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Diversity of Actinobacteria in Algerian Saharan soil and description of sixteen new taxa
Diversité des Actinobactéries dans le sol saharien algérien et description de seize nouveaux taxons

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Abstract _

The goal of this study was to investigate the biodiversity of actinobacteria in Algerian Saharan soils by using a polyphasic taxonomic approach based on the phenotypic and molecular studies (16S rRNA gene sequence analysis and DNA-DNA hybridization).

A total of 323 strains of actinobacteria were isolated from different soil samples, by a dilution agar plating method, using selective isolation media without or with 15-20% of NaCl (for halophilic strains). The morphological and chemotaxonomic characteristics (diaminopimelic acid isomers, whole-cell sugars, menaquinones, cellular fatty acids and diagnostic phospholipids) of the strains were consistent with those of members of the genus *Saccharothrix*, *Nocardiopsis*, *Actinopolyspora*, *Streptomonospora*, *Saccharopolyspora*, *Actinoalloteichus*, *Actinokineospora* and *Prauserella*.

The 16S rRNA gene sequence analysis of 15 selected strains isolated from different soil samples of Adrar (SA152, SA233 and B32), Biskra (H254), Djelfa (H27), Ouargla (H19), El-Oued (H23 and H32), Ghardaïa (H53, H55, PAL84 and H225) and Tamanrasset (SA181, SA198 and AH97) showed that each strain formed a distinct phyletic line within the radiation of the most closely related genus: SA233, SA152, SA181 and SA198 with *Saccharothrix*, B32 with *Nocardiopsis*, H19, H23, H32, H55 and H254 with *Actinopolyspora*, H27 with *Streptomonospora*, H53 with *Saccharopolyspora*, AH97 with *Actinoalloteichus*, PAL84 with *Actinokineospora* and H225 with *Prauserella*. Furthermore, the result of DNA–DNA hybridization (DDH) between each strain and the nearest species was clearly below the 70% threshold considered for the delineation of separate species. The genotypic and phenotypic data confirmed that these actinobacteria represent five novel species of the genus *Actinopolyspora* named *Actinopolyspora algeriensis*, *A. saharensis*, *A. righensis*, *A. mzabensis* and *A. biskrensis*, respectively with the type strains H19^T (DSM 45476^T), H32^T (DSM 45459^T), H23^T (DSM 45501^T), H55^T (DSM 45460^T) and H254^T (DSM 46684^T).

Moreover, four new species of *Saccharothrix*: *Saccharothrix algeriensis*, *S. saharensis*, *S. hoggarensis* and *S. tamanrassetensis*, were described, respectively with the type strains SA233^T (DSM 44581^T), SA152^T (DSM 45456^T), SA181^T (DSM 45457^T) and SA198^T (DSM 45947^T). In addition, six new species: *Nocardiopsis algeriensis* B32^T (DSM 45462^T), *Streptomonospora algeriensis* H27^T (DSM 45604^T), *Saccharopolyspora ghardaiensis* H53^T (DSM 45606^T), *Actinoalloteichus hoggarensis* AH97^T (DSM45943^T), *Actinokineospora mzabensis* PAL84^T (DSM 45961^T) and *Prauserella isguenensis* H225^T (DSM 46664^T) were also reported in this study.

On the other hand, a new strain designated H195^T was isolated from Ghardaïa. The comparative analyses of the 16S rRNA gene sequence and nucleotide signatures (along with phenotypic and chemotaxonomic properties) of this strain revealed that it is different from all known actinobacterial genera. Based on these results, this strain: H195^T (DSM 46680^T) represents a novel genus and species, named *Mzabimyces algeriensis* gen. nov., sp. nov., within a new family, *Mzabimycetaceae* fam. nov. In total, 16 new taxa were described in this study.

Keywords: Biodiversity, Actinobacteria, Saharan soil, Algeria, new taxa.

Résumé _

La biodiversité actinomycétale et la recherche de nouvelles espèces d'actinobactéries ont été étudiées dans les échantillons de sols provenant de différentes régions du Sahara algérien. La taxonomie et la diversité de la population des actinomycètes ont été évaluées en utilisant une approche polyphasique basée sur des études morphologiques, chimiotaxonomiques, physiologiques et moléculaire (séquençage de l'ARN ribosomique 16S avec une étude phylogénétique approfondie et hybridation ADN-ADN).

Au total, 323 souches d'actinobactéries ont été isolées en utilisant des milieux sélectifs, contenant ou pas 15-20% de NaCl. Les résultats ont montré une certaine diversité dans la communauté des actinobactéries au niveau des sols étudiés. Les souches ont été identifiées à huit genres peu fréquents à rares de par le monde: *Saccharothrix*, *Nocardiopsis*, *Actinopolyspora*, *Streptomonospora*, *Saccharopolyspora*, *Actinoalloteichus*, *Actinokineospora* et *Prauserella*.

Quinze souches isolées de plusieurs régions saharienne: Adrar (SA152, SA233 et B32), Biskra (H254), Djelfa (H27), Ouargla (H19), El-Oued (H23 et H32), Ghardaïa (H53, H55, PAL84 et H225) et Tamanrasset (SA181, SA198 et AH97) partagent de faibles similitudes d'ARNr 16S avec les espèces les plus proches de chaque genre ce qui suggère fortement la présence de plusieurs nouvelles espèces: *Saccharothrix* (SA233, SA152, SA181 et SA198), *Nocardiopsis* (B32), *Actinopolyspora* (H19, H23, H32, H55 et H254), *Streptomonospora* (H27), *Saccharopolyspora* (H53), *Actinoalloteichus* (AH97), *Actinokineospora* (PAL84) et *Prauserella* (H225). L'hybridation ADN-ADN a permis de confirmer la présence de 15 espèces originales. Cinq nouvelles espèces d'*Actinopolyspora*: *Actinopolyspora algeriensis* H19^T (DSM 45476^T), *A. saharensis* H32^T (DSM 45459^T), *A. righensis* H23^T (DSM 45501^T), *A. mzabensis* H55^T (DSM 45460^T) et *A. biskrensis* H254^T (DSM 46684^T), quatre nouvelles espèces de *Saccharothrix*: *Saccharothrix algeriensis* SA233^T (DSM 44581^T), *S. saharensis* SA152^T (DSM 45456^T), *S. hoggarensis* SA181^T (DSM 45457^T) et *S. tamanrassetensis* SA198^T (DSM 45947^T). D'autres nouvelles espèces ont été

également signalées: *Nocardiopsis algeriensis* B32^T (DSM 45462^T), *Streptomonospora algeriensis* H27^T (DSM 45604^T), *Saccharopolyspora ghardaiensis* H53^T (DSM 45606^T), *Actinoalloteichus hoggarensis* AH97^T (DSM45943^T), *Actinokineospora mzabensis* PAL84^T (DSM 45961^T) et *Prauserella isguenensis* H225^T (DSM 46664^T).

Une souche H195^T doit être considérée comme un nouveau genre: *Mzabimyces algerensis* (DSM 46680^T) en raison de sa très faible similitude avec tous les genres voisins. Egalement, une nouvelle famille *Mzabimycetaceae* a été proposée.

Introduction

Actinobacteria (mycelial bacteria) have been paid a great attention owing to their production of potential pharmaceutical, industrial and agricultural natural products (Bouras, 2005). Currently, about 150 antibiotics have being applied in human therapy and agriculture; from 100 to 120 of them were produced by actinobacteria (Berdy, 2005).

Mycelial bacteria are extensively distributed in soils and other terrestrial and marine environments, where they have been shown to play an important ecological role in soil nutrient turnover (Gonzàlez et al., 2005). This group of microorganisms is widespread in nature and is able to occupy several extreme ecosystems. In modern natural-product screening strategies, the isolation of the uncommon and less studied rare actinobacteria is required to improve a number of novel natural products screened (Lazzarini et al., 2001; Berdy, 2005).

Deserts are characterized by lack of moisture (annual rain less than 260 mm) as a result of which biological activities are regulated by ephemeral water availability (Bhatnagar and Bhatnaga, 2005). Microbial activity in desert soils is highly dependent on characteristics such as temperature, moisture and the availability of organic carbon.

