Nocardiopsis halotolerans sp. nov., isolated from salt marsh soil in Kuwait

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INTRODUCTION

Originally, members of the genus Nocardiopsis had been isolated from mildewed grain (Brocq-Rousseau, 1904), but the natural habitat of Nocardiopsis is soil (Mishra et al., 1987). Recent reports have shown that Nocardiopsis strains are frequently isolated from alkaline soils with high salt concentrations (Mikami et al., 1982; Al-Tai & Ruan, 1994; Yassin et al., 1993a), but they can also be isolated from clinical material (Yassin et al., 1997) and may be the cause of human infections, including conjunctivitis (Liegard & Landrieu, 1911), mycetomas (Sindhuphak et al., 1985) and skin infections (Philip & Roberts, 1984). Many of the Nocardiopsis species prefer moderately alkaline conditions (pH 8-5) (Kroppenstedt, 1992) and some grow better on media supplemented with sodium chloride. There is strong evidence that actinomycetes that are halotolerant and are isolated under alkaliphilic conditions belong to the genus Nocardiopsis. In this communication, isolation of a novel halotolerant strain from Kuwaiti salt marsh soil is reported.

METHODS

Strains and culture conditions. Strain F100T was isolated from salt marsh soil in a desert area at Al-Khiran, Kuwait. The organism was isolated by dilution plating on salt-starch-nitrate agar containing 15% NaCl. For morphological studies, strain F100T was cultivated on solidified starch mineral agar +10% NaCl and Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) medium 252...
+ 10% NaCl at 28 °C for 7–14 d. ISP media were used for melanin production (Shirling & Gottlieb, 1966). Cell material for chemotaxonomic analyses was obtained by growing the strain in liquid medium (DSMZ medium 252+10% NaCl). Cells were collected by filtration or centrifugation, washed twice with water and freeze-dried.

**Physiology.** The basal media used for physiological characterization were those described by Shirling & Gottlieb (1966) and Waksman (1961) plus 10% NaCl. For the carbon utilization tests, 1% of each substrate was added to the medium. Inoculated Petri dishes were incubated at 28 °C. Plates were checked for growth after 2 and 4 weeks.

**Analysis of cell wall amino acids and sugars.** The amino acid and sugar analysis of whole-cell hydrolysates followed previously described procedures (Staneck & Roberts, 1974).

**Extraction and analysis of isoprenoid quinones and polar lipids.** Isoprenoid quinones were extracted and purified using the small-scale integrated procedure of Minnikin et al. (1984). Dried preparations were dissolved in 200 µl 2-propanol and 1–10 µl portions were separated by HPLC without further purification. The menaquinones were separated by HPLC on Lichrosorb RP-18 at 40 °C using acetonitrile/2-propanol (65:35, v/v) as solvent (Kroppenstedt, 1985; Kroppenstedt et al., 1981). Polar lipids were extracted, examined by two-dimensional TLC and identified using published procedures (Minnikin et al., 1984).

**Extraction and analysis of fatty acids and mycolic acids.** Fatty acid methyl esters (FAMEs) were prepared from 40–80 mg wet cells (Miller, 1982; Sasser, 1990). FAME mixtures were analysed by capillary GC using a Hewlett Packard model 5898A GC run by Microbial Identification software (Microbial ID). The occurrence of mycolic acids was checked by TLC following the procedure of Minnikin et al. (1975).

**16S rDNA sequence determination and phylogenetic analyses.** Genomic DNA extraction, PCR-mediated amplification of the 16S rDNA, purification of PCR products and electrophoreses of sequence reactions were done as described previously (Rainey et al., 1996). The 16S rDNA sequence was aligned manually with published sequences from representatives of the actinomycete sublines contained in the DSMZ database of 16S rDNA sequences. The ae2 editor (Maidak et al., 1999) was used to align the 16S rDNA sequence of strain F100 against the 16S rDNA sequences of the *Nocardiopsis* type strains available from public databases. Pairwise evolutionary distances were computed using the correction of Jukes & Cantor (1969). The least squares distance method of De Soete (1983) was used in the construction of the phylogenetic dendrogram from distance matrices. Maximum-likelihood analyses and bootstrap analyses were done as described by Felsenstein (1993).

Determination of DNA–DNA similarity was performed by the spectrophotometric reassociation method as described by Kleespies et al. (1996) and the G+C content was determined by HPLC of deoxyribonucleosides according to the method of Mesbah et al. (1989).

**RESULTS AND DISCUSSION**

The morphological and chemotaxonomic characters of DSM 44410T were consistent with those described for *Nocardiopsis* species (Kroppenstedt, 1992).

