer, 1994). Standard procedures were used for the extraction and analysis of the diagnostic isomers of diaminopimelic acid (Hasegawa et al., 1983) and whole-organism sugars (Lechevalier & Lechevalier, 1980), menaquinones (Collins et al., 1987; Wu et al., 1989), mycolic acids (Minnikin et al., 1975) and polar lipids (Minnikin et al., 1984), by using appropriate controls. Fatty acids were extracted, purified, methylated and quantified by GC with the standard Fig. 1. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing relationships between strain SA6\(^T\) and representatives of recognized species of the genus *Nocardiopsis*. The sequence of *Actinomadura madurae* DSM 43067\(^T\) was used as an outgroup. Asterisks at nodes indicate branches that were also obtained in the maximum-parsimony tree. Numbers at nodes indicate percentages of bootstrap support, based on a neighbour-joining analysis of 1000 resampled datasets; only values above 50 % are given. Bar, 0.01 substitutions per nucleotide position.
Microbial Identification System (TSBA40, MIDI version 6; Sasser, 1990; Kämpfer & Kroppenstedt, 1996). The base composition of the genomic DNA of the isolate was determined by using the thermal denaturation method (Marmur & Doty, 1962) with *Escherichia coli* AS 1.365 as the control. The organism contained meso-diaminopimelic acid as the cell-wall diamino acid in the peptidoglycan and no diagnostic sugars in whole-cell hydrolysates (wall chemotype III/C sensu Lechevalier & Lechevalier, 1970). The predominant menaquinones were MK-10(H$_2$) and MK-10(H$_4$), with MK-10, MK-10(H$_6$), MK-9(H$_2$), MK-9(H$_4$) and MK-9(H$_6$) present as minor components. The phospholipid type was type PIII sensu Lechevalier et al. (1977), and phosphatidylcholine was the characteristic phospholipid. Other phospholipids, such as phosphatidylethanolamine, phosphatidylglycerol and diphasatidylglycerol, were also detected. This pattern matched quite well with those found in recognized species of the genus *Nocardiopsis* (Kroppenstedt & Evtushenko, 2002). The fatty acid pattern of strain SA6$^T$ was mainly composed of iso- and anteiso-branched components together with 10-methyl branched-chain fatty acids with 17 and 18 carbon atoms, straight-chain saturated (C16:0, C17:0, C18:0) and unsaturated (C17:1, C18:1) components (see Supplementary Table S1 available in IJSEM Online); no hydroxy fatty acids were detected. Strain SA6$^T$ lacked mycolic acids. The G+C content of the DNA of strain SA6$^T$ was 68.6 mol%. These properties were consistent with the classification of the isolate in the genus *Nocardiopsis* (Grund & Kroppenstedt, 1990; Kroppenstedt, 1992).

The organism was grown on GYM agar, trypticase soy agar, modified Sauton’s agar (Mordarska et al., 1972) and standard ISP media (Shirling & Gottlieb, 1966) for 3–14 days at 35 °C, and was examined for pigmentation, aerial mycelium and other morphological features. The colours of both substrate and aerial mycelia and any soluble pigments produced were determined by comparison with those of *Kelly* (1964). Growth over various temperature, pH and NaCl ranges was determined on GYM agar plates incubated for up to 14 days. Additional physiological and biochemical properties were determined by using standard media and standard ISP media.

### Table 1. Differential phenotypic characteristics between strain SA6$^T$ and the type strains of closely related species of the genus *Nocardiopsis*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
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<td><strong>Utilization of:</strong></td>
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<td>White/yellowish grey</td>
<td>White</td>
<td>Greys yellow</td>
<td>White</td>
<td>Yellow or colourless</td>
<td>White/yellowish white</td>
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<tr>
<td>D-Fructose</td>
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<td>+</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
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<td>+</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
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<td>+</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>myo-Inositol</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
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<td>+</td>
<td>+</td>
<td>ND</td>
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<td>+</td>
<td>+</td>
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<tr>
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<td>+</td>
<td>+</td>
<td>ND</td>
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<tr>
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<td>+</td>
<td>ND</td>
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<tr>
<td>Melibiose</td>
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<td>+</td>
<td>+</td>
<td>ND</td>
<td>+</td>
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<tr>
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<td>ND</td>
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<tr>
<td><strong>Growth at/with:</strong></td>
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<td>10 °C</td>
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<td>42 °C</td>
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<tr>
<td>5% NaCl</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>10% NaCl</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td><strong>Major menaquinone(s)</strong></td>
<td>10/2, 10/4</td>
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<td>10/6, 10/8</td>
<td>10/0, 10/2</td>
<td>10/0, 10/2</td>
<td>10/4, 10/6</td>
<td>10/4, 10/6</td>
<td>10/0, 10/2</td>
<td>10/4, 10/6</td>
<td>10/6, 10/8</td>
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<tr>
<td>Urease activity</td>
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<td>ND</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>Nitrate reductase</td>
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<td>-</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
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</table>