Many studies performed in arid regions, such as Saharan soils, have shown the richness of this special ecosystem in actinobacteria, which have led to the detection of many bioactive compounds (Boubetra et al., 2013; Meklat et al., 2012).

The aim of this study is to investigate the biodiversity of the culturable actinobacteria isolated from different regions of Algerian Sahara.

Materials and methods

Sampling site and sample collection

Seventy-two non-rhizospheric soil samples (0-20 cm of depth) were collected aseptically from different arid regions of Algerian Sahara (28 from Ghardaïa, 12 from Ouargla, 12 from Biskra, 6 from Tamanrasset, 5 from Adrar, 5 from Béchar, 2 from El Oued, 1 from Laghouat and 1 from Djelfa). The soil samples were maintained in sterile polyethylene bags closed tightly.

In general, the soil textures are loamy sand to sandy loam. Their moisture (at the time of sampling) ranged from 4 to 20%. The pH is slightly basic (7.5 to 8.9). The percentages of carbon and nitrogen are very low. The electrical conductivity (1/5 soil/water ratio [wt/vol] at 25 °C) is variable depending on the locations, and ranged between 0.02 and 7.8 mS/cm in

non-saline soils of the western and central Algerian regions, and ranged between 0.5 and 27.6 mS/cm in saline soils of the eastern Algerian regions.

Isolation of actinobacterial strains

The actinobacterial strains were isolated by a dilution agar plating method using humic acid-vitamin agar medium recommended for isolation of rare actinomycetes (Hayakawa and Nonomura, 1987). In some cases, the antibacterial agents, such as streptomycin (10 µg/ml) or rifampicin (5 µg/ml), were added to the isolation medium.

The halophilic actinobacterial strains were isolated by the serial dilution method on complex agar (Chun et al., 2000), chitin-vitamin agar (Hsu and Lockwood, 1975) and humic acid-vitamin agar media supplemented with NaCl (15-20%).

The antifungal cycloheximide (50 µg/ml) was always used to inhibit development of invasive microfungi. The plates were incubated at 30 °C for 2 to 7 weeks, and the colonies were examined directly by light microscopy, and each strain was picked-up, purified and maintained at 4 °C.

Each strain was deposited in the Germen Collection of Microorganisms and Cell Cultures (DSMZ, Germany), in the Culture Collection in the University of Göteborg (CCUG, Sweden), and/or in the Spanish Type Culture Collection (CECT, Spain) and/or in the Microbial Type Culture Collection (MTCC, India).

Morphological studies

Cultural characteristics of each strains were investigated after 1, 2, 3 and 4 weeks of incubation at 30 °C using the media of the International *Streptomyces* Project (ISP2, ISP3, ISP4 and ISP5) (Shirling and Gottlieb, 1966), Bennett's medium, nutrient agar and complex medium agar (Saker et al., 2014). For halophilic actinobacteria, the media used for morphological characteristics were supplemented with 15-20% w/v NaCl. The degree of growth, the color of the aerial and substrate mycelia and any diffusible pigments produced were determined by comparison with ISCC-NBS color charts (Kelly and Judd, 1976). The morphological characteristics, including spore chain morphology and spore size, were examined by naked eye and by light microscopy (Motic, B1 Series).

Physiological studies and numerical taxonomy

Sixty three physiological tests were used to characterize the isolated strains. The physiological characteristics were evaluated according to the methods of Locci (1989). They concern the assimilation of 23 carbohydrates and derivatives as sole carbon sources, the assimilation of 3 amino acids as sole nitrogen sources, the degradation of 9 organic acids, the degradation of adenine, arbutin, esculin, gelatin, guanine, hypoxanthine, starch, testosterone, Tween 80, tyrosine and xanthine, the production of nitrate reductase, the sensitivity to lysozyme (0.005%). Growth in the presence of different concentrations of NaCl (0, 7, 10, 15, 20, 25 and 30% w/v), at different temperatures (15, 20, 25, 30, 35, 37 and 45 °C), and various pH values (5, 7, 9 and 10, using the buffer system described by Xu et al. (2005), and in the

presence of erythromycin (15 mg/l), tetracycline (30 mg/l), and nalidixic acid (30 mg/l) were determined on nutrient agar medium. For halophilic actinobacteria, the media used were supplemented with 15-20% w/v NaCl (except for the NaCl concentration test).

Chemical studies of cell constituent

Biomass, for chemotaxonomic and molecular studies, of actinobacterial strains was obtained by cultivation in shake flasks (250 rpm) using ISP2 or complex medium broth containing 15-20% w/v NaCl (for halophilic strains). The pH of the medium was adjusted to 7. After one week of incubation at 30 °C, the biomass was harvested by centrifugation and washed twice with distilled water. Diaminopimelic acid (DAP) isomers and whole-cell sugar pattern were analyzed according to the methods of Becker and al. (1964) and Lechevalier and Lechevalier (1970), respectively.

DNA extraction, amplification and sequencing

The DNA was extracted according to the method of Liu et al. (2000). The 16S rRNA gene was amplified by PCR with a MP Biomedical Kit using two primers: 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') in a thermocycler (Bio-Rad My Cycler^{TM}). Amplification is carried out in a volume of 50 µL of reaction mixture consisting of 25 to 50 ng of genomic DNA, 0.5 µM of each primer, 1 × PCR buffer containing $MgCl_2$ (10 mM tris-HCl pH 9.0, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100, 0.2 mg/ml bovine serum albumin), 200 µM mixture of dNTP and 1.5 U *Taq* DNA polymerase. The conditions of the PCR were standardized with initial denaturation at 98 °C for 4 min followed by 30 cycles of amplification (denaturation at 94 °C for 1 min, annealing temperature at 52 °C for 1 min and extension at 72 °C for 1 min) and 10 min at 72 °C as final extension. The PCR product was analyzed using 0.8% agarose gel, and the fragment was separated at 100 volts for 60 min in TAE buffer. The bands were observed under UV light. The primers used for sequencing were listed in Coenye et al. (1999).

16S rRNA gene phylogenetic analyses

The sequences obtained were compared with sequences present in the EzTaxon-e server (Kim et al., 2012). The sequences were aligned with reference sequences. Phylogenetic analyses were conducted using MEGA version 5 (Tamura et al., 2011). Phylogenetic trees were constructed using the neighbor-joining method of Saitou and Nei (1987) with the model of Jukes and Cantor (1969). The resulting topologies of trees were evaluated by bootstrap analyses on 1000 replicates.

Results and discussion

Phenotypic and chemotaxonomic studies

Based on the morphological, chemotaxonomic and physiological characteristics, all the isolated actinobacterial strains were tentatively classified into several genera or groups.

Group I comprises 32 strains (SA152, SA233, SA181, SA198, etc.) which characterized by fragmentation of both substrate and aerial mycelia into rods and ovoid elements, type III cell-wall *meso*-diaminopimelic acid without glycine, the presence of galactose, rhamnose and small amounts of mannose as diagnostic whole-cell sugars, a phospholipid type PII (phosphatidylethanolamine) or PIV (phosphatidylethanolamine and glucosamine-containing phospholipids) pattern (Labeda and Lechevalier, 1989), the presence of MK-9(H_4) as the predominant menaquinone and the absence of mycolic acids (Labeda and Kroppenstedt, 2000). These characteristics are those of the genus *Saccharothrix*.

Group II contained only a single strain (PAL84). This strain was observed to produce pinkish-purple aerial mycelium and purplish red substrate mycelium, which fragmented into chains of non-motile elements. This strain is characterized by cell wall of type IVA (*meso*-diaminopimelic acid as a cell-wall diamino acid, and galactose and arabinose as diagnostic whole-cell sugars), a type PII phospholipid pattern (phosphatidylethanolamine), the presence of MK-9(H_4) as predominant menaquinone, and the presence of iso-$C_{16:0}$ as the major fatty acid. This strain was tentatively classified as *Actinokineospora*.

Group III contained only five strains (including AH97). The aerial mycelium was branched and fragmented into straight spore chains. The strain contained *meso*-diaminopimelic acid in the cell-wall peptidoglycan. The whole-cell hydrolysates contained galactose, glucose, mannose and ribose, typical of cell-wall type III and whole-cell sugar pattern type C (Lechevalier and Lechevalier, 1970). The phospholipid profile comprises mainly phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, phosphatidylinositolmannosides, corresponding to phospholipid type PII (Lechevalier et al., 1977). These strains seem to belong to the genus *Actinoalloteichus*.

