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ORIGINAL ARTICLE

Diversity and antagonistic properties of culturable halophilic actinobacteria in soils of two arid regions of septentrional Sahara: M'zab and Zibans

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Abstract The diversity of culturable halophilic actinobacteria in two Algerian arid ecosystems (M'zab and Zibans) of septentrional Sahara was investigated. A total of 69 halophilic strains were isolated from 19 soil samples. Taxonomy was determined using a polyphasic approach based on morphological, physiological (with the application of numerical taxonomy), chemotaxonomic and molecular investigations. Sequencing of the 16S rRNA gene revealed that the strains belonged to six clusters corresponding to six genera: *Actinopolyspora*, *Nocardiopsis*, *Prauserella*, *Saccharomonospora*, *Saccharopolyspora* and *Streptomonospora*. Some strains were found to represent known species, but others formed distinct phyletic lines. Interestingly, cluster VII, which contained four strains, represents an unknown actinobacterial genus. An assessment of the biological properties of the actinobacterial strains showed moderate to strong antimicrobial activities against fungi and bacteria, including methicillin-resistant *Staphylococcus aureus*. The bioactive potential of strains which did not show any antimicrobial activity on yeast extract-malt extract agar was analyzed based on the genetic background of these strains using polyketide synthase (type I, II and PKSE) and non-ribosomal peptide synthetase (NRPS) gene sequences. Most of the strains harbored NRPS and PKS-II genes, indicating that they might have a great potential to produce bioactive compounds if the laboratory culture conditions were changed.

Keywords Halophilic actinobacteria · Algerian arid soils · Numerical taxonomy · Phylogeny · Antagonistic activity

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Introduction

Members of the microbacterial phylum *Actinobacteria* represent the largest reservoir of producers of potentially valuable natural products, such as antitumor and immunosuppressive agents, enzymes, antiparasitics, insecticides, herbicides, antioxidants and, in particular, antibiotics (Solanki et al. 2008). Actinobacteria are extensively distributed in soils and other terrestrial environments, where they have been shown to play an important ecological role in soil nutrient turnover (González et al. 2005). This group of microorganisms is widespread in nature and is able to occupy several extreme ecosystems. For convenience, extremophilic actinobacteria can be conveniently grouped into several groups based on their tolerance/resistance to various environmental factors: acidophilic and alkaliphilic, psychrophilic and thermophilic, halophilic and haloalkaliphilic and xerophilic groups (Jiang and Xu 1993; Al-Tai and Ruan 1994; Selyanin et al. 2005).

Several new taxa (species and genera) of halophilic actinobacteria have been reported (Tang et al. 2011; Chang

et al. 2012; Guan et al. 2013a, b). Furthermore, many studies performed in arid regions, such as Algerian Sahara, have shown the metabolic richness of the halophilic (or moderately halophilic) actinobacteria which inhabit this special ecosystem. This in turn has led to the detection of many bioactive compounds (Boudjelal et al. 2011; Meklat et al. 2012, 2014).

An alternative approach is the characterization of actinobacterial strains based on metabolic potential by targeting widespread genes involved in the production of bioactive compounds (Anderson et al. 2002; Ayuso-Sacido and Genilloud 2004). For example, modular polyketide synthases [PKSs; modular PKS (PKS-I), interactive PKS (PKS-II; Shen 2003), enediyne polyketide synthase (PKSE)] and non-ribosomal peptide synthetases (NRPS; Schwarzer et al. 2003) have been extensively described as being responsible for the biosynthesis of a broad range of biologically active secondary metabolites in microorganisms such as actinobacteria. Nolan and Cross (1988) recommended exploring new soils and habitats to screen for rare microorganisms capable of producing several antibiotics. In this context, we have continued to investigate halophilic actinobacteria to increase current knowledge on this group of bacteria, particularly in terms of genetic diversity.

The aims of this study were to investigate the biodiversity of culturable halophilic actinobacteria isolated from two Algerian arid ecosystems (M'zab and Zibans) of septentrional Sahara, to evaluate their potential to exhibit different antimicrobial activities and to detect the presence of genes encoding PKS-I, PKS-II, PKSE and NRPS.

Materials and methods

Sampling site and sample collection

Soil samples were taken from arid regions of M'zab (358–555 m a.s.l.) and Zibans (110–210 m a.s.l.), which are located in the center and the center-eastern region of Algeria (septentrional Sahara), respectively (Table 1). Surface soil samples (the top 20 cm) were collected and placed in sterile polyethylene bags that were closed tightly and then stored at 4 °C until analysis. A total of 19 samples were collected during the study period.

The soil textures at M'zab and Zibans are loamy sand to sandy loam. The moisture content (at the time of sampling) ranged from 4 to 9 %, the pH was slightly basic (8.0–8.9). The percentages of carbon and nitrogen ranged from 0.32 to 0.80 %, and from 0.04 to 0.06 % at M'zab and Zibans, respectively (very low amounts). The electrical conductivity [1/5 (w/v) soil/water ratio at 25 °C] was variable depending on the sampling location and ranged between 2.5 and 4.2 mS cm^{-1} in the moderately saline soils of the M'zab region (except for the highly saline soils of Zelfana: 15.2 and 18.1 mS cm^{-1}) and between 7.8 and 11.9 mS cm^{-1} in non-saline soils of the Zibans region.

Isolation of halophilic actinobacteria

Halophilic actinobacterial strains were isolated from the arid soils by the serial dilution method and culture on selective culture media: complex medium agar (Chun et al. 2000) and chitin–vitamin agar (Hsu and Lockwood 1975) supplemented with NaCl (15 %) and actidione (50 mg L^{-1}). After 3–4 weeks of incubation at 30 °C, the actinobacterial strains were picked out, purified and preserved on complex medium agar at 4 °C.

