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Isolation, Classification and Antagonistic Properties of Alkalitolerant Actinobacteria from Algerian Saharan Soils

Atika Meklat[a,b], Noureddine Bouras[a,c], Salim Mokrane[a], Abdelghani Zitouni[a], Nadjette Djemouai[a,c], Hans-Peter Klenk[d], Nasserdine Sabaou[a], and Florence Mathieu[e]

[a]Laboratoire de Biologie des Systèmes Microbiens (LBSM), Ecole Normale Supérieure de Kouba, Alger, Algeria; [b]Département de Biologie et Physiologie Cellulaire, Faculté des Sciences de la Nature et de la Vie, Université Saâd Dahleb, Blida, Algeria; [c]Département de Biologie, Faculté des Sciences de la Nature et de la Vie et Sciences de la Terre, Université de Ghardaia, Ghardaïa, Algeria; [d]School of Biology, Newcastle University, Newcastle upon Tyne, UK; [e]Laboratoire de Génie Chimique, Université de Toulouse, CNRS, Toulouse, France

ABSTRACT

The Sahara, one of the most extreme environments on Earth, constitutes an unexplored source of alkalitolerant actinobacteria. In this work, we studied the diversity of alkalitolerant actinobacteria in various soils collected from different regions of the Algerian Sahara. A total of 29 alkalitolerant actinobacterial strains were isolated by using a complex agar medium. The diversity of these actinobacteria was evaluated using a polyphasic approach, which included morphological, chemotaxonomic, physiological (numerical taxonomy) and 16S rRNA gene analyses. The isolates which were assigned to the genus *Nocardiopsis*, shared relatively low 16S rRNA gene sequences similarities compared to closely related species suggesting that they belonged to putatively new species. All of the strains were tested for antibiotic activity against a broad range of microorganisms and screened for genes encoding polyketide synthases and non-ribosomal peptide synthetases and found to have the potential to produce secondary metabolites. Consequently, the study supports the view that extreme environments contain many novel actinobacteria, which represent an unexplored source for the discovery of biologically active compounds.

KEYWORDS

Alkalitolerant actinobacteria; Saharan soils; diversity; antimicrobial activity

Introduction

Actinobacteria are a group of microorganisms that are widespread in nature and are able to occupy diverse habitats such as soils (Li et al. 2019), plants tissues (Chankhamhaengdecha et al. 2013), lichens (Yamamura et al. 2011) and extreme aquatic and terrestrial ecosystems (Dhakal et al. 2017; Goodfellow et al. 2018; Idris et al. 2017). Extremophilic actinobacteria can be divided into acidophilic (Golinska et al. 2016), alkaliphilic (Selyanin et al. 2005; Thumar et al. 2010), haloalkaliphilic (Gohel and Singh 2018), halophilic (Al-Tai and Ruan 1994; Chun et al. 2000; Hamedi et al. 2013; Meklat et al. 2011, 2013), psychrophilic (Männistö et al. 2000), thermophilic (Zhou et al. 2012) and xerophilic (Montero-Calasanz et al. 2012) groups.

Many alkaliphilic and alkalitolerant actinobacteria have long been known to live in various habitats including neutral as well as alkaline environments (Jiang and Xu 1993). These actinobacteria are capable of growing in alkaline lakes (Yang et al. 2008), alkaline soils (Li et al. 2006; Zhang et al. 2015); salt alkaline lakes (Li et al. 2005; Mwirichia et al. 2010; Zhang et al. 2013b) and soda lakes (Duckworth et al. 1998; Grant and Sorokin 2011; Groth et al. 1997; Jones et al. 1998; Sorokin et al. 2009). This group of actinobacteria can be divided into three major categories, namely alkaliphilic, moderately alkaliphilic and alkalitolerant actinobacteria that grow optimally at pH 10–11 and pH 7–10, but show poor growth at pH 7.0; and pH 6-11, respectively (Jiang and Xu 1993).

Several novel genera and species of alkaliphilic and alkalitoleratnt actinobacteria have been described such as *Bogoriella caseilytica* (Groth et al. 1997), *Kocuria aegyptia* (Li et al. 2006), *Nesterenkonia alba* (Luo et al. 2009), *Nesterenkonia alkaliphila* (Zhang et al. 2015), *Nitriliruptor alkaliphilus* (Sorokin et al. 2009), *Nocardiopsis algeriensis* (Bouras et al. 2015), *Nocardiopsis alkaliphila* (Hozzein et al. 2004), *Nocardiopsis metallicus* (Schippers et al. 2002), *Nocardiopsis valliformis* (Yang et al. 2008), *Streptomyces aburaviensis* (Thumar et al. 2010), *Streptomyces fukangensis* (Zhang et al. 2013b) and *Streptomyces sodiiphilus* (Li et al. 2005).

All of these represent species that share 16S rRNA gene sequence similarities with their current closest phylogenetic neighbors below the 97%, 98.2% and 98.65 thresholds recommended by Wayne et al. (1987), Meier-Kolthoff et al. (2013) and Kim et al. (2014), respectively supporting thresholds used to detect prospective new species.

