Nocardiopsis mwathae sp. nov., isolated from the haloalkaline Lake Elmenteita, in the African Rift Valley

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The INSDC accession number for the 16S rRNA gene sequence of strain No.156^{T} is KF976731.

Abstract

Strain No.156^T isolated from a sediment sample from the haloalkaline lake Elmenteita in the African rift valley was studied by a polyphasic taxonomic approach. The strain produced yellow aerial and substrate mycelia, grew best over a temperature range of 30-35^{o}C in salt concentrations of 6-9% (w/v) and at pH 7-9. The DNA G+C contents of the novel strain was 71 mol%. Analysis of 16S rRNA sequences indicated that the isolate belonged to the genus *Nocardiopsis* with a sequence similarity below 98% to the type strains of all other representatives of the genus. Mycolic acids were not detected in whole cell methanolysates. The cell wall contained meso-diaminopimelic acid without diagnostic sugars. Major phospholipids included phosphatidylmethylethanolamine, phosphatidylcholine, phosphatidylglycerol and phosphatidylinositol but no diphosphatidylglycerol. The predominant menaquinones were MK-11(H_8), MK-11(H_6), MK-10(H_8) and MK-10(H_6). The fatty acid composition included *iso*- and *anteiso*-branched acids combined with tuberculostearic acid (Me18:0), straight-chain saturated (16:0, 18:0) and unsaturated fatty acids. These characteristics match those of the genus *Nocardiopsis*. Based on 16S rRNA gene sequence analysis and phenotypic characteristics, a novel species with the name *Nocardiopsis mwathae* is proposed. The type strain is No.156^T (= DSM 46659 = CECT 8552). The INSDC accession number for the 16S rRNA gene sequence is KF976731.

Key words: *Streptosporangineae*, Haloalkaline, Polyphasic taxonomy, Soda lake

Introduction

The genus *Nocardiopsis* was first described by Meyer (1976) based on morphological characteristics and the chemical composition of cells. Members of the genus *Nocardiopsis* have been isolated from wide range of sources; alkaline slag dump (Schippers et al. 2002), indoor environments (Peltola et al. 2001), the atmosphere of a composting facility (Kämpfer et al. 2002) clinical material (Bernatchez and Lebreux, 1991; Yassin et al. 1997) household waste (Yassin et al. 2009) but predominantly reported from saline or alkaline soils (Yassin et al. 1993a; Al-tai and Ruan, 1994; Al-Zarban et al. 2002; Tang et al. 2003; Li et al. 2004, 2006; Yang et al. 2008). The genus *Nocardiopsis* was proposed to include organisms with the following salient chemotaxonomic characteristics: cell wall chemotype III C (meso isomer of diaminopimelic acid and no characteristic sugars in whole-cell hydrolysates) (Lechevalier and Lechevalier, 1970), phospholipid type PIII (phosphatidylcholine and phosphatidylmethylethanolamine as diagnostic phospholipids), lack of mycolic acids (Rainey et al.

1996), menaquinone MK-10 with variable degrees of saturation as the major isoprenoid quinones (Collins et al., 1977), the fatty acids include *iso*-branched, *anteiso*-branched, and 10-methyl-branched fatty acids (Rainey et al. 1996) and a DNA G + C content ranging between 64 and 71 mol% (Grund and Kroppenstedt, 1990). At the time of writing the genus contained 45 species (with five subspecies) and the genomic analysis which generates a more in-depth insight into its genetic mechanisms of environmental adaptability covered already about half of the type strains (Li et al., 2013). Members of the genus *Nocardiopsis* are known to produce bioactive metabolites (Li et al. 2012) hence isolation from different environments may provide access to new bioactive products and contribute to an understanding of their ecological roles. In this paper we describe morphological, physiological, and chemical data which support the identification of strain No.156^T isolated from a sediment sample from Lake Elmenteita, a soda lake in the African Rift Valley, as representative of a novel species of the genus *Nocardiopsis*. The name *Nocardiopsis mwathae* sp. nov. is proposed for this species.

Materials and Methods

Isolation and phenotypic characterization

Lake Elmenteita is situated at 0º27´S, 36º15´E on the floor of the Kenyan Rift Valley at 1776 m altitude and has no direct outlet (Melack 1988). The region is characterised by a hot, dry and semi-arid climate with a mean annual rainfall of about 700 mm (Mwaura 1999). Due to the high temperatures there are very high evaporation rates during the drier seasons leading to a seasonal reduction in the total surface area of the lake. The size of Lake Elmenteita is roughly 20 km^2 and the depths rarely exceed 1.0 m (Mwirichia et al. 2010a). The alkalinity of the water is high (1200 mg Na_2CO_3/l) with a high concentration of carbonates, chlorides and sulphates (Mwirichia et al. 2010b). The water temperature ranges between 30-40ºC and the pH is above 9.