Group IV includes 180 strains. These strains (AH37, H1, B32, etc.) fragmented irregularly into long chains of rod-shaped and non-motile spores. The substrate mycelium is more or less fragmented into coocoid and bacillary elements. The cells contain in their wall DL isomer of diaminopimelic acid and in their cells ribose, glucose and sometimes galactose, which corresponds to the chemotype IIIC. These strains grew in NaCl levels between 7 and 20%. These are typical characteristics of the genus *Nocardiopsis*, with several species are known to be halophilic or halotolerant bacteria (Hozzein and Trujillo, 2012).

Group V comprises 18 strains (H27, H231 and H238, etc.) which characterized by an aerial mycelium with the same morphological characteristics as group IV, with the exception of the production of isolated spores in the substrate mycelium. These strains have a cell walls of type IIIC (*meso*-diaminopimelic acid without diagnostic sugar); a type PII phospholipid pattern (phosphatidylethanolamine); the presence of menaquinones with nine or ten isoprenoid chains and a varying degree of hydrogenation; and the presence of $C_{16:0}$, $C_{17:0}$, iso-$C_{15:0}$ and iso-$C_{16:0}$ as the major fatty acids. These strains grew at NaCl levels between 7 and

20%. These characteristics are those of the genus *Streptomonospora*, in which all species are halophilic (Cui, 2012).

Group VI contains 12 strains (H149, H137 and H225, etc.) with a nocardioform aspect, with sterile (or less fragmented) aerial mycelium, and excessively fragmented substrate mycelium into non-motile coccoid and rod elements. These strains, which are halophilic or halotolerant, have chemotype IVA (arabinose and galactose). They grew between 0 and 20% or between 7 and 25% of NaCl. These strains seem to belong to the genus *Prauserella*, which contains many species that are known to be halophilic or halotolerant (Kim and Goodfellow, 2012a).

Groups VII (51 strains including H19, H23, H32, H55 and H254), VIII (20 strains including H53) and IX (4 strains: H150, H151, H195 and H199) have an aerial mycelium which produces chains of non-motile spore (5-30 spores per chain) often in rod (group VII), ovoid (group VIII) or rounded elements (group IX). The substrate mycelium is fragmented into coccoid and rod elements. These strains have chemotype IVA (DL diaminopimelic acid, arabinose and galactose, along with glucose, ribose and sometimes mannose). They grew between 7 and 30% (group VII), 7 and 25% (group VIII) or 10 and 30% of NaCl (group IX). The characteristics of groups VII and VIII are respectively those of the genus *Actinopolyspora*, in which all species are halophilic (Trujillo and Goodfellow, 2012) and *Saccharopolyspora*, in which some species are halophilic or halotolerant (Kim and Goodfellow, 2012b). However, the strains H150, H151, H195 and H199 seem to belong to an unknown genus, closely related to the genera *Actinopolyspora* and *Saccharopolyspora*.

Assessment of diversity by 16S rRNA gene analyses

All the actinobacterial strains belonging to different groups were subjected to molecular analysis. The resulting 16S rRNA gene sequences were deposited in GenBank, and compared with EzTaxon.

The similarity of the 16S rRNA sequence of strain SA233^T to those of the other species of the genus *Saccharothrix* ranged from 97.5 to 98.8%, with *S. australiensis* DSM 43800^T having the closest match. This strain was determined to have only 55.9% genomic DNA–DNA hybridization (DDH) to *S. australiensis*.

The 16S rRNA gene sequence comparison revealed that strain SA152^T shared the highest degree of 16S rRNA gene sequence similarity with *S. xinjiangensis* DSM 44896^T (99.3%) and *S. texasensis* DSM 44231^T (98.9%). However, DDH studies showed only 16.2% relatedness with *S. xinjiangensis* and 33.9% relatedness with *S. texasensis*

The similarity of the 16S rRNA gene sequence of strain SA181^T to those of other members of the genus *Saccharothrix* ranged from 96.8 to 98.9%. Strain SA181^T displayed highest 16S rRNA gene sequence similarity with *S. longispora* DSM 43749^T (98.9%), *S. xinjiangensis* DSM 44896^T (98.4%) and *S. texasensis* DSM 44231^T (98.2%). DDH values between strain SA181^T and *S. longispora*, *S. texasensis* and *S. xinjiangensis* were 16.05%, 50.05% and 22.0%, respectively.

The 16S rRNA gene sequence analysis confirmed that strain SA198^T was a member of the genus *Saccharothrix* and showed a similarity level ranging between 97.5 and 98.9% within *Saccharothrix* species, *S. australiensis* DSM 43800^T being the most closely related. The strain SA198^T displayed highest 16S rRNA gene sequence similarity to *S. australiensis* DSM 43800^T (98.9%), *S. xinjiangensis* DSM 44896^T (98.8%), *S. algeriensis* DSM 44581^T (98.7%) and *S. espanaensis* DSM 44229^T (98.6%). Strain SA198^T was determined to have 60.5% genomic DNA-DNA relatedness with *S. australiensis*, the phylogenetically closest species of the genus *Saccharothrix*. Furthermore, DNA-DNA hybridization studies showed only 36.2% similarity with *S. xinjiangensis*, 24.0% similarity with *S. algeriensis* and 49.3% similarity with *S. espanaensis*.

All of the data support the designation of the strains SA 233^T, SA152^T, SA181^T and SA198^T as representing a four novel species of the genus *Saccharothrix*, for which we propose the names *Saccharothrix algeriensis*, *S. saharensis*, *S. hoggarensis* and *S. tamanrassetensis*, respectively with the type strains SA233^T (DSM 44581^T), SA152^T (DSM 45456^T), SA181^T (DSM 45457^T) and SA198^T (DSM 45947^T) (Fig. 1).

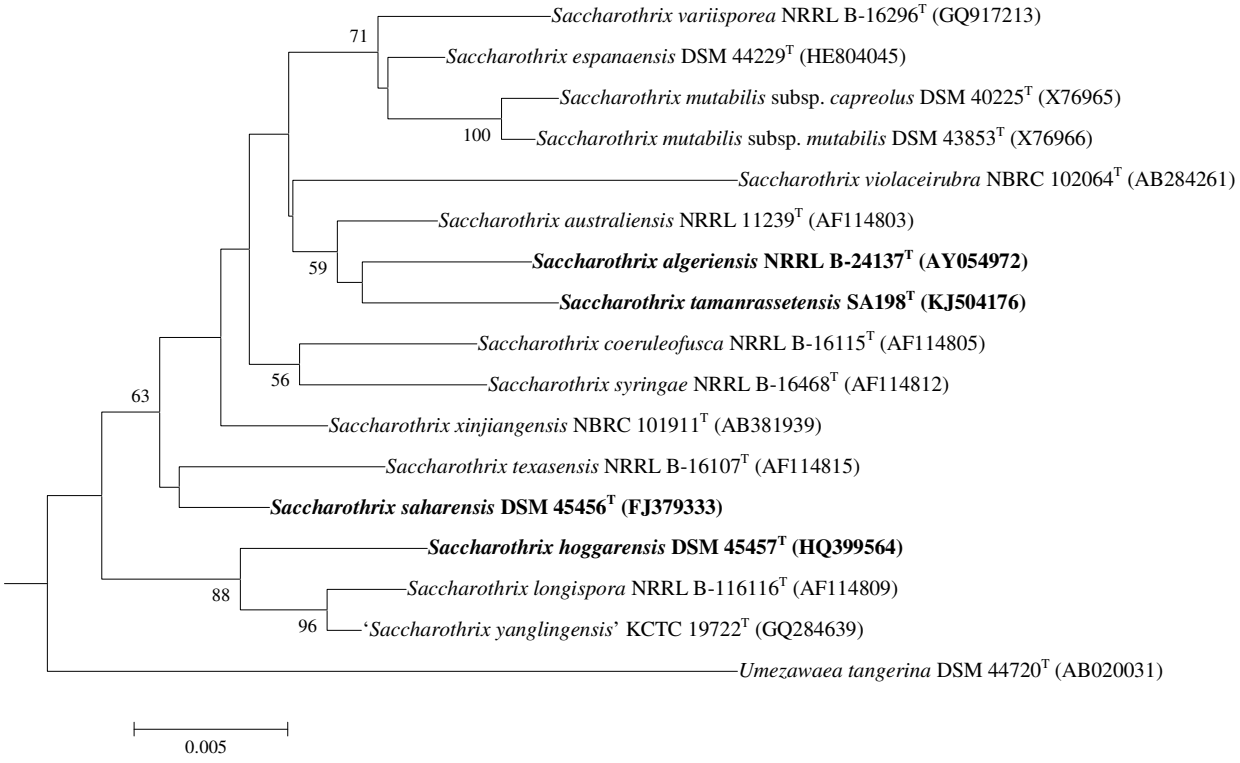

Figure 1. Neighbor-joining phylogenetic tree based on a comparison of the 16S rRNA gene sequence, showing relationships between the isolates of *Saccharothrix* and their phylogenetic neighbors.

