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Streptomyces species from the rhizosphere of the medicinal plant *Artemisia herba-alba* Asso: Screening for biological activities

Biological activities of rhizospheric *Streptomyces* strains

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***Streptomyces* species from the rhizosphere of the medicinal plant *Artemisia herba-alba* Asso: screening for biological activities**

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Abstract

This work aims to study cultivable actinobacterial isolated from the rhizosphere soil of *Artemisia herba-alba* Asso; an important component of the Mediterranean dry steppe flora. The isolates functional attributes with respect to antifungal, *in vitro* growth-promotion properties and enzymatic capacities, were studied. A total of eleven cultivable actinobacterial strains were isolated and identified as *Streptomyces* species by 16S rRNA gene sequencing. The eleven *Streptomyces* strains were positive for the production of almost all the hydrolytic enzymes tested, while the majority had ACC deaminase activity (6 strains) and exhibited ammonia (7 strains), siderophores production (9 strains) and phosphate solubilization (6 strains). Two out of the eleven strains named *Streptomyces* sp. BKS30 and BKS40 showed antifungal activities. One promising *Streptomyces* sp. strain BKS30 that was in-depth characterized morphologically and biochemically was further tested for its antifungal activity for the filtrate and the butanolic extract against nine target-fungi as well as for its antioxidant activity by DPPH and ABST tests. The obtained results demonstrate that *Streptomyces* species isolated from the rhizosphere of *Artemisia herba-alba* Asso have the potential for different biological activities including antifungal and antioxidant activities particularly for strain BKS30.

Keywords Rhizosphere · *Streptomyces* · *Artemisia herba-alba* · Antifungal · Antioxidant · Plant growth-promoting traits

Abbreviations

PGPR Plant Growth-Promoting Rhizobacteria

EC Electrical Conductivity

CEC Cation Exchange Capacity

SEM Scanning Electron Microscopy

PCR Polymerase Chain Reaction

MEGA Molecular Evolutionary Genetics Analysis

NCBI National Centre for Biotechnology Information

ACC 1-Aminocyclopropane-1-Carboxylic acid

DPPH 2,2-diphenyl-1-picrylhydrazyl

ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)

BHT Butylhydroxytoluene

ANOVA Analysis of variance

LSD Least Significant Difference

Author contributions

N.D., A.M. and R.G-T. conceived and managed the project. N.D., K.O-A., S-A.S., S.S. and A.N. performed the experiments. C.V-V., A.M. and A.N. performed molecular analysis. N.D. and N.B. drafted the manuscript which was critically reviewed by all of the authors.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no competing interests.

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Introduction

In the rhizosphere, plants can interact with different microorganisms such as fungi, viruses, archaea and bacteria. The rhizosphere microorganisms are considered key determinants of plant growth and fitness to variable environmental conditions (Prasad et al. 2019; Odelade and Babalola 2019). Plant growth is facilitated by plant growth-promoting rhizobacteria (PGPR) that display many beneficial effects on their host plants including playing roles in the biocontrol of phytopathogens (Sathya et al. 2017; Prasad et al. 2019). PGPRs like actinobacteria are reported to possess multiple benefits that can be used for improving soil and plant health (Sathya et al. 2017; El-Tarabily et al. 2020, 2021). They are also known to produce metabolites with antioxidant activity when grown in oxidative stress conditions (Dholakiya et al. 2017).

Among these, members of the *Streptomyces* genus that were associated with rhizospheric soils of different plants have been isolated from potatoes (Nabti et al. 2014) and date palms (Messaoudi et al. 2020) from Algeria, olive trees from Turkey (Dede et al. 2020) and *Salicornia bigelovii* from United Arab Emirates (Mathew et al. 2020).

The interest in *Streptomyces* species is mainly due to their ability to produce a variety of compounds that can be used for medical and agricultural purposes (Saeed et al. 2017; Kamil et al. 2018; Odelade and Babalola 2019). Several authors reported positive influences on plants treated by *Streptomyces* species highlighting the mechanisms used by these species; such as nitrogen, phosphate and potassium acquisition (Jog et al. 2014; Boubekri et al. 2021), auxins (indole acetic acid), gibberellins and ethylene phytohormone synthesis (Jaemsaeng et al. 2018; El-Tarabily et al. 2019; Sharma et al. 2020; Mathew et al. 2020) and production of siderophores (Kumar et al. 2021; Gopalakrishnan et al. 2021). In addition, many *Streptomyces* species have been reported to control phytopathogens through antifungal and antibacterial activities (Toumatia et al. 2014; Kumar et al. 2021; Al Raish et al. 2021; Al Hamad et al. 2021) and the production of hydrogen cyanide and hydrolytic enzymes as chitinase (Mukhtar et al. 2017; Gopalakrishnan et al. 2021).

Algeria harbors diverse ecosystems (semi-arid, arid and Saharan lands) that are still poorly explored and require scientific investigations to search for biologically active actinobacteria. These actinobacteria represent potential candidates for their high abundance in different environments, high resistance to salinity and drought as well as their numerous biological activities (Toumatia et al. 2014; Benhadj et al. 2018; Tata et al. 2019; Laassami et al. 2020; Messaoudi et al. 2020). Three *Streptomyces* strains that were isolated from the surface soil of the plain of Mitidja exhibited active petroleum and naphthalene degradation *via* their biosurfactants production and emulsification abilities (Ferradji et al. 2014). Benhadj et al. (2018) have published a paper on the diversity of 125 *Streptomyces* strains that were isolated from an Algerian wetland (Fetzara lake) and their antimicrobial activities. The study of Baoune et al. (2018) revealed for the first-time petroleum biodegradation by endophytic *Streptomyces* and their multiple plant growth promotion features highlighting the potential use of these *Streptomyces* species in bioremediation of polluted sites. The diversity of actinobacteria isolated from date palm rhizosphere and saline environments has revealed the dominance of *Streptomyces* species (23 species out of 40) from which eight strains were the most active against different tested microorganisms as reported by Messaoudi et al. (2020). Djinni and Djoudi (2021) revealed the potential use of *Streptomyces* sp. WR1L1S8 that was isolated from the inner tissues of the marine brown algae *Fucus* sp. as heavy metals removing strain.

Many bioactive compounds have been discovered from different *Streptomyces* species from Algerian environments. For example, an actinomycin D-producing strain, *Streptomyces* sp. IA1 was reported by Toumatia

et al. (2014) and Khebizi et al. (2018) reported the production of oligomycins A and E by *Streptomyces* sp. HG29. *Streptomyces* sp. PAL114 collected from the M'zab region in the South of Algeria showed a strong antagonistic potential against several microorganisms due to its production of saquayamycins A and C, chaetoglobosin A and vineomycin A1 and mzabimycins A and B (Tata et al. 2019).

For studies on the *Artemisia* genus, the search for actinobacteria from the coastal rhizosphere of *Artemisia* Linn. plants has been undertaken by Wang et al. (2015) who published a paper on *Nocardia rhizosphaerae* from China. Thus, this work was motivated by the promising approach of mining new niches to search for beneficial actinobacteria, particularly members of the *Streptomyces* genus. Little information is available regarding the composition and biological functions of the actinobacteria of the genus *Streptomyces* inhabiting the rhizosphere soil of *Artemisia herba-alba* Asso; one of the plants that cover immense territories of semi-arid and arid lands. It is a medicinal plant of the *Asteraceae* family that has been widely used in Algerian phytomedicine (Sekiou et al. 2021) as well as in many other countries like Morocco (Amkiss et al. 2021), Libya (Elmhalli et al. 2021), Egypt (Mohamed et al. 2019), Saudi Arabia (Aziz et al. 2018), Pakistan (Aziz et al. 2016) and Spain (Arroyo et al. 2018).

To the best of our knowledge, only two studies were published by Hamdi and Yousef (1979) on *Azobacter* sp. nitrogen fixers in the rhizosphere of desert plants, including *Artemisia herba-alba* from Iraq and the recent study of Djemouai et al. (2022) on the strain of *Streptomyces* sp. BTS40 isolated from a semi-arid environment that showed antibacterial activity against only Gram-positive bacteria and had multiple Plant Growth-Promoting (PGP) traits.

Here, using culture-dependent techniques, we characterized a fraction of the actinobacterial communities of the rhizosphere soils of *Artemisia herba-alba* Asso in Biskra Province, Algeria. The eleven strains of *Streptomyces* were tested for their *in vitro* antagonistic potential against a selection of phytopathogenic and mycotoxigenic fungi, as well as their *in vitro* PGP abilities. Furthermore, one promising strain named *Streptomyces* sp. BKS30 was functionally characterized by antifungal and antioxidant activities. This study provides a useful insight into the *Streptomyces* species associated with the rhizosphere of *Artemisia herba-alba* from the arid lands of Algeria.

