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

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A polyphasic taxonomic study of a halotolerant micro-organism, isolated from Kuwait salt marsh soil, revealed that this strain represents a novel *Nocardiopsis* species. The strain produced substrate and aerial mycelium, grew at 28–35 °C in salt concentrations of 0–15% and was slightly keratinolytic. Results of the 16S rDNA sequence comparison revealed that strain F100ᵀ clustered with strains of the genus *Nocardiopsis*. This is consistent with other data such as: (i) growth characteristics, i.e. the formation of a white to yellow aerial mycelium and the typical zig-zag form of hyphae, which fragment when ageing; (ii) the presence of DL-diaminopimelic acid and glucose plus ribose in whole-cell hydrolysates; (iii) the presence of phosphatidyl choline, phosphatidyl inositol, phosphatidyl glycerol, phosphatidyl methylethanolamine and diphosphatidyl glycerol in polar lipid extracts; (iv) the presence of menaquinones MK-10(H_{0-6}) and MK-11(H_{0-6}) in the non-polar fraction; (v) the presence of iso/anteiso-branched plus 10-methyl-branched fatty acids, showing the diagnostic combination for *Nocardiopsis* spp. of 14-methyl-hexadecanoic acid (18%), oleic acid (9%) and tuberculostearic acid (2%); and (v) the absence of mycolic acids. Analysis of 16S rDNA revealed that strain F100ᵀ represents a distinct taxon within *Nocardiopsis*. Based upon phenotypic differences to other members of the genus, a novel species, *Nocardiopsis halotolerans* sp. nov., is proposed. The type strain of the species is F100ᵀ (= DSM 44410ᵀ = NRRL B-24124ᵀ).

Keywords: *Nocardiopsis*, *Nocardiopsis halotolerans* sp. nov.

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 be 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.

Abbreviations: DPG, diphosphatidyl glycerol; FAMEs, fatty acid methyl esters; PC, phosphatidyl choline; PG, phosphatidyl glycerol; PI, phosphatidyl inositol; PME, phosphatidyl methylethanolamine.

The EMBL accession number for the 16S rDNA sequence of *Nocardiopsis halotolerans* DSM 4410ᵀ is AJ290448.

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^T 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 44410^T 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 (Labeda *et al.*, 1984).

Chemotaxonomic characteristics

The chemotaxonomic properties of strain F100^T were consistent with its classification in the genus *Nocardiopsis* (Kroppenstedt, 1992). Whole-cell hydrolysates contained *meso*-diaminopimelic acid as the only diamino 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 (Labeda *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 methylethanolamine (PME), diphosphatidyl glycerol (DPG) and three to four unknown phospholipids with high R_F 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_F 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(H_2) (18%), MK-10(H_4) (17%) and MK-10(H_6) (8%) and the minor series was MK-11 (13%), MK-11(H_2) (5%), MK-11(H_4) (4%) and MK-11(H_6) (5%). Traces (1%) of MK-9(H_4) 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

1, *N. halotolerans* sp. nov.; 2, *N. dassonvillei* subsp. *dassonvillei*; 3, *N. dassonvillei* subsp. *alborubida*; 4, *N. alba*; 5, *N. listeri*; 6, *N. prasina*; 7, *N. lucentensis*; 8, *N. trehalosi*; 9, *N. tropica*; 10, *N. synnemataformans*; 11, *N. halophila*. +, Positive; −, negative; v, variable; ND, no data. Data from Al-Tai & Ruan (1994); Evtushenko *et al.* (2000); Grund & Kroppenstedt (1990); Yassin *et al.* (1993a, b, 1997).