Levels of similarity between strain PAL84^T and the type strains of recognized *Actinokineospora* species ranged from 96.2 to 97.8% (based on 16S rRNA gene sequence). This strain was most closely related to *A. baliensis* NBRC 104211^T (97.8%) and *A. cibodasensis* NBRC 104212^T (97.7%). Meier-Kolthoff et al. (2013) showed that the DDH should be mandatory only above a similarity percentage of 98.2%. More recently, Kim et al.

(2014) proposed a threshold of 98.65% to differentiate two species without using DDH. As the closest similarities of strain PAL84^T were below these thresholds, DDH was not performed. Consequently, strain PAL84^T represents a novel species for which the name *Actinokineospora mzabensis* (DSM 45961^T) is proposed (Fig. 2).

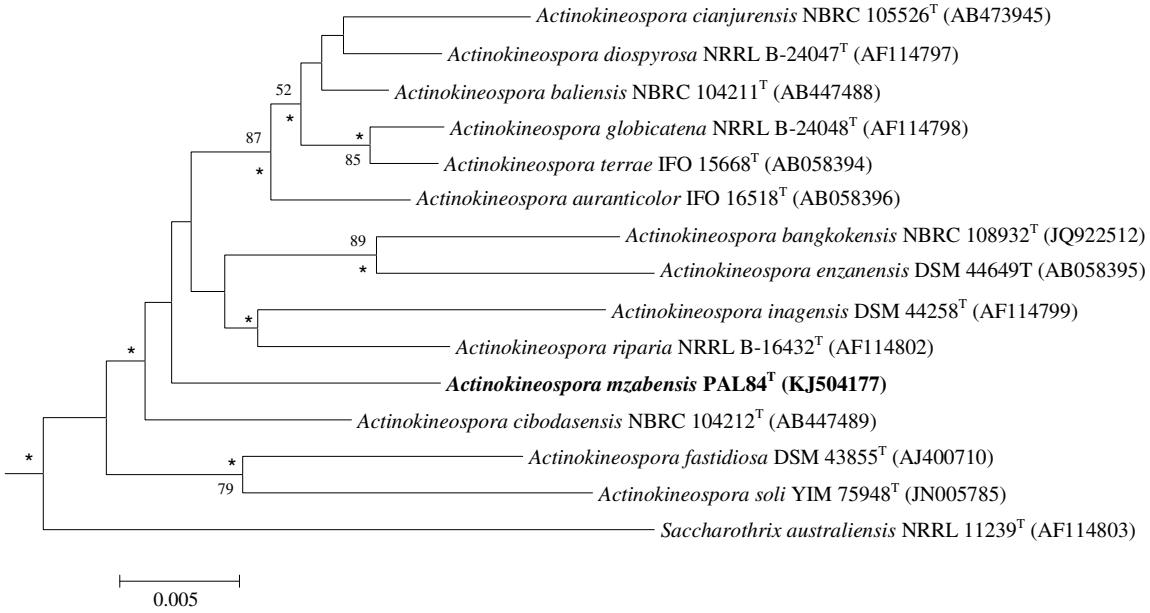

Figure 2. Neighbor-joining phylogenetic tree based on a comparison of the 16S rRNA gene sequence, showing relationships between the isolates of *Actinokineospora* and their phylogenetic neighbors.

Results of 16S rRNA gene sequence comparison revealed that strain AH97^T shared the highest degree of 16S rRNA gene sequence similarity with *Actinoalloteichus hymeniacidonis* DSM 45092^T (99.3%) and *A. nanshanensis* DSM 45655^T (98.7%). However, DDH studies showed only 26.5% relatedness with *A. hymeniacidonis* and 28.0% with *A. nanshanensis*. The genotypic and phenotypic data showed that the strain AH97^T represents a novel species for which the name *Actinoalloteichus hoggarensis* (DSM 45943^T) is proposed (Fig. 3).

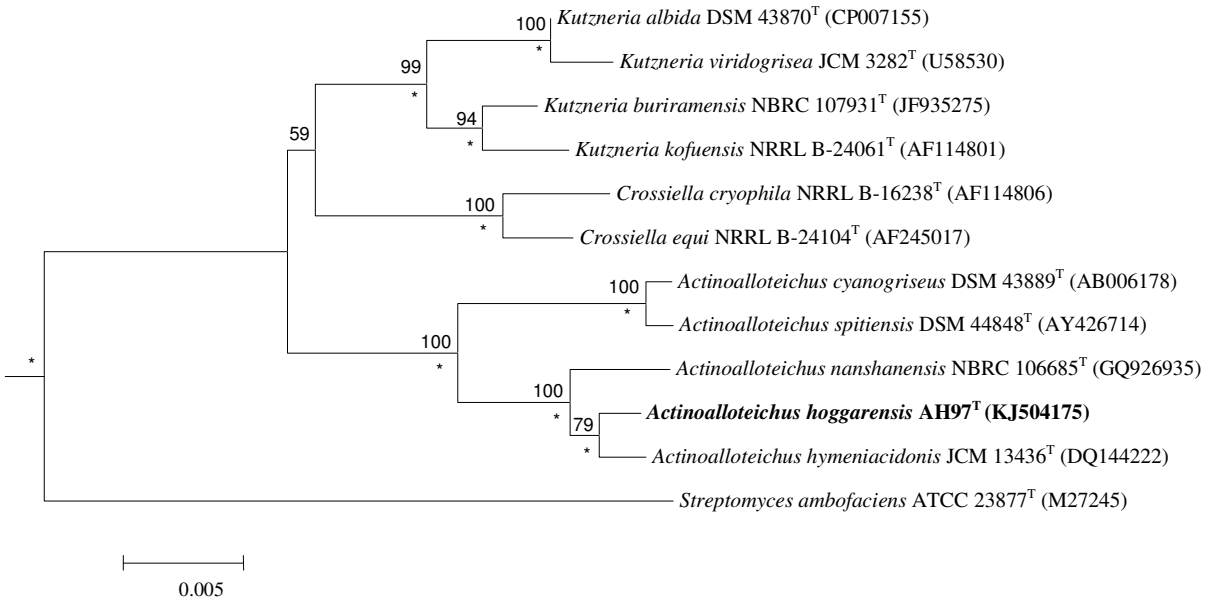

Figure 3. Neighbor-joining phylogenetic tree based on a comparison of the 16S rRNA gene sequence, showing relationships between the isolates of *Actinoalloteichus* and their phylogenetic neighbors.

The 16S rRNA gene sequence analysis indicated that strain B32^T is most closely related to *Nocardiopsis alba* DSM 43377^T (98.7%), *N. lucentensis* DSM 44048^T (98.6%), *N. aegyptia* DSM 44442^T (98.6%), *N. sinuspersici* HM6^T (98.6%) and *N. arvandica* HM7^T (98.5%). However, the DDH values between strain B32^T and the closely related type strains were 17.9, 14.6, 31.1, 27.1 and 14.1%, respectively. Based on this data, it is proposed that strain B32^T should be classified as representative of a novel species, for which the name *Nocardiopsis algeriensis* (DSM 45462^T) is proposed (Fig. 4).