Strain No.156^T was isolated from sediment collected from Lake Elmenteita in March 2013. Sediment samples (2 g) were suspended in filter sterilised Lake water (10 ml) and serially diluted using the same water. Aliquots (100 µl) of appropriate dilutions were plated onto marine agar (DSMZ medium 123), at pH 8 supplemented with cAMP (Sigma Chemical Co., St. Louis, Mo.) to a final concentration of

10_μM (Bruns and Cypionka 2002). The plates were incubated at 28°C for 14 days. Colonies appearing on the plates were streaked again on to fresh marine agar (DSMZ medium 123) for purification. The purified strain was stocked in marine broth with 15% glycerol (v/v) at -20°C.

All physiological tests were performed at 28°C using strain No.156^T and cultures of *N. rosea* DSM 44842^T (Li et al. 2006), *N. gilva* DSM 44841^T (Li et al. 2006), *N. baichengensis* DSM 44845^T (Li et al. 2006), *N. rhodophaea* DSM 44843^T (Li et al. 2006), *N. chromatogenes* DSM 44844^T (Li et al. 2006), *N. halophila* DSM 44494^T (Al-tai and Ruan 1994) and *N. composta* DSM 44551^T (Kämpfer et al. 2002) as reference strains, in parallel assays. Aerial spore mass and substrate mycelia coloration were tested on marine medium (DSMZ medium 123), ISP2 (DSMZ medium 5318), ISP3 (DSMZ medium 609), ISP4 (DSMZ medium 547), Czapek medium (DSMZ medium 83), Sabouraud medium (DSMZ medium 1429), potato dextrose medium (DSMZ medium 129), CASO medium (DSMZ medium 220) and nutrient agar (DSMZ medium 1) for 21 days. Colony features were observed after 21 days under a binocular microscope according to (Pelczar, 1957). Exponentially growing bacterial cultures were observed with an optical microscope (Zeiss AxioScope A1) with a 1000-fold magnification and phase-contrast illumination. Colours were determined using the RAL Classic K5 colour chart. Pictomicrographs of bacterial cells of strain No.156^T grown on marine agar for 21 days were taken with a field-emission scanning electron microscope (FE-SEM Merlin, Zeiss, Germany). Gram reaction was performed using the KOH test described by (Gregersen 1978).

Growth was assessed at 10-55°C (in increments of 5°C) for 14 days on marine agar, pH values from 5.0-13.0 (in increments of 1.0 pH unit) for 14 days at 28°C using the buffer system described by Xu et al. (2005) and 0, 1-15% (w/v) NaCl (in increments of 2 units) for 14 days on nutrient agar at 28°C. Biochemical characteristics such as hydrolysis of casein, degradation of xanthine, tyrosine, starch, catalase and DNase activity were determined using standard procedures (Shirling and Gottlieb 1966; Williams et al. 1983; Smibert and Krieg, 1994). Strains were additionally characterized using API 20NE and API ZYM (bioMérieux) identification systems according to manufacturer's instructions. Phenotypic features were rated as positive when signal obtained was either weak or more pronounced.

Chemotaxonomic procedures

For chemotaxonomic studies, strain No.156^T and the reference strains were grown in marine broth in a shaking incubator at 150 r.p.m, 28ºC for 4 days. The mycelia were harvested by centrifugation and washed twice with 0.9% NaCl solution and freeze dried. All chemotaxonomical analyses were conducted under standardized conditions with strain No.156^T and cultures of the same set of reference strains as listed above for the phenotypic characterisations. The presence of mycolic acids was checked by the acid methanolysis method described by Minnikin et al. (1980). Amino acids and whole-cell sugars were prepared according to (Lechevalier and Lechevalier, 1970), followed by thin layer chromatography (TLC) analysis (Staneck and Roberts, 1974). Polar lipids were extracted, separated by two-dimensional TLC and identified according to procedures outlined by (Minikin et al. 1984) with modifications proposed by (Kroppenstedt and Goodfellow 2006). The composition of peptidoglycan hydrolysates (6 N HCl, 100ºC for 16 h) was examined by TLC as described by (Schleifer and Kandler 1972). The extraction and analysis of cellular fatty acids was carried out from biomass grown in shaking incubator at 150 r.p.m for 3 days held at 28ºC. Analysis was conducted using the Microbial Identification System (MIDI) Sherlock Version 6.1 (TSBA40 database) as described by (Sasser 1990). Fatty-acid patterns were visualized as heat map using the lipid extensions of the opm package (Vaas et al. 2013).

