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Isolation, Identification and Screening of Saharan Actinomycete Strain *Streptomyces fimbriatus* AC31 Endowed with Antimicrobial Activity

Omar Khirennas[1,2*], Slimane Mokrani[3,4], Belkacem Behira[1,2], Noureddine Bouras[5,6], El Hadj Driche [5,7], Ouahiba Moumen[2]

1. Laboratory of Geo-Environment and Spatial Development (LGEDE), Faculty of Nature and Life Sciences, University Mustapha Stambouli of Mascara, Mascara, Algeria.
2. Laboratory of Geomatics, Ecology and Environment (LGO2E), Faculty of Nature and Life Sciences, University Mustapha Stambouli of Mascara, Mascara, Algeria.
3. Laboratory of Research on Biological Systems and Geomatics, Faculty of Nature and Life Sciences, University Mustapha Stambouli of Mascara, Mascara, Algeria.
4. Laboratory of Renewable Energies Management, Faculty of Natural and Life Sciences, University of Bejaia, Algeria.
5. Laboratoire de Biologie des Systèmes Microbiens (LBSM), Ecole Normale Supérieure de Kouba, Alger, Algeria.
6. Laboratoire de Valorisation et Conservation des Ecosystèmes Arides (LVCEA), Université de Ghardaia, Ghardaïa, Algeria.
7. Laboratoire de Biologie Moléculaire, Génomique et Bio-Informatique (LBMGB), Faculté des Sciences de la Nature et de la Vie (SNV), Université Hassiba Benbouali de Chlef, Hay Salem, 02000 Chlef, Algeria.

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ABSTRACT

The increasing global public health concern of antimicrobial resistance (AMR) necessitates exploration of natural antimicrobial agents as potential alternatives. This study aimed to investigate antimicrobial activities of Saharan actinomycetes, with specific focus on the strain *Streptomyces fimbriatus* AC31, that holds promising potential as an alternative to combat AMR. In this context, 32 actinomycetes were isolated from El Atteuf (Ghardaïa), Algeria. Isolates obtained were characterized morphologically and biochemically. Screened isolate was identified by 16S rRNA gene sequencing. Classification of actinomycete isolates was carried out by UPGMA (Unweighted Pair Group Method with Arithmetic Mean). Then, they were screened for their antimicrobial activity by cross-streak method. Identification of 32 isolates revealed 5 genera: *Streptomyces* (65.63%), *Nocardia* (9.38%), *Streptosporangium* (9.38%), *Nocardiopsis* (9.38%) and *Actinomadura* (6.25%). According to the biochemical and physiological characteristics, UPGMA classified the isolates in 4 phenons. A number of 24 (75.00%) isolates were active against Gram-positive bacteria, 21 (65.63%) isolates were effective against Gram-negative bacteria, and 25 (78.13%) isolates inhibited *Candida albicans*. Screened strain *Streptomyces fimbriatus* AC31 showed highest antagonistic activity and revealed an inhibition zones of 41, 38, 41, 42, and 44 mm, against *B. subtilis* (ATCC 6633), *E. coli* (ATCC 8739), *S. typhimurium* (ATCC 13331), *S. aureus* (ATCC 6538) and *C. albicans* (ATCC 10231), respectively. Phylogenetic identification of the AC 31 isolate using 16S rRNA gene sequence showed similarity of 100% with *Streptomyces fimbriatus* NBRC 15411^T. Actinomycete isolates characterized in this study were endowed with antimicrobial activity against various pathogenic microorganisms that could be used efficiently in developing new antimicrobial substances.

Keywords: Actinomycetes, antimicrobial activity, Saharan soil, screening

*Corresponding Omar Khirennas
Address: Laboratory of Geo-Environment and Spatial Development (LGEDE), Faculty of Nature and Life Sciences, University Mustapha Stambouli of Mascara, Mascara, Algeria.
E-mail: o.khirennas@univ-mascara.dz

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Introduction

In the last two decades, multidrug-resistant (MRD) bacteria are proliferating throughout the world, posing a serious public health threat (1). According to the World Health Organization assessment, one of the biggest concerns to public health in the twenty-first century is antibiotic resistance (2). Currently, several studies reported that the increase of resistant bacteria, to the most commonly used antibiotics such as *Staphylococcus aureus* (3) and *Salmonella typhi* (4). It is a major challenge that needs development of innovative, safe, and effective antimicrobial medications (5-6). Consciously, antibiotics are still being developed by researchers around the world in an attempt to develop new and effective medications to combat these MRD bacteria (7). Natural products are major source of new therapeutic molecules coming from prokaryotic and eukaryotic microorganisms, plants, and other animals. Main discovered antimicrobial compounds were derived from microbial and plant sources (8).

Particularly, actinomycetes can make different secondary metabolites with a wide range of chemical structures, they are Gram-positive bacteria with high GC% and most of them produce mycelium (9). They can generate spores, arthrospores, conidia, and sporoangia, all of which are essential in the classification of this group of microorganisms (10). They produce commercially valuable compounds in the field of biotechnology, such as anti-parasitic agents, herbicides, immuno-suppressants, pigments, larvicides, phytohormones, surfactants, etc. Also, useful substances in the food industry, such as enzymes and vitamins, could be produced (11). Antibacterial and antifungal medicines are among their most well-known products (12). Actinomycetes provide the majority of natural antibiotics, and they are the most widely distributed in nature inhabiting mostly soils (13). Around two-thirds of all natural antibiotics are estimated to come from actinomycetes (12). They account for about 45% of the 22500 physiologically active substances obtained from microorganisms (8). Exploration of ecosystems under extreme environmental conditions is an interesting strategy to discover new bioactive substance producers (14). Especially, Algerian Saharan soil, was reported to be rich in antibiotic-producing actinomycetes (15). The aim of this work is to isolate, identify and screen actinomycetes from Algerian Saharan soil and evaluate their antimicrobial activities against various human pathogenic microorganisms.

Materials and methods

Study area El Atteuf is one of the municipalities of Ghardaïa department located at 600 Km from the coast and at an altitude of 450 m above sea level (Figure 1). The municipality of El Atteuf represents an area of 750 km^2. Ghardaïa is located in the south of Algeria characterized by an arid climate and low precipitation (48.9 mm per year) with very high temperatures in summer and low temperatures in winter. It is hot and dry in summer with temperature ranging between 29 °C to 53.2 °C. In winter, temperatures range from low value of 0 °C to high value of 16.9 C. Annually, average monthly temperature is 23°C (16).