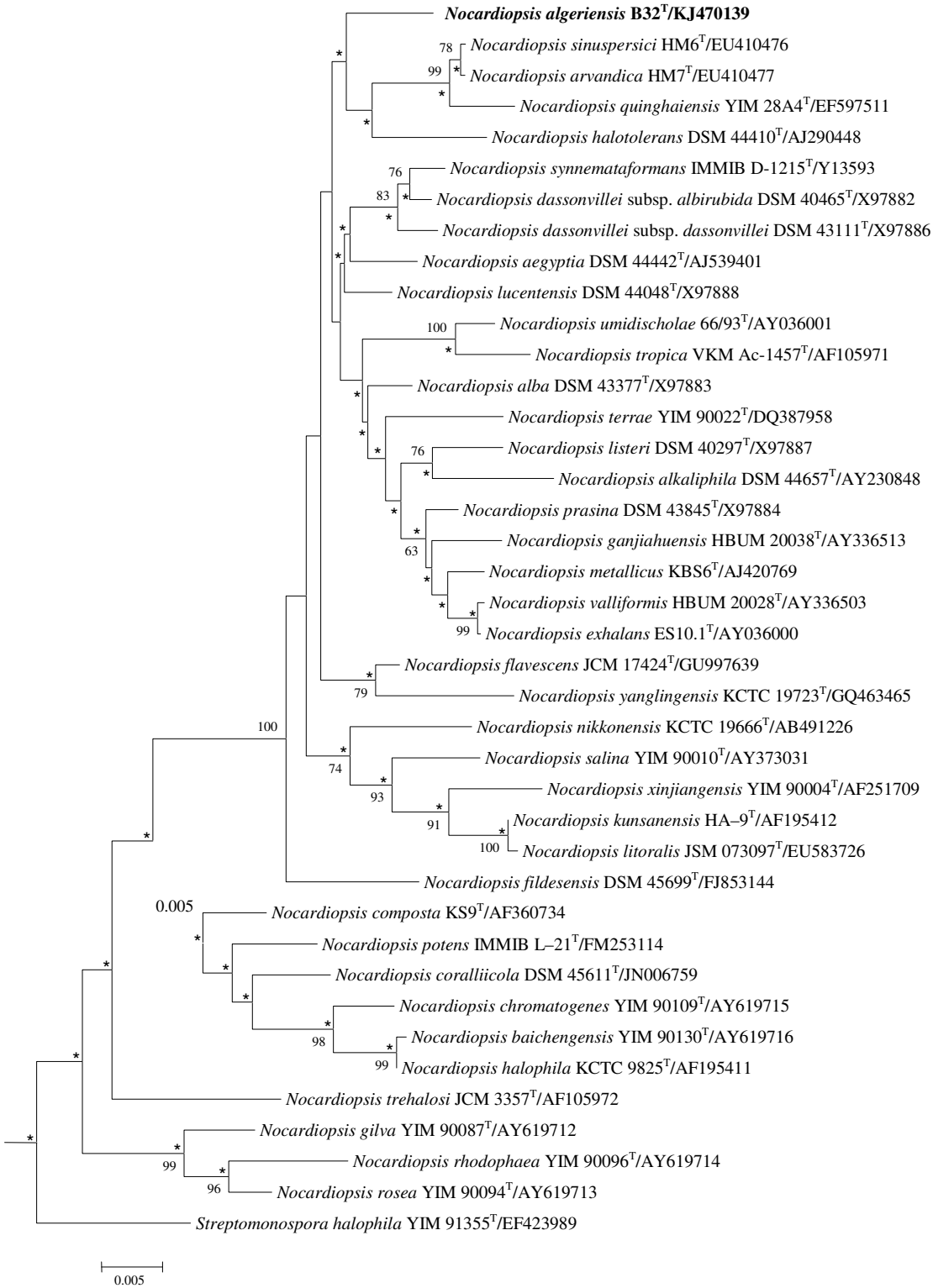

Figure 4. Neighbor-joining phylogenetic tree based on a comparison of the 16S rRNA gene sequence, showing relationships between the isolates of *Nocardiopsis* and their phylogenetic neighbors.

High levels of 16S rRNA gene sequence similarity were found between strain H225^T and its nearest neighbours, *Prauserella flava* DSM 45265^T (99.1%), *P. alba* DSM 44590^T (99.0%), *P. aidingensis* DSM 45266^T (98.8%), *P. salsuginis* DSM 45264^T (98.8%) and *P.*

sediminis DSM 45267^T (98.6%). DDH values between strain H225^T and *P. flava*, *P. alba*, *P. aidingensis*, *P. salsuginis* and *P. sediminis*, were 43.6, 65.5, 40.6, 27.9 and 45.0%, respectively. Accordingly, strain H225^T should be classified as representative of a novel species, for which the name *Prauserella isguenensis* (DSM 46664^T) is proposed (Fig. 5).

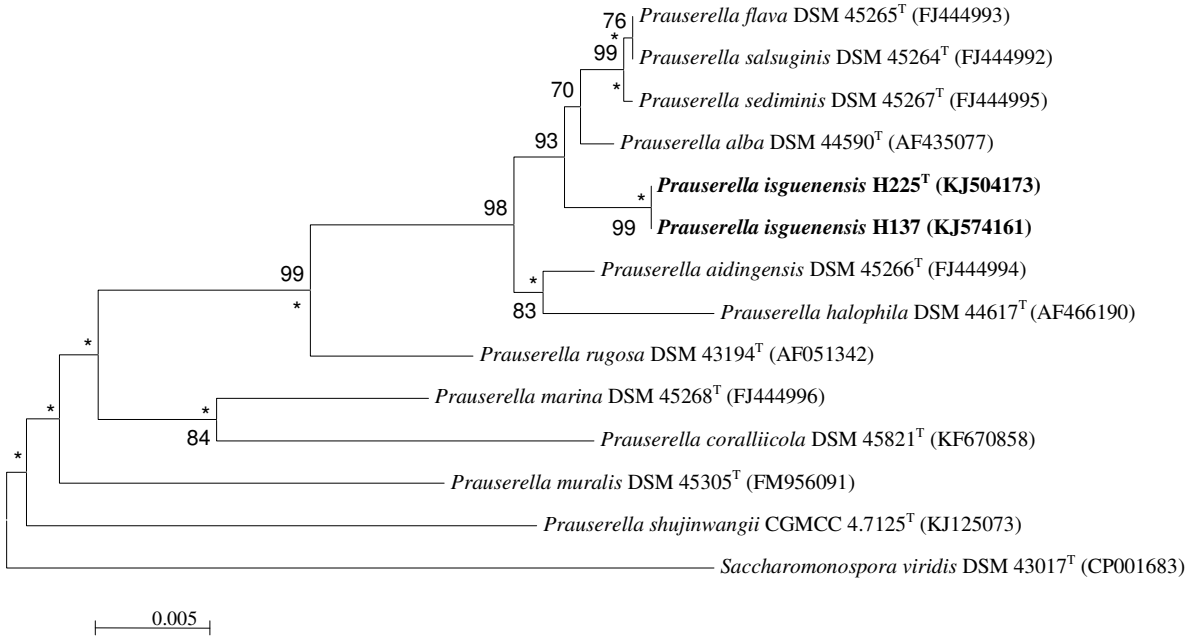

Figure 5. Neighbor-joining phylogenetic tree based on a comparison of the 16S rRNA gene sequence, showing relationships between the isolates of *Prauserella* and their phylogenetic neighbors.

The similarity level of H19^T was 98.5% to *Actinopolyspora halophila* DSM 43834^T, the most closely related species (Fig. 6). However, the 16S rRNA gene sequence similarities between strain H19^T and other remaining *Actinopolyspora* species were below 97%. The level of DDH between H19^T and *A. halophila* DSM 43834*T* was 43.6%.

The 16S rRNA gene sequence showed that strain H32^T was related to members of the genus *Actinopolyspora* and exhibited highest 16S rRNA gene sequence similarity to *A. algeriensis* DSM 45476^T (98.8%) and *A. halophila* DSM 43834^T (98.5%), whereas the sequence similarities with other members of the genus *Actinopolyspora* ranged from 96.5 to 97.2%. DDH between strain H32^T and *A. algeriensis*, *A. halophila* were respectively mean values of 30.5, 55.1%.