Materials and methods

Sample collection and soil characteristics

Three sampling sites located in Biskra Province (Southeastern Algeria) were chosen for the search of actinobacteria associated with the rhizospheric soil of *Artemisia herba-alba* Asso. In this region, *Artemisia herba-alba* Asso is widely distributed (Ayad et al. 2007) and three sites were selected based on its abundance (El Kantara, Chaiba and Djemorah).

Bulk soils as well as soil attached (rhizosphere soils) to the roots of apparently healthy *Artemisia herba-alba* Asso plants (with no marks or injuries), were collected (3 plants per site) as indicated in Table 1 and Fig. 1. Excavated root systems of the three plants at each site were placed into sterile plastic bags and transferred to laboratory and stored at 4 °C until further analysis.

The chemical analysis of the three bulk soils that were collected from the three sampling sites was carried out. Soil samples were air-dried, homogenized and sieved to remove > 2 mm debris. The soil texture was characterized as sandy loam by the United States Department of Agriculture (USDA) soil taxonomy method. The pH and Electrical Conductivity (EC) were measured in a 1:5 soil water solution. The soil organic carbon

content, total carbon and Cation Exchange Capacity (CEC) were determined as per laboratory procedures (Meklat et al. 2020). All the physico-chemical analyses were performed in triplicate.

Isolation of actinobacteria

Isolation of actinobacteria was carried out on three composite samples of rhizospheric soil associated with the roots of *Artemisia herba-alba* Asso. It was done as was recommended by Hayakawa and Nonumura (1987) on the surface of Petri dishes containing Chitin-Vitamins-B agar medium (CH-V, Hsu and Lockwood 1975) supplemented with nalidixic acid (50 µg/mL) to suppress the growth of Gram-negative bacteria and cycloheximide (80 µg/mL) to reduce fungal contamination. Eleven isolates namely BKS4, BKS7, BKS13, BKS16, BKS17, BKS20, BKS30, BKS31, BKS33, BKS34 and BKS40 were picked up and sub-cultured on International *Streptomyces* Project 2 medium (ISP2 medium, Difco Laboratories, Michigan, USA) (Shirling and Gottlieb 1966).

Morphological and physiological characterization of the actinobacterial isolates

The obtained actinobacterial isolates were the subject of macro- and micro-morphological identification using three culture media: ISP1 (Agar Tryptone Yeast Extract), ISP2 (Agar yeast-malt extract) and ISP4 (Agar Starch and inorganic salts) (Shirling and Gottlieb 1966) supplied from Difco Laboratories (Michigan, USA). The isolates were streaked on the plates and incubated at 28±2 °C for 21 days. All strains were observed by light microscopy (Motic BA210, China) and the characteristics evaluated were: growth rate, the formation and color of aerial spore mass and substrate mycelia with the ISCC-NBS color charts used to determine the colony color (Kelly 1964). For one isolate (BKS30), morphological characteristics using the Scanning Electron Microscopy (SEM, JSM 6360LV, JEOL, Akishima, Tokyo, Japan) of a culture grown on ISP2 agar at 28±2 °C for 14 days were studied.

All the actinobacterial isolates were monitored for their growth on ISP2 medium at 0 to 10% (w/v; pH 7) NaCl concentrations with an interval of 1%, different temperatures (20, 30, 40 and 50; pH 7) and at a pH range of 4 to 12. Their growth was monitored for 14 days at 28±2 °C (Anwar et al. 2016).

Biochemical characterization and antibiotic sensitivity test of strain BKS30

For the strain BKS30, biochemical tests and evaluation of acid production from carbohydrates were determined using API 20NE and API 50CH test strips (Biomérieux, France) following the manufacturer's recommendations. This strain was also tested for its sensitivity to 10 clinical antibiotics using a disc diffusion assay (Otto-Hanson et al. 2013). Commercial antibiotic discs from Biomériaux Laboratories (France) and Pasteur Institute (Algeria), containing streptomycin (10 µg), fosfomycin (50 µg), neomycin (30 µg), cefoperazone (30 µg), penicillin G (6 µg), erythromycin (15 µg), spiramycin (100 µg), rifampicin (30 µg), fusidic acid (10 µg) and trimethoprim/sulfamethoxazole (cotrimoxazole: 1.25/23.75 µg), were placed on ISP2 semi-solid medium that was seeded with a spore suspension (100 µL). After 5 days of growth at 28±2 °C, inhibition zones were measured. Strain-antibiotic interactions where inhibition zones < 2 mm were considered resistant.

Molecular identification

According to the method of Liu et al. (2000), the genomic DNA of all the isolates was extracted. The 16S rRNA gene was amplified by Polymerase Chain Reaction (PCR) primers pair 10-30F (5'-GAGTTTGATC-CTGGCTCA-3') and 1500R (5'-AGAAAGGAGGTGATCCAGCC-3'). The PCR reactions were carried out as described in Laassami et al. (2020). The sequencing was performed using the same PCR primers by Genewiz, Ltd. (Takeley, UK).

The obtained sequences were analyzed and aligned using Molecular Evolutionary Genetics Analysis; MEGA7 (Kumar et al. 2016) and were submitted to the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/, Kim et al. 2012). The 16S rRNA gene sequence of all the isolates was aligned using the "Clustal W" program (Thompson et al. 1994). The evolutionary distance matrix was generated as described by Jukes and Cantor (1969) and the phylogenetic tree was constructed using the neighbor-joining method of Saitou and Nei (1987). The topology of the tree was evaluated by a bootstrap test (Felsenstein 1985) using 1000 re-samplings. The 16S rRNA gene sequences of the identified rhizospheric actinobacterial strains have been submitted to National Centre for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/) with the GeneBank accession numbers of OK263096 to OK263105 and OL818328.

Plant growth-promoting attributes

The functional diversity of the tested actinobacterial strains was studied by the qualitative screening of their PGP attributes. The actinobacterial strains were screened for their ability to utilize 1-aminocyclopropane-1-carboxylate (ACC) as the sole nitrogen source, which is the consequence of the activity of the enzyme ACC deaminase. The strains were grown on the sterile minimal DF (Dworkin and Foster) salts media amended with 3 mM ACC (Sigma-Aldrich, Germany) or $(NH_4)_2SO_4$ (Riedel-de Haën, Germany) as the sole nitrogen sources (Dworkin and Foster 1958; Penrose and Glick 2003). The production of ammonia (NH_3) was evaluated using peptone water medium and revelation with Nessler reagent (Cappuccino and Sherman 2014). Solubilization of phosphate and siderophores production were assessed using Pikovskaya agar (Pikovskaya 1948) and CAS media (Schwyn and Neilands 1987). All assays were performed in triplicate at 28±2 °C and the obtained results were the subject of a Venn diagram produced using the Venn-Diagram software (https://bioinformatics.psb.ugent.be/webtools/Venn/).

Hydrolytic enzymes production

All the rhizospheric actinobacterial strains were screened in triplicate for their production of hydrolytic enzymes. For amylase production, actinobacterial strains were grown at 28±2 °C on 2% starch agar plates and the assessing media was cleared using an iodine solution (Passari et al. 2016).

Protease activity was determined after inoculating the obtained strains on skim milk agar medium (HiMedia Laboratories, India). After 10 days of incubation at 28±2 °C, the production of protease is indicated by the presence of a colorless zone around the cultures (Passari et al. 2016).

Cellulase production was carried out by growing the actinobacterial strains on medium plates containing Carboxy Methyl Cellulose (CMC, Acros organics, New Jersey, USA) at 28±2 °C for 10 days followed by flooding the Petri dishes with 0.1% (w/v) Congo red (Sigma-Aldrich, Germany) for 15 min and with 1M NaCl to remove unfixed dye from the medium. The appearance of a clear halo around the colonies indicates the presence of cellulases (Passari et al. 2016).

The production of chitinase was determined on chitin agar plates using prepared colloidal chitin from shrimp shells (Sigma-Aldrich, Germany) as a substrate (Mamangkey et al. 2021). Lastly, a Venn diagram was produced using the Venn-Diagram program (Bardou et al. 2014).

Primary screening for antifungal activity

All the actinobacterial strains were evaluated for their *in vitro* antagonistic abilities against nine micro-fungal strains that were obtained from the LBSM collection namely: *Aspergillus carbonarius* (M333), *A. westerdijkiae* (ATCC 3174), *A. brasiliensis* (ATCC 16404), *Penicillium expansum* (Pe), *Umbelopsis ramanniana* (NRRL

1829), *Fusarium graminearum* (Fg), *F. oxysporum* f. sp. *albedinis* (Foa), *F. oxysporum* f. sp. *lycopersici* (Fol), *F. culmorum* (Fc).

