Nocardiopsis algeriensis sp. nov., an alkalitolerant actinomycete isolated from Saharan soil

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Abstract An alkalitolerant actinomycete strain, designated B32^T, was isolated from a Saharan soil sample collected from Adrar province (South of Algeria), and then investigated using a polyphasic taxonomic approach. The strain was observed to produce short chains of spores on the dichotomous branched aerial mycelium and formed a fragmented substrate mycelium. The optimum NaCl concentration for growth was found to be 0–5 % (w/v) and the optimum growth temperature and pH were found to be 25–35 °C and 7.0–10.0 °C, respectively. The diagnostic diamino acid in the cell-wall peptidoglycan was identified as *meso*-diaminopimelic acid. The predominant menaquinones of strain B32^T were identified as MK-10 (H_4) and MK-11 (H_4). The major fatty acids were found to be iso-$C_{16:0}$ and anteiso-$C_{15:0}$. The diagnostic phospholipids detected were phosphatidylcholine, phosphatidylmethylethanolamine, diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. The chemotaxonomic properties of strain B32^T are consistent with those shared by members of the genus *Nocardiopsis*. 16S rRNA gene sequence analysis indicated that strain B32^T is most closely related to *Nocardiopsis alba* DSM 43377^T (98.7 %), *Nocardiopsis lucentensis* DSM 44048^T (98.6 %), *Nocardiopsis aegyptia* DSM 44442^T (98.6 %), *Nocardiopsis sinuspersici* HM6^T (98.6 %) and *Nocardiopsis arvandica* HM7^T (98.5 %). However, the DNA–DNA relatedness values between strain B32^T and the closely related type strains were 17.9, 14.6, 31.1, 27.1

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and 14.1 %, respectively. Based on the combined genotypic and phenotypic evidence, it is proposed that strain B32^T should be classified as representative of a novel species, for which the name *Nocardiopsis algeriensis* sp. nov. is proposed. The type strain is B32^T (=DSM 45462^T = CECT 8712^T).

Keywords *Nocardiopsis* · *Nocardiopsis algeriensis* sp. nov. · Alkalitolerant actinomycete · Saharan soil · Polyphasic taxonomy

Introduction

The genus *Nocardiopsis* was first described by Meyer (1976) and was affiliated with the family *Nocardiopsaceae* (Rainey et al. 1996). Numerous studies have shown that *Nocardiopsis* strains are ubiquitously distributed in the environment (Kroppenstedt and Evtushenko, 2006). Many of these species prefer moderately alkaline conditions (pH 8.5) (Kroppenstedt 1992) and some grow better on media supplemented with sodium chloride. At the time of writing, there were 45 species of the genus *Nocardiopsis* with validly published names. The genus *Nocardiopsis* was shown to exhibit distinct chemotaxonomic characteristics: cell-wall chemotype III/C (*meso*-diaminopimelic acid without diagnostic sugar in whole-cell hydrolysates; Lechevalier and Lechevalier 1970), phospholipid type PIII (phosphatidylcholine as characteristic phospholipid; Lechevalier et al. 1977), menaquinone MK-10 with variable degrees of saturation in the side chain as the predominant isoprenoid quinone (Kroppenstedt 1992), fatty acid type 3d containing iso-branched, anteiso-branched and 10-methyl-branched chain fatty acids (Kroppenstedt 1985) and the G+C content of the genomic DNA between 64 and 71 mol% (Grund and Kroppenstedt 1990).

During our study on the diversity and taxonomy of bacterial communities in Saharan environment, a *Nocardiopsis*-like strain designated B32^T was isolated from a sample collected from Adrar (Algerian Sahara). Based on the results of the present polyphasic taxonomic study, it is proposed that strain B32^T represents a novel species of the genus *Nocardiopsis*, named *Nocardiopsis algeriensis* sp. nov.