Alkaliphiles and alkalitolerant actinobacteria are an interesting group that produce valuable natural compounds, such as antibiotics (Ali et al. 2009; Dieter et al. 2003; Ding et al. 2012;

CONTACT Noureddine Bouras noureddine bouras@yahoo.fr Laboratoire de Biologie des Systèmes Microbiens (LBSM), Ecole Normale Supérieure de Kouba, Alger, Algeria.

Helaly et al. 2013; Nair et al. 1989; Sanghvi et al. 2014; Solanki et al. 2008; Thumar et al. 2010; Wang et al. 2013), anticancer, antitumor and immunosuppressive agents (Dieter et al. 2003; Li et al. 2007), antioxidants (Quadri and Agsar 2012), antiparasitics (Dieter et al. 2003), cytotoxic compounds (Tsujibo et al. 1990), enzymes (Ara et al. 2012; Jani et al. 2012; Mehta et al. 2006; Ningthoujam et al. 2009; Sorokin et al. 2009; Thumar and Singh 2009), herbicides (Souagui et al. 2015), insecticides (Nair et al. 1989) and nanoparticles (Ahmad et al. 2003).

A wide range of bioactive secondary metabolites are biosynthesized by the non-ribosomal peptide synthetase (NRPS) and polyketide synthase (PKS) pathways. Polyketides are structurally diverse secondary metabolites that have demonstrated efficacy in a wide range of therapeutic applications (Ayuso-Sacido and Genilloud 2005; Fischbach and Walsh 2006; Gomes et al. 2013; Metsä-Ketelä et al. 1999). In turn, NRPS gene clusters have been found to synthesize a diverse selection of biologically active non-ribosomal peptides, in particular antibiotics (Cane et al. 1998; Grünewald and Marahiel 2013). The presence of NRPS and PKS genes has been documented in many studies. From a population of 52 halophilic desert actinobacteria, half of the strains were bioactive and harbored genes encoding for NRPS and PKS compounds (Meklat et al. 2011). In another study, the PKS and NRPS genes detected in 53 actinobacteria isolated from Qinghai-Tibet Plateau indicated diversified potential bioactive products (Ding et al. 2013). In contrast, little is known about the products of NPRS or PKS genes in alkaliphilic or alkalitolerant actinobacteria; thus, the screening for these genes would allow for the generation of new drugs.

One of the main strategies in the search for new sources of bioactive compounds is the isolation of non-streptomycete actinobacteria; also known as rare actinobacteria which are often difficult to isolate and cultivate; from underexplored and uncommon habitats (Baltz 2017; Bundale et al. 2019; Goodfellow et al. 2018). In this context, the Sahara is a good example of the extreme habitats that is unfavorable for life, apart from microbial life that has become adapted to the harsh conditions. It is clear from studies on Saharan soils that they are a rich source of members of rare genera that have been shown to produce many antibiotics (Bouras et al. 2008; Meklat et al. 2011, 2012; Sabaou et al. 1998; Zitouni et al. 2005).

Nocardiopsis species are widespread in extreme habitats such as the Atacama (Goodfellow et al. 2018; Idris et al. 2017), the Qinghai-Tibet (Ding et al. 2013) and Sahara (Bouras et al. 2015; Hozzein et al. 2004; Li et al. 2006; Meklat et al. 2011; Montero-Calasanz et al. 2012; Zitouni et al. 2005) deserts. They are known to produce a variety of bioactive compounds such as tumor inducers, anticancer substances and immunomodulators and novel extracellular enzymes such as amylases, inulinases, chitinases, proteases, xylanases, glucanases and cellulases (Bennur et al. 2014).

The aim of the present study is to investigate the biodiversity of alkalitolerant actinobacteria isolated from Algerian Saharan soils, to determine the relationship of these alkalitolerant actinobacteria by using a polyphasic approach and to evaluate their potential to produce bioactive substances.

Materials and methods

Soil samples

Seventeen non-rhizospheric soil samples were collected from various Saharan regions in the South of Algeria. They were taken from depths of up to 20 cm, after removing approximately 3 cm of the soil surface. The samples were placed in sterile polyethylene bags that were closed tightly and stored at 4 °C until analysis. The geographic locations and the physicochemical properties of the soils are given in Table 1. Soil pH was determined by using a Jenway pH meter (soil: distilled water = 1: 2.5). For determination of soil organic carbon (in %), the Walkley and Black wet oxidation method (1934) was used. Soil total nitrogen to organic carbon (in %) was determined by the Kjeldahl method (Bremner 1996). Total $CaCO_3$ content was determined using a weak acid dissolution followed by measurement of the pH of the extractant

Table 1. Geographical locations of sampling sites and physicochemical properties of soils.