Soil sampling and preparation

Four samples (S1, S2, S3 and S4) of Saharan soil were collected from El Atteuf; Ghardaïa (Table 1). The points of collection were chosen in a way to ensure range of properties like, moisture content, small particle size, and avoid contamination. They were taken at depth of 15 cm under the soil surface. Soil samples were collected in clean, dry, and sterile polypropylene pots and kept at 4 °C. Soil samples were dried for seven days to reduce bacterial and fungal populations. Then, to facilitate the isolation of actinomycetes, soil samples were exposed to other physical and chemical pretreatment methods (17). First pre-treatment consisted of heating 10 g of each sample at 50 °C for 60 min to reduce majority of undesirable Gram-negative bacteria (18). The second pre-treatment consisted of treating 1 g of each soil sample with 0.1 g of CaCO3 and the mixture formed was placed in sterile and humidity-saturated chamber at 30°C for 7 days constituting dry soil samples (19).

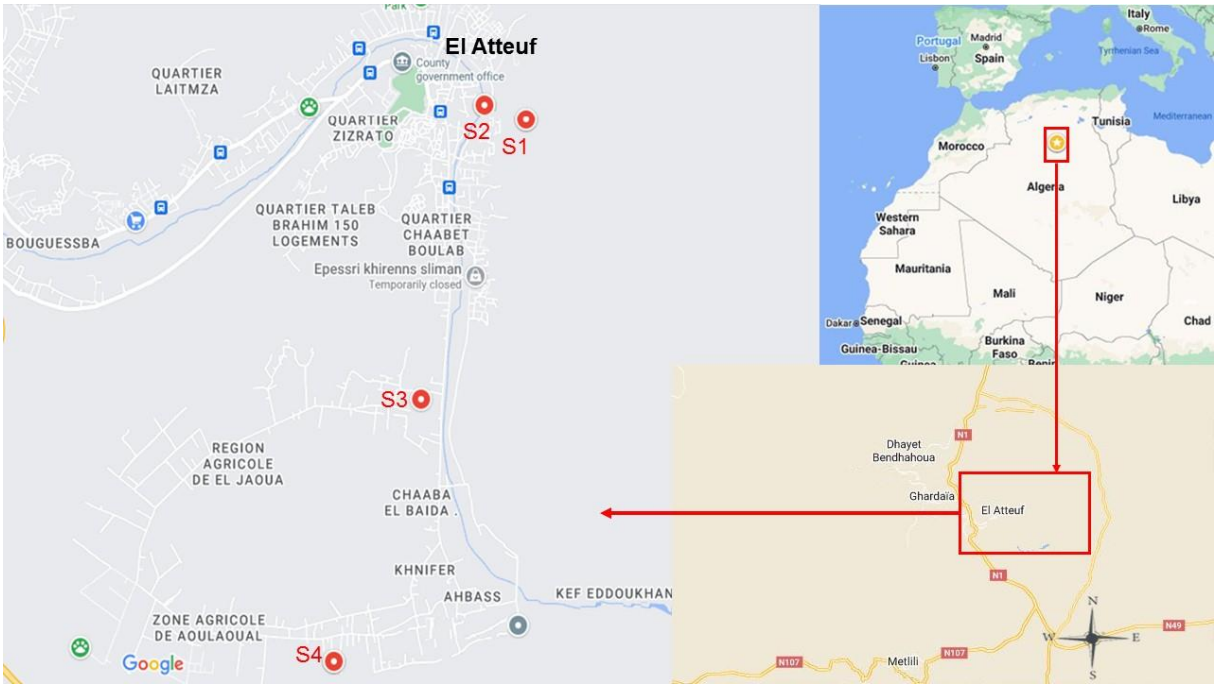

Fig.1. Map showing the sampling sites from El Atteuf (Ghardaïa, Algeria). S1: Tammou El Maleh, S2: Mzab Valley, S3: El Jaoua; S4: Aoulaoual (available at: https://www.google.dz/maps/ accessed on 10 December 2022).

Table 1. Distribution of total isolated actinomycetes from different localities of El Atteuf (Ghardaïa, Algeria).

S	Site of sampling	Geographic localization	Number and code of the isolated bacteria	Soil type
S1	Tammou El Maleh	32°28'34.9"N, 3°45'16.1"E	AC1 -AC3	Mountain
S2	Mzab Valley	32°28'38.6"N, 3°45'03.1"E	AC4 -AC9	Valley
S3	El Jaoua	32°27'25.9"N, 3°44'43.5"E	AC10 -AC17	Oasis
S4	Aoulaoual	32°26'21.2"N, 3°44'16.2"E	AC18 - AC32	Oasis

S: Sample

Isolation of actinomycetes

Dry soil samples were diluted in sterile physiological water (9 $g.L^{-1}$ NaCl). A volume of 0.2 mL aliquot of each dilution was spread on Petri plates containing chitin-vitamin agar medium (20). To inhibit growth of persistent fungi and bacteria, the medium was supplemented with cycloheximide (80 $mg.L^{-1}$), penicillin (25 $\mu g.mL^{-1}$) streptomycin (10 $\mu g.mL^{-1}$). Petri plates were incubated at 30 °C for 7-14 days and observed periodically for actinomycetes growth. Pure colonies were chosen by observing thin filaments surrounding actinomycetes under light microscopy. Pure colony cultures were kept on ISP2 (International *Streptomyces* Project) culture medium at 4 °C (21).

Identification of actinomycetes

Morphological characterization

Morphological characters of actinomycete isolates were studied by their inoculation in two selective culture media ISP (ISP2 and ISP7). The culture media were sterilized and poured into Petri dishes. After solidification, pure isolates were streaked aseptically and incubated at 30 °C for 14 days. Characterization of actinomycetes refers to study formation, shape, and structure of aerial and substrate mycelia. The colors (pigments production) of aerial and substrate mycelia were determined using ISCC-NBS (Inter-Society Color Council-National Bureau of Standards) color charts (22).

Microscopic observation

Type of spore chains

Actinomycete isolates belonging to genus *Streptomyces* were observed by light microscopy (Motic B1-253SP) after incubation at 30 °C for 14 days on ISP2 medium. Type of spore chains formed were determined, including: S (*Spira*), RF (*Rectus Flexibilis*), or RA (*Retinaculum Apertum*) according to Sabaou (23).