Morphological studies

Cultural characteristics were determined after 3–4 weeks of growth on International *Streptomyces* Project (ISP) media ISP2 (malt extract 10 g, yeast extract 4 g, glucose 4 g, agar 18 g, distilled water 1000 mL, pH 7.2) and ISP4 (starch 10 g, K_2HPO_4 1 g, $MgSO_4 \cdot 7H_2O$ 1 g, $(NH_4)_2SO_4$ 2 g, $CaCO_3$ 2 g, $FeSO_4 \cdot 7H_2O$ 1 mg, $MnCl_2 \cdot 4H_2O$ 1 mg, $ZnSO_4 \cdot 7H_2O$ 1 mg, agar 18 g, distilled water 1000 mL, pH 7.2) (Shirling and Gottlieb 1966), nutrient agar (peptone 5 g, yeast extract 2 g, meat extract 1 g, agar 18 g, distilled water 1000 mL, pH 7.2) and complex medium agar (Difco casamino acids 7.5 g, yeast extract 10 g, $MgSO_4 \cdot 7H_2O$ 10 g, sodium citrate 3 g, KCl 1 g, $FeSO_4 \cdot 7H_2O$ at 4.98 % 1 mL, agar 18 g, distilled water 1000 mL, pH 6.8). All media used for the determination of morphological characteristics were supplemented with 15 % (w/v) NaCl. The color of the substrate and aerial mycelia and any diffusible pigments produced were determined by comparison with ISCC-NBS color charts. The micromorphology and sporulation of selected strains were examined by light microscopy (B1 series; Motic, PR China).

Physiological studies and numerical taxonomy

Sixty-three physiological tests were performed to characterize the isolated strains, and physiological characteristics were evaluated according to the methods of Locci (1989). The aims of these tests were to assess the assimilation of 23 carbohydrates and derivatives as sole carbon sources; the assimilation of three amino acids as sole nitrogen sources; the degradation of adenine, arbutin, esculin, gelatin, guanine, hypoxanthine, starch, testosterone, Tween 80, tyrosine, xanthine and nine organic acids; the reduction of nitrates; sensitivity to lysozyme (0.005 %); growth at different concentrations of NaCl (0, 7, 10, 15, 20, 25, 30 % w/v), at different pH (5, 7, 9) and at two different temperatures (30, 45 °C); growth in the presence of erythromycin (15 mg L^{-1}), tetracycline (30 mg L^{-1}) and nalidixic acid (30 mg L^{-1}). All media contained 15 % (w/v) NaCl (except for the NaCl concentration tests).

Table 1 Location of sampling and the number of strains collected

Region	Collection place	Sample	Number of strains	Strains
M'zab	Ahbas (32°27′N, 3°41′E)	AHS1	19	H139, H141, H145, H147, H148, H149, H150, H151, H154, H155, H159, H195, H196, H197, H198, H199, H200, H202, H225
		AHS2	3	H142, H156, H160
		PAL3	3	H133, H137, H229
	Aberda (32°27′N, 3°40′E)	AB2	7	H208, H176, H178, H181, H182, H203, H206
	Inghid (32°25′N, 3°40′E)	PAL2	3	H243, H244, H245.
	Azrar (32°20′N, 3°44′E)	AZ1	3	H165, H215, H224
		AZ2	5	H167, H168, H212, H213, H169
	Awrir (32°21′N, 3°44′E)	AW	1	H220
	Oudjoujen (32°28′N, 3°41′E)	PAL1	2	H239, H240
		AI	1	H218
	Oued Ntissa (32°46′N, 3°70′E)	ALL1	1	H247
		ALL2	1	H248
	Djaber (32°28′N, 3°41′E)	DJ	3	H216, H217, H223.
	Zelfana (32°23′N, 4°13′E)	ZF1	1	H227
		ZF2	5	H231, H232, H233, H234, H238
		ZF5	1	H126
Zibans	Sidi Khaled (34°23′N, 4°59′E)	SK2	1	H249
		SK3	1	H250
	Lioua (34°38′N, 5°25′E)	LIO	8	H254, H255, H256, H257, H258, H259, H260, H261

The results of the physiological study were analyzed by numerical taxonomy. Data on phenetic characters were coded in a binary system (1/0). The degree of similarity between the studied halophilic strains was calculated by simple matching coefficient (S_{SM}), and clustering was performed by the unweighted-pair group method with arithmetic mean (UPGMA) using average linkages in the SPSS package (v.16.0.1; SPSS Inc., Chicago, IL) as described by Meklat et al. (2011).

Chemical studies of cell constituents

Biomass for the chemical and molecular studies of the isolated actinobacterial strains was obtained by cultivation in flasks (250 rpm) containing complex medium broth (pH 7.2) supplemented with 15 % (w/v) NaCl, and incubation with shaking (250 rpm) at 30 °C for 1 week. Diaminopimelic acid (DAP) isomers and whole-cell sugar pattern were analyzed according to the methods of Becker et al. (1964) and Lechevalier and Lechevalier (1970), respectively.

DNA extraction, amplification and sequencing

The actinobacterial colonies were removed aseptically and transferred to 1.5-mL sterile Eppendorf tubes. 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 (MP Biomedicals, USA) using the primers 27F (5′-AGAG TTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCT TGTTACGACTT-3′) in a thermocycler (My Cycler™; Bio-Rad, USA). Amplification was carried out in a final volume of 50 μL of reaction mixture consisting of 25–50 ng of genomic DNA, 0.5 μM of each primer (27F and 1492R), 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^{-1} bovine serum albumin), 200 μM mixture of desoxynucleoside triphosphate (dNTP) and 1.5 U Taq DNA polymerase. The conditions of the PCR were standardized and consisted of an initial denaturation at 98 °C for 4 min followed by 30 cycles of amplification (denaturation at 94 °C for 1 min, annealing at 52 °C for 1 min and extension at 72 °C for 1 min), with a final extension cycle for 10 min at 72 °C. The PCR product was analyzed in a 0.8 % agarose gel, and the fragment was separated by electrophoresis at 100 V for 60 min in TAE buffer. The bands were observed under UV light. The primers used for sequencing are listed in Coenye et al. (1999).

16S rRNA gene phylogenetic analyses

The sequences obtained were compared with sequences present in public sequence databases as well as with those in the EzTaxon-e database server (Kim et al. 2012). All sequences were manually aligned with reference sequences. Phylogenetic analyses were conducted using MEGA version 5 (Tamura et al. 2011). A phylogenetic tree was constructed using the neighbor-joining method of Saitou and Nei (1987)

with the model of Jukes and Cantor (1969). The resulting topology of the trees was evaluated by bootstrap analysis on 1000 replicates.