*For example, 10/4=MK-10(H$_4$).*
Nocardiopsis flavescens sp. nov.

**Description of Nocardiopsis flavescens sp. nov.**

*Nocardiopsis flavescens* [fla’ve.sens. L. part. adj. flavescens (from L. v. flavescio), becoming golden yellow in colour].

Aerobic, Gram-positive, non-motile actinomycete. A pale yellow to yellowish brown substrate mycelium is formed on GYM agar, ISP media 3, 4 and 5, trypticase soy agar and modified Sauton’s agar. The aerial mycelium is white on most media tested. Soluble pigments are not produced. Colony elevation is convex to irregular and colony margins are filamentous. Substrate hyphae are long, well developed and fragment with age. Aerial hyphae are long, and at the beginning of sporulation are more or less zigzag-shaped. The zigzag-shaped hyphae subdivide into smaller spores. Spores are rod-shaped, smooth and non-motile. Optimum growth occurs at 35 °C, at pH 7.2–7.5 and in the presence of 0–3 % (w/v) NaCl. Grows at 23–40 °C, at pH 5.5–11.0 and in the presence of 0–10% (w/v) NaCl. Hydrolases

acesculin, arbutin and gelatin, but not urea. Nitrate is reduced. Degrades casein, elastin, starch and L-tyrosine. Positive for catalase, phosphatase, β-galactosidase and β-glucosidase. Acid is formed from D-glucose, lactose (weakly), myo-inositol, D-mannitol, D-rhamnose, D-sorbitol (weakly) and D-xylene, but not from adonitol, dulcitol, meso-erythritol or methyl D-glucoside. L-Arabinose (weakly), D-fructose, D-galactose, D-glucose, glycerol, myo-inositol, lactose, maltose, D-mannitol, D-mannose, melibiose, D-rhamnose, D-ribose, sucrose, trehalose, D-xylene, acetate, citrate, L-lactate, L-malate, propionate and succinate are used as sole carbon and energy sources, whereas raffinose and oxalate are not. Resistant to lincomycin (128 μg ml⁻¹), gentamicin (64 μg ml⁻¹), penicillin G (128 μg ml⁻¹), streptomycin (64 μg ml⁻¹) and neomycin (4 μg ml⁻¹) but not to rifampicin (128 μg ml⁻¹). Chemotaxonomic properties are typical of the genus *Nocardiopsis*. Whole-cell hydrolysates contain meso-diaminopimelic acid but no diagnostic sugars. The predominant meningiaines are MK-10(H₂) and MK-10(H₄). Polar lipids comprise phosphatidylcholine, phosphatidylmethylethanolamine, phosphatidylglycerol and diphasatidylglycerol. The major fatty acids are anteiso-C₁₇:₀, iso-C₁₆:₀ and C₁₈:₀. C₁₇:₀ 10ΩC, iso-C₁₈:₀, anteiso-C₁₅:₀, C₁₇:₀, C₁₆:₀, C₁₇:₀, C₁₈:₀, C₁₈:₀ 10methyl C₁₇:₀, C₁₇:₀ 10methyl C₁₇:₀, anteiso-C₁₄:₀ and C₁₇:₀ 10OCl are present as minor components. Mycolic acids are absent. The DNA G+C content of the type strain is 68.6 mol%.

The type strain, SA6T (=CGMCC 4.5723T =JCM 17424T), was isolated from marine sediment at a depth of 20 cm on the seashore of Lianyungang, Jiangsu Province, China. The species description is based on a single strain.

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