Nocardiopsis aegyptia sp. nov., isolated from marine sediment

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An actinomycete, strain SNG49T, was isolated from marine sediment of Abu Qir Bay, on the western seashore of Alexandria, Egypt. The bacterium was aerobic and Gram-positive. It produced beige to light-yellow aerial mycelium, brown substrate mycelium and straight to flexuous hyphae, but no specific spore chains. 16S rDNA sequence analysis and chemotaxonomic markers were consistent with classification of strain SNG49T in the genus Nocardiopsis, i.e. meso-diaminopimelic acid; no diagnostic sugars; phosphatidylcholine, phosphatidylmethylethanolamine, phosphatidylinositol, phosphatidylinositol, phosphatidylglycerol and diphosphatidylglycerol as polar lipids; menaquinones of the MK-10 series from MK-10(H0) to MK-10(H8); and iso/anteiso-branched and 10-methyl-branched fatty acids, the principal fatty acids being anteiso-17 : 0 and tuberculostearic acid. Nocardiopsis lucentensis and Nocardiopsis alba are the phylogenetic neighbours of strain SNG49T, respectively showing 98.8 and 98.7 % 16S rRNA gene sequence similarity; however, moderate DNA–DNA reassociation values between these two species and strain SNG49T (44 and 60 %, respectively) showed that strain SNG49T could be clearly separated from them. These data, together with distinct physiological traits, led to the conclusion that this isolate represents a novel species within the genus Nocardiopsis, for which the name Nocardiopsis aegyptia is proposed. The type strain is SNG49T (= DSM 44442T = NRRL B-24244T).

Nocardiopsis strains are distributed ubiquitously in the environment (Kroppenstedt & Evtushenko, 2002). 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 aim of this study was to classify strain SNG49T, a novel strain isolated from marine sediment, by morphological, physiological, chemotaxonomic and molecular biological methods.

Strain SNG49T was isolated from marine sediment taken at a depth of 20 cm on the seashore of Abu Qir Bay, west of Alexandria, Egypt, using dilution plating on ISP agar supplemented with 5 % NaCl (Shirling & Gottlieb, 1966). Determination of morphological traits and colours of the aerial and substrate mycelium, as well as of soluble pigments, was done as described by Shirling & Gottlieb (1966). Biochemical tests were performed according to Kroppenstedt & Evtushenko (2002) and Al-Zarban et al. (2002). Strain SNG49T showed the typical macroscopic and microscopic appearance of most species of the genus Nocardiopsis (Meyer, 1994), with dirty white aerial mycelium, which becomes light- to dark-yellowish grey in ageing cultures grown on GYM medium (4 g glucose, 4 g yeast extract, 10 g malt extract l⁻¹). The substrate mycelium was brownish. No pigments were released into the medium. The hyphae of the aerial mycelium were straight to flexuous. In older cultures, hyphae of aerial mycelium disintegrated into spore-like structures.

Carbon sources utilized by strain SNG49T are listed in Table 1. The strain grew at 10 and 40 °C and in 5 % NaCl, but was not able to grow in the presence of 10 % NaCl. Optimal growth was observed at 25–28 °C.

Cell material used for chemotaxonomic analyses was obtained from cultures grown in trypticase soy broth (BBL) for 4 days at 28 °C on a rotary shaker. Cells were harvested by centrifugation and washed twice with distilled water. Analyses of amino acid and sugars were carried out using the methods of Staneck & Roberts (1974). Menaquinones and polar lipids were extracted following the procedure of Minnikin et al. (1984). Polar lipids and menaquinones were respectively analysed by TLC (Minnikin...
et al., 1977) and HPLC (Kroppenstedt, 1982, 1985). Fatty acids were analysed according to the methods of Miller (1982) and Sasser (1990). The presence of mycolic acids was determined by the method of Minnikin et al. (1975).

Whole-cell hydrolysates of strain SNG49T contained meso-diaminopimelic acid as the diamino acid in the peptidoglycan. Galactose, glucose and ribose were the only sugars found in the hydrolysates (cell wall type III, according to Lechevalier & Lechevalier, 1980). The diagnostic sugars arabinose, xylose and madurose could not be detected. Strain SNG49T synthesized menaquinones from MK-10(H0) to MK-10(H8) (for quantitative distribution, see species description). The fatty acids were 14-methyl hexadecanoic acid (anteiso-17:0) and 10-methyl octadecanoic acid (tuberculostearic acid). This combination of fatty acids is diagnostic for species of the genus Nocardiopsis (fatty acid pattern 3d sensu Kroppenstedt & Evtushenko, 2002). Qualitative and quantitative distributions are given in the species description. Mycolic acids were not detected. All chemotaxonomic properties of strain SNG49T were consistent with its classification in the genus Nocardiopsis (Kroppenstedt & Evtushenko, 2002).