Evaluation of antimicrobial activity

The antimicrobial activity of the halophilic strains was evaluated by the cylinder plate method against *Staphylococcus aureus* S1, methicillin-resistant *Staphylococcus aureus* 639c, *Bacillus subtilis* ATCC 6633, *Saccharomyces cerevisae* ATCC 4226, *Candida albicans* M3, *Umbelopsis ramanniana* NRRL 1829 (formerly *Mucor ramannianus*), *Penicillium expansum* PE1 and *Aspergillus carbonarius* M333. The actinobacterial strains were grown on ISP2 medium supplemented with 15 % (w/v) NaCl at 30 °C. After 12 days of incubation, cylinder agar samples of actinobacterial strains were placed on the surface of ISP2 medium (without NaCl) seeded with the target microorganisms. Inhibition zones were measured after 24 h at 30 °C for bacteria and yeasts and after 36–48 h at 30 °C for filamentous fungi. A control cylinder of ISP2 medium containing only 15 % (w/v) NaCl was used for each target microorganisms.

Detection of PKS-I, PKS-II, PKSE and NRPS

Genes for PKS-I, PKS-II, PKSE and NRPS were amplified by PCR from genomic DNA using the degenerate primer pairs K1F/M6R, KSα/KSβ, EdyA/EdyE and A3F/A7R, respectively, according to methods described previously (Metsä-Ketelä et al. 1999; Ayuso-Sacido and Genilloud 2004; Liu et al. 2003). Each reaction mixture consisted of 20–40 ng DNA template (obtained by the method of Liu et al. 2000), 0.4 μM of dNTP mixture, 2 μM of each primer, 1×buffer containing $MgCl_2$ and 2.5 U of Taq DNA polymerase and 5 % dimethylsulfoxide. The PCR amplification program consisted of an initial denaturation at 94 °C for 4 min, followed by 30 cycles of amplification (a denaturation step at 94 °C for 1 min, an annealing step of 1 min at 57 °C with K1F/M6R, at 58 °C with KSα/KSβ, at 62 °C with EdyA/EdyE and at 57 °C with A3F/A7R and an extension of 2 min at 72 °C, with a final extension at 72 °C for 10 min. All amplification products were examined by electrophoresis in agarose gel (0.8 %), and bands of 1200–1400, 600, 1400 and 700–800 bp were classified as products of the PKS-I, PKS-II, NRPS and PKSE genes, respectively.

Results and discussion

Phenotypic and chemotaxonomic studies

According to the tests on morphology, chemotaxonomy and physiology, including halophilism, the 69 isolated actinobacterial strains were tentatively classified into several genera or groups. The physiological tests (numerical taxonomy), based on the similarity S_{SM} coefficient and on UPGMA clustering classified the isolates, at the 83 % similarity (S) level, into seven clusters, designated I–VII (Fig. 1).

As shown in Fig. 1, Cluster IV includes 15 strains. These strains produced a cream or sometimes pinkish aerial mycelium which fragmented irregularly into long chains of rod-shaped and non-motile spores. The substrate mycelium, cream

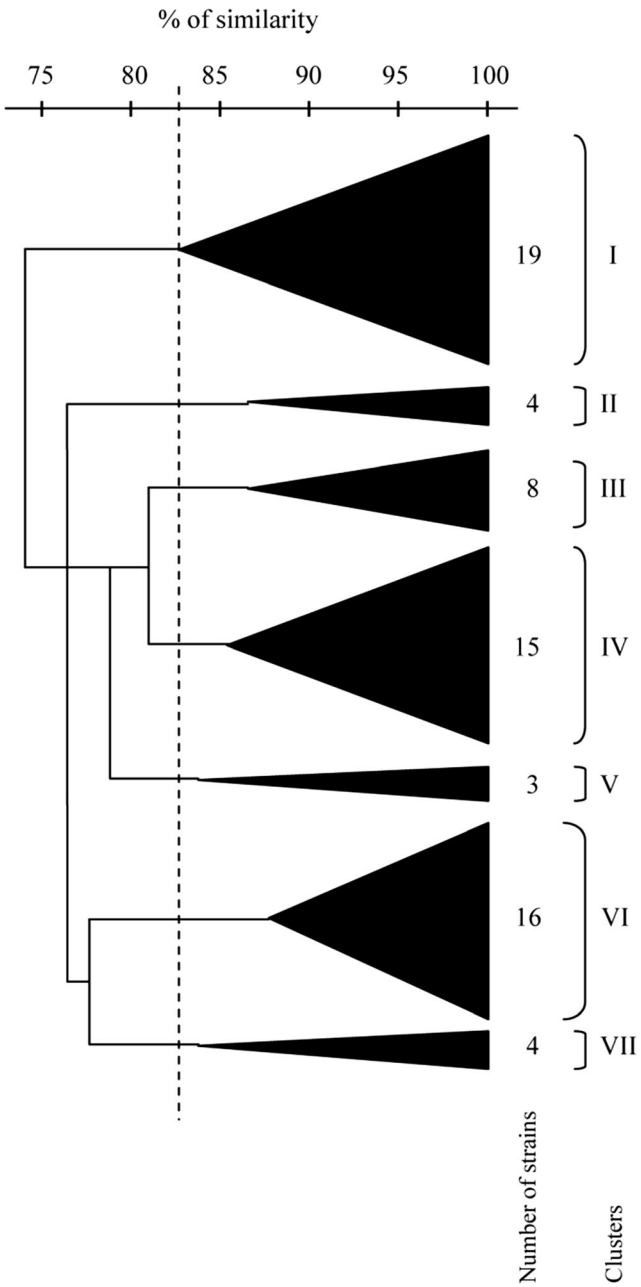

Fig. 1 Dendrogram derived from the similarity (simple matching) coefficient (S_{SM}), unweighted-pair group method with arithmetic mean (UPGMA) analysis based on 63 unit characters, showing the relationships between the 69 halophilic actinobacterial strains isolated from the M'zab and Zibans regions

to non-colored, was more or less fragmented into coccoid and bacillary elements. The peptidoglycan of these cells contained the DL-isomer of DAP, and the whole-cell hydrolysates contained glucose, ribose and sometimes galactose, indicating wall chemotype IIIC (Lechevalier and Lechevalier 1970). These properties are characteristic of *Nocardiopsis*, a genus which contains several species of halophilic or halotolerant actinobacteria (Hozzein and Trujillo 2012). These strains grew in the presence of 7–20 % NaCl and were distinguished from the other clusters by their ability to degrade adenine and by their sensitivity to erythromycin (10 μg mL^{-1}).

