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Research Article

Antagonism and plant growth promoting traits of actinomycetes isolated from the rhizosphere of halophyte *Atriplex halimus* L.

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Abstract

Biocontrol is considered as an effective alternative to the application of agrochemicals, which are harmful to the environment, human, and animal health. In this study, twenty-six strains of actinomycetes were isolated from rhizospheric arid soil of the halophyte *Atriplex halimus* L. 'Guettaf' in Biskra province, Algeria. The six isolates that have inhibited at least three phytopathogenic fungi among the five tested (*Fusarium oxysporum*, *Alternaria alternata*, *Fusarium solani*, *Aspergillus flavus* and *Botrytis cinerea*) were selected, and have been tested *in vitro* against phytopathogenic bacteria (*Pectobacterium carotovorum* and *Streptomyces scabies*). They were also evaluated for their ability to hydrolyze phosphate, elaborate siderophores, produce indole-3-acetic acid (IAA), and to antagonize *S. scabies in vivo* (on radish seedlings). Based on the physicochemical analyses, soil samples were categorized as alkaline and extremely-saline. The antagonism results revealed varying antifungal potential among the selected isolates (Act11, Act16, Act17, Act18, Act23 and Act24), about 50% were able to inhibit the growth of *F. solani* and *A. flavus*, followed by 33.33% of those having antagonized *F. oxysporum,* while *A. alternata* was found to be the most sensitive. Only Act18 has antagonized *S. scabies in vitro* with an inhibition diameter zone of 19 ± 0.41 mm. However, *in vivo* trials showed that four isolates have counteracted *S. scabies*. Among them, Act18 and Act24 have significantly and positively affected the root surface ($P = 0.0062$) and prevented common scab. IAA was detected in all selected isolates with Act24 being the highest producer (77.45 μg mL^{-1}). Additionally, degradation ability revealed that four isolates were able to hydrolyze phosphate while three exhibited the capacity of elaborating siderophores. The six isolates were assigned to *Streptomyces* genius according to their morphological, physiological and chemotaxonomical traits. Based on this study, *Streptomyces* sp. Act18 and *Streptomyces* sp. Act24 that tolerate 7.5% NaCl concentration,

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prevent common scab and exhibit some plant growth attributes, may be considered as promising biocontrol agents to be applied in arid and saline soils.

Keywords: actinomycetes; antagonism; *Atriplex halimus*; PGPR; rhizospheric soil

Introduction

Field crops production is affected by plant diseases, fungi are the main cause of damage and quality degradation of harvested products. In this regard, a range of fungal species cause diseases that affect all parts of the plants, including *F. oxysporum* and *F. solani*, which are considered as the causal of plant wilting diseases (Sudiana *et al*., 2020). On the other hand, *B. cinerea* is one of the most widespread phytopathogenic fungi, it causes a gray mold disease that affects many crops (De Angelis *et al*., 2022). Diseases caused by *Alternaria* spp. are very common such as leaf blight, which attacks a variety of crops leading to a reduction in their marketability (Wang *et al*., 2020). *Aspergillus* species are the cause of post-harvest diseases on the crop, altering the features and nutritional value of the products (Rios-Muñiz and Evangelista-Martínez, 2022).

In addition to fungal damage, bacteria are responsible for 24% of the 14% overall yield damage engendered by phytopathogens, including *S. scabies*, *P. carotovorum*, *Agrobacterium tumefaciens*, *Erwinia amylovora* and *Xanthomonas* spp. (Aouar *et al*., 2020; Le *et al*., 2022). Common radish scab disease is caused by several *Streptomyces* spp., mainly induced by *S. scabies*, it does not affect yield, but it badly impacts marketability (Kang *et al*., 2022). Usually, the control of the phytopathogenic fungi is undertaken by agrochemicals products which have harmful consequences on the environment, human and animal health. Quite recently, considerable attention has been paid to the implementation of antagonistic microorganisms to suppress plant diseases engendered by phytopathogenic bacteria and fungi, with the purpose of avoiding the use of chemical control methods and overuse of fungicides (Suárez-Moreno *et al*., 2019). Actinobacteria and other bacterial genera such as *Pseudomonas* and *Bacillus* have been greatly investigated for their biocontrol potential (Vurukonda *et al*., 2021).

In the last few years, Actinobacteria has been gaining an important interest for their essential role in controlling plant pathogens against both bacteria and fungi (Lee *et al*., 2021). *Streptomyces* is considered as the remarkable genius of Actinobacteria. They have the potential to be employed as a biopesticide and to promote plant growth (Nonthakaew *et al*., 2022). They constitute a diversified group of Gram-positive, spore-forming bacteria with comparatively sizeable genomes and a high G/C percentage of more than 70%. *Streptomyces* represent about 80% of all antibiotic producers in comparison to other genera. They are also well recognized for their capacity to produce a variety of active substances with agricultural uses, including phytohormones (Aouar *et al*., 2020). IAA is the main plant hormone assigned to the auxins class; it plays an essential role in plant growth by enhancing cell proliferation, root formation and elongation (Vurukonda *et al*., 2021). As plant growth promoting bacteria (PGP), *Streptomyces* species display several PGP traits; they can directly improve plant growth by solubilization of minerals like phosphorus and siderophores secretion (Alibrandi *et al*., 2021).