Genetic and phylogenetic analysis

G + C content of chromosomal DNA of strain No.156^T was determined by HPLC according to (Mesbah 1989). Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and purification of the PCR product was carried out as described by (Rainey et al. 1996). The resulting sequence was compared with 16S rRNA gene sequences of other type strains using the EzTaxon server (Kim et al. 2012). Closely related 16S rRNA gene sequences to strain No.156^T were downloaded from EzTaxon. Phylogenetic analysis was based on alignment of 16S rRNA gene sequences from closely related type strains with validly published names in the genus *Nocardiopsis* inferred as described by Montero-Calasanz et al. (2013a). Rooting was done using the midpoint method (Montero-Calasanz et al., 2013a). Pairwise similarities were calculated as recommended by Meier-Kolthoff et al. (2013) and

Montero-Calasanz et al. (2013b). DNA-DNA relatedness experiments were not carried out between strain No.156^T and its closest phylogenetic neighbours as the level of 16S rRNA gene sequence similarity between the strains was less than the cut-off value recommended for genomic distinction of species by Stackebrandt and Goebel (1994) and Meier-Kolthoff et al. (2013).

Results and discussion

Strain No.156^T was found to be aerobic, Gram-reaction-positive and formed a well-branched substrate mycelium with long, densely branched hyphae (Figure 1). Fragmentation of substrate mycelia did not occur as is typical of *Nocardiopsis* species (Labeda et al. 1984). Strain No.156^T grew abundantly on marine and Sabouraud agar, moderately on CASO agar, Czapek agar, nutrient agar and ISP3 agar, sparsely on ISP2 agar and potato dextrose agar, but did not grow on ISP4 agar. The substrate mycelium was yellow and formed white aerial mycelia on ISP3, ISP4 and marine agar, but not on the other media tested. No diffusible pigments were produced. Strain No.156^T grew over a temperature range of 25-45^{o}C (optimum, 30-35^{o}C), pH 7.0-12.0 (optimum, pH 7.0-9.0) and at NaCl concentrations of 1-9% (w/v) (optimum, 6-9% (w/v)). The strain was positive for catalase, DNase activity and degradation of casein but negative for xanthine, starch and tyrosine degradation. No mycolic acids were detected in whole cell methanolysates which is in line with earlier report by Rainey et al. (1996). Analysis of cell-wall components revealed the presence of meso-diaminopimelic acid and no diagnostic sugars (Lechevalier et al. 1971; Labeda et al. 1984). The polar lipid pattern revealed the presence of the diagnostic components phosphatidylmethylethanolamine (PME) and phosphatidylcholine (PC), as well as phosphatidylglycerol (PG), phosphatidylinositol (PI) and four unspecified glycolipids (GL1-4). Strain No.156^T did however not have diphosphatidylglycerol (DPG) and other additional phospholipids, which is unique for this genus (Supplementary figure 1). The predominant menaquinones were MK-11(H_8) (23%), MK-11(H_6) (22%), MK-10(H_8) (20%) and MK-10(H_6) (17%) as previously described for the reference strains *N. rosea* DSM 44842^T, *N. gilva* DSM 44841^T and *N. rhodophaea* DSM 44843^T whose predominant menaquinones consist of 11 isoprene units in the side chain and a variable degree of saturation (Table 2). This quinone system is unique among species of the genus and has also been reported for the type strains of *N. composta* (Kämpfer et al. 2002) and *N. potens* (Yassins et al. 2009) while other representatives of *Nocardiopsis* are reported to contain a quinone system dominated by menaquinone MK-10 and a variable degree of saturation of the isoprenoid side chain (Collins et al.,

1977). The following fatty acids were detected (>2%): $C_{18:1\omega9c}$ (22.2%), *iso*-$C_{16:0}$ (19.7%), 10-methyl-$C_{18:0}$ (tuberculostearic acid, 12.5%), *iso*-$C_{16:0}$ (7.6%), *anteiso*-$C_{17:1}$ (7.1%), *anteiso*-$C_{17:0}$ (4.6%), $C_{16:1\omega9c}$ (3.3%) and 10-methyl-$C_{16:0}$ (2.9%) (Table 3). Fatty acid patterns were also visualized as heat map (Supplementary figure 2). This combination of fatty acids with *iso*/*anteiso*-branched fatty acids, smaller amounts of 10-methyl-branched and unbranched fatty acids is characteristic for *Nocardiopsis* species (Fischer et al. 1983; Kroppenstedt 1992). Genomic G + C content was 71.0 mol%. In API ZYM tests the culture was positive for alkaline phosphotase, lipase C14, leucine aminopeptidase, valine aminopeptidase, cystine aminopeptidase, trypsin, chymotrypsin, acid phosphatase, phosphoamidase, α-glucosidase, and N-Acetyl-β-glucosaminidase, but no enzymatic activity was observed for α-galactosidase, β-glucosidase esterase C4, lipase C8, β-glucuronidase, α-mannosidase and α-fucosidase. In API 20 NE the culture was positive for cytochrome oxidase, urea and aesculin hydrolysis L-arginine, β-glucosidase β-galactosidase, D-glucose, L-arabinose, D-mannose, D-mannitol, D-maltose and fermentation of glucose. Reduction of nitrates to nitrites, indole production and gelatin hydrolysis was not observed. The chemotaxonomic properties of strain No.156^T were consistent with its classification in the genus *Nocardiopsis* (Meyer 1976; Kroppenstedt 1992). A summary of selected differential phenotypic characteristics are presented in Table 2.