				Soil characteristics					
Region	Latitude	Longitude	Sample	pH	Carbon (%)	Nitrogen (%)	$CaCO_3$ (%)	EC[a]	Texture[b]
El Oued	33°19′N	6°52′E	EO1	8.1	0.62	0.04	16.8	0.3	S
Ghardaïa	32°19′N	3°40′E	Gh1	8.3	0.45	0.04	12.8	4.9	SL
			Gh2	8.0	0.66	0.08	10.0	1.9	SL
			Gh6	8.2	0.19	0.01	3.6	0.2	S
Adrar	27°51′N	0°19′W	Ad3	8.6	0.80	0.04	4.0	6.5	SL
			Ad4	8.5	0.15	0.06	3.2	0.4	SL
			Ad5	8.8	0.81	0.05	6.0	0.2	SL
Ouargla	32°0′N	5°16′E	Ou3	8.8	0.80	0.04	6.0	33.9	SL
			Ou4	8.7	0.15	0.06	3.2	3.9	S
Béchar	31°34′N	2°16′W	Bc1	9.3	0.15	0.06	5.6	20.4	LS
			Bc2	8.9	0.45	0.04	6.4	0.2	S
			Bc3	8.5	0.15	0.07	10.0	0.4	S
			Bc4	8.4	0.40	0.02	6.4	10.2	LS
			Bc5	8.2	0.15	0.07	12.0	3.0	SL
			Bc6	8.9	0.32	0.07	8.4	13.3	SiL
Laghouat	33°49′N	2°55′E	La1	7.0	3.38	0.18	8.8	1.4	SiL
Djelfa	34°43′N	3°13′E	Dj1	8.9	0.88	0.04	7.2	8.4	L

[a]EC: electrical conductivity (1/5 at 25 °C) in ms/cm.
[b]Texture according to United States Department of Agriculture (USDA): L: loam; LS: loamy sand; S: sandy; SiL: silt loam; SL: sandy loam.

(Loeppert et al. 1984). The measurements of the electrical conductivity of saturated soil extracts were made using a Junway conductivity meter and soil texture was determined by the hydrometer method (Day 1982).

Isolation and distribution of alkalitolerant actinobacteria

In total, 29 actinobacterial strains were selectively isolated by the dilution plate method on Complex Medium agar (CM: 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 10) (Chun et al. 2000) supplemented with 50mg/l of cycloheximide to reduce fungal contamination. The pH was adjusted to 10.0 by using the buffer 0.1 M $NaHCO_3$/0.1 M Na_2CO_3.

After incubation at 30 °C for 1–2 weeks, the suspected colonies were picked up and purified on CM agar (pH 10.0) and incubated at 30 °C. The purified strains were stored on the same medium at 4 °C and also at −20 °C as 20% (v/v) glycerol suspensions.

Morphological characteristics

Twenty nine actinobacterial strains were grown on two International *Sptreptomyces* Project (ISP) culture media (Shirling and Gottlieb 1966), namely yeast extract-malt extract-dextrose agar (ISP2) and inorganic salts starch agar (ISP4), as well as CM agar. The cultures were grown for 3 weeks at 30 °C and pH 10.0.

All strains were observed by light microscopy (Motic; B1 Series). A 15 days culture of strain B33 that was grown on ISP2 medium was fixed in glutaraldehyde (2.5%) for 1 h and then post-fixed in osmium tetroxide vapor (2%). After using ethanol and tert-butanol, the dehydrated sample was sputter-coated with gold–palladium and then observed under a scanning electron microscope (model S-450; Hitachi).

Physiological characteristics

All actinobacterial strains were characterized by using sixty three physiological tests. *Nocardiopsis algeriensis* (Bouras et al. 2015) was examined under the same conditions for the same characters. All tests were made at pH 7.5 (except those of pH resistance). Utilization of twenty two carbohydrates and decarboxylation of nine organic acids were determined according to the methods of Gordon et al. (1974). Degradation of adenine, gelatin, guanine, hypoxanthine, milk casein, starch, testosterone, Tween 80, L-tyrosine and xanthine were studied as described by Goodfellow (1971) and Marchal et al. (1987). Lysozyme sensitivity was evaluated by the method of Gordon and Barnett (1977). Production of melanoid pigments was tested on peptone yeast extract iron agar (ISP6) and tyrosine agar (ISP7) (Shirling and Gottlieb 1966). Growth at two different temperatures (30 and 45 °C) and three NaCl concentrations (0, 7 and 10% w/v), and in the presence of chloramphenicol (25 µg/mL), erythromycin (10 µg/mL), kanamycin (5 µg/mL), penicillin (25 µg/mL) and streptomycin (10 µg/mL) were determined on nutrient agar. The following buffers were used to test the pH range of growth on nutrient broth: 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.

Table 2. PCR primers used to amplify 16S rRNA, PKSs and NRPS genes.

Primer name	Sequence (5′ 3′)	Target gene
FC27	5′ AGAGTTTGATCCTGGCTCAG 3′	
RC1492	5′ GGTTACCTTGTTACGACTT 3′	16S rRNA
K1F	5′ TSAAGTCSAACATCGGBCA 3′	
M6R	5′ CGCAGGTTSCSGTACCAGTA 3′	PKS I
KSα	5′ TSGCSTGCTTGGAYGCSATC 3′	
KSβ	5′ TGG AANCCG CCGAABCCTCT 3′	PKS II
EdyA	5′ CCGCVCACATCACSGSCCTCGCSGTGAACATGCT 3′	
EdyE	5′ GCAGGCKCCGTCSACSGTGTABCCGCCGCC 3′	PKSE
A3F	5′ GCSTACSYSATSTACACSTCSGG 3′	
A7R	5′ SASGTCVCCSGTSCGGTAS 3′	NRPS

Numerical taxonomy

The results of the physiological study were analyzed by numerical taxonomy. The data were coded in a binary system (1/0) and examined with the SPSS package (v.16.0.1), using the simple matching (S_{SM}) coefficient (Sokal and Michener 1958), which includes both positive and negative similarities. Clustering was achieved using the unweighted pair group method with arithmetic averages (UPGMA) algorithm (Sneath and Sokal 1973).