Cluster V (Fig. 1) comprises three strains, each with aerial mycelium with the same morphological characteristics and also the same wall chemotype as cluster IV, with the exception of the production of isolated spores in the substrate mycelium. These strains grew in the presence of 7–20 % NaCl. These properties are characteristic of *Streptomonospora*, a genus in which all species are halophilic (Cui 2012). Strains of this cluster were the only strains able to degrade ribose.

Cluster VI (Fig. 1) contains 16 strains, most of which produced blue-green aerial mycelium (although some formed a green aerial spore mass) and produced non-motile, single spores that were sessile or borne on very short sporophores. The non-fragmenting substrate mycelium had either a blue-green or cream coloration. All 16 strains had wall chemotype IV, i.e., peptidoglycan contained the DL-isomer of DAP and whole-cell hydrolysates contained arabinose and galactose (sometimes with ribose and glucose). These strains grew in the presence of 7–15 % or 7–20 % NaCl and probably belong to the genus *Saccharomonospora*, which includes halophilic and halolerant species (Kim 2012). These 16 strains were distinguished from other clusters by their inability to degrade hypoxanthine.

Cluster II (Fig. 1) contains four strains with a nocardioform aspect, with white to cream aerial mycelium, sterile (or less fragmented) and a whitish, cream or yellow substrate mycelium fragmented excessively into non-motile coccoid and rod elements. These strains, which are halophilic or halotolerant, have wall chemotype IVA (arabinose and galactose). They grew in the presence of 0–20 % or 7–25 % NaCl. These strains would appear to belong to the genus *Prauserella*, which contains many species that are known to be halophilic or halotolerant (Kim and Goodfellow 2012a). Strains of this cluster were the only strains able to degrade mannitol, but they were unable to degrade milk casein or to produce nitrate reductase.

Isolates classified into clusters I (19 strains), III (8 strains) and VII (4 strains) (Fig. 1) had a white to cream aerial mycelium, which produced spore chains (5–30 spores per chain), non-motile, often in rod (cluster I), ovoid (cluster III) or rounded elements (cluster VII). The substrate mycelium was whitish to cream and fragmented into coccoid and rod-like elements. These strains had wall chemotype IVA (peptidoglycan contained the DL-isomer of DAP, and whole-cell hydrolysates contained arabinose, galactose, glucose, ribose and sometimes mannose). These strains grew in the presence of 7–30 %, 7–25 % or 10–30 % NaCl. The genera that have these characteristics and which are halophilic or salt tolerant are *Actinopolyspora* (all species are halophilic) (Trujillo and Goodfellow 2012) and *Saccharopolyspora* (some species are halophilic or halotolerant) (Kim and Goodfellow 2012b). Strains of cluster I were the only strains to use adonitol as a sole carbon source, strains of cluster III were the only strains which assimilated erythritol and strains of cluster IV wee the only strains to assimilate gelatin, arabinose and xylose.

Assessment of diversity based on 16S rRNA gene analyses

From a total of 69 actinobacteria, 58 strains belonging to different physiological groups (clusters) were subjected to molecular analysis. The resulting 16S rRNA gene sequences were compared with sequences present in the public sequence databases and in the EzTaxon database server; they have been deposited in GenBank under the accession numbers KJ574145–KJ574163, KJ574165–KJ574179, KJ574181–KJ574187, KJ574189–KJ574203, KJ504173, KJ504178 and KJ504174. A phylogenetic tree was constructed using the neighbor-joining method of Saitou and Nei (1987) with the model of Jukes and Cantor (1969). The topologies of the trees were evaluated by bootstrap analysis. Almost all of the nodes leading to actinobacterial strains studied were supported by high bootstrap values and should be considered significant (Figs. 2–4). A perfect similarity between the physiological and phylogenetic analysis was observed.

Similarly, it was confirmed that the isolates in cluster IV belonged to the genus *Nocardiopsis*. The strains of *Nocardiopsis* formed a distinct phylogenetic line within the tree (Fig. 2). Strains H159 and H249 were related to *Nocardiopsis halotolerans* with 99.4 and 99.7 % similarity, respectively, strains H139 and H239 were assigned to *N. xinjiangensis* with 99.2 and 99.3 % similarity, respectively, while the remaining six strains were related to *N. litoralis* (similarity 99.7–99.9 %). High 16S rRNA similarities were found between representatives of validly published *Nocardiopsis* species, such as the type strains of *Nocardiopsis valliformis* and *N. exhalans* (99.9 %) (Yang et al. 2008), *N. sinuspersici* and *N. arvandica* (99.9 %) (Hamedi et al. 2011), *N. halophila* and *N. baichengensis* (99.9 %) (Li et al. 2006), *N. litoralis* and *N. kunsanensis* (99.6 %) (Chun et al. 2000) and *N. metallicus* and *N. exhalans* (99.4 %) (Schippers et al. 2002). Thus, three strains of *Nocardiopsis* isolated from Algerian arid soils (H159, H139 and H239) that have similarities of <99.5 % may belong to a new species. All *Nocardiopsis* strains were isolated from the moderately saline soils of the M'zab region, with the exception of H249 (closely related to *N. halotolerans*)