The agar diffusion method was used in this screening to evaluate the antifungal activity. The method consists of inoculating the actinobacterial isolates by tight streaks on the surface of the ISP2 medium and incubating at 28±2 °C for 10 days. At the end of the incubation period, agar cylinders of 10 mm in diameter were taken from the culture of actinobacteria (top sporulating actinobacteria and bottom agar medium) and were placed on the surface of Potato Dextrose Agar (PDA, Oxoid, UK) semi-solid medium (12 g/L) which was previously seeded with one of the tested micro-fungi (10^7 spores/mL). ISP2 plates without actinobacteria, but seeded with the test micro-fungi were maintained as the negative control. The inhibition zones were then determined after 72 h of incubation at 25±2 °C (Bauer et al. 1966).

Secondary screening for antifungal activity

The antagonistic potential of the strain *Streptomyces* sp. BKS30 was evaluated against the same list of micro-fungi used in the primary screening. The strain *Streptomyces* sp. BKS30 was grown on the solid medium ISP2 for 10 days at 28±2 °C at first. Then, three agar plugs of 6 mm diameter from each culture were added to 250 mL flasks containing 50 mL of ISP2 broth medium. The flasks were incubated for 3 days in shaking conditions at 250 rpm and 28±2 °C (New Brunswick Scientific Co., NJ, USA). From the 3 days culture, 3 mL were used to inoculate 500 mL flasks containing 100 mL ISP2 broth medium and put to shaking with the same prior conditions for 7 days. After incubation, the culture media were centrifuged using Hettich Universal 320R centrifuge (Germany) for 15 min at 13000 *g* with the separation of the pellet and the supernatant. The obtained crude supernatant was passed through sterile 0.22 µm Millipore filters. The cell-free culture filtrate was then used to check the antagonistic activity against all the target micro-fungi using the agar well diffusion method (Aszalos 1986).

Briefly, PDA semi-solid medium seeded with 10^7 spores/mL was poured into Petri dishes. Then, holes with a diameter of 10 mm were punched aseptically with a sterile cork borer and a volume of 200 µL of the filtrate in triplicate was introduced into the wells. Finally, agar plates were incubated at 28±2 °C for 72 h. The filtrate possessing antimicrobial agents diffuse in the agar medium and inhibit the growth of the tested microorganism with the detection of inhibition zones around the wells.

Extraction and recovery of antifungal compounds

The isolated strain BKS30 was cultured in 100 mL of ISP2 medium in 500 mL flasks under fermentation conditions, they were kept on a rotary shaker (250 rpm, New Brunswick Scientific Co., NJ, USA) at 28±2 °C for 7 days. After fermentation, the medium was harvested and centrifuged to remove cell debris. The antifungal compounds were recovered from the filtrate by solvent extraction method. The *n*-butanol was added to the filtrate in the ratio of 1:1 (v/v) (Saadi et al. 2021). The organic phase was separated from the aqueous phase under reduced vacuum (Rotavapor R-210, Buchi, Switzerland) and the obtained extract was dissolved in 1 mL of methanol (LiChrosolv, Germany).

The antifungal activity was determined by the paper disc method on PDA medium (12 g/L agar) against all the previously tested micro-fungi, 80 µL of the extract was pipetted onto a paper disc (6 mm, Pasteur Institute, Algeria) (Hayes and Markovic 2002). Discs were then sterilized by exposing them to hood UV (CACHAN 94230, France) for 45 min on each side. The plates were incubated at 28±2 °C for 72 h and the antifungal activity was estimated by measuring the diameters of zones of inhibition (in mm) around the discs.

Antioxidant assays

The obtained filtrate as well as the butanolic extract of strain BKS30 that was obtained after liquid-liquid extraction after 7 days of fermentation culture (as previously prepared in section secondary antifungal activity) were further evaluated for their antioxidant and radical scavenging activities by two different methods. The experiments were conducted in triplicate.

DPPH radical scavenging assay

The free radical scavenging activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay described by Blois et al. (1958) with the use of butylhydroxytoluene (BHT, Sigma-Aldrich, Germany) as the positive control. From the filtrate and the butanolic extract of strain BKS30, 40 µL were each diluted to the concentrations of 6.25, 12.5, 25, 50, 100, 200 and 400 µg/mL), then they were mixed with 160 µL of a methanolic DPPH (Sigma-Aldrich, Germany) solution (6 mg in 100 mL of methanol). The mixture was incubated at room temperature for 30 min. Then, the absorbance was measured at 517 nm using a 96-well microplate reader (En Spire, PerkinElmer, MA, USA). Results were expressed as % Inhibition and as IC_{50} values in µg/mL.

% Inhibition = [(Ac – As / As)] × 100.

Where; Ac and As where the absorbance of the negative control and the absorbance of the sample at 30 min, respectively. The IC_{50} (µg/mL) corresponds to half-maximal inhibitory concentration.

$ABTS^{\bullet+}$ scavenging activity

ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical-scavenging activities of the filtrate as well as the butanolic extract of strain BKS30, were determined according to the method described by Re et al. (1999). Different concentrations of sample solutions were made using distilled water (6.25, 12.5, 25, 50, 100, 200 and 400 µg/mL). Then 40 µL of sample solutions were mixed with 160 µL of $ABTS^{\bullet+}$ solution (Sigma-Aldrich, Germany) in a microplate of 96 wells with the use of BHT as a positive control. After 10 min of incubation, the absorbance was recorded at 734 nm in the 96-well microplate reader (En Spire, PerkinElmer, MA, USA). Results were expressed as inhibition percentage (%) and as IC_{50} values (µg/mL).

% Inhibition = [(A0 – A1 / A0)] × 100.

Where; A0 is the absorbance of the negative control, A1 is the absorbance of the sample at 10 min. The IC_{50} (µg/mL) corresponds to the half-maximal inhibitory concentration.

Statistical analysis

All analyses were carried out in triplicate and the data are presented as a mean value ± standard deviation (SD). Statistical analysis of the data was performed with Statistica software using analysis of variance for factorial designs (ANOVA). The significance of differences was tested using a Tukey least significant difference (LSD) test ($p \leq 0.05$).

3. Results

Soil analysis

The results of the physico-chemical analysis of the three collected soils are summarized in Table 1. The results revealed a slightly alkaline pH varying from 7.45 to 8.35 with significant differences between the different localities. The obtained results showed that these sites present sandy loam soils with low organic matter and carbon contents. Comparisons between the three localities showed that there was a significant difference in CEC results whereas, the results of EC from El Kantara and Djemorah were non-significant compared to Chaiba.

Identification and characterization of the isolated actinobacteria

Eleven actinobacterial strains were isolated from the rhizosphere soil associated with *Artemisia herba-alba* Asso from Biskra Province (Algeria) by a culture-dependent method. Of these, 5 strains were obtained from El Kantara, 3 from Chaiba and 3 from Djemorah.

All the actinobacterial isolates were characterized by extensively branched hyphae and spore chains, which indicates that they belong to *Streptomyces* genus. From the morphological characteristics shown in Table 2 and Fig. 2, all the tested isolates exhibited variations in the substrate color and aerial mycelia. Some similarities were noticed for two groups of isolates (BKS4 and BKS34) and (BKS17, BKS31 and BKS33), whereas the rest of the isolates were morphologically different. Many of the isolates were able to produce diffusible pigments depending on the tested media.

All the actinobacterial strains were cultured on ISP2 medium at different temperatures, pH and NaCl concentrations. The obtained results show that the strains were able to grow at 20, 30 and 40 °C but not at 50 °C and the optimum temperature was 28°C. The tested strains were all halotolerant and alkalitolerant with optimum growth at 0% of NaCl and 7.2 of pH (Table 3). BKS17 was found to have good growth on ISP2 medium supplemented with NaCl up to the 10% and at pH from 4 to 12, which makes it the most resilient strain in this collection.

Identification and characterization of strain BKS30

Streptomyces sp. BKS30 is a Gram-positive aerobic strain with a positive catalase reaction and spiny spores forming spiral chains (Fig. 3). Based on the API results presented in Table 4, this strain hydrolyzes starch, gelatin but not tyrosine, and uses several compounds as the sole source of carbon including D-xylose, rhamnose, D-fructose, D-arabinose, D-glucose, D-galactose, D-trehalose, D-mannose and cellulose.

Antibiotic sensitivity and resistance pattern for strain BKS30

The results of the antibiotic sensitivity test that was conducted against some of the commonly used antibiotics for bacterial infections are presented in Table 4. Strain BKS30 exhibited a high sensitivity towards six of the tested antibiotics namely streptomycin, neomycin, erythromycin, spiramycin, rifampicin, fusidic acid and resistance to the remaining four tested antibiotics.