Chemical analysis of cell constituents

For the chemical analysis, the biomass of each strain was harvested by centrifugation at 3.500 rpm of cultures growing on CM broth (pH 7.5) at 30 °C for 6 days on a 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 using the procedure of Minnikin et al. (1977).

Phylogenetic studies

Genomic DNA of 19 selected strains, belonging to different clusters established by numerical taxonomy, was extracted for 16S rRNA gene analysis according to the method of Liu et al. (2000). 16S rRNA genes were PCR-amplified in 50 µL of reaction mixture, using 25–50 ng of genomic DNA, 0.5 µM of the forward FC27 and reverse RC1492 primers (Table 2), 1X PCR buffer, 10 µM deoxynucleoside triphosphate mixture and 0.4 U *Taq* DNA polymerase. The amplification was performed according to the following conditions: initial denaturation of template DNA at 98 °C for 4 min followed by 30 cycles of denaturation at 94 °C for 1 min, primer annealing at 52 °C for 1 min and extension at 72 °C for 2 min. At the end of cycling, the reaction mixture was held at 72 °C for 10min to achieve the final elongation and then

cooled to 4 °C. The PCR products were analyzed by agarose gel electrophoresis, and then submitted to the Beckman Coulter Genomics Company (United Kingdom) for purification and sequencing.

The sequences obtained were compared with sequences present in the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; Kim et al. 2012). Phylogenetic analysis was conducted using MEGA version 5 (Tamura et al. 2011). The 16S rRNA gene sequences of the 19 strains were aligned against neighboring nucleotide sequences using the CLUSTAL W (with default parameters) (Thompson et al. 1994). A phylogenetic tree was constructed by using the neighbor-joining method (Saitou and Nei 1987) with the Jukes and Cantor (1969) model. Bootstrap analysis (Felsenstein 1985) was performed to evaluate the reliability of the tree topology.

Antimicrobial assay on solid medium

The 29 strains of actinobacteria were tested against four bacteria (*Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* CIP 7625, *Klebsiella pneumoniae* CIP 8291 and *Micrococcus luteus* ATCC 9314), four fungi (*Umbelopsis ramanniana* NRRL 1829, *Penicillium expansum* B831P, *P. glabrum* and *Aspergillus carbonarius* M333) and one yeast (*Saccharomyces cerevisiae* ATCC 4226) to evaluate their antimicrobial activities after cultivation on ISP 2 agar.

The experiment was done firstly by streaking a straight line of the actinobacterium inoculum across the surface of the medium in 90 mm diameter plates and incubating at 30 °C for 10 days. After the growth of the strains, target microorganisms were seeded in streaks crossing the actinobacterium culture. The antimicrobial activity was recorded by measuring the length of inhibition between target microorganisms and the actinobacterial colony margins.

Detection of PKS-I, PKS-II, enediyne PKS and NRPS sequences

The series of primers listed in Table 2 were used to detect the genes encoding polyketide synthases I and II (PKS-I and PKS-II), enediyne polyketide synthase (PKSE) and non-ribosomal peptide synthetase (NRPS) genes for all the isolated strains. The 50-µL PCR reaction mixture contained 20–40 ng of DNA template, 0.4 µM of deoxynucleoside triphosphate mixture, 2 µM of each primer, 10X reaction buffer containing $MgCl_2$, 2.5 U of *Taq* DNA polymerase and 5% dimethyl sulfoxide (DMSO). The PCR thermal cycle program included an initial denaturation at 98 °C for 4 min, followed by 30 cycles, with a denaturation step at 94 °C for 1 min, an annealing step of 1 min, at 57.5 °C with K1F/M6R (Ayuso-Sacido and Genilloud 2005), at 58 °C with KSα/KSβ (Metsä-Ketelä et al. 1999), at 62 °C with EdyA/EdyE (Liu et al. 2003) and at 57 °C with A3F/A7R (Ayuso-Sacido and Genilloud 2005), followed by an extension step during 1 min at 72 °C. Final extension was performed at 72 °C for 10 min.

All of the amplification products were examined by agarose gel electrophoresis (0.8%), and bands of 1200–1400 pb, 600 pb, 1400 pb and 700–800 pb were classified as products of PKS-I, PKS-II, PKSE and NRPS genes, respectively.

Results

Isolation of actinobacterial strains and distribution in soils

On the basis of characteristic colonial morphology, mainly the ability to form aerial and substrate mycelia, microorganisms putatively identified as actinobacteria were selected.