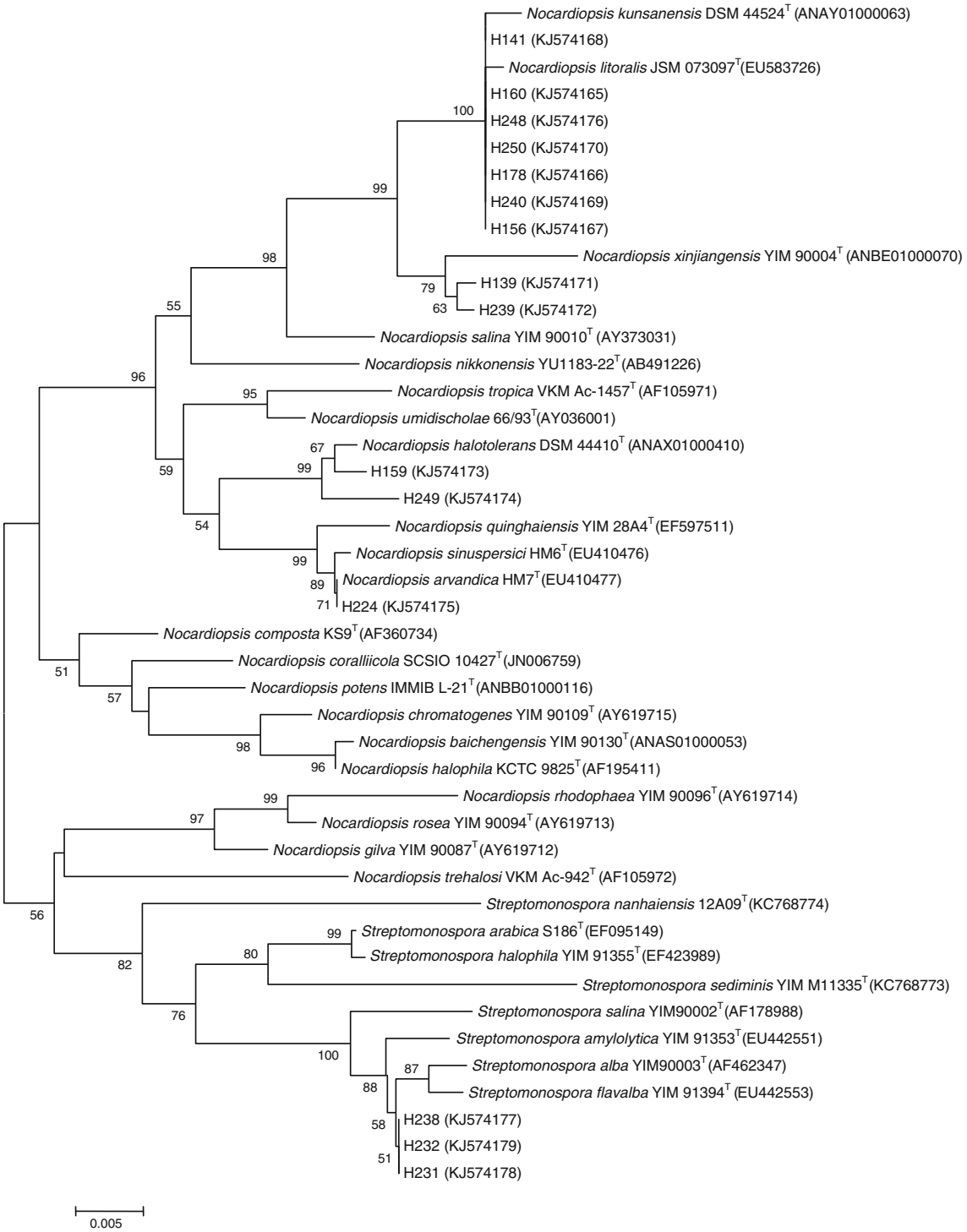

Fig. 2 Neighbor-joining (NJ) tree based on 16S rRNA gene sequences showing relationships between *Nocardiopsis* and *Streptomonospora* isolates, and between these and their nearest phylogenetic neighbors. Bootstrap values of >50 % are indicated at *nodes*. *Bar*: 0.005 substitution per nucleotide position

and H250 (closely related to *N. kunsanensis* and *N. litoralis*) which were isolated from saline soils of the Zibans region.

Phylogenetic analysis showed that cluster V represents the genus *Streptomonospora* (Fig. 2). Two strains of *Streptomonospora* (H238, H231) were assigned to *Streptomonospora amylolytica* with 99.4 % similarity, and strain H232 was related to *S. alba* with 99.5 % similarity. These 16S rRNA sequence similarities are below or approximately the same as the similarities between closely related *Streptomonospora* species, such as *Streptomonospora halophila* and *S. arabica* (99.9 %),*S. flavalba* and *S. alba* (99.7 %), *S. flavalba* and *S. amylolytica* (99.4 %) and

S. amylolytica and *S. alba* (99.3 %) (Cai et al. 2009). This result suggests that strains H238, H231 and H232 belong to new species. All *Streptomonospora* strains were isolated from highly saline soils of Zelfana (M'zab region).