Atriplex (Chenopodiaceae) is a halophyte genus with significant phenotypical diversity. It comprises numerous halophytes and is largely spread in arid and saline ecosystems around the world. It has been found that the rhizospheric soil of halophytes is a potential source of plant growth-promoting bacteria (Chaudhary *et al*., 2022). *Atriplex halimus* L. has been the subject of numerous recent studies around the world, by exploring its related endophytic, rhizospheric, and non-rhizopspheric microbial populations (Tahtamouni *et al*., 2016; Bona *et al*., 2021). From the Algerian arid rhizosphere of *Atriplex halimus*, Dif *et al*. (2022) isolated Gram-negative bacteria able to promote tomato growth through phosphate solubilization, phytohormones and siderophores production.

Arid and saline soils may be promising ecosystems to isolate new actinomycetes strains with antagonistic potential against soil-born phytopathogenic fungi and bacteria. Biocontrol agents from this ecosystem could be interesting as they are already adapted to the salinity and aridity conditions. The diversity of actinomycetes colonizing the arid-salty ecosystem of *Atriplex halimus* L. has not been extensively explored. To the author's knowledge, in Algeria few or no studies have been focused on the investigation of actinomycetes possibly PGPR and antagonistic, isolated from the rhizosphere of this halophyte, widely distributed in the Algerian arid regions and Sahara.

In this paper, we explore the isolation of actinomycetes from the rhizosphere of *Atriplex halimus* L. collected from the arid zone in Biskra province. Collected soils from the three rhizospheric sites of the halophyte were analyzed for some physico-chemical properties. Selected isolates were identified according to their morphological, physiological, and chemotaxonomical traits, and characterized for their ability to inhibit phytopathogenic agents (fungi and bacteria) *in vitro* and *S. scabies in vivo* (in growth poutches). Also, they were explored for their PGPR features: IAA production, siderophores release, and phosphate solubilization.

Materials and Methods

Study site, soil samples collection and their physico-chemical traits

Actinomycetes strains were isolated from the rhizospheric soil of three different sites of the halophyte *Atriplex halimus* L. 'Guettaf', collected from the region of Biskra, located in the South-Eastern of Algeria. This zone is distinguished by an annual index of aridity of De Martonne I = 0.34 that corresponds to a hyperarid climate (I < 5) (Lebourgeois and Piedallu 2005). The locations of the three explored sites are: site-1 /Chaiba (34°78'20"N, 5°04'97"E), site-2 /Chegga (34°45'19"N, 5°89'18"E) and site-3 /El Feidh, (34°46'15"N, 6°57'06"E). From each site, the root systems of *Atriplex halimus* L. (roots and soil surrounding the halophyte) were taken from the soil and gently scraped to separate rhizospheric soil, remove debris (roots and stones) and collect about 100-150 g. The soil samples were then filled into sterile container and transported to the laboratory for analysis (Aouar *et al*., 2020).

pHs were measured directly, on 1: 2.5 soil to water mixtures. Conductivity was measured in the limpid filtrate obtained by adding 1:5 distilled water to each soil sample (Khenaka *et al*., 2019). In order to calculate the humidity rate, ten grams of the soil were oven-dried for two days at 105 °C to reach a steady weight. Each dried sample was burnt for 16 h at 450 °C, the difference between the dry weight and the ash weight yields the organic matter content (Lee and Hwang, 2002). For each test, the experiments were repeated thrice.

Isolation of actinomycetes

One selective media GBA (Agar 20 g; starch 20 g; meat extract 5 g; peptone 10 g; glycerol 20 g; $CaCo_3$ 3 g) was used for actinomycetes isolation. The latter was mixed with 75 µg mL^{-1} amphotericin B to prevent filamentous fungi growth and 10 µg mL^{-1} of polymixin to inhibit Gram-negative bacteria. The sampled soil was dried in the laboratory at room temperature for 24 hours. For each sample, 1 g of soil was introduced into a tube containing 9 mL of sterile physiological water. Then, the tubes were vortexed for 4 to 5 minutes. These suspensions were considered as the stock solutions. Decimal dilutions were ranging from 10^{-1} to 10^{-6}. Afterward, an aliquot of 0.1 mL of 10^{-3} to 10^{-6} dilutions was poured on the surface of GBA plates and incubated for 21 days at 28 °C. Experiments were performed in triplicate.

The actinomycetes colonies were identified according to their macroscopic and microscopic aspects. Regular observations were conducted, during the incubation period, to determine the appearance of the isolated colonies. The examined macroscopic characters included: growth rate, spore mass color, as well as their size, shape, texture, and border (regular, irregular, jagged). Actinomycetes were recognised by their typical filamentous and powdery appearance. These observations were assisted by using optical microscope at 10x

magnification (Williams and Cross, 1971). Colonies exhibiting the macroscopic characteristics of actinomycetes were subjected to Gram staining. Then, strains were maintained on ISP2 slants at 4 °C, and stored at - 20 °C in 20% glycerol for long-term use (Aouar *et al*., 2012).