Materials and methods

Isolation and maintenance of strain

Strain B32^T was isolated from a non-rhizospheric soil sample collected from Aougrout (28°69′16″N, 0°31′62″E), Adrar province, Touat region (Algeria). Isolation was carried out by a dilution-agar plating method on complex medium (CM) agar (Chun et al. 2000) containing 0.5 % (w/v) NaCl supplemented with cycloheximide (50 μg ml^{-1}) to reduce fungal growth. The pH was adjusted to pH 10.0 by using autoclaved NaOH. After incubation at 30 °C for 1 week, the *Nocardiopsis*-like strain was picked up and purified on CM agar (pH 10.0) containing 0.5 % (w/v) NaCl and incubated at 30 °C. The strain was stored at 4 °C on the same medium, and also at −20 °C as 20 % (v/v) glycerol suspensions.

Strain B32^T was deposited in the German Collection of Microorganisms and Cell Cultures (DSMZ), Germany, as strain DSM 45462^T and in Spanish Type Culture Collection (CECT), Spain, as strain CECT 8712^T.

Phenotypic characterization

Cultural characteristics were investigated after 7, 14 and 21 days of incubation at 30 °C on media of the International *Streptomyces* Project, ISP 2 and ISP 4 (Shirling and Gottlieb 1966), and also on CM agar (Chun et al. 2000) containing 0.5 % (w/v) NaCl. The degree of growth was determined and the colours of the substrate and aerial mycelia and any soluble pigments produced were determined by comparison with ISCC-NBS colour charts (Kelly and Judd 1976). The morphological characteristics of strain B32^T, including spore-chain morphology, spore size and surface ornamentation, were observed by using a model B1 Motic light microscope and a model Hitachi S-450 scanning electron microscope.

Strain B32^T was characterized by using sixty-three physiological tests. All tests were made at pH 7.5 (except those of pH test). Production of acid from twenty-two carbohydrates and decarboxylation of nine organic acids was determined according to the methods of Gordon et al. (1974). Degradation of adenine, gelatin, guanine, hypoxanthine, milk casein, starch, testosterone, Tween 80, tyrosine and xanthine was studied as described by Goodfellow (1971) and

Marchal et al. (1987). Lysozyme sensitivity and production of nitrate reductase were determined according to the methods of Gordon and Barnett (1977) and Marchal et al. (1987), respectively. Production of melanoid pigments was tested on ISP 6 and ISP 7 media (Shirling and Gottlieb 1966). Growth in the presence of chloramphenicol (25 μg ml^{-1}), erythromycin (10 μg ml^{-1}), kanamycin (5 μg ml^{-1}), penicillin (25 μg ml^{-1}) and streptomycin (10 μg ml^{-1}), at different temperatures (20, 25, 30, 35, 40 and 45 °C) and at different NaCl concentrations (0, 5, 7.5, 10 % w/v) was determined on nutrient agar medium. The following buffers were used to test the pH range of growth on nutrient broth medium: pH 6.0, 7.0 and 8.0, 0.1 M KH_2PO_4/0.1 M NaOH; pH 9.0 and 10.0, 0.1 M $NaHCO_3$/0.1 M Na_2CO_3; pH 11.0, 0.05 M Na_2HPO_4/0.1 M NaOH and pH 12.0, 0.2 M KCl/0.2 M NaOH. After the basic medium was sterilized, the pH was adjusted to pH 6.0, 7.0, 8.0, 9.0, 10.0, 11.0 or 12.0 using autoclaved buffer solutions before pouring the medium onto plates.

Chemical analysis of cell constituents

For chemical analysis, the biomass of strain B32^T was harvested by centrifugation at 3,500 rpm of cultures growing in complex medium (CM) broth (pH 7.5) (containing only 0.5 % (w/v) NaCl) at 30 °C for 6 days on rotary shaker (250 rpm). The isomeric form of diaminopimelic acid and the presence (or not) of glycine in the cell wall were ascertained as described by Becker et al. (1964). The composition of whole-cell sugars was determined as described by Lechevalier and Lechevalier (1970). Phospholipids were analyzed according to the method of Minnikin et al. (1977). The fatty acid profile was determined by the method of Sasser (1990), using the Microbial Identification System (MIDI). Menaquinones were isolated according to Minnikin and O'Donnell (1984) and were analyzed by HPLC (Kroppenstedt 1982, 1985).