The similarity levels between strain H23^T and the type strains of recognized *Actinopolyspora* species ranged from 94.8 to 97.8%. Strain H23^T was related most closely to *A. xinjiangensis* TRM 40136^T (97.8%), *A. erythraea* DSM 45583^T (97.7%) and *A. alba* DSM 45004^T (97.5%). DDH experiments were not required in this case (Kolthoff et al., 2013).

The similarity of the 16S rRNA gene sequence of strain H55^T to those from other species of the genus *Actinopolyspora* ranged from 93.3 to 98.0%. Strain H55^T displayed the highest 16S rRNA gene sequence similarity to *A. erythraea* DSM 45583^T (98.0%) and *A. alba* DSM

45004^T (97.6%). The mean levels of DDH with these two type strains were respectively 44.5 and 42.35%.

The comparative analysis of the 16S rRNA gene sequences revealed that the strain H254^T was most closely related to *A. saharensis* DSM 45459^T (99.2%), *A. halophila* DSM 43834^T (99.1%) and *A. algeriensis* DSM 45476^T (99.0%). Nevertheless, the strain had relatively lower mean values for DDH with the above strains (57.2, 68.4 and 45.6%, respectively).

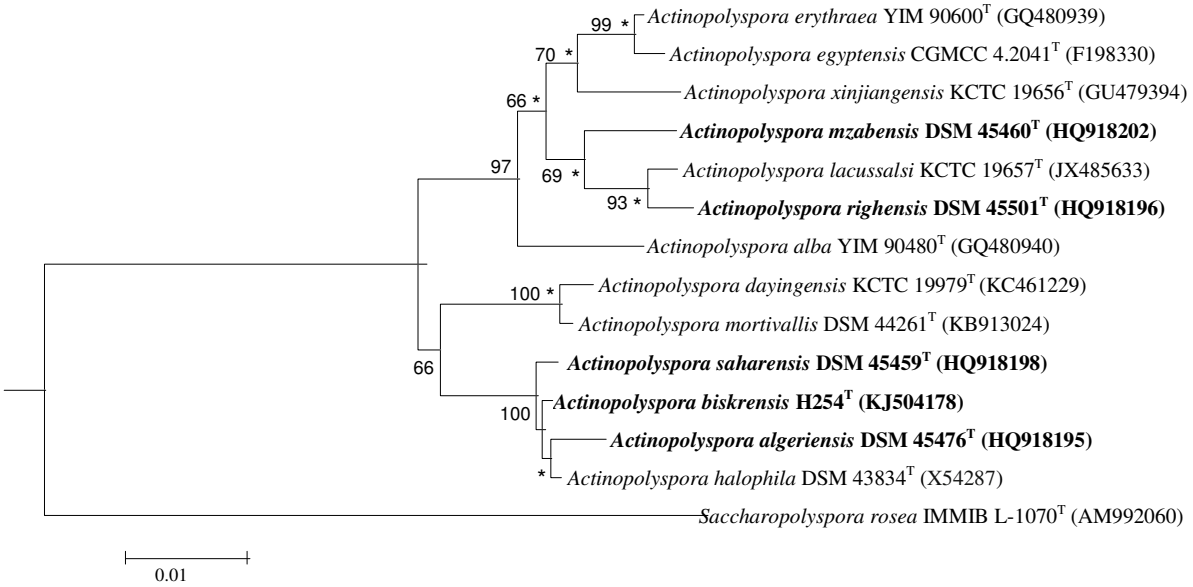

Figure 6. Neighbor-joining phylogenetic tree based on a comparison of the 16S rRNA gene sequence, showing relationships between the isolates of *Actinopolyspora* and their phylogenetic neighbors.

The 16S rRNA sequence similarities between strain H53^T and other members of the genus *Saccharopolyspora* ranged from 92.1 to 94.3%. DDH experiments were not required in this case (Kolthoff et al., 2013). As a result, strain H53^T represents a novel species for which the name *Saccharopolyspora ghardaiensis* (DSM 45606^T) is proposed (Fig. 7).

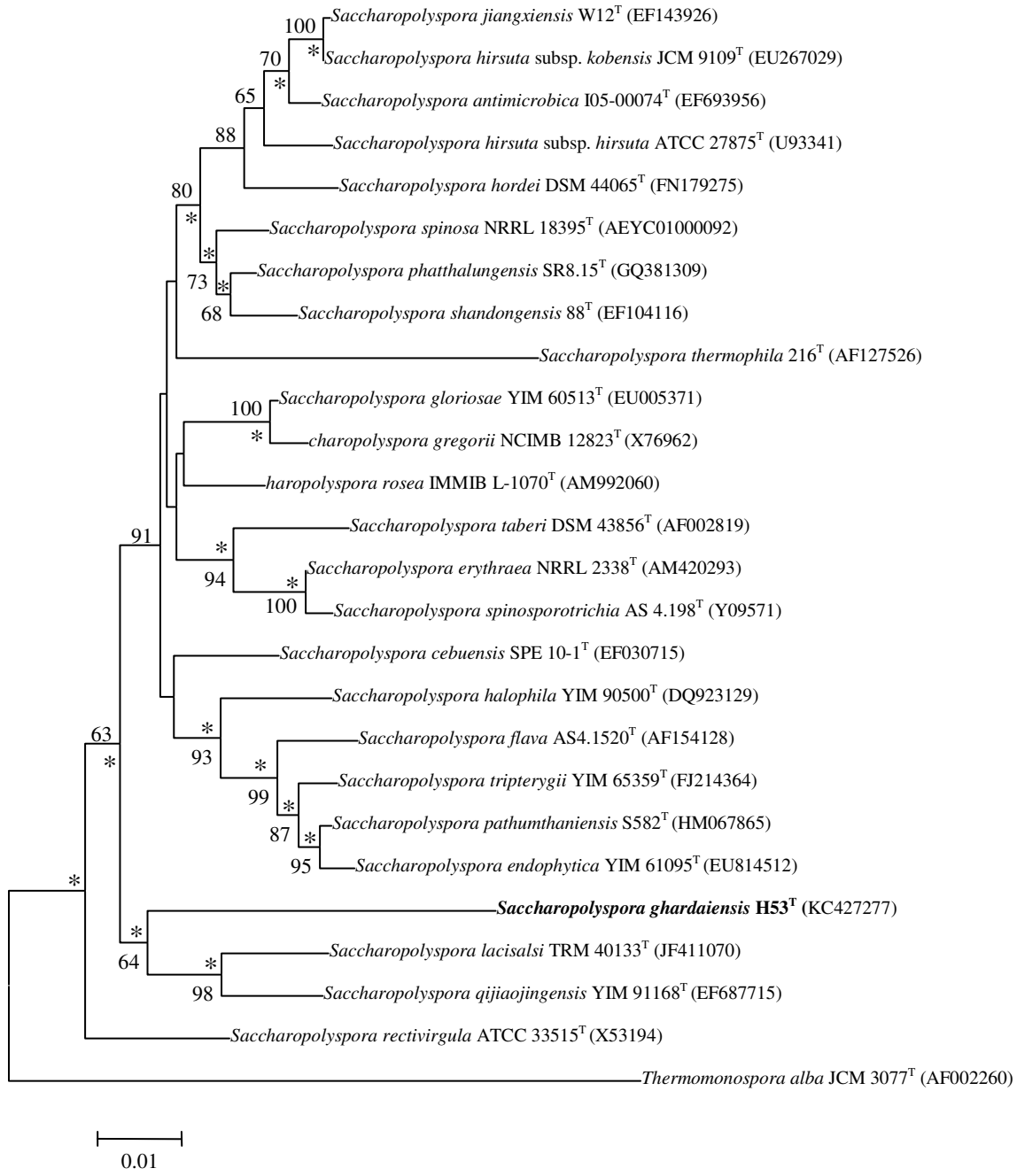

Figure 7. Neighbor-joining phylogenetic tree based on a comparison of the 16S rRNA gene sequence, showing relationships between the isolates of *Saccharopolyspora* and their phylogenetic neighbors.

The 16S rRNA gene sequence analysis indicated that strain H27^T is most closely related to *Streptomonospora alba* DSM 44588^T (98.8%) and *S. flavalba* DSM 45155^T (98.7%) whereas the DDH values between strain H27^T and the two type strains were 17.1 and 57.9%, respectively. Consequently, strain H53^T represents a novel species for which the name *Streptomonospora algeriensis* (DSM 45604^T) is proposed (Fig. 8).