Except for the neutral pH of the soil sample La1 of Laghouat, all of the samples had a basic pH ranging from 8.0 to 9.3. In addition, very low amounts of carbon and nitrogen were observed in the analyzed soil samples, and some of them such as Gh1 (Ghardaïa), Ad3 (Adrar) Ou3 (Ouargla) Bc1, Bc4 and Bc6 (Béchar) and DJ1 (Djelfa) were saline soils (Table 1).

In the adopted cultural conditions, moderate levels of cultivable actinobacteria were observed ranging from 1.0 to 63.0 10^2 CFU per g (dry weight) of soil. Actinobacteria were found in all the soil samples. In total, 29 actinobacteria were isolated from 17 analyzed soil samples (Table 3). There was no correlation between the distribution of actinobacteria and pH as the highest number (63.0 10^2) was found at neutral pH (the soil sample La1 of Laghouat).

Morphological and chemotaxonomic studies

The preliminary investigation based on morphological and chemical analysis (diaminopimelic acid isomers, sugars and phospholipids) of the 29 actinobacteria allowed these strains to be tentatively classified at the genus level. All strains formed a branched substrate mycelium that fragmented into coccoid and rod-shaped elements. Aerial hyphae were zigzag shaped at the beginning of sporulation and fragmented at maturity into branched and straight to flexuous spore chains with rod-shaped spores (Figure 1, Supplementary Figure S1). These strains contained *meso*-diaminopimelic acid in the cell wall, phosphatidylcholine as the diagnostic

Table 3. Distribution of alkalitolerant actinobacterial strains in Saharan soils.

Soil sample	Number of actinobacterial colonies (10^2 CFU/g of soil dry weight)	Number of strains isolated (isolate code)
EO1	1.0	1 (B33)
Gh1	8.3	2 (B12, B14)
Gh2	47.0	1 (B30)
Gh6	1.0	1 (B20)
Ad3	2.0	1 (B5)
Ad4	2.0	1 (B6)
Ad5	4.5	3 (B9, B10, B11)
Ou3	13.0	1 (B28)
Ou4	4.0	4 (B15, B16, B17, B18)
Bc1	1.0	1 (B25)
Bc2	1.0	1 (B4)
Bc3	3.0	3 (B1, B2, B3)
Bc4	2.7	3 (B22, B23, B24)
Bc5	1.0	1 (B29)
Bc6	54.2	2 (B26, B27)
La1	63.0	2 (B7, B8)
Dj1	1.0	1 (B19)

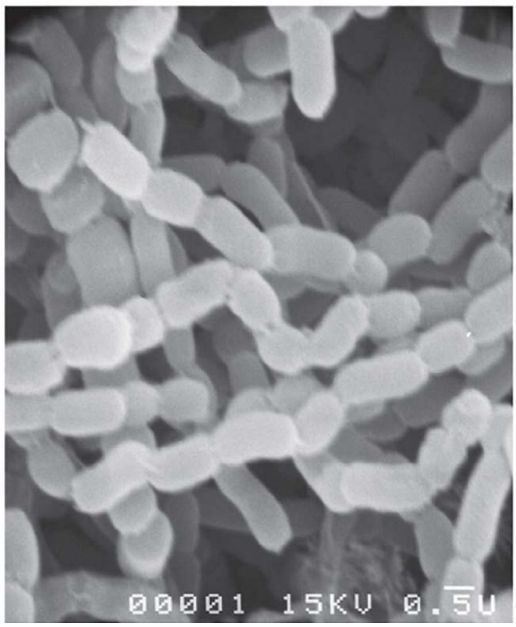


Figure 1. Scanning electron micrograph of strain B33^T grown on ISP 2 medium for 15 days at 30 °C. *Bar* 5 µm.

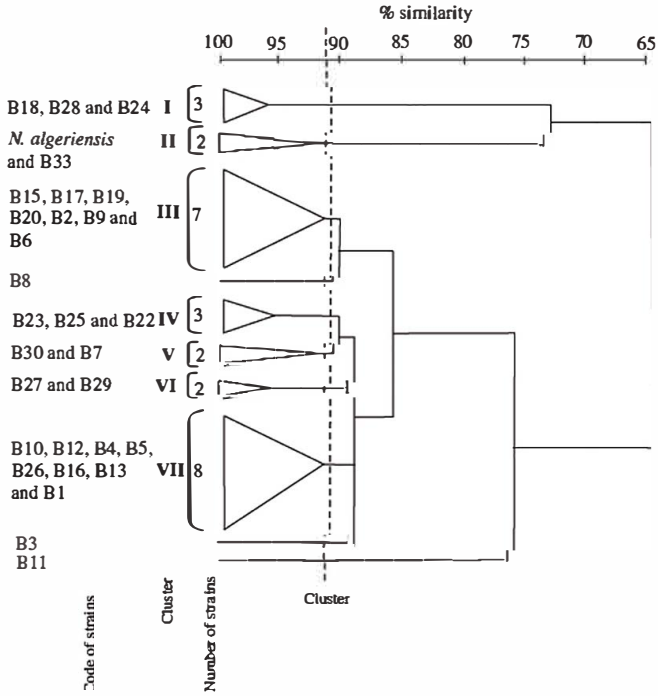


Figure 2. Dendrogram derived from UPGMA cluster analysis of 63 phenotypic characters showing the relationship between 29 strains of alkalitolerant actinobacteria isolated from Saharan soils.

phospholipid in the cell membrane, and ribose and glucose (no diagnostic sugars) in the whole-cells. All of these properties are consistent with the isolates being assigned to the genus *Nocardiopsis* (Meyer, 1989).