Molecular identification

According to the phylogenetic identification, eight different species were identified with similarity percentages of 98.9 to 100% (Table 5 and Fig. 4). These results confirm the morphological identification and resemblances noticed for some strains. The three isolates BKS17, BKS31 and BKS33 were identified as *Streptomyces lateritius* while, the two isolates (BKS4 and BKS34) were found similar to *S. plumbiresistens*. The other strains belonged to *S. coerulescens* (BKS7), *S. europaeiscabiei* (BKS13), *S. albidochromogenes* (BKS16), *S. bungoensis* (BKS20), *Streptomyces polymachus* (BKS30) and *S. cyaneochromogenes* (BKS40).

Screening for PGP traits and enzymatic activities

The *in vitro* abilities of the isolated *Streptomyces* species to promote growth (ACC deaminase activity, solubilization of inorganic phosphate, production of siderophores and ammonia) and produce enzymes (amylase, cellulase, protease and chitinase) were studied. The results are given in Table 6 and Fig. 5.

The obtained results revealed that all the strains had at least one PGP trait, like strain BKS40 that was only positive for ACC deaminase activity while the four strains BKS7, BKS13, BKS16 and BKS31 had at least two PGP traits. Furthermore, four strains had three PGP traits (BKS4, BKS17, BKS30 and BKS33) and only two

strains possessed the four PGP traits (BKS20 and BKS34). In addition, six strains were positive for ACC deaminase activity with two strains out of three for the group of *S. lateritius* (BKS17 and BKS33) and only the strain BKS34 for the group of *S. plumbiresistens*. Moreover, six strains were positive for phosphate solubilization and seven strains were ammonia producers.

From the results of the Venn diagram, we obtained different groups depending on the results of the PGP traits. Out of the eleven strains, only two were positive for the four tested PGP traits (BKS20 and BKS34). For hydrolytic enzymes production, nine strains exhibited the four PGP traits while two strains namely BKS7 and BKS20 had amylase, cellulase and chitinase hydrolysis.

Antifungal activity

The eleven isolated strains were first screened for their antifungal activity against several micro-fungi from the collection of the LBSM laboratory. The results indicate that seven strains were not at all active against any of the tested micro-fungi; the strain BKS4 was active only against Pe and Fg; whereas, in addition to the two previously cited micro-fungi, the strain BKS34 was active against Ur and Fc. Two strains had interesting antifungal activities with the strain BKS40 being active against all the tested micro-fungi with variable inhibition diameters from 11.66 mm against Ur to 29.67 mm against Fc. Furthermore, strain BKS30 had a broad-spectrum of antifungal activity with the least activity was against Ur (13.00 mm) and the most important activity was recorded for Fc (34.33 mm) (Table 7, Fig. 6). This last strain was selected for a secondary screening for its antifungal activity. After seven days of fermentation culture, the filtrate and the organic phase that was obtained after liquid-liquid extraction with *n*-butanol of the same filtrate were used for the antifungal activity against all the target-fungi. The inhibition zones are illustrated in Table 8.

The filtrate of strain BKS30 showed antifungal activity against all the target-fungi, with a strong activity for Fc (30.33 mm) followed by a moderate activity against Fol (25.33 mm) and low activity against the rest of the tested micro-fungi. The butanolic extract, on the other hand, showed also an antifungal activity against all the tested micro-fungi with a moderate activity with inhibition diameters towards Fol of 27 mm followed by Fc with 26.67 mm and Aw as well as Foa each with inhibition zones of 22.33 mm. Low antifungal activity was recorded for the remaining target-fungi.

Antioxidant activity

The IC_{50} values of the filtrate and the butanolic extract of strain BKS30 and the positive controls were determined and listed in Table 9. These results show that the filtrate possessed the highest antioxidant activity than the butanolic extract. The highest DPPH scavenging activity was obtained by the filtrate of BKS30 with an IC_{50} value of <12.5 µg/mL while the butanolic extract gave an IC_{50} value of 40.02±5.02 µg/mL, these two results were comparable to the BHT that was used as a positive control.

On the other hand, the $ABTS^{\bullet+}$ scavenging activity results show all of the filtrate and butanolic extract gave IC_{50} values of 22.86±2.06 µg/mL and 34.34±1.6 µg/mL, respectively which were higher than those obtained by the positive control BHT with an IC_{50} value of <12.5 µg/mL.

Discussion

Among actinobacteria, *Streptomyces* species are the most studied as many researchers revealed the biological activities of *Streptomyces* species isolated from Algerian ecosystems. For example, Oubeira's Lake sediments in the North-East of Algeria were a source of *Streptomyces* SLO-105, *Streptomyces* sp. S72 and *Streptomyces* sp. AA13 that showed potential antimicrobial, antifungal properties and activity against *Candida albicans*,

respectively (Morakchi et al. 2009; Ayari et al. 2011; Ayari et al. 2016). The filtrate of the endophytic strain *Streptomyces* sp. PT_2 showed a PGP effect on seed germination and root elongation of tomato cv. Marmande (Goudjal et al. 2013). Ghadbane et al. (2015) reported four *Streptomyces* strains isolated from the rhizosphere of *Ononis angustissima* Lam. that had interesting antimicrobial bioactive substances and was a potential biocontrol agent against chickpea wilt disease.

To the best of our knowledge, the cultivable actinobacterial species associated with the rhizosphere of *Artemisia herba-alba* Asso, one of the medicinal plants that are widely distributed in semi-arid, arid and Saharan lands and studied for its ethnopharmacological properties in Algeria has been poorly studied. We hypothesized that the rhizosphere of *Artemisia herba-alba* Asso potentially harbors actinobacteria with biological activities.

No novel species were found in this study after comparison of the obtained 16S rRNA gene sequences of the *Streptomyces* isolates with sequences of type strains in the EzBioCloud server database (Yoon et al. 2017) as we had similarity values above 98.65%. This value that was proposed by Kim et al. (2014) is considered the cutoff value for species identity. Eight strains were identified with three strains belonging to *S. lateritus*, two strains were affiliated to *S. plumbiresistens* and the remaining were identified as different strains. Searching for the origin or place of isolation, we brose the NCBI site, (https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi) and we found that the majority of the strains that are similar to ours were first isolated from soil samples of different areas of the world. For example, *Streptomyces bungoensis* isolated from soil samples collected in the Yabakei area of Ohita Prefecture, Japan was found to produce an antibiotic by Eguchi et al. (1993). The novel species of *S. plumbiresistens* was first isolated from lead-polluted soil in Gansu province, north-west China (Guo et al. 2009). Also, the novel species of *Streptomyces polymachus* strain $T258^T$ was isolated from forest soil at Bongnae Falls, South Korea (Nguyen and Kim 2015) whereas, *S. cyaneochromogenes* strain MK-45T was isolated from soil sampled at a manganese-contaminated area in Xiangtan, China (Tang et al. 2019).

Other origins of isolation were also stated for some of the strains that were reported in our study. A strain named *S. albidochromogenes* E2 was isolated from soil sample of Tanah Rata (Cameron Highlands, Malaysia) showed decolorization potential of many toxic triphenylmethane dyes (Adenan et al. 2020). In addition, *Streptomyces lateritius* CSF09, an endophyte strain that was isolated from *Eleusine indica* (L.) Gaertn was a producer of 4-methoxysalicylaldehyde an insecticidal ingredient (Chen et al. 2017). A survey that was conducted to isolate and characterize *Streptomyces* species from common scab lesions of potato in Norway has shown that *S. europaeiscabiei* are among the most well-known common scab-causing species worldwide (Dees et al. 2012).

Globally, the tested strains exhibited noticeable PGP capacities. The production of the ACC deaminase, which hydrolyzes ACC, the precursor of ethylene in plants to ammonia and α-ketobutyrate is a key factor in the alleviation of stress associated with high salinity, drought, flooding, heavy metal contamination and organic pollutants in plants. These PGPR strains reduce stress caused by ethylene by converting ACC in root exudates, thereby reducing the endogenous level of ACC in plants, which is in homeostasis with the exterior (Glick 2005; Glick et al. 2007).

Ammonia production by actinobacteria has been reported by Thilagam and Hemalatha (2019) for *Streptomyces violaceoruber* isolated from rhizospheric garden soil of India and for the endophyte *Streptomyces hainanensis* strain S4303 obtained from *Aquilaria crassna* Pierre ex Lec (Nimnoi et al. 2010). Ammonia production is a direct mechanism of plant growth promotion. As was reported by Marques et al. (2010),

ammonia produced by bacteria can accumulate and provide nitrogen that will promote shoot and root elongation. In addition, ammonia can act in defense by suppressing phytopathogens (Avalos et al. 2019).