Physiological and biochemical analysis

The utilization of carbon sources was assessed by culturing isolates on basal carbon medium (ISP 9) supplemented with 1% of each carbon source. Cultures were incubated at 28 °C for 10 days. Tested carbon sources included D-glucose, D-fructose, D-arabinose, D-mannitol, mannose, maltose, lactose, D-galactose, sorbitol, and ribose (21). Growth evaluation of isolates in the presence of amino acids as sole nitrogen source was conducted using the following amino acids: L-alanine, asparagine, histidine, tryptophan, and tyrosine. These amino acids were added separately and aseptically at the concentration of 0.1% to ISP9 basal medium. Isolates were streaked and incubated at 30 °C for 7 and 14 days. Results were expressed by evaluating growth that should exceed negative controls without amino acids (24). Melanoid pigment production was tested on tyrosine agar medium (ISP 7) following the procedure described by Shirling and Gottlieb (21). Degradation of milk casein by actinomycete isolates was indicated by the appearance of clear halo around colonies, according to Goodfellow (24). Starch hydrolysis by actinomycete isolates was assessed by streaking them onto starch agar plates, followed by incubation at 30 °C for 7 days. After incubation, iodine solution was poured onto agar plates to check for starch hydrolysis that was indicated by formation of clear zone around growth. Aesculin degradation was characterized by the development of brown to black pigment around colonies, and catalase production according to Marchal's method (25). To assess lipolytic activity, Tween 80 assay was conducted, positive result was indicated by the presence of opaque halo surrounding colonies (26). Identification of actinomycete isolates was carried out according to Bergey's Manual of Systematic and determinative Bacteriology (27). Dendrogram Unweighted Pair Group Method

with Arithmetic Mean (DendroUPGMA) (28) and Interactive Tree Of Life (iTOL) (29) were employed to construct dendrogram of physiological and biochemical characteristics of actinomycete isolates. For this, pattern was a score specified with binary characters (1 = presence of growth, and 0 = absence of growth) for the assimilation of carbohydrates (sugars), nitrogen sources (amino acids) and enzymatic degradation (30).

Molecular identification

GF-1 Nucleic Acid Extraction Kit was used to extract genomic DNA of screened strain in accordance with manufacturer's instructions (Vivantis Technologies SdnBhd, Selangor DE, Malaysia). Extracted DNA was kept at 4 °C. PCR amplifications were performed using the following primers: Forward 27F (5'–AGAGTTTGATCCTGGCTCAG–3') and reverse 1492R (5'–CCGTCAATTCCTTTGAGTTT-3') for 16S rRNA gene (31). Thermal cycler (iCycler Bio-Rad, USA) was used for amplification as follows: initial denaturation at 94 °C for 12 min, denaturation at 94 °C for 30 s, hybridization at 55 °C for 30 s, and elongation at 72 °C for 1 min 40 s. A total of 30 cycles were applied and final elongations at 72 °C for 7 min. PCR products were verified using agarose gel (1.5%, Sigma-Aldrich, USA). 100 base pair DNA ladder (Solis Biodyne, Estonia) was used as an indicator for DNA molecular weight. Electrophoresis was applied (80 V for 1 h and 30 min). Obtained gel was stained with Midori Green Advance (Nippon Genetics, Japan) before examination under UV light. Generated sequences were compared using BLASTn from NCBI website (available at: http://blast.ncbi.nlm.nih.gov). Phylogenetic tree was carried out using Molecular Evolution Genetics Analysis (MEGA) software version 10.2.2 (32), and the neighbor-joining method (33) with the Kimura 2-parameter model (34). Alignment of sequence was realized using Clustal W algorithm (35).

Target strains

Target microorganisms used in the present study include: *Candida albicans* (ATCC 10231), *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 8739), and *Salmonella typhimurium* (ATCC 13331), that were obtained from Pasteur Institute located in Algiers (Algeria). They were mostly human pathogenic and antibiotic resistant microorganisms.

Antimicrobial activity

Cross streaking-plate technique was used to screen antimicrobial activity of actinomycete isolates against pathogenic bacteria and yeast. Single streak of each actinomycete culture was streaked at the corner of Petri plates containing ISP2 medium and incubated at 30 °C for 8 days. Then, fresh cultures of target microorganisms were inoculated in crossed streaks to actinomycete cultures and incubated at 37 °C for 24 h. Antimicrobial activity was evaluated by measuring inhibition distance between pathogenic microorganisms and colony margins of actinomycete cultures formed (36).

Results

Identification of actinomycetes

Morphological characterization

Morphological characteristics of actinomycete isolates on ISP2 medium were presented in Table 2. Examination of aerial and substrate mycelia showed that their color varied among isolates. Color of aerial mycelia for most isolates was white (15 isolates representing (46.88%) or light gray 9 isolates representing

(28.13%). For the remaining 8 isolates (25.00%), varied colors can be observed including pink, light yellowish brown, beige, light blue and deep grey. Colors of substrate mycelia were deep orange-yellow for 12 isolates (37.50%) and light orange-yellow for 11 isolates (34.38%) of total isolated actinomycetes. For the remaining isolates, color varied between yellow, brown, black, and dark green. Fifteen (46.88%) isolates showed very good growth and 8 (25.00%) isolates showed excellent growth on ISP2 medium. Moreover, 9 (28.13%) isolates revealed good growth. Colony surfaces observed of 30 (93.75%) isolates were rough and 2 (6.25%) isolates were smooth. Colony textures formed were filamentous for 15 (46.88%) isolates, powdery for 13 (40.63%) isolates and rhizoids for 4 (12.50%) isolates.

Table 2. Morphological characteristics of actinomycete isolates.

Isolates	Genus	CS	Growth	Texture	AM	SM	Pig	TSC	Gram
AC1	*Nocardiopsis*	Rough	Very good	Filamentous	White	Black	-	nd	+
AC2	*Streptosporangium*	Rough	Very good	Rhizoids	White	Deep orange yellow	-	nd	+
AC3	*Streptomyces*	Rough	Excellent	Powdery	White	Light orange yellow	+	*S*	+
AC4	*Streptomyces*	Rough	Very good	Powdery	Light grey	Deep orange yellow	-	*S*	+
AC5	*Streptomyces*	Rough	Very good	Filamentous	Light grey	Brown	-	*S*	+
AC6	*Streptomyces*	Rough	Very good	Filamentous	White	Deep orange yellow	-	*RA*	+
AC7	*Streptomyces*	Rough	Very good	Filamentous	White	Light orange yellow	+	*S*	+
AC8	*Streptomyces*	Rough	Very good	Powdery	White	Light orange yellow	+	*RA*	+
AC9	*Streptomyces*	Rough	Excellent	Powdery	Light grey	Deep orange yellow	-	*S*	+
AC10	*Streptomyces*	Rough	Excellent	Powdery	Pink	Light orange yellow	-	*RF*	+
AC11	*Streptomyces*	Rough	Good	Powdery	Pink	Yellow	-	*RF*	+
AC12	*Streptomyces*	Rough	Very good	Powdery	Light grey	Deep orange yellow	-	*RF*	+
AC13	*Streptomyces*	Rough	Good	Rhizoids	Light grey	Deep orange yellow	+	*RA*	+
AC14	*Streptomyces*	Rough	Excellent	Filamentous	Light yellowish brown	Brown	-	*S*	+
AC15	*Streptomyces*	Rough	Excellent	Powdery	Light grey	Deep green	-	*S*	+
AC16	*Streptomyces*	Rough	Very good	Filamentous	White	Light orange yellow	-	*RA*	+
AC17	*Streptomyces*	Rough	Very good	Powdery	Deep grey	brown	-	*S*	+
AC18	*Actinomadura*	Rough	Good	Powdery	White	Yellow	-	nd	+
AC19	*Nocardia*	Smooth	Excellent	Powdery	White	Deep orange yellow	+	nd	+