Phylogenetic analyses

Strain B32^T was grown in CM broth containing only 0.5 % (w/v) NaCl and genomic DNA was extracted with a DNA extraction kit (MasterPure Gram Positive DNA Purification kit; Epicentre Biotechnologies). PCR amplification of the 16S rRNA gene was performed as described by Rainey et al. (1996). PCR products were purified with a PCR product purification kit (Qiagen). The primers used for sequencing were as listed in Coenye et al. (1999). The sequence obtained was compared with sequences present in the public sequence databases as well as with the EzTaxon-e server (Kim et al. 2012). Phylogenetic analyses were conducted using MEGA version 5 (Tamura et al. 2011). The 16S rRNA gene sequence of strain B32^T was aligned against neighbouring nucleotide sequences using CLUSTAL W (with default parameters) (Larkin et al. 2007). Phylogenetic trees were reconstructed by using the neighbour-joining method (Saitou and Nei 1987) with the model of Jukes and Cantor (1969). The topology of the trees was evaluated by bootstrap analysis based on 1,000 replicates (Felsenstein 1985).

DNA–DNA hybridization

For DNA–DNA hybridizations, cells were disrupted by using a French pressure cell (Thermo Spectronic). The DNA in the crude lysate was purified by chromatography on hydroxyapatite as described by Cashion et al. (1977). DNA–DNA hybridization was carried out as described by De Ley et al. (1970) under consideration of the modifications described by Huss et al. (1983), using a model Cary 100 Bio UV/Vis spectrophotometer equipped with a Peltier-thermostatted 666 multicell changer and a temperature controller with in situ temperature probe (Varian). DNA–DNA hybridization experiments were done in duplicate in 2× SSC in the presence of 10 % formamide at 71 °C.

Results and discussion

Morphological observation of a 3 weeks old culture of strain B32^T revealed that the substrate mycelium was well fragmented into oval spores. The strain was observed to produce short chains of one to six (or more) non-motile spores on the dichotomous well developed branched aerial mycelium (Fig. 1). Strain B32^T was found to show good growth on CM agar (with 0.5 % NaCl) and ISP 2 media and poor growth on ISP 4 medium. Yellowish white aerial mycelium was found to be produced on CM agar medium, but not well developed on ISP 2 and ISP 4 media. The substrate mycelium was observed to be cream yellow

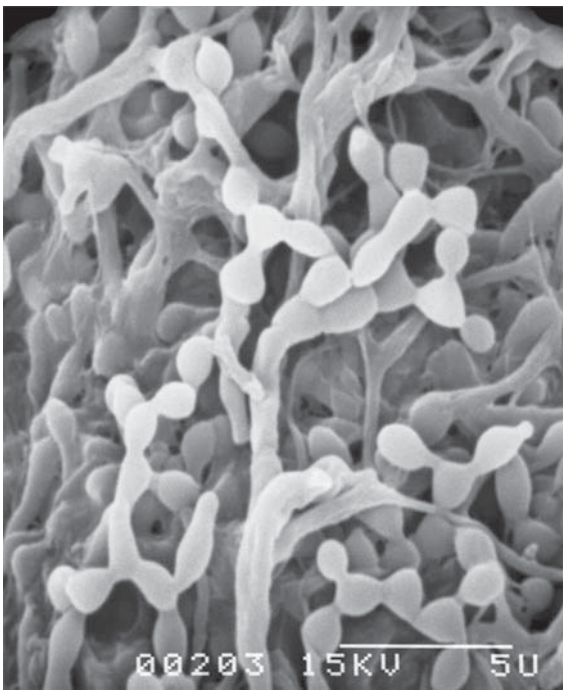

Fig. 1 Scanning electron micrograph of aerial mycelium of strain B32^T grown on complex medium agar (pH 10.0) containing 0.5 % (w/v) NaCl for 1 week and incubated at 30 °C. *Bar* 5 μm

to light yellow on CM agar medium, pale yellow on ISP 2 medium and pale yellow to greenish yellow on ISP 4 medium. No diffusible pigment was detected on any tested media.