The almost complete (1463 bp) 16S rRNA gene sequence revealed the strain was closely related to the representatives of the genus *Nocardiopsis* and showed the highest degree of similarities with the type strains of *N. rosea* (97.9%), *N. composta* (97.7%), *N. gilva* (97.5%), *N. rhodophaea* (97.3%) and *N. halophila* (97.2%). Strain No.156^T and the closely related type strains clustered with members of the genus *Nocardiopsis* by both maximum likelihood and maximum-parsimony estimations (Figure 2). Based on its 16S rRNA sequences, it is clear that the isolate strain No.156^T belongs to the genus *Nocardiopsis* and represents a novel species.

The presented data demonstrates that strain No.156^T is a representative of a novel species of the genus *Nocardiopsis*. The strain can be differentiated from other *Nocardiopsis* species by cultural and phenotypic characteristics given in Table 1 and 2 respectively. The distinctness of this organism is further seen by the distinct phylogenetic position within the genus *Nocardiopsis* (Figure 2). The name *Nocardiopsis mwathae* is therefore proposed for the novel species with strain No.156^T as the type strain.

Description of *Nocardiopsis mwathae* sp. nov.

Nocardiopsis mwathae (N.L. gen. fem. n. *mwathae*, of mwatha, named after Kenyan microbial ecologist, the late Professor Wanjiru Mwatha in recognition of her pioneering work on Kenyan soda lakes).

Aerobic, Gram-reaction-positive bacterium. Forms well-branched substrate mycelium on marine agar. Grows abundantly on marine and Sabouraud agar, moderately on CASO, Czapek, nutrient and ISP3 agar, sparsely on ISP2 agar and potato dextrose agar but not on ISP4 agar. Forms yellow substrate mycelia on all media tested and white aerial mycelia on ISP3, ISP4 and marine agar. Diffusible pigments are not produced. Optimum growth is at 30-35^{o}C, pH 7.0-9.0 with 6-9% (w/v) NaCl. Positive for catalase, DNase activity and degradation of casein, negative for starch, xanthine and tyrosine degradation. Mycolic acids were not detected. Cell wall contains meso-diaminopimelic acid and no diagnostic sugars. The main polar lipids are phosphatidylmethylethanolamine, phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol and four unspecified glycolipids, but no diphosphatidylglycerol and uncharacterised phospholipids. The predominant menaquinones are MK-11(H_8), MK-11(H_6), MK-10(H_8) and MK-10(H_6). Major cellular fatty acids are $C_{18:1\omega9c}$ (22.2%), *iso*-$C_{16:0}$ (19.7%), 10-methyl-$C_{18:0}$ (tuberculostearic acid, 12.5%). The type strain has a genomic DNA G + C content of 71.0 mol %. In API ZYM tests, the culture is positive for alkaline phosphotase, lipase C14, leucine aminopeptidase, valine aminopeptidase, cystine aminopeptidase, trypsin, chymotrypsin, acid phosphatase, phosphoamidase, α-glucosidase and N-Acetyl-β-glucosaminidase. No enzymytic activity is observed for α-galactosidase, β-glucosidase, esterase C4, lipase C8, β-glucuronidase, α-mannosidase and α-fucosidase. In API 20 NE, the culture is positive for cytochrome oxidase, urea and aesculin hydrolysis, L-arginine, β-glucosidase, β-galactosidase, D-glucose, L-arabinose, D-mannose, D-mannitol, D-maltose and fermentation of glucose. Reduction of nitrates to nitrites, indole production and gelatin hydrolysis is not observed. The INSDC accession number for the 16S rRNA gene sequences of the type strain No.156^{T} (= DSM 46659 = CECT 8552) is KF976731.