AC20	*Nocardiopsis*	Rough	Very good	Powdery	Light grey	Deep orange yellow	-	nd	+
AC21	*Nocardia*	Rough	Good	Filamentous	White	Light orange yellow	-	nd	+
AC22	*Streptomyces*	Rough	Excellent	Rhizoids	Light grey	Deep orange yellow	-	*RA*	+
AC23	*Streptosporangium*	Rough	Good	Powdery	White	Light orange yellow	-	nd	+
AC24	*Streptomyces*	Rough	Very good	Filamentous	White	Yellow	-	*RF*	+
AC25	*Streptomyces*	Smooth	Good	Filamentous	White	Light orange yellow	-	*S*	+
AC26	*Streptosporangium*	Rough	Good	Filamentous	White	Light orange yellow	-	nd	+
AC27	*Nocardiopsis*	Rough	Good	Filamentous	Light brown	Light orange yellow	-	nd	+
AC28	*Streptomyces*	Rough	Very good	Rhizoids	Light grey	Deep orange yellow	-	*RA*	+
AC29	*Actinomadura*	Rough	Very good	Filamentous	Grayish white	Deep orange yellow	+	nd	+
AC30	*Streptomyces*	Rough	Very good	Filamentous	Light yellowish brown	Light orange yellow	-	*S*	+
AC31	*Streptomyces*	Rough	Excellent	Filamentous	White	Yellow	+	*RF*	+
AC32	*Nocardia*	Rough	Good	Filamentous	Light brown	Deep orange yellow	-	nd	+

CS: Colony surface; AM: Aerial mycelium; SM: Substrate mycelium; Pig: Pigmentation on ISP7 medium; TSC: Type of Spore Chains; *S*: *Spira*; *RF*: *Rectus Flexibilis*; *RA*: *Retinaculum Apertum*; - Negative reaction; + Positive reaction; nd: not determined.

Microscopic observation

Microscopic observation revealed that all isolates formed filamentous structures and belonged to Gram-positive bacteria (Table 2). Aerial mycelia of 21 isolates (65.63%) (presumed belonging to Streptomyces genus) produced various types of spore chains including: 10 isolates (47.62%) formed S (*Spira*), 5 isolates (23.81%) revealed RF (*Rectus Flexibilis*) and 6 isolates (28.57%) formed RA (*Retinaculum Apertum*) of total presumed *Streptomyces* isolates (Figure 2). Identification of the total 32 isolates from soil sampling sites revealed 5 actinomycete genera: 21 *Streptomyces* (65.63%), 3 *Nocardia* (9.38%), 3 *Streptosporangium* (9.38%), 3 *Nocardiopsis* (9.38%), and 2 *Actinomadura* (6.25%) (Table 2). Morphological characterization and microscopic observation of the main 5 actinomycete genera were summarized in (Figure 3).

Physiological and biochemical analysis

Biochemical and physiological identification of 32 actinomycete isolates showed high metabolic diversity (Table 3). Firstly, the majority of isolates (96.88%) were catalase positive. Starch hydrolysis showed positive results for 28 (87.50%) isolates, as they produced an opaque zone around growth, and negative result for only 4 (12.50%) isolates. Out of a total 32 isolates, 26 (81.25%) isolates were found to be positive for casein hydrolysis. Secondly, 30 (93.75%) isolates were able to use three amino acids tested (L-alanine, asparagine, and histidine). Thirdly, they also used various carbohydrates tested: 27 (84.38%) isolates were able to

assimilate D-glucose, 26 isolates (81.25%) used D-fructose, D-mannitol, and maltose as unique carbon sources. Fourthly, only 7 (21.88%) isolates, produced melanin pigment on ISP7 medium, among them, 5 were presumed to belong to *Streptomyces* genus. Numerical analysis of phenotypic characteristics revealed high polymorphism among actinomycete isolates. Four major phenons were identified among the 32 total isolates (Figure 4). Numbers and percentages of isolates including phenons 1, 2, 3 and 4 were 3 (9.00%), 11 (34.00%), 8 (25.00%) and 10 (31.00%), respectively. Two most prevalent and diversified phenons were 2 and 4.

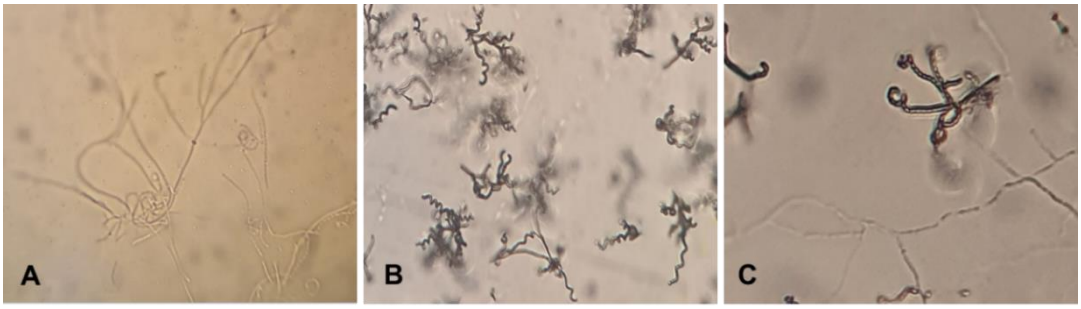

Fig.2. Spore chain morphology of *Streptomyces* (Light microscopy, 1000 x). A: *Rectus Flexibilis* type; B: *Spira* type; C: *Retinaculum Apertum* type.