Six strains of *Streptomyces* (BKS4, BKS7, BKS17, BKS20, BKS30 and BKS34) were found to be phosphate solubilizers. In literature, there have been many studies on *Streptomyces* species that can solubilize phosphate and promote plant growth (Jog et al. 2014; Boubekri et al. 2021).

Concerning the production of siderophores, the majority of the tested *Streptomyces* strains tested positive with the exception of BKS40. Siderophores which are low-molecular-weight molecules, act as scavengers making iron unavailable for phytopathogens (Jog et al. 2014).

On the other hand, all the strains had interesting enzymatic activities for amylase, protease, cellulase and chitinase except for the strains BKS7 and BKS20, which were only negative producers of proteases. *Streptomyces* species are known for their production of hydrolytic enzymes that play major roles in fighting phytopathogens (Mukhtar et al. 2017; Gopalakrishnan et al. 2021).

Of the tested strains, *Streptomyces* spp. BKS40 and BKS30 exhibited the strongest activities against all the tested micro-fungi. This is consistent with many studies that revealed that *Streptomyces* species produce antimicrobial compounds against pathogens (Toumatia et al. 2014). The first strain BKS40 exhibited a 99.79% resemblance with *Streptomyces cyaneochromogenes*, an actinobacterium that was first isolated from a manganese-contaminated soil in Xiangtan (China) by Tang et al. (2019). The second strain BKS30 showed a 98.95% similarity with the strain *Streptomyces polymachus* that was first isolated by Nguyen and Kim (2015) from forest soil in South Korea. This strain showed multiple antifungal and antibacterial activities hence the designation *polymachus* (from *polymachos* fighting many). Thus, it would be interesting to further identify and characterize the antifungal compounds produced by the two strains.

Based on all the obtained results of PGP traits, enzymatic and antifungal activities, we selected the strain BKS30 to further test its biological activities in depth. Our results showed that strain BKS30 had a broad spectrum of antifungal activity. Many authors have reported similar results for antimicrobial activity of different species of *Streptomyces* from different sources (Laskaris and Karagouni 2021; Kumar et al. 2021). Many *Streptomyces* species that were isolated from arable soil, compost and soil amended with compost in the study of Kovácsová et al. (2015), demonstrated activity against at least two of the following test phytopathogens: *Xanthomonas campestris* (CCM 22), *Pseudomonas syringae* (CCM 2868), *Erwinia amylovora* (CCM 1114), *Clavibacter michiganensis* subsp. *sepedonicus* (CCM 7014), *Botrytis cinerea*, *Fusarium poae*, *Alternaria tenuissima* and *Alternaria arborescens*. The study of Tistechok et al. (2021), revealed that the strain *Streptomyces* sp. Je 1–6 that was isolated from rhizosphere soil of *Juniperus excelsa* Bieb from Ukraine had an interesting activity against Gram-positive bacteria, including polyresistant clinical isolates of *Staphylococcus* sp. In another study, strain *Streptomyces atacamensis* (ACG1) that was isolated from a soil sample in India, was active against several Gram-positive and negative bacteria of uropathogens (Singh and Singh 2021). Fahmy and Abdel-Tawab (2021) reported that, the ethyl acetate extract of strain *Streptomyces* sp. NMF6 associated with the marine sponge *Diacarnus ardoukobae* collected from Safaga at the Egyptian Red Sea coast showed an interesting antimicrobial activity against *Staphylococcus aureus*, *Enterococcus faecalis*, *Vibrio damsela* and *Candida albicans*.

Nowadays, there is great interest in the discovery of antioxidant compounds derived from natural sources with *Streptomyces* isolates appearing to be a promising source of these antioxidants (Tan et al. 2019). Based on our

findings, the filtrate as well as the butanolic extract of strain BKS30 possessed antioxidant by exerting free radical scavenging properties with IC_{50} <12.5 and 22.86 µg/mL for the filtrate when screened using the ABTS and DPPH assays, respectively. These findings are in line with previous studies highlighting that different *Streptomyces* species from different habitats as antioxidant-producing strains (Tan et al. 2019; Larasati et al. 2020).

Conclusion

As a whole, the results of this study highlighted that the *Streptomyces* associated with rhizosphere soil of *Artemisia herba-alba* in particular strain BKS30 possess immense potential to synthesize antioxidative and antifungal metabolites. This strain displayed multiple biological traits which make them good candidates to further be tested as potential fertilizers, biostimulants and for plant protection and hence could be exploited for future development of functional ingredients in different applications. Further investigations are required to identify the compounds responsible for the antifungal activity as well those having antioxidant potential of the studied *Streptomyces* sp. strain BKS30.

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FIGURES AND TABLES

Fig. 1 Sampling site locations for isolation of rhizospheric actinobacteria from *Artemisia herba-alba* Asso. **(a)**: Map of Algeria, **(b)**: Map of Biskra Province showing the three sampling sites of El Kantara (black), Chaiba (orange) and Djemorah (blue). **(c)**: Morphology of *Artemisia herba-alba* Asso, **(d)**: Example of the vegetation and landscape of one of the study areas. (Maps have been adapted from https://d-maps.com/)

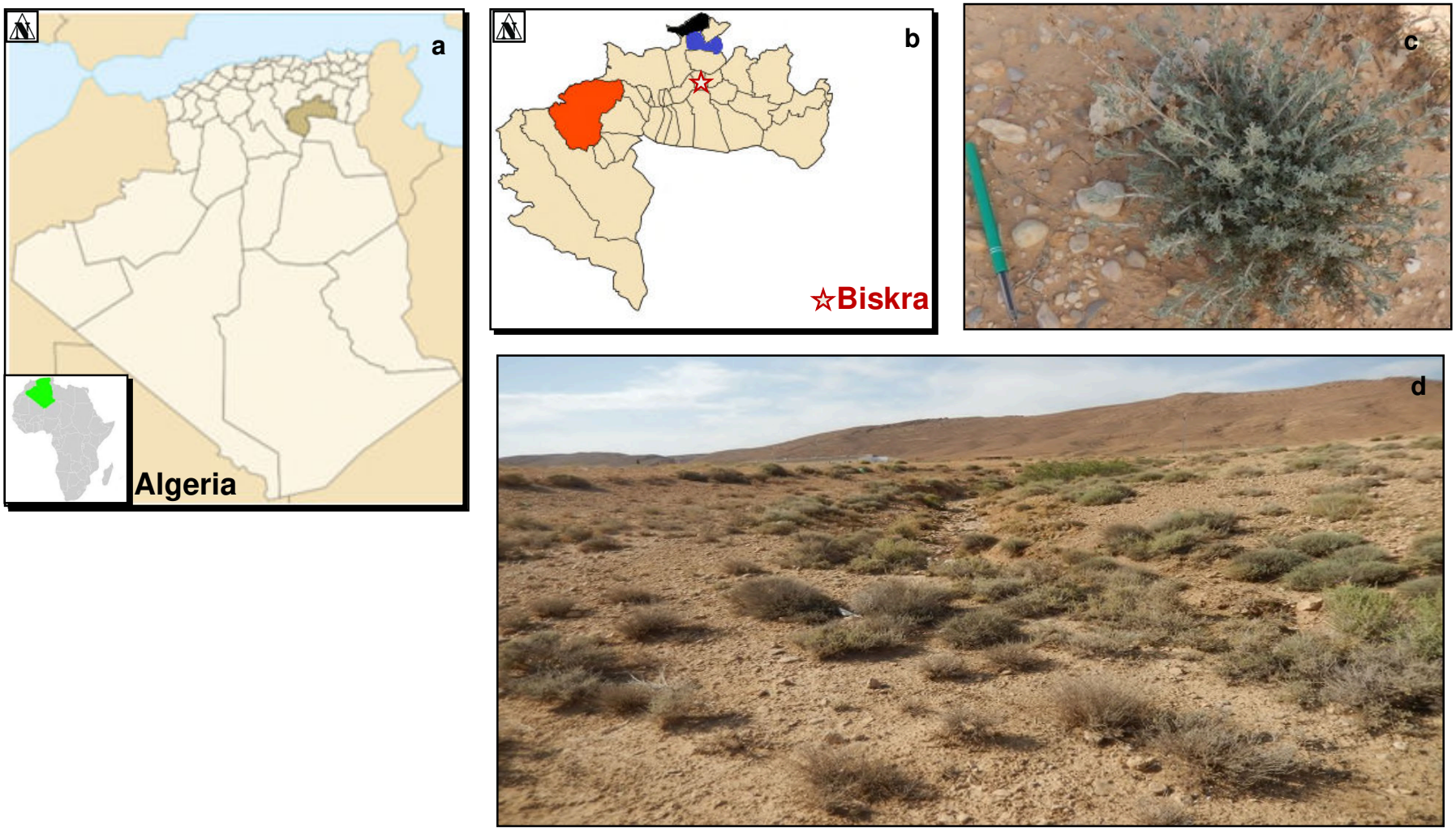

Fig. 2 Isolates of actinobacterial strains isolated from the rhizosphere of *Artemisia herba-alba* Asso. The isolates were grown on ISP2 plates for 14 days at 28±2 °C.