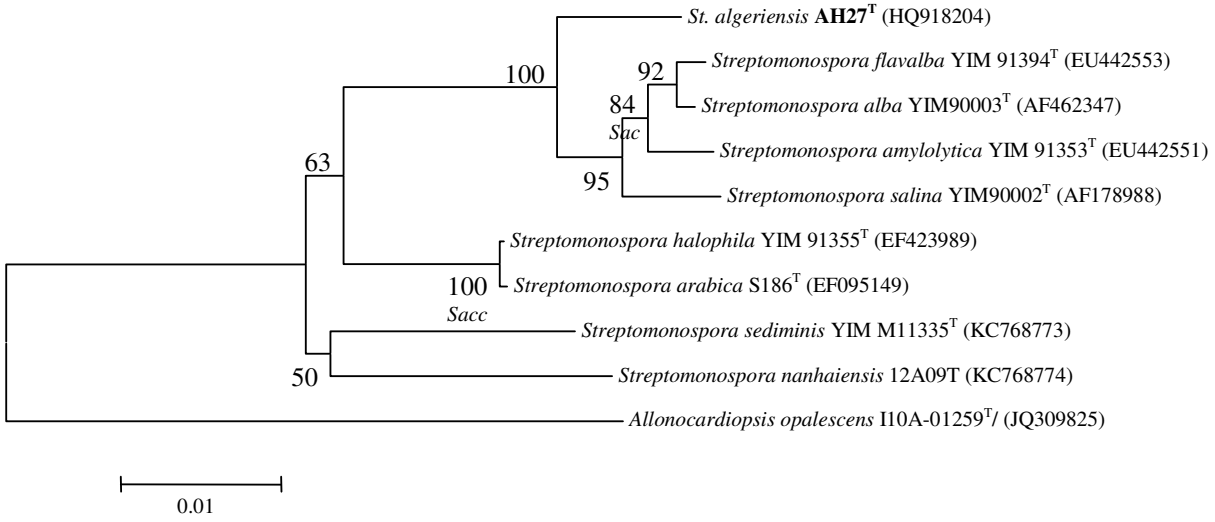

Figure 8. Neighbor-joining phylogenetic tree based on a comparison of the 16S rRNA gene sequence, showing relationships between the isolates of *Streptomonospora* and their phylogenetic neighbors.

On the other hand, the similarity percentages of strains H195^T, H150, H151 and H199 are 91.78–92.18% with *Saccharopolyspora qijiaojingensis* YIM 91168^T, 91.73–92.21% with *S. lacisalsi* TRM 40133^T and 92.13–92.42% with *S. rectivirgula* ATCC 33515^T (from the family *Pseudonocardiaceae*); and 91.00-91.34% with *Actinopolyspora halophila* ATCC 27976^T, 90.70-91.41% with *A. xinjiangensis* TRM 40136^T and 90.61–91.70% with *A. righensis* DSM 45501^T (from the family *Actinopolysporaceae*). On the basis of its phenotypic features and phylogenetic position, we propose that strain H195^T represents a novel genus and species, *Mzabimyces algeriensis* gen. nov., sp. nov., within a new family, *Mzabimycetaceae* fam. nov. The type strain of *M. algeriensis* is strain H195^T (=DSM 46680^T) (Fig. 9).

In this study, fifteen new species of actinobacteria were isolated from Algerian Saharan soil. These new species belonging to *Saccharothrix*, *Actinokineospora*, *Actinoalloteichus*, *Nocardiopsis*, *Prauserella*, *Actinopolyspora*, *Streptomonospora* and *Saccharopolyspora* genera. More interestingly, a new genus named *Mzabimyces* was also isolated in this stud, and the new family *Mzabimycetaceae* was proposed. The results obtained showed also the evidence of the high potential of actinobacteria, isolated from arid environments. The extreme environments are an important reservoir of new species of actinobacteria that could represent a promising source of a large number of biologically active compounds.

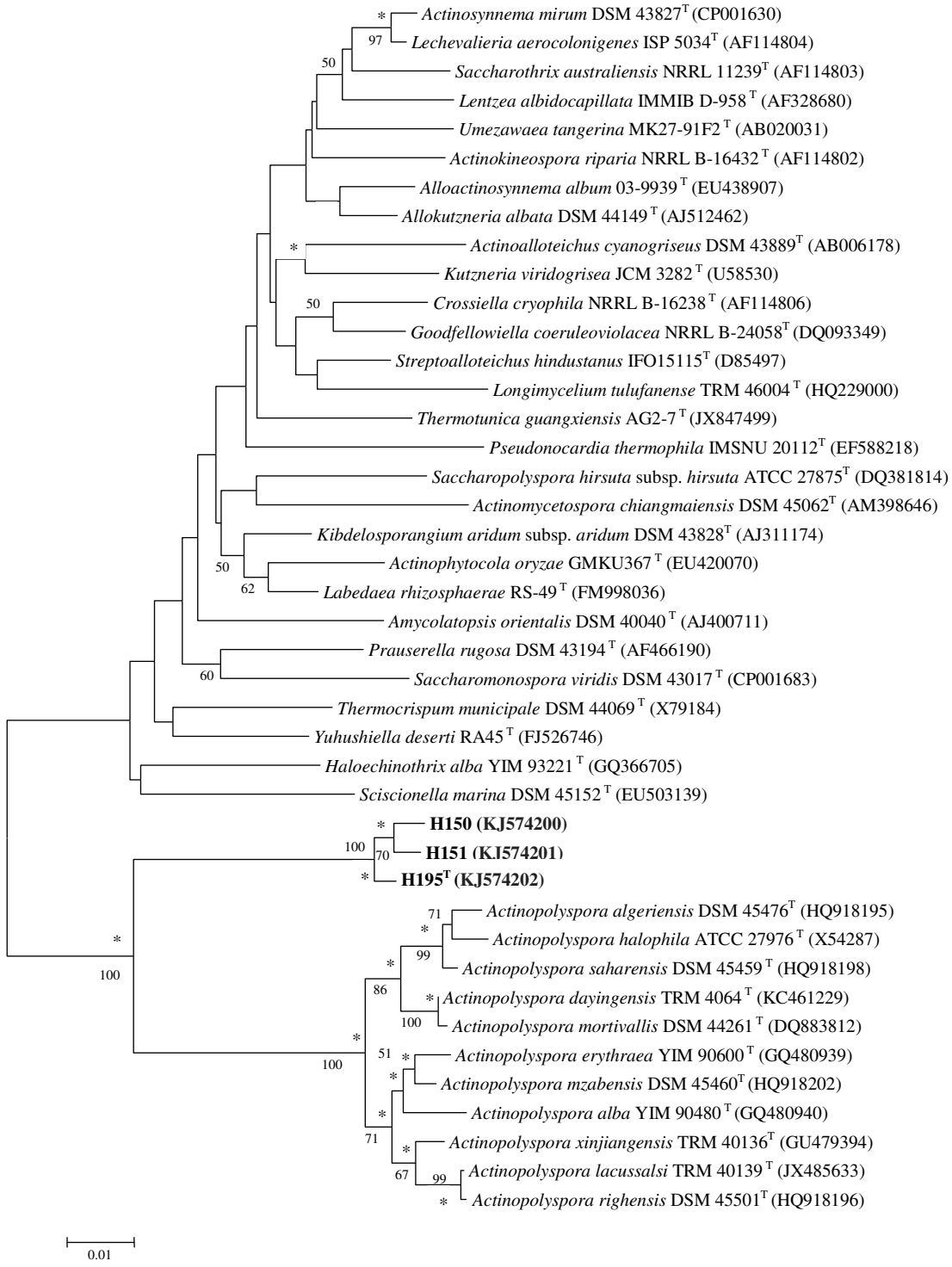

Figure 9. Neighbor-joining phylogenetic tree based on a comparison of the 16S rRNA gene sequence, showing relationships between the isolates H150, H151 and H195 and the most closely related genera (belonging to *Actinopolysporaceae* and *Pseudonocardiaceae*).