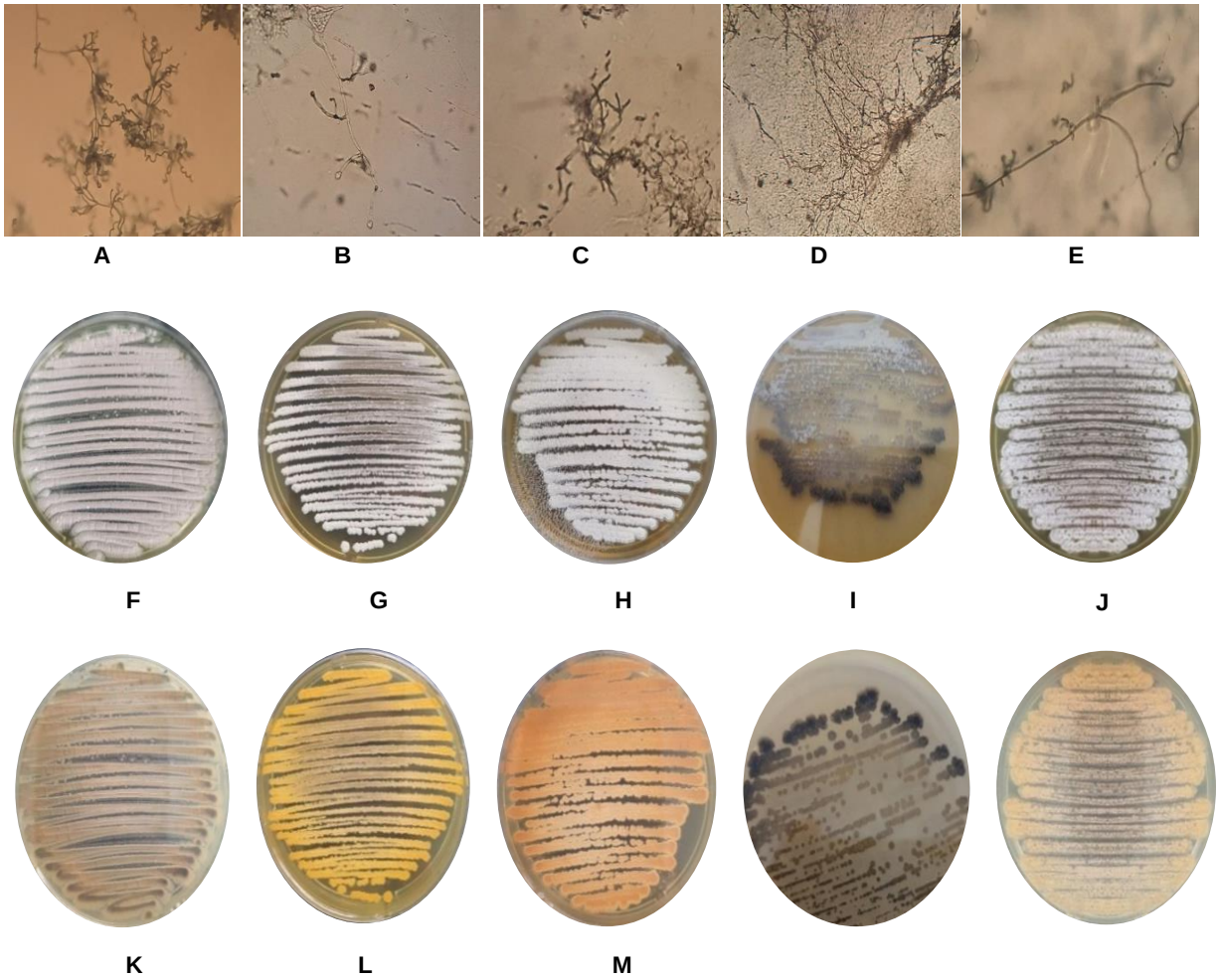

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Fig.3. Morphological characterization and microscopic observation of actinomycete genera. A-E show microscopic observation of actinomycetes isolates; F-J show areal mycelium morphology on ISP2 medium; K-O show substrate mycelium morphology on ISP2 medium. From left to right: isolate *Streptomyces* AC14, isolate *Streptosporangium* AC2, isolate *Nocardia* AC19, isolate *Nocardiopsis* AC1, isolate *Actinomadura* AC29, respectively.

Table 3. Physiological and biochemical characteristics of actinomycete isolates.

Isolates	CAT	CAS	AES	STA	TWE	ALA	ASP	HIS	TRY	TYR	FRU	GLU	LAC	GAL	ARA	MAL	MAN	SOR	MANN	RIB
AC1	+	+	-	+	+	+	+	+	+	+	+	-	+	-	+	+	-	+	-	-
AC2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
AC3	+	+	-	-	+	+	+	+	+	-	+	+	-	+	-	+	+	+	+	+
AC4	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
AC5	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
AC6	+	+	+	+	-	+	+	+	+	+	-	+	-	+	-	+	+	-	+	-
AC7	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
AC8	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
AC9	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
AC10	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+
AC11	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
AC12	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
AC13	+	-	+	-	+	+	+	+	+	-	+	+	+	-	-	-	+	-	+	-
AC14	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
AC15	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
AC16	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
AC17	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
AC18	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
AC19	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-	-	-	+	-	-
AC20	+	+	-	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
AC21	+	+	-	+	+	+	+	+	-	-	-	+	-	-	-	-	-	-	-	-
AC22	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
AC23	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
AC24	+	+	+	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	+	-

AC25	+	+	+	-	+	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+
AC26	+	+	+	-	+	+	+	+	-	-	+	+	+	+	+	+	+	-	+	+
AC27	+	+	-	+	+	-	-	-	-	-	+	+	+	-	+	+	-	+	+	+
AC28	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
AC29	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
AC30	+	+	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	+	+	-
AC31	+	+	-	+	-	+	+	+	+	-	-	+	+	+	+	+	+	-	+	+
AC32	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

CAT: Catalase; CAS: Casein; AES: Aesculin; STA: Strach; TWE: Tween 80; ALA: L-Alanine; ASP: Asparagine; HIS: Histidine; TRY: Tryptophan; TYR: Tyrosin; FRU: D-Fructose; GLU: D-Glucose; LAC: Lactose; GAL: D-Galactose; ARA: D-Arabinose; MAL: Maltose; MAN: D-Mannitol; SOR: Sorbitol; MAN: Mannose; RIB: Ribose; −: Negative reaction; +: Positive reaction.