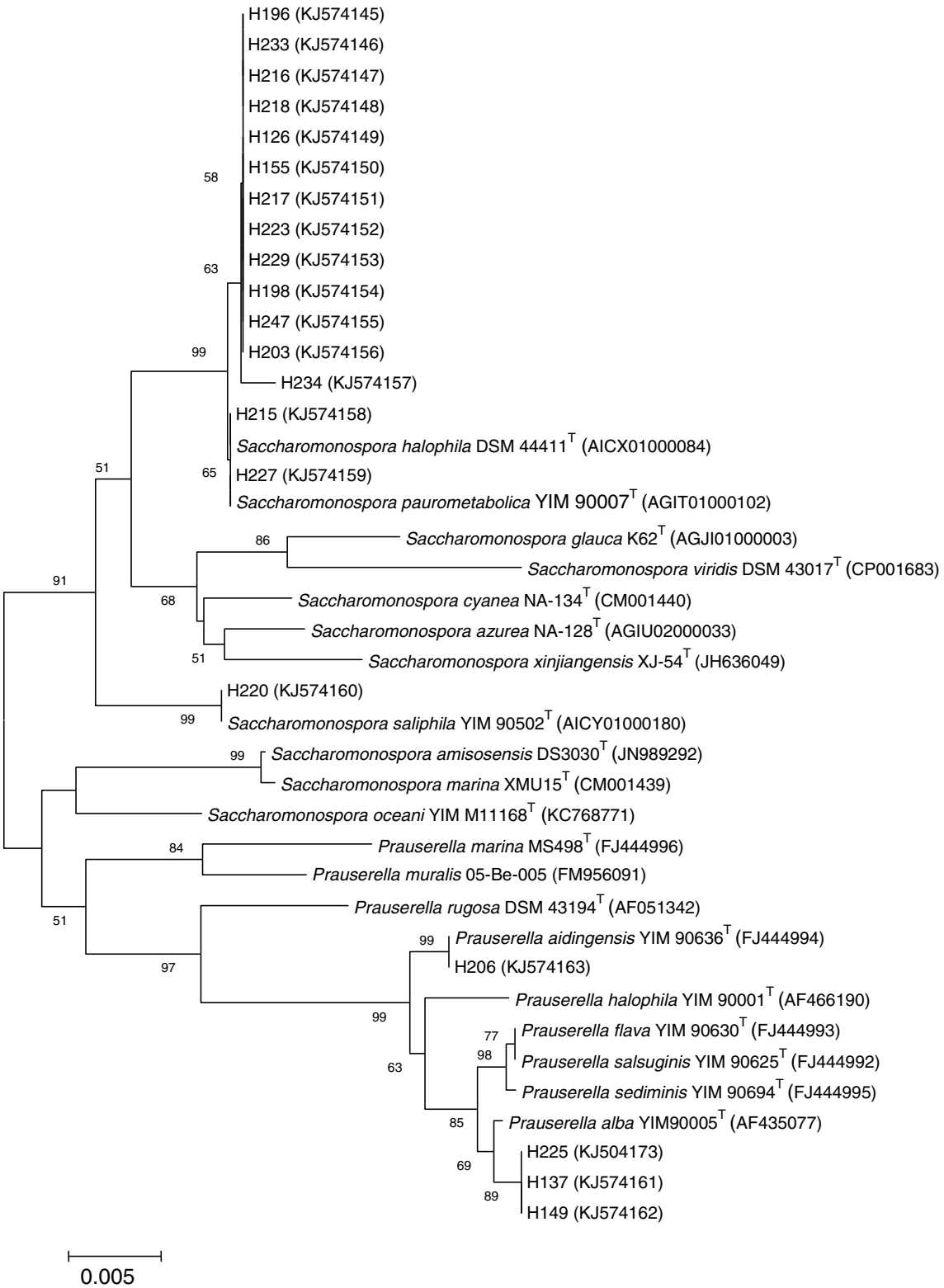

Fig. 3 NJ tree based on 16S rRNA gene sequences showing relationships between *Saccharomonospora* and *Prauserella* isolates, and between these and their nearest phylogenetic neighbors. Bootstrap values of >50 % are indicated at *nodes*. *Bar*: 0.005 substitution per nucleotide position

The 16S rRNA gene sequences of *Saccharomonospora* strains (cluster VI) formed a distinct cluster with the type strain of *Saccharomonospora paurometabolica* (Fig. 3) and shared a 16S rRNA gene similarity with the latter of 99.4–99.7 %. Strain H220, however, was 100 % identical to *S. saliphila*. Some species of *Saccharomonospora* have high similarities between them, as in the case of *Saccharomonospora amisosensis* and *S. marina* (99.6 %)

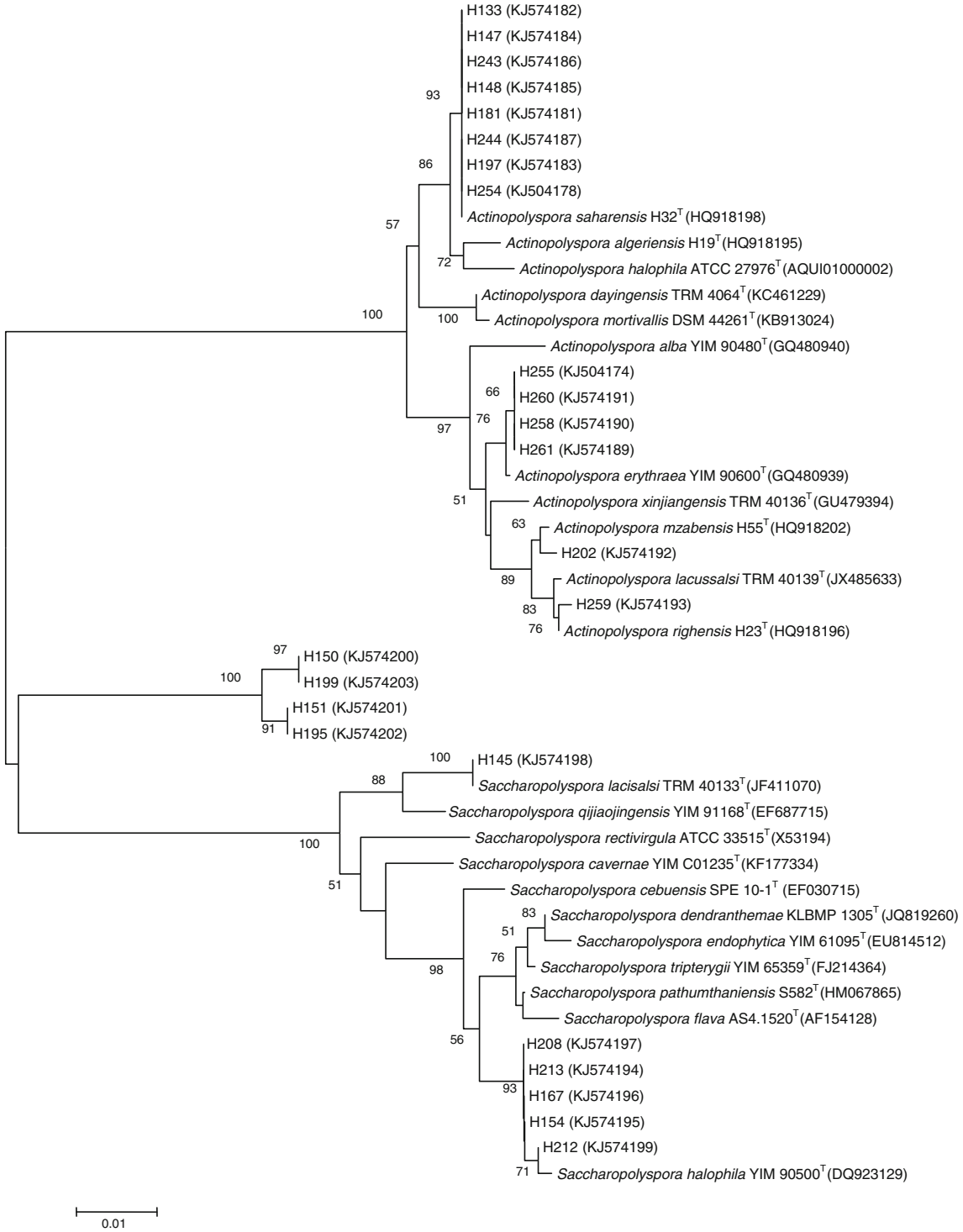

Fig. 4 NJ tree based on 16S rRNA gene sequences showing relationships between *Actinopolyspora*, *Saccharopolyspora* and isolates from an unknown genus, and between these and their nearest phylogenetic neighbors. Bootstrap values of >50 % are indicated at nodes. *Bar*: 0.01 substitution per nucleotide position