Physiological and numerical study

The physiological classification based on the S_{SM} coefficient and UPGMA clustering method was examined in detail as it gave the most compact aggregate groups. The 30 strains (29 isolated strains and *Nocardiopsis algeriensis* DSM 45462^T) were assigned to 7 cluster-groups, designated I to VII, and three single strains at the 91% similarity (S) level (Figure 2). This dendrogram showed that strain B33 was grouped in cluster II with *N. algeriensis* DSM 45462^T.

All the isolated strains shared the following characteristics: they grew within the pH range of 7–11, and in the presence of 0–7% of NaCl. Furthermore, most strains (except B1, B3, B13, B24, B25 and B26) were able to grow at pH 12. However, only strains B3, B9, B13, B15, B20 and B28 were able to grow at pH 6. All of the strains were resistant to penicillin (25 µg/mL), degraded adenine, casein, gelatin, glucose, tyrosine and Tween 80, were sensitive to kanamycin (5 µg/mL) but did not use adonitol, benzoate, butyrate, erythritol, inositol, melezitose, testosterone and starch as a sole carbon sources.

Molecular taxonomy

From a total of 29 actinobacteria, 19 strains belonging to different physiological groups (clusters) were subjected to molecular analysis. The phylogenetic tree (Figure 3) based on the 16S rRNA gene sequences displayed high consistency regarding relationships between the actinobacteria included. Most nodes leading to the isolates were supported by high bootstrap values. A comparison of the nearly complete 16S rRNA gene sequences of the selected strains for phylogenetic study against sequences in the GenBank database showed that the phylogeny of these strains corresponded well with the chemotaxonomic and phenotypic data. All strains were assigned to the *Nocardiopsis* genus. The 16S rRNA gene sequence similarities ranged from 98.4% and 99.9%. Strains B18 and B24 from cluster I were related to *N. listeri* showing a similarity of 99.9% with the later. Strain B33, which grouped with *N. algeriensis* DSM 45462^T in the cluster II (numerical taxonomy), shared a relatively low 16S rRNA similarity with this species and with *N. lucentensis* (98.4% of similarity). The representative strains of cluster III (B2, B9, B19 and B20), cluster IV (B22 and B23), cluster VI (B27 and B29), cluster VII (B1, B5 and B12) and strains B3 and B11 (which composed two single isolates) were related to *N. dassonvillei* subsp. *dassonvillei*; they shared similarities within the range 99.1 to 99.9%. Strain B15, which belonged to cluster III, was closely related to *N. dassonvillei* subsp. *albirubida* (the 16S rRNA gene sequence similarity was 99.1%). Strain B7 (representative of cluster V) was assigned to *N. umidischolae* with 99.7% similarity. The single isolate B8 was closely related to *N. ganjiahuensis* (99.7% of similarity).

Antimicrobial activity and detection of biosynthetic gene sequences

Antimicrobial activity of all stains was evaluated on CM agar (at pH 7.5) using the streak method against various microorganisms (Table 4). The results showed that 96.5% of the strains were active against at least one of the tested target microorganisms. The most frequent activity was