References

BERDY J. 2005. Bioactive microbial metabolites. Journal of Antibiotics, 58: 1-26.

BHATNAGAR A. and BHATNAGAR M. 2005. Microbial diversity in desert ecosystems. Current Science, 89: 91-100.

BOUBETRA D., SABAOU N., ZITOUNI A., BIJANI C., LEBRIHI A. and MATHIEU F. 2013. Taxonomy and chemical characterization of new antibiotics produced by *Saccharothrix* SA198 isolated from a Saharan soil. Microbiological Research, 103: 771-776.

Bouras, N. 2005. Régulation de la production d'antibiotiques dithiolopyrrolones chez *Saccharothrix algeriensis* NRRL B-24137. Ph.D. Thesis. Institut National Polytechnique, ENSAT-INP, Toulouse, 238 p.

BECKER B., LECHEVALIER M. P., GORDON R. E. and LECHEVALIER H. A. 1964. Rapid differentiation between *Nocardia* and *Streptomyces* by paper chromatography of whole-cell hydrolysates. Applied Microbiology, 12: 421-423.

CHUN J., BAE K. S., MOON E.Y., JUNG S.O., LEE H. K. and KIM S. J. 2000. *Nocardiopsis kunsanensis* sp. nov., a moderately halophilic actinomycete isolated from a saltern. International Journal of Systematic Evolutionary and Microbiology 50: 1909-1913.

COENYE T., FALSEN E., VANCANNEYT M., HOSTE B., GOVAN J. R., KERSTERS K., and VANDAMME P. 1999. Classification of *Alcaligenes faecalis*-like isolates from the environment and human clinical samples as *Ralstonia gilardii* sp. nov. International Journal of Systematic Bacteriology, 49:405-413

CUI X-L. 2012. Genus III. *Streptomonospora*. In: Goodfellow M, Kampfer P, Busse H-J, Trujillo ME, Suzuki K-I; Ludwig W, Whitman WB (eds), Bergey's Manual of Systematic Bacteriology, The Actinobacteria, 2nd ed, Vol. 5, New York Dordrecht Heidelberg. London, Springer, pp 1908–1914.

GONZALEZ I., AYUSO-SACIDO A., ANDERSON A. and GENILLOUD O. 2005. Actinomycetes isolated from lichens: evaluation of their diversity and detection of biosynthetic gene sequences. FEMS Microbiology Ecology 54:401-415.

HAYAKAWA M. and NONOMURA H. 1987. Humic acid-vitamin agar, a new medium for the selective isolation of soil actinomycetes. Journal of Fermentation Technology, 65: 501-509.

HOZZEIN W. N., TRUJILLO M. E. 2012. Genus I. *Nocardiopsis*. In: Goodfellow M, Kampfer P, Busse H-J, Trujillo ME, Suzuki K-I; Ludwig W, Whitman WB (eds), Bergey's Manual of Systematic Bacteriology, The Actinobacteria, 2nd ed, Vol. 5, New York Dordrecht Heidelberg. London, Springer, pp 1891–1906.

HSU S. C. and LOCKWOOD J. L. 1975. Powdered chitin agar as a selective medium for enumeration of actinomycetes in water and soil. Applied Microbiology, 29:422–426.

SHIRLING E. B. and GOTTLIEB D. (1966). Methods for characterization of *Streptomyces* species. International Journal of Systematic Bacteriology 16: 313-340.

JUKES T. H. and CANTOR C. R. 1969. Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.

KIM S. B. and GOODFELLOW M. 2012a. A Genus XII. *Prauserella*. In: Goodfellow M, Kampfer P, Busse H-J, Trujillo ME, Suzuki K-I; Ludwig W, Whitman WB (eds), Bergey's Manual of Systematic Bacteriology, The Actinobacteria, 2nd ed, Vol. 5, New York Dordrecht Heidelberg. London, Springer, pp 1384-1390.

KIM S. B. and GOODFELLOW M. 2012b. Genus XIV. *Saccharopolyspora*. In: Goodfellow M, Kampfer P, Busse H-J, Trujillo ME, Suzuki K-I; Ludwig W, Whitman WB (eds), Bergey's Manual of Systematic Bacteriology, The Actinobacteria, 2nd ed, Vol. 5, New York Dordrecht Heidelberg. London, Springer, pp 1396-1414.

KIM M., OH H. S., PARK S. C., CHUN J. 2014. Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. International Journal of Systematic Evolutionary and Microbiology 64:346-351.

KIM, O. S., CHO, Y. J., LEE, K., YOON, S. H., KIM, M., NA, H., PARK, S. C., JEON, Y. S., LEE, J. H. & OTHER AUTHORS (2012). Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. International Journal of Systematic Evolutionary and Microbiology 62: 716–721.

KELLY K. L. and JUDD D. B. 1976. Color: Universal Language and Dictionary of Names (National Bureau of Standards Special Publication 440). Washington, DC: US Department of Commerce.

LABEDA D. P. and LECHEVALIER M. P. 1989. Amendment of the genus *Saccharothrix* Labeda *et al*. 1984 and descriptions of *Saccharothrix espanaensis* sp. nov., *Saccharothrix cryophilis* sp. nov., and *Saccharothrix mutabilis* comb. nov. International Journal of Systematic Bacteriology, 39: 420-423.

LABEDA D. P. and KROPPENSTEDT R. M. 2000. Phylogenetic analysis of *Saccharothrix* and related taxa: proposal for *Actinosynnemataceae* fam. nov. International Journal of Systematic Evolutionary and Microbiology 50: 331-336.

LAZZARINI A., CAVALETTI L., TOPPO G. and MARINELLI F. 2001. Rare genera of actinomycetes as potential producers of new antibiotics. Antonie Leeuwenhoek. 78: 399-405.

LECHEVALIER M. P., DE BIÈVRE C. and LECHEVALIER H. A. 1977. Chemotaxonomy of aerobic actinomycetes: phospholipid composition. Biochemical Systematics and Ecology, 5: 249-260.

LECHEVALIER M. P. and LECHEVALIER H. A. 1970. Chemical composition as a criterion in the classification of aerobic actinomycetes. International Journal of Systematic Bacteriology 20: 435-443.

LIU D., COLOE S., BAIRD R. and PEDERSEN J. 2000. Rapid mini-preparation of fungal DNA for PCR. Journal of Clinical Microbiology 38:471.

LOCCI R. 1989. Streptomycetes and related genera. In: Williams ST, Sharpe ME, Holt JG (eds), Bergey's manual of systematic bacteriology. Baltimore: Williams and Wilkins, pp 2451-2493.

MEIER-KOLTHOFF JP, GÖKER M, SPRÖER C and KLENK H-P. 2013. When should a DDH experiment be mandatory in microbial taxonomy? Archives of Microbiology, 195:413-418.

MEKLAT A., SABAOU N., BOURAS N., ZITOUNI A., SPRÖER C., KLENK H-P., MATHIEU F. and LEBRIHI A. 2012. A novel strain of *Actinopolyspora mortivallis* with antibacterial activity isolated from a Saharan soil. Annals of Microbiology**,** 62: 1049-1057.

SAITOU N. and NEI M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution, 4: 406–425.

SAKER R., BOURAS N., ZITOUNI A., GHOUL M, RHODE M., SCHUMANN P., SPRÖER C., SABAOU N. and KLENK HP. 2014. *Mzabimyces algeriensis* gen. nov., sp. nov., a halophilic filamentous actinobacterium isolated from a Saharan soil, and proposal of *Mzabimycetaceae* fam. nov. Antonie van leeuwenhoek**,** 106: 1021–1030.

TAMURA K, PETERSON D, PETERSON N, STECHER G, NEI M and KUMAR S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution, 28:2731-2739.

TRUJILLO M. E. and GOODFELLOW M. 2012. Genus I. *Actinopolyspora*. In: GOODFELLOW M., KAMPFER P., BUSSE H. J., TRUJILLO M. E., SUZUKI K. I., LUDWIG W and WHITMAN W. B. (eds), Bergey's Manual of Systematic Bacteriology, The Actinobacteria, 2nd ed, Vol. 5, New York Dordrecht Heidelberg. London, Springer, pp 163-170.

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. International Journal of Systematic Evolutionary and Microbiology, 55:1149-1153.