Nocardiopsis aegyptia sp. nov., isolated from marine sediment

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An actinomycete, strain SNG49^T, 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 SNG49^T in the genus *Nocardiopsis*, i.e. meso-diaminopimelic acid; no diagnostic sugars; phosphatidylcholine, phosphatidylmethylethanolamine, 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 SNG49^T, respectively showing 98.8 and 98.7 % 16S rRNA gene sequence similarity; however, moderate DNA–DNA reassociation values between these two species and strain SNG49^T (44 and 60 %, respectively) showed that strain SNG49^T 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 SNG49^T (= DSM 44442^T = NRRL B-24244^T).

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 SNG49^T, a novel strain isolated from marine sediment, by morphological, physiological, chemotaxonomic and molecular biological methods.

Strain SNG49^T 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 SNG49^T 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 SNG49^T 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 Stanek & 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

Abbreviations: DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PME, phosphatidylmethylethanolamine.

The GenBank accession number for the 16S rRNA gene sequence of strain DSM 44442^T is AJ539401.

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

Strains: 1, DSM 44442^T; 2, *N. prasina* DSM 43845^T; 3, *N. metallicus* DSM 44598^T; 4, *N. exhalans* DSM 44407^T; 5, *N. listeri* DSM 40297^T; 6, *N. alba* DSM 43377^T; 7, *N. tropica* DSM 44381^T; 8, *N. umidischolae* DSM 44362^T; 9, *N. lucentensis* DSM 44048^T; 10, *N. dassonvillei* subsp. *dassonvillei* DSM 43111^T. Data were taken from Kroppenstedt & Evtushenko (2002), Peltola *et al.* (2001) (*N. exhalans* and *N. umidischolae*) and Schippers *et al.* (2002) (*N. metallicus*). +, Positive; –, negative; v, variable; ND, no data. None of the strains grew at 45 °C.

Characteristic	1	2	3	4	5	6	7	8	9	10
Aerial hyphae	+	+	+	+	–	+	+	+	+	+
Growth in/at:										
10 °C	+	–	+	+	+	–	v	+	+	v
10 % NaCl	–	+	+	+	–	–	+	–	+	+
Major menaquinones	10/6, 10/8*	10/4, 10/6	ND	10/6, 19/8, 19/4, 9/6, 9/8	10/0, 10/2	10/4, 10/6	10/6, 10/8	10/6, 10/4, 10/8, 11/6	10/6, 10/8	10/4, 10/6
Carbon source utilization:†										
L-Arabinose	+	+	ND	+	+	–	+	+	–	+
Galactose	+	–	+	–	+	–	+	+	–	+
Glycerol	+	+	ND	ND	–	+	+	ND	+	+
Inositol	+	–	ND	–	–	–	–	–	+	–
D-Lactose	+	–	ND	–	+	–	–	+	–	–
Mannitol	+	+	+	+	–	+	+	+	+	+
D-Mannose	+	–	ND	+	–	–	+	+	+	–
L-Rhamnose	+	–	+	+	+	–	+	+	+	+
Sucrose	+	+	+	+	–	+	+	+	+	+
D-Xylose	+	+	+	+	+	–	+	+	–	+

*Significant amounts of other menaquinones are also found (see species description).

†Determined using carbon sources at 1 %.

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 SNG49^T 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 SNG49^T synthesized menaquinones from MK-10(H₀) to MK-10(H₈) (for quantitative distribution, see the species description). The polar lipids comprised the diagnostic phospholipids phosphatidylcholine (PC) and phosphatidylmethylethanolamine (PME) and the non-diagnostic phosphatidylinositol (PI), phosphatidylglycerol (PG) and diphosphatidylglycerol (DPG) (i.e. phospholipid type III, according to Lechevalier *et al.*, 1977). This pattern matched quite well with those found in *Nocardioopsis* species (Kroppenstedt & Evtushenko, 2002). The fatty acid composition revealed mainly iso/anteiso-branched and 10-methyl-branched fatty acids. The principal 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 *Nocardioopsis* (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 SNG49^T were consistent with its classification in the genus *Nocardioopsis* (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 SNG49^T (1487 nt) against the 16S rDNA gene sequences of representatives of the main actinobacterial lineages and then against sequences of members of the genus *Nocardioopsis*. Phylogenetic analyses followed described methods (De Soete, 1983; Felsenstein, 1993). With intragenetic sequence similarities ranging between 95.9 and 98.8 %, strain SNG49^T was most closely related to members of the genus *Nocardioopsis*, especially to *Nocardioopsis lucentensis* (98.8 % similarity) and *Nocardioopsis alba* (98.7 %). These similarity values are lower than those found for some other individual pairs of *Nocardioopsis* species, e.g. *Nocardioopsis tropica* DSM 44381^T and *Nocardioopsis umidischolae* DSM 44362^T (99.2 %); *Nocardioopsis metallicus* DSM 44598^T and *Nocardioopsis exhalans* DSM 44407^T (99.5 %); or *N. metallicus* DSM 44598^T and *Nocardioopsis prasina* DSM 43845^T (99.4 %).

Fig. 1. 16S rDNA dendrogram (De Soete, 1983) displaying the phylogenetic position of strain DSM 44442^T and phylogenetically related members of the genus *Nocardiopsis*. Numbers at nodes indicate percentages of bootstrap samplings, derived from 1000 resamplings; values less than 50% are not shown. Bar, 2% sequence divergence. More distantly related members of the genus served as a root.

Both distance-matrix and maximum-likelihood analyses gave consistent results in that strain SNG49^T clustered adjacent to the type strains of *N. lucentensis*, *N. alba* and related strains, but bootstrap values for the branching points of most *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 × 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^T to *N. lucentensis* DSM 44048^T was 44%, whereas the value for *N. alba* DSM 43377^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 *Pvu*II to generate restriction fragments. The RiboPrint pattern of strain SNG49^T confirmed that this strain differed from the type strains of both phylogenetic neighbours and representatives of other *Nocardiopsis* species (Fig. 2).

Fig. 2. RiboPrint patterns of *Nocardiopsis* strains. Fragments were generated from DNA by *Pvu*II.

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

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

Description of *Nocardiopsis aegyptia* sp. nov.

Nocardiopsis aegyptia (ae.gyp.ti'a. L. fem. adj. *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(H₀), 6 %; MK-10(H₂), 7 %; MK-10(H₄), 19 %; MK-10(H₆), 38 %; and MK-10(H₈), 30 %. Traces of MK-9(H₄) and MK-9(H₆) are also found. Polar lipids are PC, PME, PI, PG and DPG. The fatty acid composition (>1 % of total) is as follows: iso-C_{14:0} (12.12 %), iso-C_{15:0} (1.70 %), iso-C_{16:0} (19.14 %), iso-C_{17:0} (5.10 %), iso-C_{18:0} (3.16 %), anteiso-C_{15:0} (6.49 %), anteiso-C_{17:0} (17.85 %), anteiso-C_{17:1} (1.35 %), 10-methyl C_{16:0} (1.50 %), 10-methyl C_{17:0} (2.58 %), 10-methyl C_{18:0} (12.19 %), C_{16:0} (4.21 %), C_{16:1} (1.10 %), C_{17:0} (1.72 %), C_{17:1} (1.72 %), C_{18:0} (7.32 %) and C_{18:1} (7.76 %). Mycolic acids are absent.

The type strain is SNG49^T (=DSM 44442^T=NRRL B-24244^T), 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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