In vitro antagonism trials against pathogens

The phytopathogenic fungi: *B. cinerea*, used in this study was obtained from the Laboratory of Mycology at the Faculty of Sciences, University of Bejaia. *A. alternata* and *F. oxysporum* were acquired from the Laboratory of Mycology at the National Center for Biotechnology Research in Constantine (Algeria). Additionally, more phytopathogenic fungi (*F. solani* and *A. flavus*) and bacteria (*S. scabies* and *P. carotovorum*) were procured from the Laboratory of Biomolecules and Plant Improvement, University of Oum El Bouaghi, Algeria (Table 1).

Table 1. Origins and plants diseases of pathogenic fungi and bacteria

Strains	Pathologies	Host plants
Phytopathogenic fungi: *F. oxysporum*, *F. solani* *A. flavus* *A. alternata* *B. cinerea*	 Fusariosis Wilting, yellowing Brown spot, black rot Grey rot	*Solanum lycopersicum* L.
Phytopathogenic bacteria: *P. carotovorum* *S. scabies*	 Fire blight Common scab	 *Pyrus communis* *Solanum tuberosum* L., *Raphanus sativus* L.

In vitro antagonism assays involved a confrontation of actinomycetes isolates and plant pathogens: *A. alternata*, *F. oxysporum*, *F. solani*, *A. flavus* and *B. cinerea* on Patato Dextrose Agar (PDA). They were performed as reported by Aouar *et al*. (2020). Thus, actinomycete discs were taken and cultured on PDA plates for 4 days at 30 °C. Then, agar plugs were cut from fungi colonies (aged of 5 days), placed on the PDA plate at 3 cm distance from the actinomycetes disc and incubated at 25 °C for 5 days in the dark. Another fungus culture without actinomycete disc was realized to be served as control. After the incubation period, colonies diameters of essays and controls were scored. Each test was repeated in five replicates.

The antibacterial efficiency against *P. carotovorum* and *S. scabies* was assessed by the disc diffusion procedure (Aouar *et al*., 2020). The antagonistic strains were first grown on ISP2 at 30 °C for 7days. Discs from each antagonist were deposited on Muller-Hilton agar plates previously inoculated with the phytopathogenic bacteria. The prepared Petri dishes were stored at 4 °C for 4 h, before incubation at 30 °C for 24 h. Antibacterial activity was evaluated by measuring the diameter of the inhibition zone encircling the actinomycete discs in millimeters (mm). Each trial was performed in triplicates.

Morphological, physiological and chemotaxonomy characterization

Identification of actinomycetes was conducted using standard procedures of morphological, physiological, and chemotaxonomic characteristics, as described by Shirling and Gottlieb (1966) and Bergey's Manual of Systematic Bacteriology (Goodfellow *et al*., 2012). Morphological characterization was based on macroscopic description of cultures streaked on ISP2 medium for 21 days at 28 ± 2 °C. It includes colony morphology, produced pigments, color of spore mass, and reverse of the colony. Microscopic morphology was examined by observation of mycelium growth using cover-slip method. Thus, spore chains morphology was determined by direct microscopic examination (100x) (a minimum of 10 microscope fields should be examined). Their morphology may be verticillate or simple: rectiflexibles (RF), retinaculum apertum (RA) (open loops) and spirals (S).

The selected isolates were evaluated on ISP2 medium as a basal medium for three physiological features, the capacity to grow at different temperatures (15 °C, 25 °C, 30 °C, 40 °C and 50 °C), as well as the tolerance to NaCl concentrations (2.5, 5, 7.5 and 10%) and pHs (5, 7, 9, 11 and 13) by incubation at 28 ± 2 °C for 5 days. The rating scale for all these tests was estimated according to a range from 0 to 3, in the following way: 0 = no growth; 1 = slight growth; 2 = moderate growth and 3 = good growth (Sreevidya *et al*., 2016).

Diaminopimelic acid (DAP) isomers (*L*-DAP or *meso*-DAP) were revealed according to the Staneck and Roberts (1974) protocol through analysis of the total cell acid hydrolysate by thin layer chromatography. To carry out this test, 500 µL of 6N HCl was added to cryotubes containing glass beads and a seed loop of each isolate taken from ISP2 actinomycetes cultures. The cryotubes were vortexed for 6 minutes and incubated in a Thermoblock at 100 °C for 4 hours. Then, they were centrifuged at 4,000 rpm for 5 minutes and the supernatant was dried in a Thermoblock at 100 °C. Once dry, it was dissolved in 500 µL of sterile distilled water. This step was repeated twice. Then a volume of 3 µL of each sample and 1 µL of the control (1.9 mg of DAP standard in 10 mL of water) were applied to the TLC cellulose plate, which were placed in a TLC separation chamber containing 50 mL of the solvent system: Methanol-water-6 N HCl-pyridine (33.3: 11: 1.6: 4.1 v/v). After migration, the plates were allowed to dry and sprayed with ninhydrin (in 0.2% acetone w/v) and dried at 100 °C for 5-10 minutes before reading. The diaminopimelic acid stains were olive green in color turning to yellow and *L*-DAP migrates further than *meso*-DAP.