Strain B32^T was determined to contain *meso*-diaminopimelic acid in its cell wall. Whole-cell hydrolysates were found to contain non-diagnostic sugars, including ribose, galactose, mannose and glucose. The diagnostic sugars arabinose, xylose and madurose were not detected. Therefore, strain B32^T is classified in cell-wall type III and whole-cell sugar pattern type C (Lechevalier and Lechevalier 1970). Strain B32^T was found to possess phosphatidylcholine, phosphatidylmethylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and four unknown phospholipids (Fig. S1). The predominant menaquinones were determined to be MK-10 (H_4) (61.9 %) and MK-11 (H_4) (16.0 %); MK-10 (H_6) (7.5 %), MK-10 (H_2) (4.4 %), MK-9 (H_4) (3.3 %), MK-11 (H_6) (1.9 %) and MK-10 (H_8) (1.1 %) were also detected. The fatty acids profile was composed as follows: iso-$C_{16:0}$ (36.5 %), anteiso-$C_{15:0}$ (12.0 %), anteiso-$C_{18:1}$ *w*9*c* (8.7 %), anteiso-$C_{14:0}$ (7.6 %), $C_{16:1}$ *w*9*c* (7.0 %) and anteiso-$C_{17:0}$ (6.7 %). The morphological and chemical characteristics described above clearly support the placement of strain B32^T within the genus *Nocardiopsis*.

Good growth was found to occur at 25–35 °C, pH 7.0–10.0 and in the presence of 0–5 % of NaCl. The microorganism was found to be resistant to penicillin (25 μg ml^{-1}), but sensitive to kanamycin (5 μg ml^{-1}), erythromycin (10 μg ml^{-1}), streptomycin (10 μg ml^{-1}), chloramphenicol (25 μg ml^{-1}) and lysozyme (0.005 % w/v). Detailed results of the physiological and biochemical analyses are given in Table 1 and in the species description below. It is evident from Table 1 that there are several phenotypic characteristics that clearly separate strain B32^T from the nearest recognized species in the genus *Nocardiopsis*.

Phylogenetic analysis of an almost complete 16S rRNA gene sequence (1,460 bp, GenBank accession number KJ470139) showed that strain B32^T is related to members of the genus *Nocardiopsis* and exhibits highest 16S rRNA gene sequence similarity to *N. alba* (98.7 %), *N. lucentensis* (98.6 %), *N. aegyptia* (98.6 %), *N. sinuspersici* (98.6 %) and *N. arvandica* (98.5 %). The phylogenetic relationship between strain B32^T and the other *Nocardiopsis* species is seen in the neighbour-joining (Fig. 2), maximum parsimony (Fig. S2) and maximum-likelihood (Fig. S3) dendrograms.

DNA–DNA relatedness between strain B32^T and the type strains *N. alba* DSM 43377^T, *N. lucentensis* DSM 44048^T, *Nocardiopsis aegyptia* DSM 44442^T, *Nocardiopsis sinuspersici* DSM 45277^T and *N. arvandica* DSM 45278^T were 17.9, 14.6, 31.1, 27.1 and 14.1 %, respectively. These hybridization values are clearly below the 70 % threshold proposed by Wayne et al. (1987) for the delineation of separate species. Thus, on the basis of polyphasic taxonomic evidence, it is suggested that strain B32^T represents a novel species of the genus *Nocardiopsis*, for which the name *Nocardiopsis algeriensis* sp. nov. is proposed.

Description of *Nocardiopsis algeriensis* sp. nov

Nocardiopsis algeriensis (al.ger.i.en'sis. N.L. fem. adj. *algeriensis* referring to Algeria, the country from where the type strain was isolated).

Alkalitolerant filamentous actinomycete that produces short chains of one to six (or more) non-motile

Table 1 Differential physiologic and chemotaxonomic characteristics of strain B32^T (*Nocardiopsis algeriensis* sp. nov.) compared with its closest relative recognized species of the genus *Nocardiopsis*