Table 2 Antimicrobial activities of the halophilic actinobacterial strains isolated in this study

Strain	Activity[a] (mm) against:							
	Staphylococcus aureus S1	Methicillin-resistant *Staphylococcus aureus* 639c	*Bacillus subtilis* ATCC 6633	*Candida albicans* M3	*Saccharomyces cerevisiae* ATCC 4226	*Penicillium expansum* PE1	*Umbelopsis ramanniana* NRRL 1829	*Aspergillus carbonarius* M333
H126	–	–	–	–	–	–	16	18
H133	–	–	–	16	–	–	20	20
H137	–	–	–	18	20	–	20	28
H139	–	–	14	20	30	–	24	38
H141	–	–	15	21	17	14	28	30
H142	–	–	16	18	16	–	24	26
H148	–	–	–	18	30	–	21	22
H149	–	–	–	17	40	18	34	22
H154	–	–	–	–	28	15	28	19
H156	18	20	18	21	26	–	28	22
H159	–	–	–	18	17	16	29	20
H160	18	20	18	19	18	–	27	15
H165	–	–	–	24	21	17	26	30
H167	–	–	–	18	28	–	30	23
H168	–	–	–	–	28	–	33	20
H169	–	–	–	15	19	–	36	20
H178	–	–	–	18	18	15	28	28
H180	–	–	–	22	24	16	25	28
H181	–	–	–	19	–	–	20	26
H182	–	–	–	18	–	–	21	26
H197	–	–	–	17	21	–	18	19
H199	–	–	–	–	–	–	–	20
H206	–	–	–	17	24	22	24	19
H208	–	–	–	–	24	14	19	18
H212	–	–	–	–	–	–	34	19
H220	–	–	–	18	20	16	28	22
H224	–	–	–	20	15	20	23	29
H225	–	–	–	20	28	24	28	22
H227	–	–	–	–	–	–	–	18
H233	–	–	–	–	–	–	20	18
H239	–	–	–	17	–	15	23	19
H240	17	17	17	18	24	17	23	19
H243	–	–	–	–	28	–	28	17
H244	–	–	–	16	–	–	25	18
H245	–	–	–	14	–	–	25	20
H247	–	–	–	–	18	–	–	19
H248	–	–	–	17	–	–	22	19
H249	–	–	16	20	20	19	24	24
H250	21	18	21	20	16	20	20	20
H254	–	–	–	17	17	19	20	24
H255	46	36	32	–	14	–	–	18
H256	44	36	34	–	–	–	–	18
H258	42	34	34	–	–	–	–	18
H259	–	–	14	19	21	24	23	25
H260	36	–	33	18	23	22	21	22

Table 2 (continued)

Strain	Activity[a] (mm) against:							
	Staphylococcus aureus S1	Methicillin-resistant *Staphylococcus aureus* 639c	*Bacillus subtilis* ATCC 6633	*Candida albicans* M3	*Saccharomyces cerevisiae* ATCC 4226	*Penicillium expansum* PE1	*Umbelopsis ramanniana* NRRL 1829	*Aspergillus carbonarius* M333
H261	34	37	31	17	22	19	18	19
H262	34	37	31	17	22	18	18	21

[a] Activity was estimated by measuring the diameter of the clear zone of growth inhibition. -, No activity. Strains H145, H147, H150, H151, H155, H195, H196, H198, H 201, H203, H213, H215, H216, H217, H218, H221, H223, H229, H231, H232, H234 and H238 are inactive against all indicator microorganisms tested

and *S. halophila* and *S. paurometabolica* (99.6 %). The possibility of new species of *Saccharomonospora* being present in Algerian arid soils cannot not ruled out. Strains H126, H227, H233 and H234 (closely related to *S. halophila* and *S. paurometabolica*) were isolated from highly saline soils of Zelfana (M'zab region). The other strains (closely related to *S. halophila* and *S. paurometabolica*, and also to *S. saliphila*) were isolated from moderately saline soils of the M'zab region.

Cluster II, representing the genus *Prauserella*, exhibits a physiological heterogeneity, suggesting the presence of three different species. Based on the phylogenetic study (Fig. 3), strains H149 and H206 were assigned to *Prauserella alba* and *P. aidingensis*, with a similarity of 99.8 and 100 %, respectively. Nevertheless, strains H225 and H137 showed only a 98.9 and 99.4 % similarity to *P. flava*. We noted that three members of this genus, *Prauserella salsuginis*, *P. flava* and *P. sediminis*, have been reported to be very close phylogenetically, with 99.7–99.9 % similarity (Li et al. 2009). These results suggest that our strains represent more than one novel species based on their phylogenetic and physiological heterogeneity. This is the first report of the isolation of *Prauserella* from Algerian arid ecosystems. All *Prauserella* strains were isolated from moderately saline soils of the M'zab region.