Analysis of the major predominant sugars in the cell wall of actinomycetes is of taxonomic importance was performed using thin layer chromatography. To conduct this analysis, 0.1 mL of 0.25 N HCl was added to each cryotube. Subsequently, from the pure cultures of each isolate on ISP2 medium, a seeding loop was added to each cryotube and autoclaved for 15 minutes at 121 °C. After cooling to room temperature, 1 µL of the standard preparation of the sugars (arabinose and galactose) and 3 µL of each cell wall extract were deposited on the cellulose TLC plate, which was introduced in a TLC chamber containing solvent system: n-butanol-H_2O-pyridine-toluene (10: 6: 6: 1 v/v) and allowed to migration until the front was 1 cm from the end of the plate. Subsequently, it was dried and sprayed in the gas extractor chamber, with the aniline and phthalate reagent (aniline 0.093 g, phtalic acid 0.166 g and water-saturated butanol 100 mL). Finally, it was incubated at 105 °C for 4 minutes. Aldo-pentoses and aldo-hexoses give a bright red and brown color, respectively (Hasegawa *et al.*, 1983; Goodfellow *et al.*, 2012).

Indole-3-acetic acid (IAA) detection and quantification

IAA production was assessed according to the procedure reported by Khenaka *et al*. (2019) and Benadjila *et al*. (2022) with slight modifications. For each isolate, a volume of 10 µL of spore suspension (10^8 UFC mL^{-1}) was inoculated on yeast malt extract medium (YM) supplemented with two concentrations of L-Tryptophan (L-Trp) (1.25 and 5 mM) and agitated on rotary shaker (125 rpm) for 4 days at 30 °C in darkness. The cultures were then centrifuged at 11,000 rpm for 15 min. A volume of 100 µL of each supernatant was mixed with 100 µL of Salkowski's reagent (1 mL of 0.5 M $FeCl_3$ in 50 mL of 35% $HClO_4$) and kept for 30 min in the dark. The deviation of the color to the pink showed the production of IAA. Its quantification was performed at 530 nm according to an OD standard curve. Each test was performed in five replicates.

Phosphate solubilization

Phosphate solubilization essay was conducted using Pikovskaya medium (PVK) complemented with $Ca_3 (PO_4)_2$ as described by Khenaka *et al*. (2019). After 8 days of incubation, the development of a clean circle around the colonies indicates phosphate solubilization. Halo diameters are calculated by deducting the colony diameter from the whole diameter. The phosphate dissolution capacity was determined positive as the halo diameter ≥ 5 mm. The experiment was repeated five times.

Siderophores secretion

The selected isolates were tested for their siderophores production potential by the universal CAS assay. Prior to undertaking the experiment, the glassware was washed with 3 mol L^{-1} hydrochloric acid to eliminate iron. This test was carried out using a modified procedure described by Arora and Verma (2017). CAS agar was obtained by adding 100 mL of CAS reagent to 900 mL of sterilized LB agar medium. The inoculated plates were incubated at 30 °C for 5-7 days. In addition, an un-inoculated plate was considered as control. The appearance of an orange halo around the colony was regarded as positive. Five repetitions were performed.

In vivo antagonistic trails towards S. scabies on radish seedlings

Experiment was carried out in growth pouches (125 X 75 mm, Mega International) with radish seedlings (*Raphanus sativus*) according Aouar *et al*. (2021). For inoculums preparation, 10 µL of spore stock (10^8 UFC mL^{-1}) of *S. scabies* and antagonists were grown in 50 mL of tryptic soy broth (TSB) under agitation for 48 h at 30 °C. After centrifugation, the obtained pellets were suspended in 5 volumes of fresh TSB. On the other hand, sterilized radish seeds were germinated on water plate agar in darkness at 25 °C for 48 h. In each pouch previously sterilised and filled with 2 mL saline, six seedlings were placed. Negative control consisted only of seedlings and saline solution; positive control was prepared by mixing 1 mL of the pathogen inoculum (*S. scabies*) and 1 mL of saline solution. Confrontation test consists of mixing 1 mL of each inoculum (antagonist and pathogen) at the same time, followed by the incubation in a growth chamber for 6 days at 21 °C and 68% humidity. After that, pouches have been emptied of their liquid and scanned, before being analysed with WhinRhizo software (V. 2002c) for measurement of the seedling's roots surface. Trials were carried out in five replicates.

Data analysis

Each experiment was carried out in replicates and data were expressed as mean ± standard deviation. The data of the physico-chemical analysis of the soil samples and the IAA production were analysed using Student's test. While a one-way analysis of variance (ANOVA) was performed on the antagonism measurements followed by Tukey's HSD post-hoc test for the antagonism against fungi and least significant difference (LSD) for the *in vivo* antagonism against *S. scabies* using SPSS software (Version 23). Results were considered statistically significant at the $p < 0.05$.

Results

Physico-chemical characterization of the soil samples and actinomycetes isolation

The pHs values are summarized in Table 2. Rhizospheric samples presented pHs that range between 8.03 and 8.28. The collected soil from El Feidh region presented the highest values of conductivity (5.54 ms cm^{-1}) compared with those of Chegga and Chaiba (3.19 and 4.65 ms cm^{-1}, respectively).

From the three samples, 26 actinomycetes were isolated. The isolation process was achieved following two to three consecutive inoculations until the pure actinomycete colonies were obtained. Overall, 12 isolates were isolated from Chegga's soil (46.15%), 9 isolates from Chaiba's soil (34.62%), while only 5 isolates were obtained from El Feidh's soil (19.23%).