Fig. 3 Scanning electron microphotograph of strain *Streptomyces* sp. BKS30 after cultivation on ISP2 agar medium at 28±2 °C for 14 days, a photograph of the spores was taken by SEM. Bar 1 µm

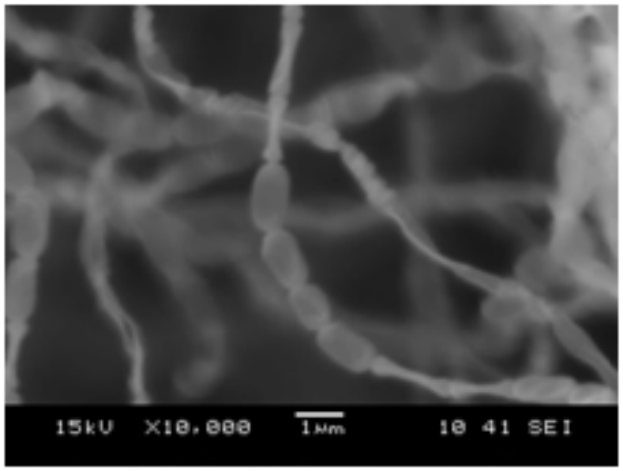

Fig. 4 Phylogenetic tree based on the 16S rRNA gene sequences, obtained by the neighbor-joining method analysis (Saitou and Nei 1987) for *Streptomyces* strains isolated from the rhizosphere of *Artemisia herba-alba* Asso and their closely related type strains by MEGA7 (Kumar et al. 2016). *Actinomadura rifamycini* was used as an outgroup for this study.

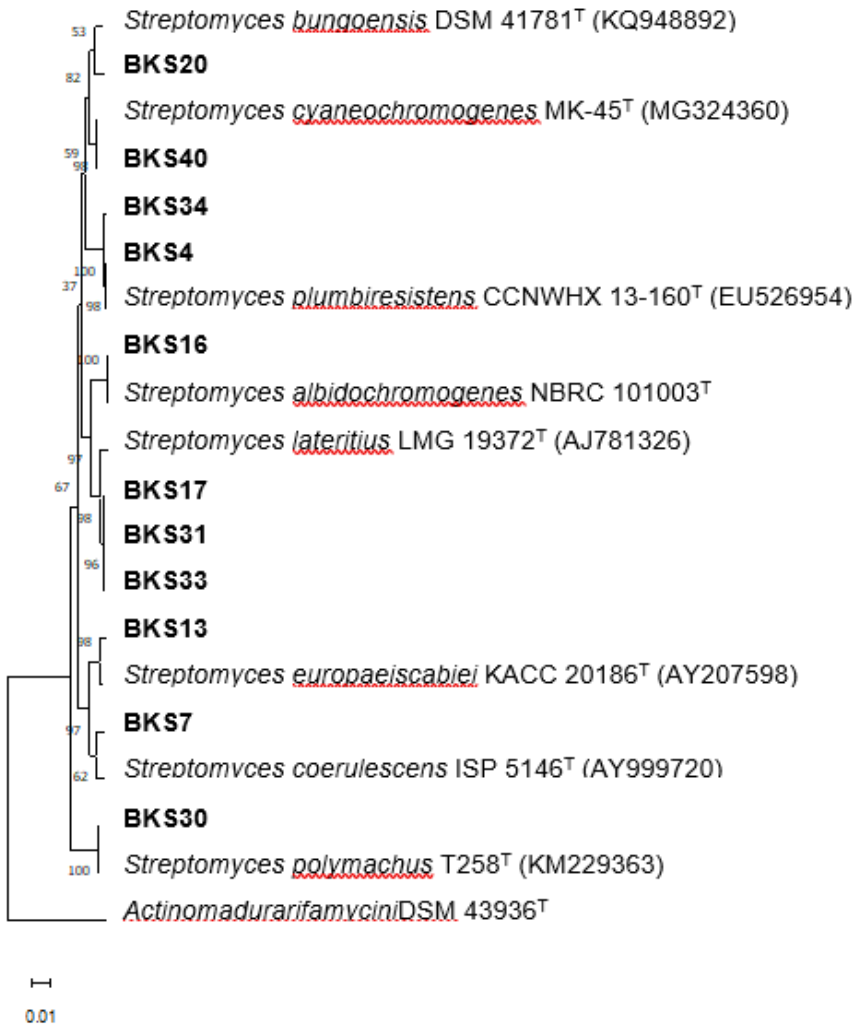

Fig. 5 Venn diagram showing the distribution of *Streptomyces* strains with their PGP traits **(a)** and hydrolytic enzymes production **(b)**. Characters are represented by different colored lines forming circles. Numbers falling within a colored circle represent that particular trait or traits.

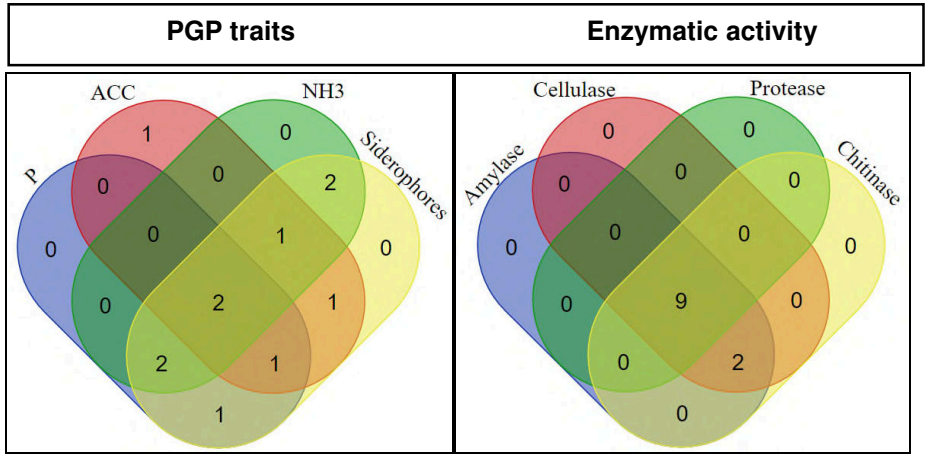

Fig. 6 Antifungal activity of *Streptomyces* sp. strain BKS30 against different fungi, **(a)**: *Aspergillus carbonarius* (M333), **(b)**: *A. westerdijkiae* (ATCC 3174), **(c)**: *A. brasiliensis* (ATCC 16404), **(d)**: *Penicillium expansum* (Pe), **(e)**: *Umbelopsis ramanniana* (NRRL 1829), **(f)**: *Fusarium graminearum* (Fg), **(g)**: *F. oxysporum* f. sp. *albedinis* (Foa), **(h)**: *F. oxysporum* f. sp. *lycopersici* (Fol) and **(i)**: *F. culmorum* (Fc)