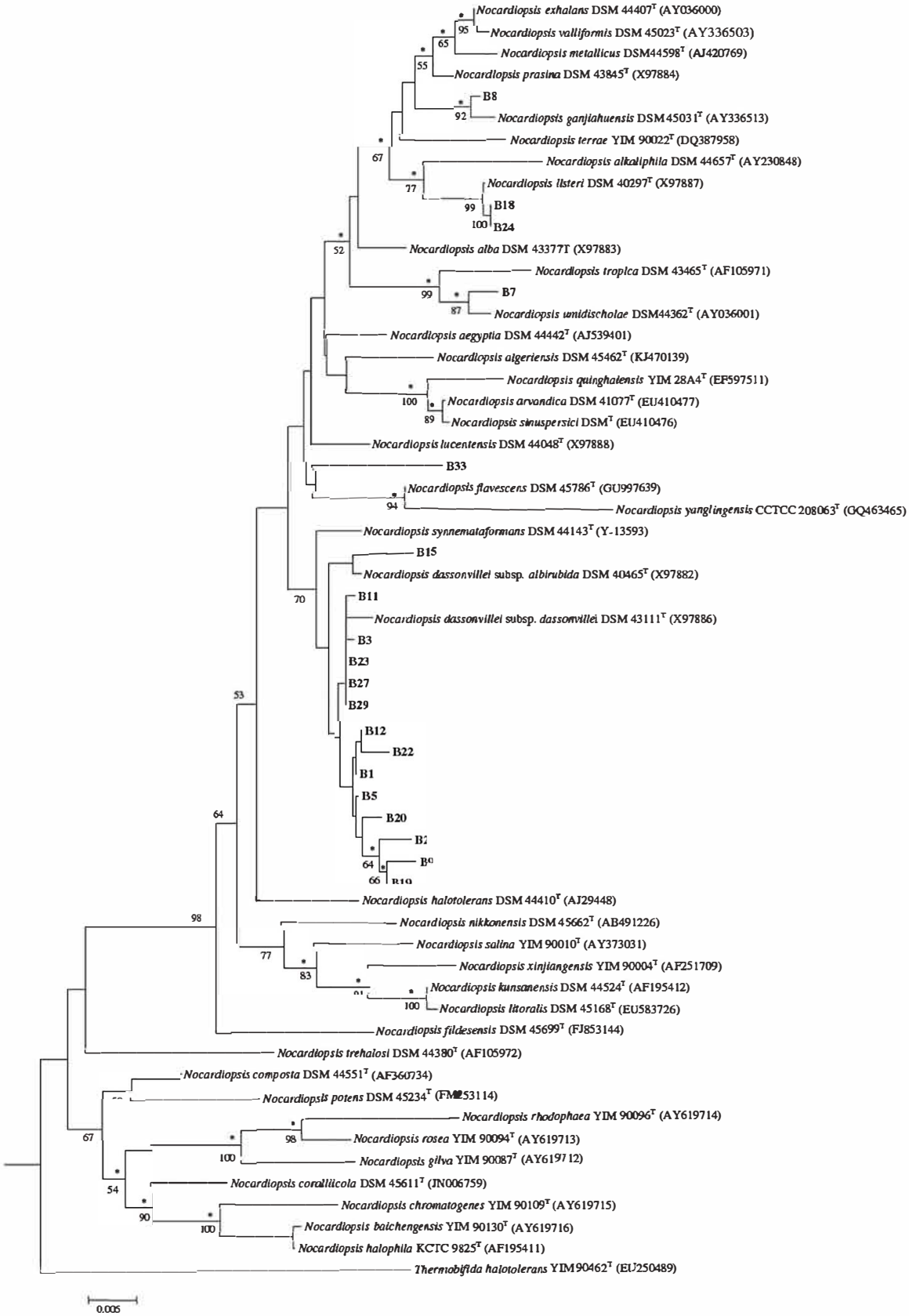


Figure 3. Phylogenetic tree derived from nearly complete 16S rRNA gene sequences showing relationships between the isolates of alkaliphilic actinobacteria and their phylogenetic neighbors. The tree was constructed using the neighbor joining method (Saitou and Nei 1987). Asterisks indicate branches of the tree that were also found using maximum parsimony (Fitch, 1977) tree making algorithm. Bootstrap values (≥ 50 %) based on 1000 resamplings are shown at branch nodes. *Thermobifida halotolerans* YIM 90462^T was used as an outgroup. Bar, 0.005 nt substitutions per site.

Table 4. Antimicrobia activities and PKSs/NRPS genes of a ka ito erant actinobacteria iso ated from Saharan soi s.

	Activity against[a]								Presence of gene[b]			
Strains	*Umbelopsis ramanniana*	*Aspergillus carbonarius*	*Penicillium expansum*	*Penicillium glabrum*	*Saccharomyces cerevisiae*	*Bacillus subtilis*	*Micrococcus luteus*	*Staphylococcus aureus*	PKS-	PKS-	PKSE	NRPS
B1	5	18	–	–	4	7	6	–	–	–	–	+
B2	20	20	22	21	10	–	–	–	–	–	+	–
B3	–	–	–	–	–	15	21	15	–	+	–	+
B4	21	–	–	–	10	5	7	–	–	–	–	–
B5	20	–	–	–	3	3	–	–	–	–	–	–
B6	10	–	3	2	–	2	–	–	–	–	–	–
B7	21	–	16	7	–	–	–	–	–	+	–	+
B8	–	11	–	–	–	–	–	–	–	–	–	+
B9	17	15	20	–	15	34	7	28	–	+	–	+
B10	–	–	–	4	–	3	17	–	–	–	–	–
B11	22	–	–	–	–	–	–	–	–	–	–	–
B12	18	–	9	2	16	–	–	–	–	–	–	–
B13	–	–	–	–	–	–	–	–	–	+	–	–
B15	–	–	3	–	–	–	–	–	–	–	–	+
B16	–	–	–	–	–	–	22	–	–	–	–	–
B17	8	–	–	–	–	–	–	–	–	+	–	–
B18	19	–	–	–	8	3	–	–	–	–	–	–
B19	16	16	–	–	10	19	18	14	–	–	–	+
B20	29	16	21	16	15	13	–	–	–	+	+	+
B22	17	–	–	–	–	8	23	–	–	–	–	–
B23	9	–	–	–	–	–	14	–	–	–	–	–
B24	4	–	–	–	–	–	16	–	+	+	–	–
B25	7	–	–	–	6	6	–	–	–	+	–	–
B26	7	–	8	10	6	6	2	–	–	+	–	–
B27	29	–	–	–	–	–	–	–	–	–	–	–
B28	4	–	3	–	–	–	14	–	+	+	–	+
B29	–	–	–	–	–	–	13	–	–	–	–	–
B30	–	–	–		15	4	30	–	–	–	–	–
B33	–	–	6	–	–	–	–	–	–	–	–	–

[a]Estimated by measuring the c ear zone of growth inhibition, –: no activity.
[b]+: present; –: absent.

observed against *Umbelopsis ramanniana* (69%), and the Gram-positive bacteria *Bacillus subtilis* and *Micrococcus luteus* (51.7 and 48.3%, respectively). Three strains designated B2, B9 and B20 showed relatively good activity with respect to intensity and breadth. However none of the alkalitolerant actinobacteria showed antibacterial activity against *Klebsiella pneumoniae*.