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Bibliography

Al Tai AM, Ruan JS (1994) *Nocardiopsis halophila* sp. nov., a new halophilic actinomycete isolated from soil. Int J Syst Bacteriol 44:474-478

Al-Zarban SS, Abbas I, Al Musallam AA, Steiner U, Stackebrandt E, Kroppenstedt RM (2002) *Nocardiopsis halotolerans* sp. nov., isolated from salt marsh soil in Kuwait. Int J Syst Evol Microbiol 52:525–529

Bernatchez H, Lebreux E (1991) *Nocardiopsis dassonvillei* recovered from a lung biopsy and a possible cause of extrinsic alveolitis. Clin Microbiol Newsl 6:47–55

Bruns A, Cypionka H (2002) Cyclic AMP and Acyl Homoserine Lactones Increase the Cultivation Efficiency of Heterotrophic Bacteria from the Central Baltic Sea. Appl Environ Microbiol 68:3978–3987

Collins MD, Pirouz T, Goodfellow M, Minnikin DE (1977) Distribution of menaquinones in actinomycetes and corynebacteria. J Gen Microbiol 100:221-230

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

Gregersen T (1978) Rapid method for distinction of gram-negative from positive bacteria. Appl Microbiol Biotechnol 5:123-127

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

Kämpfer P, Busse H, Rainey FA (2002) *Nocardiopsis compostus* sp. nov., from the atmosphere of a composting facility. Int J Syst Evol Microbiol 52:621–627

Kim OS, Cho YJ, Lee K, Yoon SH, Kim M, Na H, Park SC, Jeon YS, Lee JH, Yi H, Won S, Chun J (2012) Introducing EzTaxon-e: a prokaryotic 16S rRNA Gene sequence database with phylotypes that represent uncultured species. Int J Syst Evol Microbiol 62:716–721

Kroppenstedt RM (1982) Separation of bacterial menaquinones by HPLC using reverse phase (RP18) and a silver loaded ion exchanger. J Liq Chromatogr 5:2359-2387

Kroppenstedt RM, Goodfellow M (2006) The family *Thermomonosporaceae*: *Actinocorallia, Actinomadura, Spirillispora* and *Thermomonospora*. In: Dworkin M, Falkow S, Schleifer KH, Stackebrandt E (eds) The prokaryotes, 3rd edn, vol 3, Archaea and Bacteria: *Firmicutes, Actinomycetes*. New York, Springer, pp 682-724

Kroppenstedt RM (1985) Fatty acid and menaquinone analysis of actinomycetes and related organisms. In: Goodfellow M, Minnikin E (eds) Chemical Methods in Bacterial Systematics. London, Academic Press, pp 173-199

Kroppenstedt RM (1992) The genus *Nocardiopsis*. In: Balows A, Trüper HG, Dworkin M, Harder W, Schleifer K-H (eds) The Prokaryotes, 2nd edn. New York, Springer, pp 1139-1156

Labeda DP, Testa RT, Lechevalier MP, Lechevalier HA (1984) *Saccharothrix*: a new genus of the *Actinomycetales* related to *Nocardiopsis*. Int J Syst Bacteriol 34:426-431

Lechevalier MP, Lechevalier HA (1970) Chemical composition as a criterion in the classification of aerobic actinomycetes. Int J Syst Bacteriol 20:435-443

Lechevalier HA, Lechevalier MP, Gerber NN (1971) Chemical composition as a criterion in the classification of actinomycetes. Adv Appl Microbiol 14:47-72

Lechevalier MP, DeBiévre C, Lechevalier HA (1977) Chemotaxonomy of aerobic actinomycetes: phospholipid composition. Biochem Syst Ecol 5:249–260

Li HW, Zhi XY, Yao JC, Thou Y, Tang SK, Klenk HP, Zhao J, Li WJ (2013) Comparative genomic analyses of the genus *Nocardiopsis* provides new insights into its genetic mechanisms of environmental adaptability. PLoS ONE 8:e61528

Li J, Yang J, Zhu WY, He J, Tian XP, Xie Q, Li WJ (2012) *Nocardiopsis coralliicola* sp. nov., isolated from the gorgonian coral, Menella praelonga. Int J Syst Evol Microbiol 62:1653–1658

Li WJ, Kroppenstedt RM, Wang D, Tang SK, Lee JC, Park DJ, Jiang CL (2006) Five novel species of the genus *Nocardiopsis* isolated from hypersaline soils and emended description of *Nocardiopsis salina* Li et al. 2004. Int J Syst Evol Microbiol 56:1089–1096

Li WJ, Park DJ, Tang SK, Wang D, Lee JC, Xu LH, Kim CJ, Jiang CL (2004) *Nocardiopsis salina* sp. nov., a novel halophilic actinomycete isolated from saline soil in China. Int J Syst Evol Microbiol 54:1805–1809

Meier-Kolthoff JP, Göker M, Spröer C, Klenk HP (2013) When should a DDH experiment be mandatory in microbial taxonomy? Arch Microbiol 195:413-418

Melack JM (1988) Primary producer dynamics associated with evaporative concentration in a shallow, equatorial soda lake (Lake Elmenteita, Kenya). Hydrobiologia 158:1–14

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

Meyer J (1976) Nocardiopsis, a new genus of the order Actinomycetales. Int J Syst Bacteriol 26:487–493

Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M, Schaal K, Parlett JH (1984) An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. J Microbiol Methods 2:233-241

Minnikin DE, Hutchinson IG, Caldicott AB, Goodfellow M (1980) Thin layer chromatography of methanolysates of mycolic acid-containing bacteria. J Chromatogr 188:221-233

Montero-Calasanz MC, Göker M, Pötter G, Rohde M, Spröer C, Schumann P, Gorbushina AA, Klenk HP (2013a) *Geodermatophilus africanus* sp. nov., an halotolerant actinomycete isolated from Saharan desert sand. Antonie van Leeuwenhoek 104:207-216

Mwaura F (1999) A spatio-chemical survey of hydrogeothermal springs in Lake Elmenteita, Kenya. Int J Salt Lake Res 8:127–138

Mwirichia R, Cousin S, Muigai AW, Boga HI, Stackebrandt E (2010a) Archaeal diversity in the haloalkaline Lake Elmenteita in Kenya. Curr Microbiol 60:47–52

Mwirichia R, Muigai AW, Tindall B, Boga HI, Stackebrandt E (2010b) Isolation and characterisation of bacteria from the haloalkaline Lake Elmenteita, Kenya. Extremophiles14:339–348

Pelczar MJ (1957) Manual of Microbiological Methods. New York, NY: McGraw-Hill Book Co

Peltola JSP, Andersson MA, Kämpfer P, Auling G, Kroppenstedt RM, Busse HJ, Salkinoja-Salonen MS, Rainey FA (2001) Isolation of toxigenic Nocardiopsis strains from indoor environments and description of two new *Nocardiopsis* species, *N. exhalans* sp. nov. and *N. umidischolae* sp. nov. Appl Environ Microbiol 67:4293–4304

Rainey FA, Ward-Rainey N, Kroppenstedt RM, Stackebrandt E (1996) The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of *Nocardiopsiaceae* fam. nov. Int J Syst Bacteriol 46:28-96

Sasser M (1990) Identification of bacteria by gas chromatography of cellular fatty acids, MIDI Technical Note 101.MIDI Inc., Newark

Schippers A, Bosecker K, Willscher S, Spröer C, Schumann P, Kroppenstedt RM (2002) *Nocardiopsis metallicus* sp. nov., a metal-leaching actinomycete isolated from an alkaline slag dump. Int J Syst Evol Microbiol 52:2291–2295

Schleifer KH, Kandler O (1972) Peptidoglycan types of bacterial cell walls and their taxonomic implications. Bacteriol Rev 36:407-477

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

Smibert RM, Krieg NR (1994) Phenotypic characterization. In: Gerhardt P, Murray RGE, Wood WA, Krieg NR (eds) Methods for General and Molecular Bacteriology. Washington DC, American Society for Microbiology, pp 607–654.

Stackebrandt E, Goebel BM (1994) Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. Int J Syst Bacteriol 44: 846–849

Staneck JL, Roberts GD (1974) Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. Appl Microbiol 28:226-231

Tang SK, Li WJ, Wang D, Zhang YG, Xu LH, Jiang CL (2003) Studies of the biological characteristics of some halophilic and halotolerant actinomycetes isolated from saline and alkaline soils. Actinomycetologica 17:6–10

Williams ST, Goodfellow M, Alderson G, Wellington EMH, Sneath PHA, Sackin MJ (1983) Numerical classification of *Streptomyces* and related genera. J Gen Microbiol 129:1743–1813

Xu P, Li WJ, Tang SK, Zhang YQ, Chen GZ, Chen HH, Xu LH, Jiang CL (2005) *Naxibacter alkalitolerans* gen. nov., sp. nov., a novel member of the family '*Oxalobacteraceae*' isolated from China. Int J Syst Evol Microbiol 55:1149–1153

Yang R, Zhang LP, Guo LG, Shi N, Lu Z, Zhang X (2008) *Nocardiopsis valliformis* sp. Nov., an alkaliphilic actinomycete isolated from alkali lake soil in China. Int J Syst Evol Microbiol 58:1542–1546

Yassin AF, Galinski EA, Wohlfarth A, Jahnke K-D, Schaal KP Trüper HG (1993a) A new actinomycete species, *Nocardiopsis lucentensis* sp. nov. Int J Syst Bacteriol 43:266–271

Yassin AF, Rainey FA, Burghardt J, Gierth D, Ungerechts J, Lux I, Seifert P, Bal C, Schaal KP (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

Yassin F, Spröer C, Hupfer H, Siering C, Klenk HP (2009) *Nocardiopsis potens* sp. nov., isolated from household waste. Int J Syst Evol Microbiol 59:2729–2733

Vaas LAI, Sikorski J, Hofner B, Buddruhs N, Fiebig A, Klenk HP, Göker M (2013) opm: An R package

for analysing OmniLog® Phenotype MicroArray Data. Bioinformatics 29:1823-1824

Figure 1. Scanning electron micrograph of strain No.156^T grown on marine agar for 21 days at 28°C.