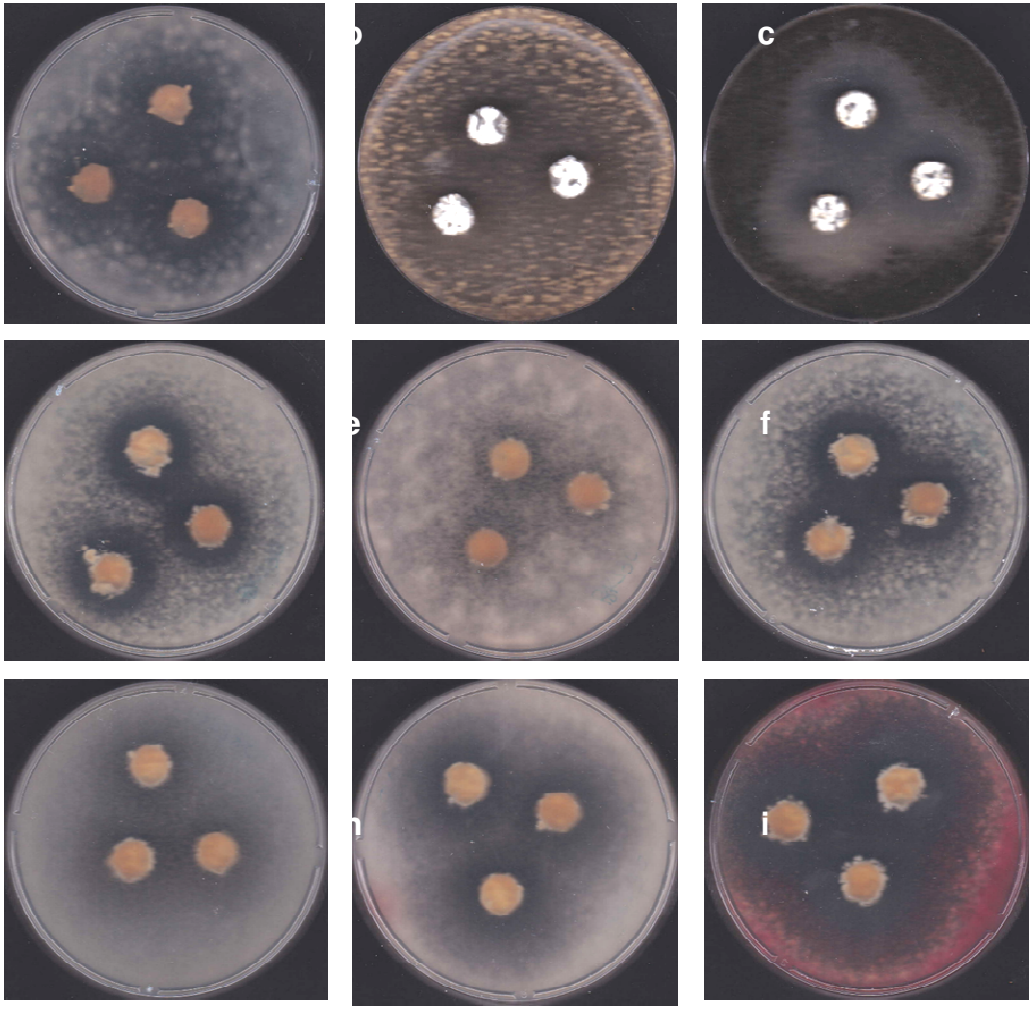

Table 1 Geographical locations, characteristics of the sampling sites and physico-chemical properties of soil samples from Biskra province

Site name	Latitude	Longitude	Altitude (m)	Soil characteristics						Bioclimatic stage
				pH (1/5)	Carbon (%)	Organic matter (%)	Cation Exchange Capacity (meq/100 g)	Electrical conductivity (mmhos/cm)	Texture*	
El Kantara	35. 23456	05. 69911	526.1	7.64±0.05^{b}	0.84±0.05^{c}	1.44±0.08^{c}	9.47±0.17^{c}	0.27±0.02^{a}	SL	Arid to Saharan
Chaiba	34. 84485	04. 89772	419.1	7.45±0.04^{a}	0.41±0.01^{a}	0.70±0.01^{a}	8.07±0.28^{a}	2.36±0.02^{b}	SL	
Djemorah	35. 07870	05. 75226	470.4	8.35±0.04^{c}	0.57±0.04^{b}	0.99±0.07^{b}	8.97±0.10^{b}	0.28±0.01^{a}	SL	

* Texture according to United States Department of Agriculture (USDA): L = loam; S = sandy; SL = sandy loam

Results are presented as means ± SD, values with different letters within the same column shows a significant difference (p<0.05) at 95% interval (one way ANOVA followed by Tukey's test)

Table 2 Morphological characteristics of actinobacterial isolates acquired from the rhizosphere of *Artemisia herba-alba* Asso

	N°	Isolate code	Medium	Growth rate	Aerial mycelium (AM)	Substrate mycelium (SM)	**Pigmentation**
El Kantara	1	BKS4	ISP1	++++	Medium yellow	Dark brown	Dark brown
			ISP2	++++	Pale grey	Dark brown	Dark brown
			ISP4	++	-	Brown	Dark brown
	2	BKS7	ISP1	+++	White to beige	Brown	Orange
			ISP2	++	White to beige	Brown to pale pink	Orange
			ISP4	++	-	Pale beige	Orange
	3	BKS13	ISP1	+++	White to blue	Brown	-
			ISP2	++	White	Light brown	Medium brown
			ISP4	+++	White to beige	Beige with pink borders	-
	4	BKS16	ISP1	++	Beige	Yellow	-
			ISP2	++++	Beige with very pale grayish borders	Medium yellow orange	-
			ISP4	++	Yellow grayish	Light beige brown	-
	5	BKS17	ISP1	+++	Beige	Medium brown	-
			ISP2	++++	Pale pink	Medium orange brown	Dark orange
			ISP4	++	Beige with pink borders	Light brown	-
Chaiba	6	BKS20	ISP1	+++	Grayish beige	Brown	Yellow
			ISP2	++++	Light pink and pale grayish pink	Dark orange brown	Yellow
			ISP4	+ to ++	Grayish green	Medium brown to yellow	Yellow
	7	BKS30	ISP1	+++	Beige white	Light brown	-
			ISP2	+++	White to non colored	Medium brown	-
			ISP4	++	Beige greyish	Light brown	-
	8	BKS31	ISP1	+++	White	Light beige	-
			ISP2	+++	White	Light beige brown	Medium brown
			ISP4	++ to +++	White	Light beige with light orange brown at borders	-
Djemorah	9	BKS33	ISP1	+++	Beige with light pink borders	Light brown	-
			ISP2	++ to +++	Beige with light pink borders	Medium brown orange	Medium brown orange
			ISP4	++	Beige with light pink borders	Medium beige brown	-
	10	BKS34	ISP1	++++	White with green	Dark brown	Dark brown
			ISP2	++++	White traces	Dark brown purple	Dark brown

		ISP4	++	White	Medium purple brown	Purple pink
11	BKS40	ISP1	++++	White with light blue	Brown	Brown
		ISP2	++++	Light blue to white purple	Dark brown	Brown
		ISP4	+ to ++	White blue	Light purple	Brown

-: no aerial mycelium or pigmentation, +: poor growth, ++: moderate growth, +++: good growth, ++++: very good growth

Table 3 Cultural characteristics of actinobacterial isolates acquired from the rhizosphere of *Artemisia herba-alba* Asso cultivated on ISP2 medium after 14 days at 28±2 °C

N°	Strain code	T °C (ISP2 agar medium)				pH (ISP2 agar medium)									NaCl concentration (%) of ISP2 agar medium									
		20	30	40	50	4	5	6	7	8	9	10	11	12	0	1	2	3	4	5	6	7	8	9	10
1	BKS4	+	++	+	-	+	+	++	++	++	++	+	-	-	++	++	++	++	++	+	+	+	-	-	-
2	BKS7	+	++	+	-	+	++	++	++	++	++	+	+	+	++	++	++	++	++	+	+	+	-	-	-
3	BKS13	+	++	+	-	+	++	++	++	++	++	+	+	-	+	+	+	+	+	+	-	-	-	-	-
4	BKS16	+	++	+	-	-	+	++	++	++	++	+	+	-	++	++	++	+	+	+	+	+	-	-	-
5	BKS17	+	++	+	-	++	++	++	++	++	++	++	+	+	++	++	++	++	++	++	++	+	+	+	+
6	BKS20	+	++	+	-	-	+	++	++	++	++	+	+	+	++	++	++	++	++	+	+	+	-	-	-
7	BKS30	+	++	+	-	-	+	+	++	++	++	++	+	-	++	++	++	++	+	-	-	-	-	-	-
8	BKS31	+	++	+	-	-	-	+	+	++	++	++	+	-	++	++	++	+	+	+	-	-	-	-	-
9	BKS33	+	++	+	-	-	-	+	++	++	++	++	-	-	++	++	++	++	+	+	+	-	-	-	-
10	BKS34	+	++	+	-	+	++	++	++	++	++	+	-	-	++	++	++	++	++	+	+	-	-	-	-
11	BKS40	+	++	+	-	-	+	++	++	++	++	+	-	-	+++	+	+	+	+	+	-	-	-	-	-

-: no growth, +: poor growth, ++: moderate growth, +++: good growth

Table 4 Biochemical characteristics of the strain *Streptomyces* sp. BKS30 isolated from the rhizosphere of *Artemisia herba-alba* Asso in Chaiba, Biskra (Algeria)