Many antibiotics produced by actinobacteria have been synthesized through a pathway involving NRPS and/or PKS, hence the 29 strains were screened by PCR for the presence of PKS-I, PKS-II, PKSE and NRPS sequences (Table 4). In total, 15 strains (51.7%) yielded at least one type of these biosynthetic sequences. NRPS and PKS-II were the most frequent genes; they were detected in 10 and 9 strains, respectively (34.5 and 31%). However, PKSE and PKS-I genes were detected only in 2 strains (6.9%).

Discussion

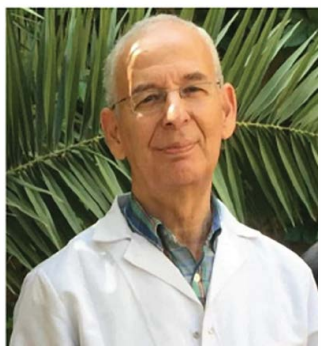
A small number of alkalitolerant actinobacteria were isolated from 17 different Saharan soils; similar results were obtained by Meklat et al. (2011) for Saharan halophilic actinobacteria. Nevertheless, previous studies have reported the abundance of non-extremophilic actinobacteria (10^6 to 10^7 CFU per g of soil) in Saharan soils (Sabaou et al. 1992, 1998).

The assignment of the isolates to genera was based on morphological and chemical characteristics while the numerical taxonomic data permitted their separation but not their identification. Nineteen isolates representing the numerically defined clusters were the subject of 16S rRNA gene sequencing studies. Only one strain (B33) was distinguished from the type strain of its nearest validly named species (Kim et al. 2014) whereas the remaining 18 strains showed similarities above the 98.65% hence these isolates need to be the subject of more detailed molecular systematic studies based on DNA-DNA reassociation or whole-genome sequencing to determine their taxonomic status.

The phylogenetic study revealed that strain B33 was distinct from other strains and the validly published *Nocardiopsis* species. This strain exhibited a similarity value of 98.4% with *N. algeriensis* DSM 45462^T and *N. lucentensis*, a value below the 98.65% threshold (Kim et al. 2014) hence this isolate can be considered to be a presumptive new *Nocaridiopsis* species. The results of 16S rRNA gene sequence similarity of the remaining 18 sequenced strains ranged from 99.1% to 99.9% (supplementary Table S1) and hence are below or approximately the same as the similarities between the closely related species of validly published *Nocardiopsis* species and are related to known species as in the case of isolates B2, B9, B15 and B22. However in these cases, additional studies are needed to confirm these deductions. In this context it is interesting to note that very high 16S rRNA gene sequence similarities have been found between the type strains of validly published *Nocardiopsis* species, as exemplified by *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%) (Chen et al. 2009), *N. metallicus* and *N. exhalans* (99.4%) (Schippers et al. 2002), *N. metallicus* and *N. prasina* (99.3%) (Schippers et al. 2002) and *N. synnemataformans* and *N. dassonvillei* (99.3%) (Yassin et al. 1997).

Most of the tested isolates showed antibacterial and antifungal activities which are in line with earlier findings (Bennur et al. 2016; Ibrahim et al. 2018; Zitouni et al. 2005). Strains B2, B9 and B20 showed relatively strong inhibition against the test microorganisms and may well be the source of novel antibiotics. To date, *Nocardiopsis* strains have been shown to be the source of new secondary metabolites, as shown by the production of 3-trehalosamine (Evtushenko et al. 2000), griseusin D (Li et al. 2007), macrolide WA52-A (Ali et al. 2009), thiopeptide (Engelhardt et al. 2010), nocapyrone derivatives (Schneemann et al. 2010), diketopiperazine derivatives (Zhang et al. 2013a), 4-oxo-1,4-dihydroquinoline-3-carboxamide and N-acetyl-anthranilic acid (Tian et al. 2014), angucyclinones (Hadj Rabia-Boukhalfa et al. 2017) and others (Ibrahim et al. 2018). The screening of NRPS, PKS-I, PKS-II and PKSE genes revealed a low presence in *Nocardiopsis* strains. NRPS sequences are extensively distributed among actinobacterial taxa, whereas PKS-I genes are concentrated in fewer genera (Ayuso-Sacido and Genilloud 2005; Meklat et al. 2011; Metsä-Ketelä et al. 1999).

Dedication



This paper is dedicated to the late Dr. Nasserdine Sabaou (1956-2019) for his genuine love, dedication, and service to science. He was a superb researcher and professor of Microbiology at the *École Normale Supérieure de Kouba, Alger (Algeria)* and the Ex-head of the *Laboratoire de Biologie des Systèmes Microbiens (LBSM)*. He published many papers on Saharan actinobacteria and their metabolites.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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