Genomic DNA extraction, PCR-mediated amplification of the 16S rDNA and purification of PCR products were carried out as described previously (Rainey et al., 1996). The ae2 editor (Maidak et al., 1999) was used to align the almost complete 16S rDNA sequence of strain SNG49T (1487 nt) against the 16S rDNA gene sequences of representatives of the main actinobacterial lineages and then against sequences of members of the genus Nocardiopsis. Phylogenetic analyses followed described methods (De Soete, 1983; Felsenstein, 1993). With intragenic sequence similarities ranging between 95-9 and 98-8 %, strain SNG49T was most closely related to members of the genus Nocardiopsis, especially to Nocardiopsis lucentensis (98-8 % similarity) and Nocardiopsis alba (98-7 %). These similarity values are lower than those found for some other individual pairs of Nocardiopsis species, e.g. Nocardiopsis tropica DSM 44381T and Nocardiopsis umidislocha DSM 44362T (99-2 %); Nocardiopsis metallica DSM 44598T and Nocardiosis exhalans DSM 44407T (99-5 %); or N. metallicus DSM 44598T and Nocardiosis prasina DSM 43845T (99-4 %).

Table 1. Diagnostic characteristics of Nocardiopsis species that are phylogenetically related to strain DSM 44442T and of the type strain of the type species, N. dassonvillei subsp. dassonvillei DSM 43111T.

<table>
<thead>
<tr>
<th>Characteristic</th>
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<th>7</th>
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<tr>
<td>Aerial hyphae</td>
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<td>Growth in/at:</td>
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<td>10 °C</td>
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<td>V</td>
<td>+</td>
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<td>V</td>
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<tr>
<td>10% NaCl</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>Major menaquinones</td>
<td>10/6, 10/4, ND</td>
<td>10/6, 19/8, 10/0, 10/4, 10/6, 10/6, 10/4, 10/6, 10/4, 10/8*</td>
<td>10/6/</td>
<td>10/6</td>
<td>19/4, 9/6, 9/8</td>
<td>10/2</td>
<td>10/6</td>
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<td>10/8, 11/6</td>
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<td>Carbon source utilization:†</td>
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<td>L-Arabinose</td>
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<td>ND</td>
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<td>+</td>
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<td>Galactose</td>
<td>+</td>
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<td>Glycerol</td>
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<td>ND</td>
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<td>+</td>
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<tr>
<td>L-Rhamnose</td>
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*Significant amounts of other menaquinones are also found (see species description).
†Determined using carbon sources at 1%.
Both distance-matrix and maximum-likelihood analyses gave consistent results in that strain SNG49\textsuperscript{T} clustered adjacent to the type strains of \textit{N. lucentensis}, \textit{N. alba} and related strains, but bootstrap values for the branching points of most \textit{Nocardiopsis} type strains were low ( < 50 \%) (Fig. 1).

DNA was isolated by chromatography on hydroxyapatite by the procedure of Cashion et al. (1977). DNA–DNA hybridization was carried out as described by Huß et al. (1983) using a Gilford 2600 spectrophotometer equipped with a Gilford 2527-R thermoprogrammer and plotter. Reassociation was performed under optimal conditions [2 x SSC + 10 % (v/v) DMSO at 68 °C] and was recorded using a Gilford 2600 spectrophotometer (Huß et al., 1983; Jahnke, 1992). The DNA–DNA relatedness of strain SNG49\textsuperscript{T} to \textit{N. lucentensis} DSM 44048\textsuperscript{T} was 44 \%, whereas the value for \textit{N. alba} DSM 43377\textsuperscript{T} was 60 \%. These values are below the threshold value of about 70 \%, recommended by Wayne et al. (1987) for assigning strains to the same species.