Table 2. Physicochemical properties of the sampled soils and number of isolates

Sites	Soil characteristics				Isolates
	pH	Moisture content (%)	Total organic matter	EC ms cm^{-1}	
Site1/Chaiba	8.03 ± 0.03 b	12.60 ± 0.03 b	3.20 ± 0.01 b	4.65 ± 0.04 b	9 (34.62%)
Site2/Chegga	8.28 ± 0.02 a	11.80 ± 0.02 c	5.54 ± 0.02 a	3.19 ±0.02 c	12 (46.15%)
Site3/El Feidh	8.25 ± 0.04 a	19.23 ± 0.07 a	2.24 ±0.005 c	5.54 ± 0.03 a	5 (19.23%)

EC: Conductivity of aqueous extracts 1/5th. Different letters between sites denote significant differences (Student test, $p < 0.05$).

The twenty-six isolates were evaluated for their potential to inhibit *in vitro* plant pathogenic fungi. *F. oxysporum*, *F. solani*, *A. alternata*, *A. flavus* and *B. cinerea*. Confrontation has allowed the screening of six promoting isolates (Act11, Act16, Act17, Act18, Act23, and Act24) this isolates showed a potential inhibitory effect against the growth of at least three fungi. Among the previously mentioned isolates, 50% were able to inhibit the growth of *F. solani*, and *A. flavus*, while 33.33% antagonized *F. oxysporum*. Accordingly, these six isolates were selected for further experiments (Figure 1; Figure 2). It is worth mentioning that the origins of these isolates are as follows: isolates Act11, Act24 and Act17 are from Chaiba's sample, isolates Act16 and Act23 are from Chegga's soil, while Act18 is isolated from El Feidh's sample.

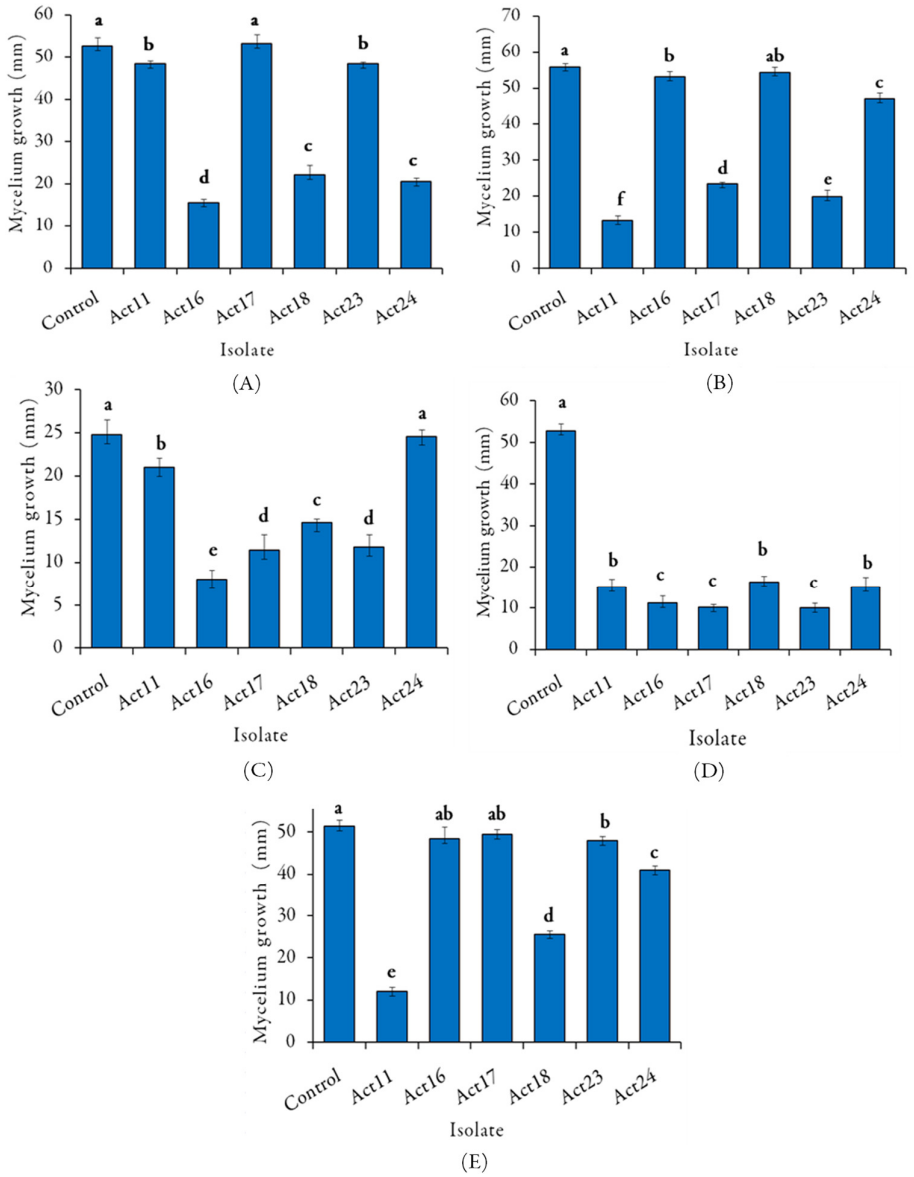

Figure 1. *In vitro* bioassay activity of actinomycetes isolates in mm against fungi; (A): *A. flavus*; (B): *F. solani*; (C): *B. cinerea*; (D): *A. alternata*; (E): *F. oxysporum*

Data are reported as the mean ± SD of five parallel measurements.