**Macroscopic and microscopic features**

The strain showed the typical dirty-white aerial mycelium which changed in older cultures to grey-yellow (griseus colour). This appearance matched with that of most *Nocardiopsis* species and can easily be confused with *Streptomyces griseus* strains (Gordon & Horan, 1968). The substrate mycelium was yellow-brown, but did not develop any specific colour, like green, red or blue. Soluble pigments were not produced on any of the ISP media tested. Melanin was not produced on either peptone-iron agar or tyrosine agar (Shirling & Gottlieb, 1966). The mature aerial mycelium showed the typical zig-zag formation that fragments in older cultures. The same morphology was also observed in submerged cultures. This morphology is diagnostic for *Nocardiopsis* and related taxa (Labeleda et al., 1984).

**Chemotaxonomic characteristics**

The chemotaxonomic properties of strain F100T were consistent with its classification in the genus *Nocardiopsis* (Kroppenstedt, 1992). Whole-cell hydrolysates contained *meso*-diaminopimelic acid as the only di-amino acid of the peptidoglycan, as well as ribose and glucose, but no diagnostic sugars like arabinose, xylose and madurose (Lechevalier et al., 1971) or rhamnose (Labeleda et al., 1984). Mycolic acids could not be detected. The polar lipid pattern revealed the presence of the diagnostic phosphatidyl choline (PC), phosphatidyl inositol (PI), phosphatidyl glycerol (PG), phosphatidyl methylthanolamine (PM), diphasphatidyl glycerol (DPG) and three to four unknown phospholipids with high *R* value (above that for DPG). The detection of PC leads to phospholipid pattern III according to Lechevalier et al. (1977). This phospholipid pattern is found in species of the genera *Nocardiopsis*, *Actinopolyspora*, Saccharopolyspora and *Pseudonocardia*. *Nocardiopsis* strains, however, can easily be differentiated from these taxa by the occurrence of PME, high amounts of PG and the lack of hydroxy-phosphatidyl ethanolamine. In addition, three to four unknown phospholipids with high *R* value (above DPG) can be detected. These unknown phospholipids are of diagnostic value and have, until now, only been found in *Nocardiopsis* species (Kroppenstedt, 1992). The taxonomic value of other ‘non-diagnostic’ phospholipids has been mentioned by Yassin et al. (1993b).

The strains synthesized a very complex pattern of menaquinones which revealed two homologous series. The main series was MK-10 (30%), MK-10(H2) (18%), MK-10(H2) (17%) and MK-10(H2) (8%) and the minor series was MK-11 (13%), MK-11(H2) (5%), MK-11(H2) (4%) and MK-11(H2) (5%). Traces (1%) of MK-9(H2) could also be found.

The fatty acid pattern of this strain was composed of iso/anteiso-branched fatty acids. Smaller amounts of 10-methyl-branched and unbranched fatty acids were...
Table 1. Diagnostic characteristics of Nocardiopsis species

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* Nomenclature, e.g. 10/2, MK-10(H2). For N. halotolerans, significant amounts of other menaquinones are also found.

also found. The high amount of anteiso fatty acids in combination with 10-methyl-branched fatty acids (fatty acid type 3d) is diagnostic for species of the genus Nocardiopsis (Kroppenstedt, 1985). The following fatty acids were detected: terminally branched iso/anteiso fatty acids, i.e. iso-C14:0 (2%), iso-C15:0 (4%), iso-C16:0 (23%), iso-C17:0 (4%), iso-C18:0 (1%), anteiso-C15:0 (17%) and anteiso-C17:0 (18%); 10-methyl-branched 10-methyl-C17:0 (2%) and 10-methyl-C18:0 (2%); and the unbranched saturated and unsaturated fatty acids C16:0 (3%), C16:1 (1%), C17:0 (3%), C17:1 (7%), C18:0 (4%) and C18:1 (9%). This combination of fatty acids is unique among Nocardiopsis spp. (Fischer et al., 1983; Kroppenstedt, 1992).