Figure 2. Maximum likelihood phylogenetic tree inferred from 16S rRNA gene sequences, showing the phylogenetic position of strain No.156^T relative to the type strains within the genus *Nocardiopsis*. The branches are scaled in terms of the expected number of substitutions per site (see size bar). Support values from maximum-likelihood (left) and maximum-parsimony (right) bootstrapping are shown above the branches if equal to or larger than 60%.

Supplementary figure 1. Polar lipids profile of *Nocardiopsis mwathae* sp. nov. No.156^T, after separation by two-dimensional TLC. Plate was sprayed with molydatophosphoric acid for detection of total polar lipid. PC, phosphatidylcholine; PME, phosphatidylmethylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol and GL, unspecified glycolipids

Supplementary figure 2. Heatmaps generated using the lipid extensions of the opm package (Vaas et al. 2013), showing un-transformed and log-transformed measurements respectively, under default settings after importing the result files from the MIDI system. Strains: *N. mwathae* sp. nov. No.156^T DSM 46659^T, *N. rosea* DSM 44842^T, *N. gilva* DSM 44841^T, *N. baichengensis* DSM 44845^T, *N. rhodophaea* DSM 44843^T, *N. chromatogenes* DSM 44844^T, *N. halophila* DSM 44494^T and *N. composta* DSM 44551^T.

Table 1. Differential cultural characteristics of strain No.156^T and the reference strains: 1, *N. mwathae* sp. nov. No.156^T; 2, *N. rosea* DSM 44842^T; 3, *N. gilva* DSM 44841^T; 4, *N. baichengensis* DSM 44845^T; 5, *N. rhodophaea* DSM 44843^T; 6, *N. chromatogenes* DSM 44844^T; 7, *N. halophila* DSM 44494^T and 8, *N. composta* DSM 44551^T. All strains were examined after growing on each of nine different media for 21 days at 28^{o}C. All data are from the present study, unless indicated otherwise.

Cultural characteristics	1	2	3	4	5	6	7	[a]8
CASO								
Growth	++	++	+++	+++	+++	++	++	
Aerial spore-mass	-	-	-	-	white	-	-	
Substrate mycelium	yellow	light red	yellow	yellow	light red	light red	yellow	
Czapek								
Growth	++	++	++	+++	++	+++	+++	
Aerial spore-mass	-	white	-	white	white	white	white	
Substrate mycelium	yellow	light red	yellow	yellow	light red	beige red	yellow	
ISP 2								
Growth	+	++	++	+++	++	++	+++	
Aerial spore-mass	white	white	white	white	white	white	white	
Substrate mycelium	yellow	light red	yellow	cream	light red	black red	pale yellow	
ISP 3								
Growth	++	++	++	++	++	+	+	
Aerial spore-mass	white	-	white	-	white	-	-	
Substrate mycelium	yellow	light red	yellow	yellow	light red	beige red	yellow	nd
ISP 4								
Growth	-	++	+	++	++	+	-	
Aerial spore-mass	-	-	-	white	-	-	-	
Substrate mycelium	-	light red	yellow	yellow	light red	light red	-	
Marine agar								
Growth	+++	++	+++	+++	+++	++	++	
Aerial spore-mass	white	white	-	white	-	white	white	
Substrate mycelium	yellow	light red	yellow	yellow	light red	light red	yellow	
Nutrient agar								
Growth	++	++	++	+++	++	++	++	
Aerial spore-mass	-	-	-	white	-	-	-	
Substrate mycelium	yellow	light red	yellow	yellow	light red	beige red	yellow	
Potato dextrose								
Growth	+	++	++	+++	++	++	++	
Aerial spore-mass	-	-	white	white	-	white	white	
Substrate mycelium	yellow	light red	yellow	yellow	light red	black red	pale yellow	
Sabouraud								
Growth	+++	+++	++	++	++	+++	++	
Aerial spore-mass	-	-	white	white	-	-	-	
Substrate mycelium	yellow	light red	yellow	yellow	light red	black red	yellow	

-, no growth; +, sparse; ++, moderate; +++, abundant. All data are from this study. [a]; data obtained from Kämpfer et al. 2002

Table 2. Differential phenotypic characteristics of strain No.156^{T} and the reference strains. Order of strains as above. All data are from the present study, unless indicated otherwise.