Values with different superscripts (a, b, c, d, or e) in the same column differ significantly (ANOVA test, $p < 0.05$).

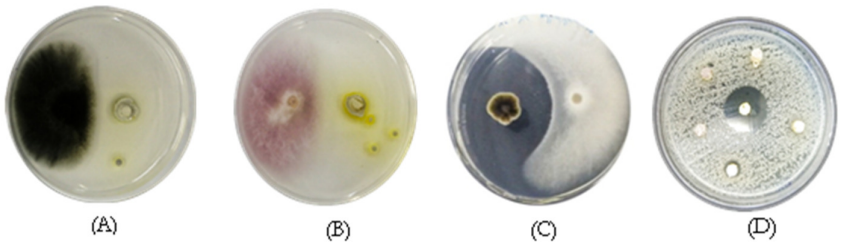

Figure 2. Antifungal and antibacterial activity of actinomycete isolates against phytopathogenic fungi and bacteria; (A): *A. alternata*; (B): *F. oxysporum*; (C): *F. solani*; (D): *P. carotovorum*

The *in vitro* assays performed against phytopathogenic bacteria revealed only two antagonistic isolates. Results are shown in Table 3. Interestingly, Isolate Act18 has inhibited both tested bacteria. Nevertheless, *S. scabies* was more resistant than *P. carotovorum*, which showed an area of 21 mm (Figure 2). However, Act24 has antagonized only *P. carotovorum* (20 mm).

Table 3. Inhibition zone diameters (mm) of the phytopathogenic bacteria by the actinomycetes

Pathogens	Diameter of inhibition zone (mm)					
	Act11	Act16	Act17	Act18	Act23	Act24
S. scabies	n.i	n.i	n.i	19.00 ± 0.41	n.i	n.i
P. carotovorum	n.i	n.i	n.i	21.66 ± 0.47	n.i	20.00± 0.82

n.i.: no inhibition.

Genera determination according to morphology, physiology and chemotaxonomy

All the isolates are fast growing, which suggest the lack of mycolic acids in their cell walls. The cell wall hydrolysate of all the isolates, revealed *L*-DAP isomer as the main component of the peptidoglycan. Furthermore, no characteristic sugar (galactose or arabinose) has been noticed in the whole-cell hydrolysate. Actinomycetes colonies are distinguished by their typical filamentous aspect. The morphological description of the six isolates is summarized in Table 4. Except for Act24, the colonies were dry and showed sporulated aerial mycelium, with varied pigmentation ranging from beige, purple, gray or pink. Similarly, the reverse of the colonies showed brown, beige, and yellow pigmentation. For Act18 and Act23 colonies, the aspects were rough in comparison with the smooth surface of the others. Among the six isolates, only Act11 has produced diffusible pigments. Moreover, all isolates were Gram-positive. Moreover, microscopic observation of the cover-slip cultures revealed long chains with three types: spiral (S), retinaculum apertum (RA) or rectiflexibile (RF). Physiological traits are shown in Table 5. The entire isolates grew between pH 5 and 11 with an optimum growth at pH 7. In contrast, no growth was noted at pH 13. Regarding the ability to develop at different temperatures, the five isolates were able to grow under a range of temperatures between 25 °C and 40 °C, none of the isolates show growth in high temperature (50 °C). Moreover, three isolates resisted well at 7.5% of NaCl concentration.

Table 4. Morphological and physiological characteristics of the selected isolates

Features/Isolates	Isolates					
	Act11	Act16	Act17	Act18	Act23	Act24
Morphological characteristics:						
Colony surface	Smooth	Smooth	Smooth	Rough	Rough	Smooth
Spore mass color	Beige	Purple	Gray	Beige	Pink	-
Colony reverse	Yellow	Beige	Brown	Beige	Beige	Brown
Diffusible pigments	Yellow	-	-	-	-	-
Chain morphology	RA	RA	RF	S/RA	RF	RA
Physiological characteristics						
NaCl						
2.5%	3	3	3	3	3	3
5%	3	3	3	3	3	3
7.5%	3	2	2	3	1	3
10%	0	0	0	0	0	0

Temperatures						
15 °C	1	2	2	1	3	1
25 °C	3	3	3	3	3	3
30 °C	3	3	3	3	3	3
40 °C	1	2	2	2	1	2
50 °C	0	0	0	0	0	0
pHs						
5	1	1	1	1	1	1
7	3	3	3	3	3	3
9	2	2	3	2	2	2
11	1	2	2	2	2	2
13	0	0	0	0	0	0

RA: Retinaculum apertum; 0 = no growth; 1 = slight growth; 2 = moderate growth and 3 = good growth.