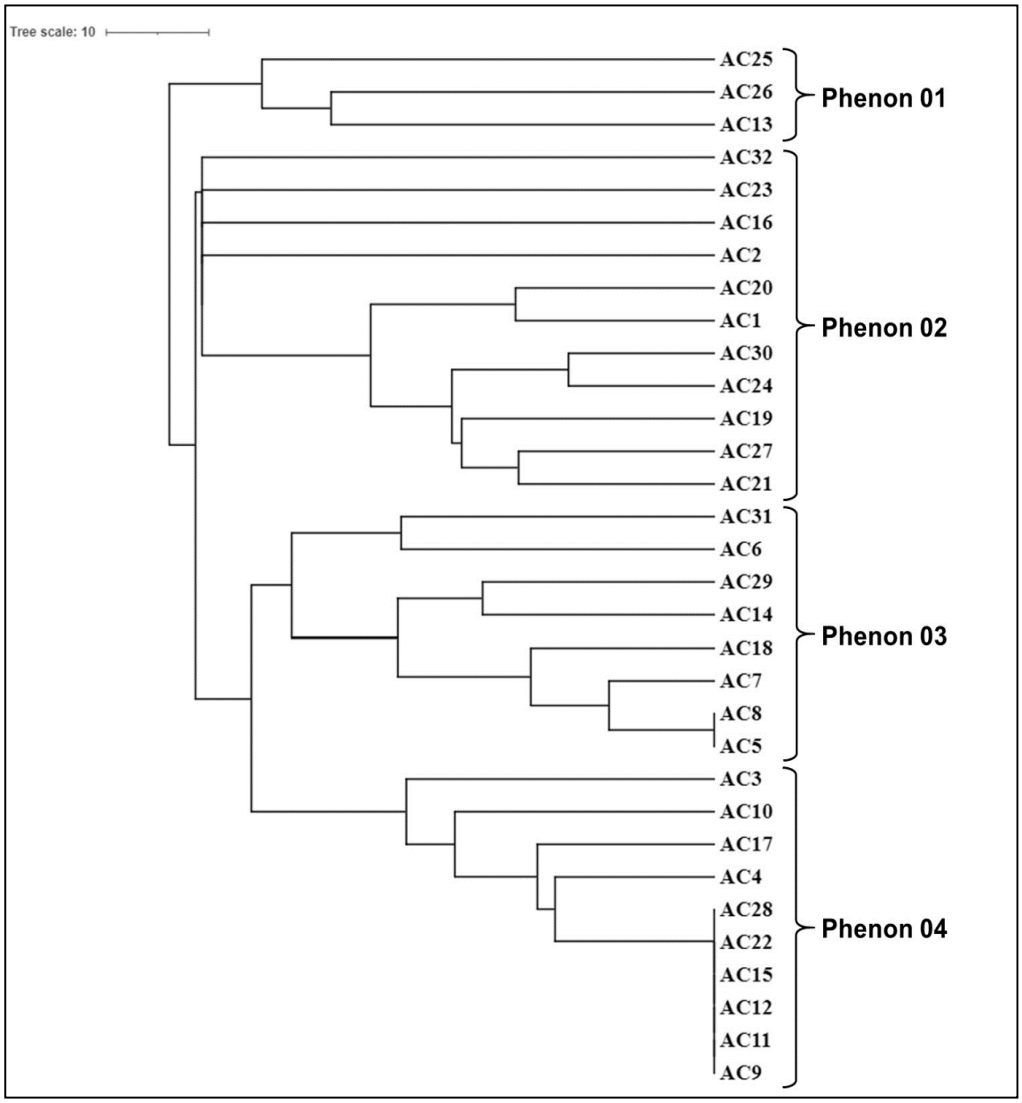

Fig.4. Dendrogram obtained by comparing physiological and biochemical characteristics of the 32 isolated actinomycetes.

Molecular identification

Constructed phylogenetic tree using 16S rRNA gene sequences showed 100% homology with partial 16S ribosomal RNA gene sequence of *Streptomyces fimbriatus* strain NBRC 15411^{T} (AB184659). 16S rRNA gene sequence of AC31 strain was assigned to the GenBank accession number OP647314 (Figure 5).

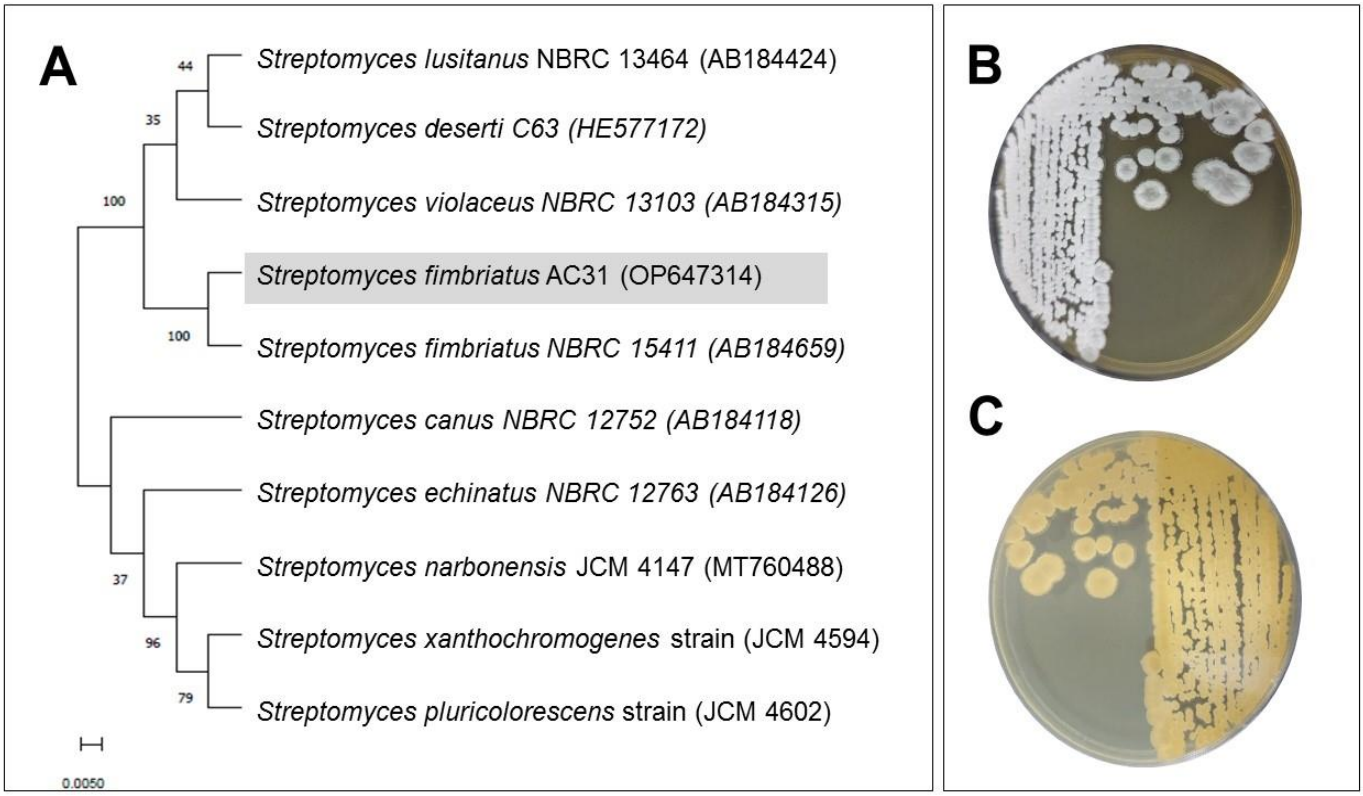

Fig.5. Phylogenetic tree and morphological characterization of strain *Streptomyces fimbriatus* AC31 A neighbor-joining phylogenetic tree based on 16 S rRNA gene sequences, showing the relationships between strain AC31 and related species of the genus *Streptomyces*; B: aerial mycelium morphology of strain AC31 on ISP2 medium; C: substrate mycelium morphology of strain AC31 on ISP2 medium.