Characteristics	Type strains					
	1	2	3	4	5	6
Utilization of						
L-Arabinose	−	−	−	+	−	−
D-Galactose	w	w	−	+	+	+
Glycerol	−	+	+	+	+	+
meso-Inositol	−	−	+	+	−	−
D-Lactose	−	−	−	w	+	−
D-Mannitol	−	w	+	+	+	−
D-Mannose	−	−	+	+	+	−
α-D-Melibiose	−	−	−	w	+	+
Raffinose	−	−	+	−	−	−
L-Rhamnose	−	−	+	+	+	−
D-Ribose	−	+	−	−	−	+
Sucrose	−	w	+	+	+	−
D-Xylose	−	w	−	+	+	−
Decomposition of						
Adenine	+	+	+	−	+	+
Starch	−	+	+	−	+	+
Tween 80	+	+	−	+	+	+
Decarboxylation of						
Sodium propionate	−	w	+	+	+	−
Production of nitrate reductase	+	−	+	+	+	−
Growth at						
10 % NaCl	−	−	+	−	+	+
45 °C	+	−	−	−	−	w
pH 12	+	−	−	+	+	w
Major menaquinones	10/4, 11/4	10/4, 10/6	10/6, 10/8	10/6, 10/8, 10/4	10/0, 10/2, 9/0	10/2, 10/4, 10/0, 9/2
Polar lipid composition						
PME	+	+	+	+	−	−
DPG	+	+	+	+	−	+
PE	+	−	−	−	+	+
PI	−	−	−	+	+	+

All data presented in this table were obtained under the same conditions

Strains: 1, *N. algeriensis* B32^T; 2, *N. alba* DSM 43377^T; 3, *N. lucentensis* DSM 44048^T; 4, *N. aegyptia* DSM 44442^T; 5, *N. sinuspersici* DSM 45277^T; 6, *N. arvandica* DSM 45278^T

+, Positive; −, negative; w, weakly positive; 10/4, MK-10(H_4); PME, phosphatidylmethylethanolamine; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PI, phosphatidylinositol

spores on the dichotomous branched aerial mycelium and produces a fragmented substrate mycelium. Aerial mycelium is abundant and shows a yellowish white colour on CM agar medium containing 0.5 % (w/v) NaCl. Diffusible pigments are not produced on CM agar, ISP 2, ISP 4, ISP 6 and ISP 7 media. Temperature and pH ranges for growth are 20–45 °C and pH 7.0–12.0, with optima at 25–35 °C and pH 7.0–10.0. The NaCl concentration range for growth is 0–7.5 %, with optimal growth occurring at 0–5 %. Utilizes D-glucose and D-galactose (weakly), but not, D-cellobiose, erythritol, D-fructose, maltose, D-mannose, D-trehalose,