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Morphology:											
Aerial hyphae	+	+	+	+	−	+	+	+	+	+	+
Synnemata	−	−	−	−	−	−	−	−	−	+	−
Growth at:											
10 °C	+	v	−	−	+	−	+	−	v	−	ND
45 °C	−	−	−	−	−	−	−	−	−	−	+
10 % NaCl	+	+	+	−	−	+	+	−	+	+	+
15 % NaCl	+	ND	ND	−	−	ND	ND	−	−	ND	+
20 % NaCl	−	ND	ND	−	−	ND	ND	−	−	ND	+
Major menaquinone(s)*	10/0, 10/2	10/4, 10/6	10/0	10/4, 10/6	10/0, 10/2	10/4, 10/6	10/6, 10/8	10/4, 10/6	10/6, 10/8	10/0, 10/2	10/6, 10/8
Growth with (1 %):											
D-Glucose	+	+	+	+	+	+	+	+	+	+	+
L-Arabinose	−	+	+	−	+	+	−	+	+	−	+
Melibiose	+	−	−	−	+	−	−	+	+	ND	ND
D-Xylose	−	+	+	−	+	+	−	+	+	+	+
Adonitol	−	−	−	−	+	−	−	−	−	ND	ND
Galactose	+	+	+	−	+	−	−	−	+	ND	−
Glycerol	+	+	+	+	−	+	+	+	+	ND	ND
D-Mannose	+	−	+	−	−	−	+	+	+	ND	−
Sucrose	+	+	−	+	−	+	+	−	+	ND	−

* Nomenclature, e.g. 10/2, MK-10(H_2). 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-$C_{14:0}$ (2 %), iso-$C_{15:0}$ (4 %), iso-$C_{16:0}$ (23 %), iso-$C_{17:0}$ (4 %), iso-$C_{18:0}$ (1 %), anteiso-$C_{15:0}$ (17 %) and anteiso-$C_{17:0}$ (18 %); 10-methyl-branched 10-methyl-$C_{17:0}$ (2 %) and 10-methyl-$C_{18:0}$ (2 %); and the unbranched saturated and unsaturated fatty acids $C_{16:0}$ (3 %), $C_{16:1}$ (1 %), $C_{17:0}$ (3 %), $C_{17:1}$ (7 %), $C_{18:0}$ (4 %) and $C_{18:1}$ (9 %). This combination of fatty acids is unique among *Nocardiopsis* spp. (Fischer *et al.*, 1983; Kroppenstedt, 1992).

Physiology

F100^T was able to degrade feathers. Good growth occurred on starch-nitrate agar supplemented with 0–10 % NaCl and, to a lesser extent, with 15 % NaCl. No growth was obtained with 20 % NaCl. The strain grew at 10 °C, but not at 40 °C. The strain could use D-glucose, D-mannose, galactose, sucrose, melibiose and glycerol for growth; L-arabinose, D-xylose and adonitol could not be used as sole carbon sources (Table 1).

16S rDNA sequence analysis

The almost complete 16S rDNA sequence of strain F100^T, 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^T) 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 *Nocardiopsis alba* and *N. prasina* (99·2 %). Distance matrix analyses placed strain F100^T 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^T and the other members of the genus. Strain F100^T 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

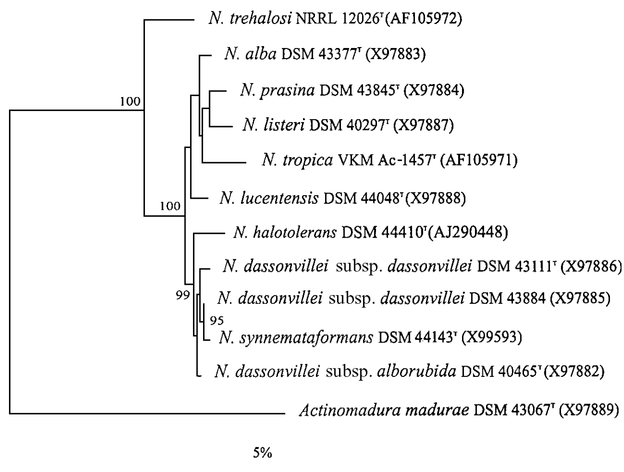

Fig. 1. Phylogenetic dendrogram obtained by distance matrix analysis showing the position of *Nocardiopsis halotolerans* DSM 44410^T among members of the genus *Nocardiopsis*. No 16S rDNA sequence was available for the type strain of *Nocardiopsis halophila*. The sequence of *Actinomadura madurae* served as outside reference. Bootstrap values > 85 % are indicated at the relevant branching points. Bar, 0·02 estimated nucleotide changes per position.

GGC ACC UCAU GGU GGA. The DNA–DNA relatedness between strain F100^T and the type strain of *N. dassonvillei* subsp. *dassonvillei* was only 47 %.