The strains of *Actinopolyspora* (cluster I) had lineages that were distinct from each other and from other members of this genus (Fig. 4). Eight of the isolated strains of this genus were most closely related to *Actinopolyspora saharensis* with a similarity of 99.4–99.9 %. Strain H202 was linked to *Actinopolyspora mzabensis* with a similarity of 99.4 %, however strain H259 showed a similarity of only 98.8 % with *A. laccussalsi* and four strains (H255, H258, H260 and H261) were most closely related to *A. erythraea* with a similarity of 99.1 %. Some of *Actinopolyspora* species showed a relatively high similarity percentage between each other (99.2 % between *A. dayingensis* and *A. mortivallis* and 99.3 % between *A. lacussalsi* and *A. righensis*) (Guan et al. 2013a, b; Meklat et al. 2013), suggesting the possible presence of two new species of this genus, particularly for strains H259 on the one hand and H255, H258, H260 and H261 on the other hand. Strains H255, H258, H260 and H261 (closely related to *A. erythrea*), H254 (closely related to *A. saharensis*) and H259 (closely related to *A. lacussalsi*) were isolated from salty soils of the Zibans region. The other strains of *Actinopolyspora* (closely related

Table 3 Distributions of the genes encoding modular polyketide synthases and non-ribosomal peptide synthetases in strains not showing any antimicrobial activity on ISP2 solid medium

Strain	Presence of indicated gene			
	PKS-I	PKS-II	PKSE	NRPS
H145	+	+	+	+
H147	+	+	–	+
H150	–	+	+	+
H151	–	–	–	+
H155	–	–	–	+
H195	–	–	–	–
H196	–	–	–	–
H198	–	–	–	+
H201	–	+	–	+
H202	–	+	–	+
H203	–	+	–	+
H213	–	–	–	+
H215	–	–	+	+
H216	–	–	–	+
H217	–	–	–	+
H218	–	–	–	–
H223	–	–	–	+
H229	–	+	–	+
H231	–	+	–	+
H232	–	+	–	+
H234	–	+	–	+
H238	–	+	–	+

PKS-I, modular polyketide synthase; PKA-II, iterative PKA ; PKSE, enediyne polyketide synthase; NRPS, non-ribosomal peptide synthetases

–, Gene not present; +, gene present

to *A. saharensis* and *A. mzabensis*) were isolated from moderately saline soils of the M'zab region.

The results of the phylogenetic tree (Fig. 4) confirmed the membership of cluster III to the genus *Saccharopolyspora* and also showed that many strains in cluster III were closely related to *Saccharopolyspora halophila* with similarities ranging between 99.3 and 99.8 %. A single strain, H145, was related to a *Saccharopolyspora lacisalsi* with a similarity of 99.5 %. High 16S rRNA similarities have occasionally been found between closely related species of *Saccharopolyspora*, as in the case of *S. erythraea* and *S. spinosporotrichia* (99.7 %), *S. pathumthaniensis* and *S. endophytica* (99.3 %) and *S. dendranthemae* and *S. endophytica* (99.2 %). Furthermore, the large physiological difference observed between our strains suggests that they belong to one or two novel species of *Saccharopolyspora*. All *Saccharopolyspora* strains were isolated from moderately saline soils of the M'zab region.

The analysis of physiological cluster VII revealed four strains (H150, H151, H195, H199) with a shared similarity within the range of 91.4 and 92.2 %. These 16S rRNA sequence similarity values are very low. Indeed, the four strains of cluster VII represent a very distinct phylogenetic lineage of the neighbor genera *Actinopolyspora* and *Saccharopolyspora*, suggesting a high probability of the presence of new genus. The four strains were isolated from moderately saline soils of the M'zab region.

In general, we found no correlation between the degree of soil salinity and the degree of resistance of actinobacterial strains to NaCl. For example, some strains of *Saccharomonospora* growing in the presence of 7–15 % NaCl were isolated from highly saline soils, while other strains of *Actinopolyspora* that grew in the presence of 7–30 % NaCl were isolated from moderately saline soils.

Antimicrobial activity

Forty-seven of the isolated strains showed activities against one or many of the target microorganisms: Gram-positive bacteria (such as *Bacillus subtilis* ATCC 6633, methicillin-resistant *Staphylococcus aureus* 639c and *S. aureus* S1), filamentous fungi (such as *Umbelopsis ramanniana* NRRL 1829, *Penicillium expansum* PE1 and *Aspergillus carbonarius* M333) and yeasts (such as *Saccharomyces cerevisae* ATCC 4226 and *Candida albicans* M3). The diameter of the inhibition zones varied from 14 to 46 mm (Table 2). Halophilic actinobacteria proved to be an interesting source of bioactive molecules. Hamedi et al. (2013) reported the results of several studies on bioactive molecules secreted by halophilic and halotolerant actinobacterial strains (terrestrial and marine) belonging to the genera of *Actinopolyspora*, *Nocardiopsis*, *Saccharopolyspora*, *Salinispora*, *Streptomyces*, among others. Meklat et al. (2012) isolated, from Saharan soil, a new species of *Actinopolyspora* which shows strong antibacterial (against Gram-positive and Gram-negative bacteria) and fungal activities (against filamentous fungi and yeast). Dhanasekaran (2014) reported the anti-infective potential of halophilic actinobacteria against drug-resistant human pathogenic microorganisms.

Detection of PKS-I, PKS-II, PKSE and NRPS genes in selected strains

The culture media used in this study may not have provided all of the physical conditions (such as temperature, pH, agitation, etc.) and chemical conditions (nutrients, minerals, etc.) needed to stimulate the production of bioactive secondary metabolites in some of the halophilic strains. Therefore, the potential of all 22 non-productive actinobacterial strains to produce bioactive compounds was evaluated by PCR for the detection of the genes encoding polyketide synthases (type I, II and PKSE) and NRPS using the primers listed in the Materials and methods. In general, high frequencies of positive PCR amplification were obtained for NRPS (87.5 %) and PKS-II (50 %), as shown in Table 3. In contrast, PKSE and PKS-I sequences were detected only in 16.6 and 8.3 % of the studied strains, respectively. The high frequency of NRPS genes in the studied actinobacterial strains may be evidence of the high potential of halophilic actinobacteria to produce a large number of bioactive secondary metabolites. Furthermore, the unusually high detection of NRPS genes may be explained in part by the efficiency of the primers A3F–A8R, which were developed to amplify the NRPS adenylation domains of the mannopeptimycin biosynthetic gene cluster, as reported by Magarvey et al. (2006) and Janso and Carter (2010). The low occurrence of PKS-I genes in actinobacterial strains has been reported by Qin et al. (2009).

In conclusion, we have identified isolates from six known halophilic genera (*Actinopolyspora*, *Prauserella*, *Streptomonospora*, *Nocardiopsis*, *Saccharopolyspora* and *Saccharomonospora*). Some of the isolates are likely to be new species within these genera. More interestingly, an unknown genus was also isolated. The results obtained support the notion that halophilic actinobacteria isolated from arid environments have a high potential to produce a large number of biologically active compounds.

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