Properties	*Streptomyces* sp. strain BKS30	*Streptomyces polymachus* T258^T (Nguyen and Kim 2015)
Biochemical tests		
Starch hydrolysis	**+**	+
Gelatin hydrolysis	**+**	ND
Tyrosine hydrolysis	**+**	+
Nitrate reduction	**-**	-
H_2S production	**-**	-
Urease	**-**	ND
Catalase	**+**	ND
Carbon-source utilization		
D-Xylose	+	+
Rhamnose	+	+
D-Fructose	+	+
D-Raffinose	-	W
Myo-inositol	-	+
D-Mannitol	-	+
L-Arabinose	-	+
D-Arabinose	+	ND
D-Glucose	+	+
D-Galactose	+	ND
D-Trehalose	+	ND
D-Mannose	+	ND
Maltose	-	ND
Cellulose	+	-
Antibiotic sensitivity test		
Streptomycin (10 µg)	S	ND
Fosfomycin (50 µg)	R	ND
Neomycin (30 µg)	S	ND
Cefoperazone (30 µg)	R	ND
Penicillin G (6 µg),	R	ND
Erythromycin (15 µg)	S	ND
Spiramycin (100 µg)	S	ND
Rifampicin (30 µg)	S	ND
Fusidic acid (10 µg)	S	ND
Trimethoprim/sulfamethoxazole (cotrimoxazole: 1.25/23.75 µg)	R	ND

- : negative, + : positive, W: week, S: sensitive, R: resistant, ND: not determined

Table 5 GenBank accession numbers and 16S rDNA taxonomic affiliation of the *Streptomyces* associated with the rhizosphere soil of *Artemisia herba-alba* Asso

Strain code	GenBank accession number	Sequence length (pb)	Closest relative by EzTaxon	% sequence similarity
BKS4	OK263097	1450	*Streptomyces plumbiresistens* CCNWHX 13-160^T	100
BKS7	OK263096	1450	*Streptomyces coerulescens* ISP 5146^T	99.86
BKS13	OK263105	1448	*Streptomyces europaeiscabiei* KACC 20186^T	99.38
BKS16	OK263104	1448	*Streptomyces albidochromogenes* NBRC 101003^T	100
BKS17	OK263103	1448	*Streptomyces lateritius* LMG 19372^T	99.45
BKS20	OK263102	1448	*Streptomyces bungoensis* DSM 41781^T	98.90
BKS30	OL818328	1448	*Streptomyces polymachus* T258^T	98.95
BKS31	OK263101	1448	*Streptomyces lateritius* LMG 19372^T	99.38
BKS33	OK263100	1448	*Streptomyces lateritius* LMG 19372^T	99.38
BKS34	OK263099	1450	*Streptomyces plumbiresistens* CCNWHX 13-160^T	99.65
BKS40	OK263098	1448	*Streptomyces cyaneochromogenes* MK-45^T	99. 79

Table 6 Plant growth promotion features of *Streptomyces* strains isolated from the rhizosphere of *Artemisia herba-alba*

Strain code	PGP traits				Enzymatic activity			
	ACC deaminase	NH_3 production	Phosphate solubilization	Siderophores	Amylase	Cellulase	Protease	Chitinase
BKS4	-	+	+	+	+	+	+	+
BKS7	-	-	+	+	+	+	-	+
BKS13	-	+	-	+	+	+	+	+
BKS16	+	-	-	+	+	+	+	+
BKS17	+	-	+	+	+	+	+	+
BKS20	+	+	+	+	+	+	-	+
BKS30	-	+	+	+	+	+	+	+
BKS31	-	+	-	+	+	+	+	+
BKS33	+	+	-	+	+	+	+	+
BKS34	+	+	+	+	+	+	+	+
BKS40	+	-	-	-	+	+	+	+

-: negative, +: positive

Table 7 *In vitro* antifungal activity of the actinobacteria isolated from *Artemisia herba-alba* Asso rhizosphere by the disc diffusion method against test micro-fungi

N°	Strain code	Zone of growth inhibition (mm) against test micro-fungi								
		Ac	Aw	Ab	Pe	Ur	Fg	Foa	Fol	Fc
1	BKS4	-	-	-	14±1	-	14.67±0.58	-	-	-
2	BKS7	-	-	-	-	-	-	-	-	-
3	BKS13	-	-	-	-	-	-	-	-	-
4	BKS16	-	-	-	-	-	-	-	-	-
5	BKS17	-	-	-	-	-	-	-	-	-
6	BKS20	-	-	-	-	-	-	-	-	-
7	BKS30	29±1	14.66±0.58	28.33±1.53	24.76±0.58	13±1	27±1	15.67±0.58	22.67±1.15	35.33±1.15
8	BKS31	-	-	-	-	-	-	-	-	-
9	BKS33	-	-	-	-	-	-	-	-	-
10	BKS34	-	-	-	14.67±0.58	12±1	14.67±0.58	-	-	19.33±1.15
11	BKS40	25.33±0.58	20.33±0.58	24.33±0.58	27.33±1.15	11.66±0.58	25±1	12.33±1.15	15.66±1.15	29.67±0.58

Aspergillus carbonarius (M333) (Ac), *A. westerdijkiae* (ATCC 3174) (Aw), *A. brasiliensis* (ATCC 16404) (Ab), *Penicillium expansum* (Pe), *Umbelopsis ramanniana* (NRRL 1829) (Ur), *Fusarium graminearum* (Fg), *F. oxysporum* f. sp. *albedinis* (Foa), *F. oxysporum* f. sp. *lycopersici* (Fol), and *F. culmorum* (Fc). Zones of inhibition values are given as mean values ± SD (*n=3*) with the value of the disc (10mm) is included, -: no inhibition

Table 8 *In vitro* antimicrobial activity of the strain *Streptomyces* sp. BKS30 by the disc diffusion method on PDA medium

Test fungi	Zone of growth inhibition (mm) after 7 days of growth	
	Filtrate of strain BKS30	Butanolic extract of strain BKS30
Ac	15.33±0.58	18.00±1.00
Aw	17.33±0.58	22.33±0.58
Ab	15.67±0.58	16.33±0.58
Pe	15.33±0.58	14.67±0.58
Ur	15.67±0.58	13.67±0.58
Fg	15.33±0.58	15.67±1.54
Foa	15.67±1.54	22.33±0.58
Fol	25.33±0.58	27.00±0.00
Fc	30.33±0.58	26.67±0.58

Aspergillus carbonarius (M333) (Ac), *A. westerdijkiae* (ATCC 3174) (Aw), *A. brasiliensis* (ATCC 16404) (Ab), *Penicillium expansum* (Pe), *Umbelopsis ramanniana* (NRRL 1829) (Ur), *Fusarium graminearum* (Fg), *F. oxysporum* f. sp. *albedinis* (Foa), *F. oxysporum* f. sp. *lycopersici* (Fol), and *F. culmorum* (Fc)
Zones of inhibition values are given as mean values ± SD (*n=3*)

Table 9 Radical scavenging activity of the filtrate and butanolic extract of *Streptomyces* sp. BKS30 evaluated using DPPH and ABTS assays

	Concentration (µg/mL)	BHT	Filtrate of BKS30	Butanolic extract of BKS30
% Inhibition in DPPH assay	12.5	17.8 ± 0.6^{a}	57.25 ± 0.9^{b}	49.12 ± 0.9^{g}
	25	31.8 ± 1.3^{c}	56.68 ± 0.3^{d}	49.30 ± 0.9^{g}
	50	64.4 ± 0.4^{e}	58.01 ± 0.9^{f}	50.99 ± 0.7^{gh}
	100	73.55 ± 1.5^{g}	58.46 ± 0.3^{h}	54.64 ± 0.7^{h}
	200	88.66 ± 1.3^{i}	58.46 ± 0.7^{j}	63.006 ± 1.8^{ij}
	400	91.37 ± 0.06^{k}	58.46 ± 0.6^{l}	71.69 ± 1.8^{k}
	800	90.23 ± 0.1^{l}	58.46 ± 0.9^{m}	80.60 ± 1.12^{l}
	IC_{50}	**38.97±0.1**	**<12.5**	**40.02±5.02**
% Inhibition in ABTS assay	12.5	71.24 ± 5.1^{a}	47.49 ± 0.9^{b}	34.15 ± 1.09^{c}
	25	76.66 ± 1.5^{c}	49.93 ± 1^{c}	43.011 ± 3.3^{d}
	50	81.56 ± 0.2^{d}	57.64 ± 0.6^{e}	61.48 ± 0.5^{e}
	100	82.49 ± 0.7^{f}	65.63 ± 5.6^{g}	88.54 ± 2.1^{ij}
	200	88.16 ± 0.3^{h}	85.006 ± 0.6^{i}	94.26 ± 0.2^{k}
	400	90.69 ± 0.3^{j}	90.35 ± 0.5^{k}	94.37 ± 0.04^{k}
	800	94.33 ± 0.1^{l}	93.13 ± 0.1^{m}	93.89 ± 0.1^{jk}
	IC_{50}	**<12.5**	**22.86± 2.06**	**34.34±1.6**

IC_{50} (µg/mL): the concentration at which 50% is inhibited, BHT: butylated hydroxytoluene

Data represent mean ± SD ($n=3$). Means in each column followed by a different letter are significantly different ($p<0.05$) (one-way ANOVA followed by Tukey's test)