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

Based on the phenotypic and genotypic data, it is concluded that F100^T merits species status in the genus *Nocardiopsis*. The phylogenetic position of this organism is within the cluster defined by *N. dassonvillei* and *N. synnemataformans* (Fig. 1). F100^T can be differentiated from the *Nocardiopsis* species by a combination of morphological, physiological and chemotaxonomic data: by morphology from *N. synnemataformans*, which produces synnemata, 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. dassonvillei* subsp. *dassonvillei*, *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 F100^T 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 F100^T (= DSM 44410^T = NRRL B-24124^T).

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. *halotolerans* 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-xylose 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(H_2) (18 %), MK-10(H_4) (17 %) and MK-10(H_6) (8 %); and MK-11 (13 %), MK-11(H_2) (5 %), MK-11(H_4) (4 %) and MK-11(H_6) (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 R_F value. The fatty acid composition is iso-$C_{14:0}$ (2 %), iso-$C_{15:0}$ (4 %), iso-$C_{16:0}$ (23 %), iso-$C_{17:0}$ (4 %), iso-$C_{18:0}$ (1 %), anteiso-$C_{15:0}$ (17 %), anteiso-$C_{17:0}$ (18 %), 10-methyl-$C_{17:0}$ (2 %), 10-methyl-$C_{18:0}$ (2 %), $C_{16:1}$ (1 %), $C_{16:0}$ (3 %), $C_{17:1}$ (7 %), $C_{17:0}$ (3 %), $C_{18:1}$ (9 %) and $C_{18:0}$ (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 F100^T (= DSM 44410^T = NRRL B-24124^T).

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REFERENCES

Al-Tai, A. M. & Ruan, J.-S. (1994). *Nocardiopsis halophila* sp. nov., a new halophilic actinomycete isolated from soil. *Int J Syst Bacteriol* **44**, 474–478.

Brocq-Rousseau, D. (1904). Sur un Streptothrix. *Ref Gen Botanique* **16**, 20–29.

De Soete, G. (1983). A least squares algorithm for fitting additive trees to proximity data. *Psychometrika* **48**, 621–626.

Evtushenko, L. I., Taran, V. V., Akimov, V. N., Kroppenstedt, R. M., Tiedje, J. M. & Stackebrandt, E. (2000). *Nocardiopsis tropica* sp. nov., *Nocardiopsis trehalosi* sp. nov., nom. rev. and *Nocardiopsis dassonvillei* subsp. *albirubida* subsp. nov., comb. nov. *Int J Syst Bacteriol* **50**, 73–81.

Felsenstein, J. (1993). PHYLIP (phylogenetic inference package) version 3.5c. Department of Genetics, University of Washington, Seattle, USA.

Fischer, A., Kroppenstedt, R. M. & Stackebrandt, E. (1983). Molecular-genetic and chemotaxonomic studies on *Actinomadura* and *Nocardiopsis*. *J Gen Microbiol* **129**, 3433–3446.

Gordon, R. E. & Horan, A. S. (1968). *Nocardia dassonvillei*, a macroscopic replica of *Streptomyces griseus*. *J Gen Microbiol* **50**, 235–240.

Grund, E. & Kroppenstedt, R. M. (1990). Chemotaxonomy and numerical taxonomy of the genus *Nocardiopsis* Meyer 1976. *Int J Syst Bacteriol* **40**, 5–11.

Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.

Kleespies, M., Kroppenstedt, R. M., Rainey, F. A., Webb, L. E. & Stackebrandt, E. (1996). *Mycobacterium hodleri* sp. nov., a new member of the fast-growing mycobacteria capable of degrading polycyclic aromatic hydrocarbons. *Int J Syst Bacteriol* **46**, 683–687.

Kroppenstedt, R. M. (1985). Fatty acid and menaquinone analysis of actinomycetes and related organisms. In *Chemical Methods in Bacterial Systematics*, pp. 173–199. Edited by M. Goodfellow & E. Minnikin. London: Academic Press.

Kroppenstedt, R. M. (1992). The genus *Nocardiopsis*. In *The Prokaryotes*, 2nd edn, pp. 1139–1156. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K.-H. Schleifer. New York: Springer.

Kroppenstedt, R. M., Korn-Wendisch, F., Fowler, V. J. & Stackebrandt, E. (1981). Biochemical and molecular genetic evidence for transfer of *Actinoplanes armeniacus* into the family *Streptomycetaceae*. *Zbl Bakt Hyg Abt Orig C* **2**, 254–262.