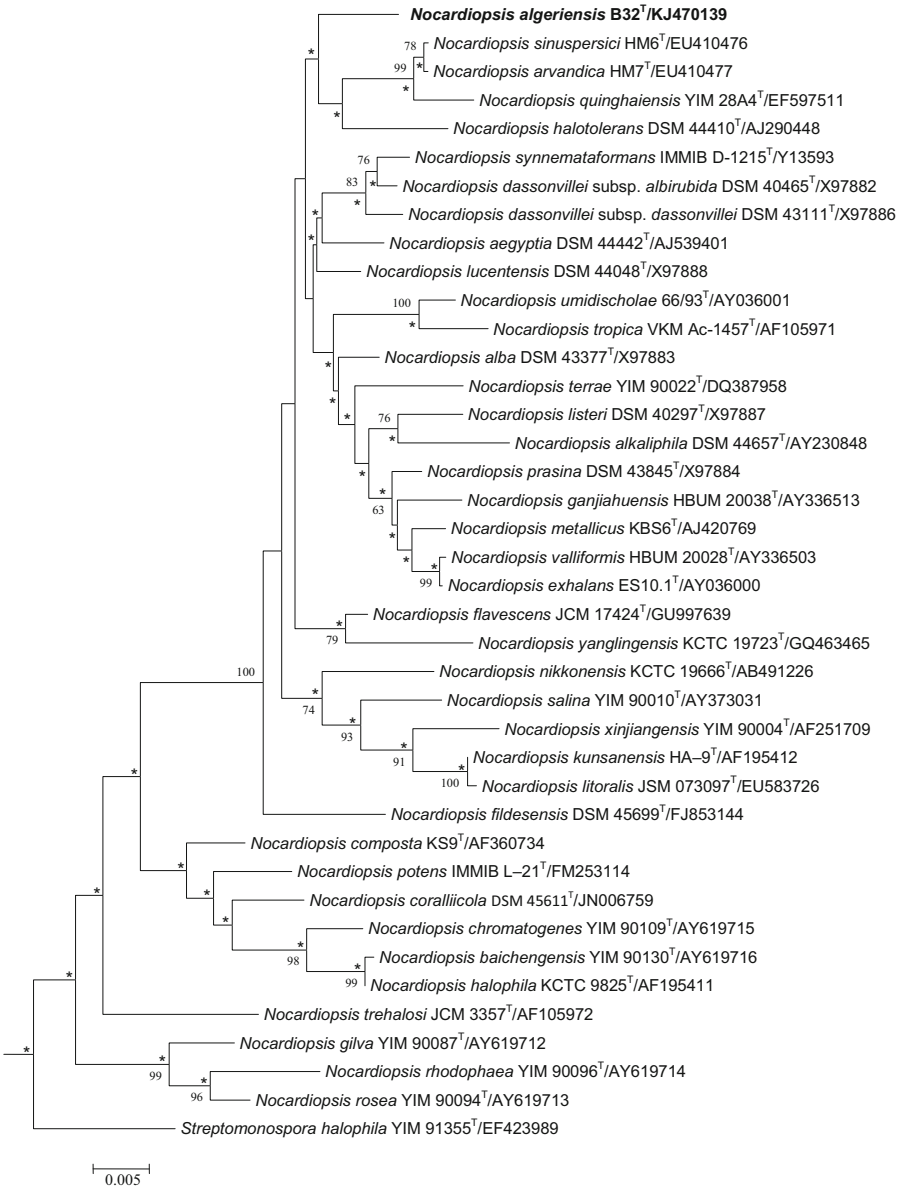

Fig. 2 Phylogenetic tree for species of the genus *Nocardiopsis* calculated from almost complete 16S rRNA gene sequences using Jukes and Cantor (1969) evolutionary distance methods and the neighbour-joining method of Saitou and Nei (1987). This illustrates the taxonomic position of strain B32^T relative to the other species of the genus. *Asterisks* indicate branches that are conserved when the neighbour-joining, maximum-parsimony and maximum-likelihood methods were used in constructing phylogenetic trees. *Numbers* at the nodes are bootstrap values, expressed as a percentage of 1,000 resamplings (only values >50 % are shown). *Streptomonospora halophila* YIM 91355^T was used as the outgroup. *Bar* 0.005 nucleotide substitution per site

adonitol, L-arabinose, glycerol, *meso*-inositol, D-lactose, D-mannitol, D-melezitose, melibiose, D-ribose, D-raffinose, L-rhamnose, salicin, D-sorbitol, sucrose, and D-xylose. Positive for casein, adenine, gelatin, tyrosine and Tween 80 hydrolysis, but negative for guanine, xanthine, hypoxanthine, testosterone and starch hydrolysis. Acetate, citrate, pyruvate, benzoate, butyrate, oxalate, propionate, succinate and tartrate are not decarboxylated. L-alanine, L-serine and L-proline are not used as a source of nitrogen. Nitrate reductase is

produced. Growth occurs in the presence of penicillin (25 μg ml^{-1}), but not in the presence of kanamycin (5 μg ml^{-1}), streptomycin (10 μg ml^{-1}), chloramphenicol (25 μg ml^{-1}), erythromycin (10 μg ml^{-1}) and lysozyme (0.005 %).

Whole-cell hydrolysates contain *meso*-diaminopimelic acid, ribose, galactose, mannose and glucose. The diagnostic phospholipid is phosphatidylcholine. The predominant menaquinones are MK-10 (H_4) and MK-11 (H_4). Major fatty acids are iso-$C_{16:0}$ and anteiso-$C_{15:0}$.

The type strain is B32^T (=DSM 45462^T = CECT 8712^T), isolated from an Algerian soil sample collected from Adrar province (Touat region). The GenBank accession number for the 16S rRNA gene sequence of strain B32^T is KJ470139.

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