16S rDNA sequence analysis

The almost complete 16S rDNA sequence of strain F100T, consisting of 1439 nt, was compared to sequences of members of the order Actinomycetales. Members of the genus Nocardiopsis were the closest phylogenetic neighbours. Binary similarity values ranged between 96.3% (Nocardiopsis trehalosi NRRL 12026) and 98.9% (Nocardiopsis dassonvillei subsp. dassonvillei DSM 43884). Similarly high or even higher values separated the type strains of Nocardiopsis species, such as those of N. dassonvillei and Nocardiopsis synnemataformans, (99.8%), Nocardiopsis prasina and Nocardiopsis listeri (98.7%), and Nocardia alba and N. prasina (99.2%). Distance matrix analyses placed strain F100T in a separate line of descent, showing no close relatedness to any other of the Nocardiopsis type strains. The phylogenetic distinctness of the novel isolate was much more obvious in the maximum-likelihood analyses (not shown) in which it branched even more separately, i.e. between N. trehalosi NRRL 12026 and the other members of the genus. Strain F100T exhibited a 16S rDNA nucleotide stretch between position 183 and 193 (Escherichia coli nomenclature), i.e. UUG GCC UCCU GGC CGG, whereas all other members of Nocardiopsis possess the homologous composition

http://ij.sgmjournals.org
Ggc ACC UCAU GGU GGA. The DNA–DNA relatedness between strain F100T and the type strain of *N. dasonvillei* subsp. *dasonvillei* was only 47%.

**Differentiation of Nocardiopsis halotolerans** sp. nov. from other Nocardiopsis species

Based on the phenotypic and genotypic data, it is concluded that F100T merits species status in the genus *Nocardiopsis*. The phylogenetic position of this organism is within the cluster defined by *N. dasonvillei* and *N. synnemataformans* (Fig. 1). F100T can be differentiated from the *Nocardiopsis* species by a combination of morphological, physiological and chemotaxonomic data: by morphology from *N. synnemataformans*, which produces synnema, and from *N. listeri*, which does not produce a well-developed aerial mycelium; by physiology, showing a unique carbon utilization pattern that differs from the other *Nocardiopsis* species (see Table 1); and by chemotaxonomy from *N. dasonvillei* subsp. *dasonvillei*, *N. alba*, *N. prasina*, *Nocardiopsis lucentensis*, *N. trehalosi*, *Nocardiopsis tropica* and *Nocardiopsis halophila*, all of which have major menaquinones that are highly saturated. Based on these results, it is concluded that isolate F100T is a strain of a novel species of the genus *Nocardia*. The name *Nocardiopsis halotolerans* sp. nov. is therefore proposed for the isolate; the type strain is F100T (= DSM 44410T = NRRL B-24124T).

**Description of Nocardiopsis halotolerans** sp. nov.

*Nocardiopsis halotolerans* (ha.lo.to.le.rans. Gr. n. hals salt; L. part. tolerans tolerating; N.L. pres. part. haloterolans referring to the ability to tolerate high salt concentrations).

Aerobic nocardioform actinomycete which produces dirty-white to yellow-grey aerial mycelium. The colour of substrate mycelium is beige to brown. Diffusible pigments are not produced. Melanin is not observed on either peptone-yeast-iron agar or tyrosine agar (ISP media 6 and 7). Aerial hyphae show the typical zig-zag formation prior to sporulation. The long-branched substrate hyphae fragment into non-motile elements. Optimal growth is obtained on starch mineral agar supplemented with 10% NaCl at 28 °C. Range for growth is 28–35 °C and NaCl concentration 0–15%. D-Glucose, D-mannose, galactose, sucrose, melibiose and glycerol are used as sole carbon sources, whereas L-arabinose, D-xyllose and adonitol cannot be used for growth. Whole-cell hydrolysates contain the cell wall diamino acid, meso-diaminopimelic acid and the sugars glucose and ribose. Two menaquinone series are found: MK-10 (30%), MK-10(H4) (18%), MK-10(H4) (17%) and MK-10(H4) (8%); and MK-11 (13%), MK-11(H4) (5%), MK-11(H4) (4%) and MK-11(H4) (5%). The polar lipid pattern is composed of eight phospholipids, i.e. PC, PI, PG, PME, DPG and three unknown *Nocardiopsis* diagnostic phospholipids with high *Rf* value. The fatty acid composition is iso-C14:0 (2%), iso-C15:0 (4%), iso-C16:0 (23%), iso-C17:0 (4%), iso-C18:0 (1%), anteiso-C15:0 (17%), anteiso-C17:0 (18%), 10-methyl-C17:0 (2%), 10-methyl-C18:0 (2%), C16:0 (3%), C17:0 (7%), C17:1 (3%), C18:2 (9%) and C19:0 (4%). DNA G+C content is 68.0 mol%. Able to degrade feathers. Isolated from salt marsh soil at Al-Khiran, Kuwait. Type strain is F100T (= DSM 44410T = NRRL B-24124T).

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Nocardiopsis halotolerans sp. nov.