Labeda, D. P., Testa, R. T., Lechevalier, M. P. & Lechevalier, H. A. (1984). *Saccharothrix*: a new genus of the *Actinomycetales* related to *Nocardiopsis*. *Int J Syst Bacteriol* **34**, 426–431.

Lechevalier, H. A., Lechevalier, M. P. & Gerber, N. N. (1971). Chemical composition as a criterion in the classification of actinomycetes. *Adv Appl Microbiol* **14**, 47–72.

Lechevalier, M. P., DeBievre, C. & Lechevalier, H. A. (1977). Chemotaxonomy of aerobic actinomycetes: phospholipid composition. *Biochem Syst Ecol* **5**, 249–260.

Liegard, H. & Landrieu, M. (1911). Un case de mycose conjunctivale. *Ann Ocul* **146**, 418–426.

Maidak, B. L., Cole, J. R., Parker, C. T., Jr & 11 other authors (1999). A new version of the RDP (Ribosomal Database Project). *Nucleic Acids Res* **27**, 171–173.

Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.

Mikami, Y., Miyashita, K. & Arai, T. (1982). Diaminopimelic acid profiles of alkalophilic and alkaline-resistant strains of actinomycetes. *J Gen Microbiol* **128**, 1709–1712.

Miller, L. T. (1982). A single derivatization method for routine analysis of bacterial whole-cell fatty acid methyl esters, including hydroxy acids. *J Clin Microbiol* **16**, 584–586.

Minnikin, D. E., Alshamaony, L. & Goodfellow, M. (1975). Differentiation of *Mycobacterium*, *Nocardia*, and related taxa by thin-layer chromatographic analysis of whole-cell methanolysates. *J Gen Microbiol* **88**, 200–204.

Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A. & Parlett, J. H. (1984). An integrated procedure for the extraction of isoprenoid quinones and polar lipids. *J Microb Methods* **2**, 233–241.

Mishra, S. K., Keller, J. E., Miller, J. R., Heisey, R. M., Nair, M. G. & Putnam, A. R. (1987). Insecticidal and nemacidal properties of microbial metabolites. *J Ind Microbiol* **2**, 267–276.

Philip, A. & Roberts, G. D. (1984). *Nocardiopsis dassonvillei* cellulitis of the arm. *Clin Microbiol Newsl* **46**, 1088–1092.

Rainey, F. A., Ward-Rainey, N., Kroppenstedt, R. M. & Stackebrandt, E. (1996). The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of *Nocardiopsaceae* fam. nov. *Int J Syst Bacteriol* **46**, 1088–1092.

Sasser, M. (1990). Identification of bacteria by gas chromatography of cellular fatty acids. *USFCC Newsl* **20**, 1–6.

Shirling, E. B. & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* **16**, 313–340.

Sindhuphak, W., MacDonald, E. & Head, E. (1985). Actinomycetoma caused by *Nocardiopsis dassonvillei*. *Arch Dermatol* **121**, 1332–1334.

Staneck, J. L. & Roberts, G. D. (1974). Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* **28**, 226–231.

Waksman, S. A. (1961). *The Actinomycetes*, vol. 2. *Classification, Identification and Description of Genera and Species*. Baltimore: Williams & Wilkins.

Yassin, A. F., Galinski, E. A., Wohlfarth, A., Jahnke, K.-D., Schaal, K. P. & Trüper, H. G. (1993a). A new actinomycete species, *Nocardiopsis lucentensis* sp. nov. *Int J Syst Bacteriol* **43**, 266–271.

Yassin, A. F., Haggenei, B., Budzikiewicz, H. & Schaal, K. P. (1993b). Fatty acid and polar lipid composition of the genus *Amycolatopsis*: application of fast atom bombardment-mass spectrometry to structure analysis of underivatized phospholipids. *Int J Syst Bacteriol* **43**, 414–420.

Yassin, A. F., Rainey, F. A., Burghardt, J., Gierth, D., Ungerechts, J., Lux, I., Seifert, P., Bal, C. & Schaal, K. P. (1997). Description of *Nocardiopsis synnemataformans* sp. nov., elevation of *Nocardiopsis alba* subsp. *prasina* to *Nocardiopsis prasina* comb. nov., and designation of *Nocardiopsis antarctica* and *Nocardiopsis alborubida* as later subjective synonyms of *Nocardiopsis dassonvillei*. *Int J Syst Bacteriol* **47**, 983–988.