Indole-3-acetic acid production

In this investigation, the overall measurements of IAA are summarized in Table 5. They ranged from 4.18 to 77.45 µg mL^{-1} and all isolates need L-Trp as precursor. Isolate Act24 was the best producer when the medium was supplemented with 5 mM of L-Trp. Furthermore, statistical analysis revealed that IAA production was positively correlated ($P < 0.05$) with L-Trp concentrations for isolates Act17, Act18, Act23 and Act24. Conversely to isolates Act11 and Act16, which did not show any correlation with L-Trp concentration ($P = 0.179$, $P = 0.986$, respectively).

Table 5. Results of PGPR traits

Isolate	IAA production (µg mL^{-1})		Phosphate solubilization (mm)	Siderophores production
	1.25 mM	5 mM		
Act11	19.82 ± 0.73 a	18.76 ± 0.57 a	-	-
Act16	14.37 ± 0.40 a	14.39 ± 1.70 a	13.67 ± 0.94	+
Act17	16.27 ± 0.27 b	29.55 ± 0.98 a	11.67 ± 0.47	-
Act18	4.18 ± 0.59 b	25.03 ± 3.07 a	12.33 ± 0.47	+
Act23	7.79 ± 0.38 b	57.85 ± 1.63 a	-	-
Act24	10.39 ± 0.36 b	77.45 ± 1.42 a	16 ± 0.82	+

Data are reported as the mean ± SD of five parallel measurements. For each isolate, values with different superscripts (a, b) differ significantly (Student test, $p < 0.05$)

Solubilization of phosphate and siderophores production

Among the six tested isolates, clear zones were scored for four isolates on PVK plates (Table 5). Diameters were ranging from 11.67 ± 0.47 to 16 ± 0.82 mm. The isolate Act24 was the most active. Relating to the CAS assay, three isolates (Act16, Act18 and Act24) have showed ability to produce siderophores (Table 5).

In vivo bioassay against S. scabies on radish seedling

Root surface were estimated by WinRhizo software, values were then analysed by ANOVA followed by LSD test, which provide results shown in Table 6. Root surface of negative and positive controls were 8.13 and 3.69 cm^2, respectively. From the six isolates, only Act18 and Act24 has inhibited significantly ($P = 0.0062$) the pathogen *in vivo*, this antagonistic activity was revealed by the increase of the root surface of the seedlings compared to the positive control in which seedlings were inoculates only by the *S. scabies* (Figure 3). Moreover, Ac18 and Act24 allowed the disappearance of common scab symptom, which was characterised by root

necrosis. Isolates Act16, Act17 were also able to inhibit *S. scabies in vivo* ($P < 0.05$) but with a reduced effect, values were 6.21 and 5.82 cm^2, respectively.

Table 6. Biocontrol of actinomycetes against *S. scabies* on radish seedlings

Essays	Root surface (cm^2)
Negative control	8.13 ± 1.15 a
Positive control (*S. scabies*)	3.69 ± 0.44 c
Co-inoculation of *S. scabies* with:	
Act18	8.05 ± 0.66 a
Act16	6.21 ± 1.26 ab
Act17	5.82 ± 1.44 b
Act11	3.91 ± 0.55 c
Act23	3,76 ± 0.13 c
Act24	7.88 ± 1.03 a

Data are reported as the mean ± SD of five parallel measurements. Values with different superscripts (a, b, c) in the same column differ significantly (LSD test, $p < 0.05$).

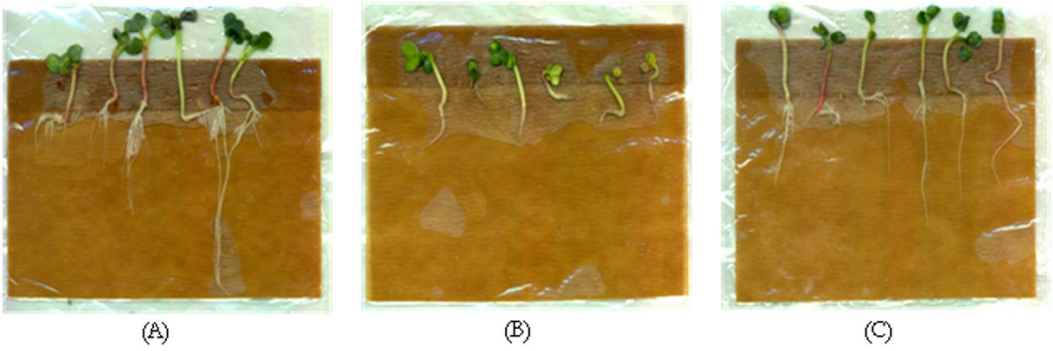

Figure 3. *In vivo* confrontation against *S. scabies* on radish seedling; (A): negative control; (B): positive control; (C): seedlings inoculation with both *S. scabies* and Act18

Discussion

It has been discovered that microorganisms were more numerous in the soil immediately adjacent to the roots (the rhizosphere) than in the loose soil distant from the roots (Ding *et al*., 2019). The major influences that the rhizospheric microorganisms have, nowadays, become an important tool to preserve plant health by an eco-friendly approach. It has been established that actinobacteria are the major microorganism in the rhizosphere and are the most ideal for controlling plant pathogens (Putrie *et al*., 2020). For these reasons, rhizospheric actinomycetes have been chosen to be explored for antagonists screening.