Antimicrobial activity

Most of actinomycete isolates were active against different target strains including yeast and bacteria: 24 (75.00%) isolates showed antibacterial activity against Gram-positive bacteria and 21 (65.63%) isolates were active toward Gram-negative bacteria. Pathogenic yeast *C. albicans* (ATCC 10231) was inhibited by 25 isolates (78.13%). Globally, diameters of inhibition zones ranged from 5 to 44 mm (Table 4). Results revealed that among 32 actinomycetes, 29 (90.63%) isolates were active against at least one of 5 target strains. Highest inhibition zones observed were 41 mm for AC9 and AC31, 41 mm for AC10, 43 mm for AC10 and AC5, and 44 mm for AC31 against target strains: *B. subtilis* (ATCC 6633), *E. coli* (ATCC 8739), *S. typhimurium* (ATCC 13331), *S. aureus* (ATCC 6538), and *C. albicans* (ATCC 10231), respectively. Twenty-

seven (84.00%) isolates were active against *B. subtilis* (ATCC 6633). Followed by 25 (78.00%) isolates toward *C. albicans* (ATCC 10231), 25 (78.00%) isolates against *S. aureus* (ATCC 6538), 24 (75.00%) isolates against *E. coli* (ATCC 8739), and 23 (72.00%) isolates toward *S. typhimurium* (ATCC 13331). Strain AC31 Streptomyces fimbriatus exhibited highest activity toward all the target strains tested. Inhibition diameters recorded of strain AC31 were 41, 38, 41, 42, and 44 mm against target strains *B. subtilis* (ATCC 6633), *E. coli* (ATCC 8739), *S.typhimurium* (ATCC 13331), *S. aureus* (ATCC 6538), and *C. albicans* (ATCC 10231), respectively.

Table 4. Antagonistic activity of actinomycete isolates against pathogenic microorganisms by cross streak method.

Isolates	Inhibition zone (mm)				
	C. albicans (ATCC 10231)	*S. aureus* (ATCC 6538)	*B. subtilis* (ATCC 6633)	*E. coli* (ATCC 8739)	*S. typhimurium* (ATCC 13331)
AC1	0	0	0	0	22
AC2	6	4	0	13	7
AC3	14	15	16	15	13
AC4	22	9	10	10	18
AC5	13	43	14	40	0
AC6	0	7	8	0	0
AC7	35	35	21	25	42
AC8	16	15	26	12	30
AC9	36	8	41	9	10
AC10	25	35	32	41	43
AC11	0	0	0	0	0
AC12	10	19	16	15	22
AC13	5	0	5	0	12
AC14	18	18	15	38	24
AC15	19	18	9	0	0
AC16	18	37	28	17	33
AC17	15	10	16	32	9
AC18	12	30	17	14	32
AC19	13	10	7	39	10
AC20	9	11	24	8	26
AC21	0	0	11	9	0
AC22	7	28	19	28	23
AC23	0	0	0	0	0
AC24	0	0	0	0	0
AC25	24	22	23	30	28
AC26	0	8	10	9	0
AC27	12	28	17	31	10
AC28	19	30	15	36	18
AC29	18	21	24	20	16
AC30	5	14	12	0	0
AC31	44	42	41	38	41
AC32	9	0	7	10	11

Discussion

Actinomycetes seem to be very promising alternative as source of antimicrobial agents against pathogenic microorganisms. Particularly, actinomycete strains isolated from Saharan soil, as an extreme and unexpected environment from El Atteuf region; Ghardaïa (Algeria).

Actinomycetes are Gram-positive, fungus-like hyphal bacteria that are the most prolific natural producers of antimicrobial agents (37). Actinomycete isolates characterized in this study, showed good to excellent growth and abundant mycelia on ISP2 culture medium after incubation at 30 °C for 7 days. Firstly, among 32 isolates identified in this current study, most of them, 21 (65.63%) isolates belonged to *Streptomyces* genus as it was reported in several studies (37). Species of this genus are generally identifiable by color of their aerial and substrate mycelia, soluble pigment, and shape and ornamentation of their spore surface due to their stability (38). Microscopic examination revealed also that three types of *Streptomyces* mycelium (*S*, *RF*, and *RA*) characterizing this genus were observed (23). Secondly, isolates AC2, AC23, and AC26 identified as belonging to *Streptosporangium* genus, that were distinguished based on white color of aerial mycelia. Substrate mycelia can take deep or light orange-yellow colors; also the presence of sporangia (39). Third, cultures of isolates AC19, AC21, and AC32 on ISP2 medium belonging to *Nocardia* genus were macroscopically abundant with rough or smooth colony surfaces, length of the hyphae in aerial mycelium was variable, and substrate mycelium was septate (40). Fourth, isolates AC1, AC27, and AC20 share the same morphology characteristics of *Nocardiopsis* genus. Colonies of this genus are numerous on organic media, aerial and substrate mycelia are both well-developed, and hyphae are long and densely branched (41). Fifth, morphological features of isolates AC29 and AC18 were compatible with description of *Actinomadura* genus that is described as non-fragmented and widely branched (42). Physiological and biochemical tests on *Streptomyces* isolates revealed diverse metabolic capabilities. Positive reactions were observed for catalase, alanine, asparagine, histidine, and tryptophan degradation, and 65.63% of isolates degrading mannose. Aesculin hydrolysis was less common (43). Specific isolates, including AC3, AC7, AC8, and AC31, produced melanin pigments, suggesting its potential use as taxonomic criterion (44). Physiological tests on *Streptosporangium* isolates revealed consistent utilization of various carbon sources, including D-fructose, D-glucose, lactose, D-galactose, D-arabinose, maltose, D-mannitol, mannose, and ribose, except for AC26 that did not utilize sorbitol. Starch hydrolysis was positive for AC2 and AC23 isolates, while tests for DL-alanine, asparagine, histidine, and tryptophan revealed negative results. Tyrosine hydrolysis was observed, nevertheless AC26 isolate was negative for tryptophan and tyrosine hydrolysis (45). Physiological tests for *Nocardiopsis* isolates revealed distinct characteristics. Positive tests included catalase reaction and the ability to hydrolyze casein and starch. Utilization of fructose, lactose, arabinose, maltose and sorbitol as carbon sources was observed. Notably, isolate AC20 showed negative lipolytic activity, which is consistent with previous research (46). Additionally, both AC20 and AC27 isolates demonstrated inability to degrade tyrosine, supporting earlier findings (47). Physiological tests showed distinct characteristics for the *Nocardia* isolates AC19, AC21, and AC32. Catalase was common, nevertheless differences in starch and Tween hydrolysis were observed compared to the study of Yang et al. (48). AC32 exhibited positive reactions for fructose and mannose, while AC21 was positive for glucose. However, AC19 and AC21 were negative for several sugars, aligning with findings of Ding et al. (49). These similarities and variations highlight the