Phenotypic characteristic	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Optimum growth								
Temperatures (^{0}C)	30-35	30-40	30-40	35-40	30-40	30-40	25-30	[a]30-40
pH	7-9	7-8	7-8	7-8	7-8	7-8	7-9	[a]7-9
NaCl% (w/v)	6-9	5-7	6-8	6-8	5-8	4-9	6-12	[a]5-10
Degradation of								
DNA	+	+	+	+	+	-	+	-
Xanthine	-	-	-	-	-	-	+	-
Enzymatic activity								
Lipase C14	+	+	-	+	-	+	+	+
Trypsin	+	+	+	-	+	-	-	-
α-galactosidase	-	-	-	+	-	+	+	-
β-galactosidase	-	-	-	+	-	+	+	+
β-glucosidase	-	-	+	+	+	+	+	-
API 20NE								
Aesculin	+	+	-	-	+	+	-	+
Nitrate reduction	-	-	+	-	-	-	-	-
Gelatin	-	-	-	+	-	-	-	+
Urea	+	-	+	+	+	+	+	-
Polar lipids (*PIII)	PIII; PME, PC, PG, PI, GL1-4	[b]PIII; DPG, PME, PG, PC, PI, PE, PL, GL	[b]PIII; DPG, PME, PG, PC, PI, PE, PL, GL	[b]PIII; DPG, PME, PG, PC, PI, PIM, PL, PE, GL	[b]PIII; DPG, PME, PG, PC, PI, PIM, PL, PE, GL	[b] PIII; DPG, PG, PC, PI, PME, PL, PIM, PE, GL	[b]PIII	[a]PIII; PME, PC, DPG, PG, PL1, PL2
Major menaquinones	MK-11($H_{6,}$ H_8) MK-10($H_{6,}$ $H_{8,}$)	[b]MK-11($H_{0,}$ $H_{2,}$ H_4)	[b]MK-11($H_{4,}$ $H_{6,}$ $H_{8,}$)	[b]MK-10(H_2, $H_{4,}$ $H_{6,}$)	[b]MK-11($H_{6,}$ H_8)	[b]MK-10(H_0, $H_{2,}$ $H_{4,}$)	[b]MK-10($H_{6,}$ $H_{8,}$)	[a]MK-11(H_8) MK-10($H_{6,}$ $H_{8,}$)
DNA G+C content (mol%)	71.0	[b]67.9[b]	[b]68.1	[b]73.2	[b]69.0	[b]71.8	[b]71.5	[a]74.7

+, positive reaction; –, negative reaction; DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PME, phosphatidylmethylethanolamine; PC, phosphatidylcholine; PIM, phosphatidylinositolmannosides; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PL, unspecified phospholipid; GL, unspecified glycolipid; [a]; data obtained from Kämpfer et al. 2002, [b]; data obtained from Li et al. (2006). *; PIII refers to the phospholipid pattern as described by Lechevalier et al. (1977)

Table 3. Cellular fatty acid compositions of strain No.156^T and the reference strains. Strain order as above. Values are (>1) percentages of total fatty acids. All data are from the present study.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Saturated (straight chain) fatty acids								
15:0								1.84
16:0	1.43	1.05		1.65	1.48	3.44	1.59	4.32
17:0					1.56			4.46
18:0	2.01	2.09	2.76	2.63	3.62	2.87	2.56	6.35
Saturated (branched chain) fatty acids								
*i*14:0		1.33			1.47	1.01		2.47
*i*15:0		1.87	1.46		3.85			2.43
*i*16:0	7.59	27.10	5.33	22.74	16.25	28.66	33.55	11.68
*i*17:0	1.60	3.94	3.29	2.83	7.47	1.95	2.06	2.12
*i*18:0	1.30	3.15	3.65	1.63	2.43	1.69	1.51	
*ai*15:0	2.64	5.71	2.80	3.87	10.52	5.59	4.60	12.18
*ai*17:0	4.55	14.76	4.87	19.11	20.29	13.97	16.43	13.23
*ai*17:1c			10.68	2.53			2.34	
*ai*17:1a	7.14	1.30			1.18	1.34		1.91
16:0 10METHYL	2.93		5.06	1.96		1.70	2.12	
17:0 10METHYL	2.09	10.96	8.52	7.03	4.05	5.09	5.01	3.04
TBSA 18:0 10METHYL	12.47	7.94	9.15	22.94	2.48	22.20	20.16	5.74
monounsaturated fatty acids								
16:1 *iso* G	19.68	2.57	20.99	1.44		1.38	2.05	1.03
18:1 *iso* G	2.67		2.21					
16:1 ω_9c	3.28		2.18	1.70	2.15	2.18	1.74	3.12
17:1 ω_9c		3.22	3.84	1.09	6.06			9.00
18:1 ω_9c	22.19	9.52	7.97	3.84	11.62	3.23	1.92	11.85
Sum in feature 7	2.51							

i-, *iso*-branched, *ai*-, *anteiso*-branched; Sum in feature 7: 18:1cis 11/4 9/t 6