Automated ribotyping was carried out with the RiboPrinter microbial characterization system (Qualicon; DuPont). Sample preparation and analysis were performed according to the manufacturer’s instructions using \textit{PvuII} to generate restriction fragments. The RiboPrint pattern of strain SNG49\textsuperscript{T} confirmed that this strain differed from the type strains of both phylogenetic neighbours and representatives of other \textit{Nocardiopsis} species (Fig. 2).

Based on the phenotypic, chemotaxonomic and genotypic data, it is concluded that SNG49\textsuperscript{T} represents a novel species in the genus \textit{Nocardiopsis}; the name \textit{Nocardiopsis aegyptia} sp. nov. is proposed. Isolate SNG49\textsuperscript{T} is currently the only strain of this species.

The phylogenetic position of this organism is within the cluster defined by \textit{N. lucentensis} and \textit{N. alba} (Fig. 1). Strain SNG49\textsuperscript{T} can be differentiated from described\textit{Nocardiopsis} species by a combination of morphological, physiological and chemotaxonomic data: by morphology from \textit{Nocardiopsis synnemataformans}, which produces synnemata, and \textit{Nocardiopsis listeri}, which does not produce a well-developed aerial mycelium; by physiology from \textit{Nocardiopsis halophila} and \textit{Nocardiopsis halotolerans}, which can grow at 15 \% NaCl or higher; and by the ability of strain SNG49\textsuperscript{T} to use nearly all of the carbon sources investigated, except L-arabinose (see Table 1). Chemotaxonomically, \textit{Nocardiopsis} species fall into two groups: those that synthesize mainly menaquinones with highly saturated isoprenoid side-chains, i.e. SNG49\textsuperscript{T}, \textit{Nocardiopsis dassonvillei} subsp. \textit{dassonvillei}, \textit{N. alba}, \textit{Nocardiopsis kunsanensis}, \textit{N. prasina}, \textit{N. lucentensis}, \textit{Nocardiopsis trehalosi}, \textit{N. tropica} and \textit{N. halophila}; and others, including \textit{N. halotolerans}, \textit{N. dassonvillei} subsp. \textit{albirubida}, \textit{N. listeri} and \textit{N. synnemataformans}, that contain non-saturated isoprene units (Kroppenstedt & Evtushenko, 2002).

\textbf{Description of \textit{Nocardiopsis aegyptia} sp. nov.}

\textit{Nocardiopsis aegyptia} (ae.gyp.ti’a. L. fem. adj. \textit{aegyptia} from Egypt, referring to the country of isolation).

Aerobic, Gram-positive, non-motile actinomycete that forms dirty white aerial mycelium, becoming light-yellowish grey in ageing cultures. No endo- or exopigments produced. Hyphae of the aerial mycelium are straight to flexuous. In older cultures, hyphae of aerial mycelium disintegrate into spore-like structures. Optimal growth obtained on GYM at 28 °C. Grows at 10 °C and in 5 \%
NaCl, but not at 45°C or in 10% NaCl. Physiological reactions are indicated in Table 1. Whole cell hydrolysates contain meso-diaminopimelic acid, but no diagnostic sugars. Menaquinones are: MK-10(H₆), 6%; MK-10(H₄), 7%; MK-10(H₆), 19%; MK-10(H₈), 38%; and MK-10(H₈), 30%. Traces of MK-9(H₄) and MK-9(H₆) are also found. Polar lipids are PC, PIME, PI, PG and DPG. The fatty acid composition (>1% of total) is as follows: iso-C₁₄:0 (12-12%), iso-C₁₅:0 (1-70%), iso-C₁₆:0 (19-14%), iso-C₁₇:0 (5-10%), iso-C₁₈:0 (3-16%), anteiso-C₁₅:0 (6-49%), anteiso-C₁₇:0 (17-85%), anteiso-C₁₇:1 (1-35%), 10-methyl C₁₆:0 (1-50%), 10-methyl C₁₇:0 (2-58%), 10-methyl C₁₈:0 (12-19%), C₁₆:0 (4-21%), C₁₆:1 (1-10%), C₁₇:0 (1-72%), C₁₇:1 (1-72%), C₁₈:0 (7-32%) and C₁₈:1 (7-76%). Mycolic acids are absent.

The type strain is SNG49T (= DSM 44442T = NRRL B-24244T), isolated from marine sediments at a depth of 20 cm on the seashore of Abu Qir Bay, west of Alexandria, Egypt.

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References