importance of understanding diversity and ecology of *Nocardia*, considering culture condition and actinomycete isolation. Isolates of *Actinomadura* AC18 and AC29 exhibited ability to grow on specific carbon sources, including D-fructose, D-glucose, lactose, arabinose, and mannitol. Positive reactions for aesculin, starch, and Tween 80 degradation, as well as catalase activity, were observed. However, AC29 isolate did not exhibit casein hydrolysis (50). These combined findings contribute to deeper understanding of *Streptomyces*, *Streptosporangium*, *Actinomadura*, *Nocardiopsis*, and *Nocardia* diversity and their potential ecological roles. The isolates' ability to produce industrial enzymes makes them of significant importance. *Streptomyces* species have been found to produce important enzymes such as amylase, protease, and cellulose that have diverse commercial applications across various industries (51). Amylase, being one of the most important industrial enzymes, finds applications in numerous fields worldwide, including food industry, paper production, and textile processing (52). Additionally, lipases were reported to play a crucial role in bioremediation of lipid, pesticide, plastic, and other environmental waste (53). On the other hand casein, a vital protein component in milk, is widely used in industries producing paper, textile, paint, leather, and other materials (54). The development of enzymatic processes has garnered increasing attention as more environmentally-friendly alternative to traditional chemical process. These chemical methods have significant environmental drawbacks due to the use of hazardous chemicals and generation of substantial waste. Actinomycete enzymes offer distinct advantages over those produced by other microorganisms or chemical processes, making them promising candidates for more sustainable and efficient production of valuable compounds. Various unexplored habitats and less explored environments are the great source of actinomycetes that produce industrially useful enzymes which have great commercial significance (55). Despite their potential as catalysts for diverse industrial applications, actinomycete enzymes face specific challenges in production and commercialization. Primary challenge in meeting growing market demand for actinomycete enzymes is establishing large-scale production facilities that necessitate substantial investments in capital, equipment, and technology. This is similar to challenges faced in production of caseinates, where its high drying cost, low bulk density, and high packaging, storage, and transportation costs have hindered its widespread adoption in many dairying countries (56). Developing efficient production methods is essential to ensure continuous and cost-effective manufacturing for actinomycete enzymes. Biofuel production holds significant promise as a sustainable alternative to conventional fossil fuels. Actinomycetes, as natural sources, also show promising potential in this field. The ability of the isolates to convert carbohydrates into simple sugars opens opportunities for production of bioethanol and biodiesel, offering sustainable alternatives to nonrenewable-based ethanol and diesel fuels (57). Moreover, advantages of using these enzymes over traditional chemical processes include their eco-friendly nature, reduced waste generation, and potential for higher efficiency and stability. Actinomycetes were also used to biodegrade several types of plastic polymers and convert them into carbon sources for production of biodegradable bioplastics (58). There are only a few enzymes and biologically active compounds available for industrial purposes, and further research is required in this area to produce enzymes and biologically active compounds at low cost. This would help to identify cost-effective and sustainable solutions for various industrial applications, thereby improving production efficiency and environmental impact. Potential for further optimization of enzyme production by actinomycetes is extended, and it includes exploring advanced

techniques such as genetic engineering and/or fermentation process optimization, such as nutrient composition, pH, and temperature, which can lead to increased enzyme yields and cost-effective production (59). Collaboration between academic researchers and industry partners holds promise in developing and commercializing actinomycetes enzymes for diverse applications. This synergy leverages scientific expertise and industry resources to push research boundaries, discover unique enzymes, and adapt them to efficient industrial processes, offering cost-effective and environmentally friendly solutions across various industries. Challenges associated with extraction and purification of bioactive compounds from natural sources are manifold. High cost of these processes limits utilization of high-value bioactive compounds. Moreover, immaturity of cultivation, harvesting, extraction, and purification techniques hampers their commercial scalability, making large-scale production more difficult. Additionally, chemical safety is another concern, as the use of chemicals may raise worries about safety of the final products. Besides, limited clinical research also restricts widespread use of bioactive products, as clinical data is crucial to demonstrate their efficacy and safety (60). Complexity of natural compounds in these sources makes extraction and purification challenging. Moreover, the mixture of bioactive compounds plays a crucial role in preserving their efficacy, underscoring the significance of strategies to maintain this stability throughout the extraction process. Complexity of mixtures further is a further challenge, as achieving selectivity and fractionation of individual compounds require careful consideration to ensure that extraction and purification methods are appropriate and effective. Another critical issue is limited production of bioactive compounds, constituting a major challenge in meeting market demand, especially when intended for therapeutic applications. Constraint of limited quantity is further compounded by presence of non-expressed genes that restrict production of these bioactive compounds. Overcoming this genetic limitation to increase production is a crucial challenge in the field of extraction and purification of bioactive metabolites from natural sources. Identifying innovative strategies and exploring new strategies to stimulate expression of these genes can open new possibilities to meet growing demand for bioactive compounds for therapeutic and other applications (61). Actinomycetes present wide range variability of biochemical and physical properties that revealed 4 phenotypes. This was probably the result of the characteristics of soil samples from which they were isolated. The 16S rRNA of the screened strain AC31 showed 100% of homology with partial 16S rRNA gene sequence of *Streptomyces fimbriatus* strain NBRC 15411T. Similar percentage was reported by Ashraf *et al*. (62) who isolated closely related species to *Streptomyces fimbriatus*.

In the present study, actinomycete isolates exhibited antimicrobial activity against various target microorganisms. Inhibition percentage calculated (90.63%) was higher than values reported by other studies like inhibition percentage of 34% (63). Moreover, strain *Streptomyces fimbriatus* AC31 showed high activity against all target strains. Antibacterial properties of this *Streptomyces* species are widely reported (64). Several studies claimed that actinomycetes, including *Streptomyces*, exhibit generally good activity against Gram-positive bacteria that corroborate with current findings (24 isolates, representing 75.00%). Whereas, they exert weak activity toward Gram-negative bacteria (65). This can be explained by their double cell membrane that serves as a protective barrier to antibiotics (66). Other investigations reported that actinomycete isolates exhibit more antibacterial activity against Gram-negative than Gram-positive bacteria (67), which was confirmed in this study mentioning good activity against Gram-negative bacteria

representing 21 (65.63%) isolates. This refers to the cell walls of Gram-negative bacteria that are easier to break than those of Gram-positive bacteria (68).

Furthermore, in this study 25 (78.13%) isolates inhibited growth of *C. albicans* (ATCC 10231). According to Ibnouf *et al*. (69), screening of antifungal activity against *Candida* revealed a higher inhibition percentage of 91%. Proteolytic compounds are among the mechanisms that cause antifungal activity against *C. albicans* (70).

Morphological, physiological and biochemical identification highlighted the richness and diversity of actinomycete bacteria from Saharan soils of the region El Atteuf (Ghardaïa, Algeria). Genus *Streptomyces* was relatively the most dominant. Screened strain *Streptomyces fimbriatus* AC31 was the most active among all isolates tested which is endowed with antimicrobial activity against various pathogenic microorganisms. This strain can constitute promising source of antimicrobial substances to treat various infectious and nosocomial diseases.

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