As indicated by Sreevidya *et al*. (2016) salinity and pH ranges in the rhizosphere are crucial criteria for the competitivity and persistence of microorganisms. Furthermore, resistance to high salinity and pH should be a criterion for the screening of microorganisms for biological control. In this regard, arid rhizospheric soil of the halophyte *Atriplex halimus* L. has been chosen for actinomycetes isolation. This plant is well adapted to arid soil; it was collected from Biskra region, which is characterized by a hyperarid climate. Regarding to physic-chemical results and referring to the pH and EC soil interpretation scale (Richards, 1954), soils samples are categorized as alkaline ($pH \geq 7$) and very salty (EC varies from 2.4 to 6). These results are confirmed by the hyperarid bioclimatic stage of the Biskra region based on the De Martonne aridity index. Similarly, the pHs

values are approximately close to those obtained by Khenaka *et al*. (2019), who studied samples from rhizospheric soil of the same region, and revealed that pH varied from 7.9 to 8. Furthermore, according to the scale of Lee and Hwang (2002), the percentages of organic matter of the three soils were very low (less than 4%). The Chegga soil gave the higher number of isolates, it is worth noting that this soil sample is characterized by a relatively low conductivity, compared to the others samples, furthermore, it has a low moisture rate. These findings are in accordance with those of Lee and Hwang (2002), which reported that the rate of organic matter is a very important factor; it directly affects the distribution of actinomycetes. Thus, this group of bacteria mostly colonizes soils rich in organic matter, but conversely, they prefer soils with low moisture content.

The twenty-six isolates displayed sensitivity towards at least three pathogens. *A. alternata* was recorded as the most sensitive towards all antagonistic isolates, followed by *B. cinerea*. Similarly, Wang *et al*. (2020) have demonstrated that *A. alternata* was also very sensitive towards *Streptomyces lydicus* M01. With regard to *F. oxysporum* and *B. cinerea*, Rios-Muñiz and Evangelista-Martínez (2022) have also reported the *in vitro* sensitivity of these pathogens when facing rhizospheric *Streptomyces* sp. CACIS-2 15CA isolate. From our findings, it appears that the two isolates *Streptomyces* sp. Act18 and *Streptomyces* sp. Act24 are of interest, since they showed both antibacterial and antifungal activities, so they have a broad spectrum, especially Act18 that has inhibited Gram-negative and Gram-positive bacteria in addition to fungi. Similarly, the study conducted by Le *et al*. (2022) has revealed that *Streptomyces* sp. AN090126, isolated from agricultural soil in Korea, showed a broad-spectrum against various phytopathogenic bacteria and fungi. Our results are promising, because according to Lee and Hwang (2002), in most cases antagonists with *in vitro* antifungal activity are also active *in vivo*.

Following the recommendations of Goodfellow *et al*. (2012), the presence of the *L*-DAP isomer and the lack of characteristic sugars suggest the presence of the parietal I chemotype, which is typical of *Streptomyces* and related genera. All isolates presented the characteristic odor of wet soil, typical of actinomycetes, especially of the genus *Streptomyces*, as specified by other studies. Considering spore mass color, the isolates could be classified into four actinomycetes series: beige, gray, purple and pink. In general, microscopic morphology showed un-fragmented vegetative mycelium, abundant aerial mycelium, and well-developed bearing long chains of spores either RA, RF or S type. According to the descriptions provided by Bergey's Manual of Systematic bacteriology (Goodfellow *et al*., 2012), this being a morphological characteristic of the genus *Streptomyces*. Thereby, the combination of macroscopic and microscopic morphology, and the parietal chemotype type I allow us to assign these isolates to the genus *Streptomyces*. In actinomycetes group, chemotaxonomical, morphological, and physiological characters are of interest, they may allow the determination of the genus. Similar studies, relying on such features to identify *Streptomyces* genus, have been already reported by several researchers (Qadir and Atalan, 2019; Aouar *et al*., 2020). In the present investigation, all isolates were assigned to *Streptomyces* genus, which is compatible with earlier studies that reported the prevalence of the streptomycetes among soil-borne actinomycetes. In addition, rhizospheric streptomycetes display an antagonistic potential and produce antifungal substances due to the high input of organic matter from plant root exudates (Lee and Hwang, 2002).

All the isolates were able to grow in a pH ranged from 5 to 11, in addition to being able to tolerate a temperature up to 40 °C and to have a tolerance to NaCl till 7.5%. These results were expected since these isolates come from an arid soil. Interestingly, Act18 and Act24 showed broad-spectrum activity and exhibit a good growth at 7.5% NaCl. These strains are originated from soils with the highest conductivity values. It has been demonstrated in several studies that actinomycetes comprise species that are resistant to high NaCl levels (Dif *et al*., 2022). Considering this capacity, Sreevidya *et al*. (2016) have demonstrated the capacity of rhizospheric *Streptomyces* isolates to grow at 8% NaCl and exclusively the isolate SAI-13 to tolerate a concentration of 10% NaCl. Such characteristics can be considered as advantages for a potential biocontrol agent.

IAA belongs to the group of phytohormones, and it is commonly regarded as the main native auxin. It is a signaling molecule involved in cell division and root extension (Vurukonda *et al*., 2021). Moreover, several studies have reported the capacity of rhizospheric *Streptomyces* to synthesize IAA and other indolic derivates in liquid media (Khenaka *et al*., 2019; Oleńska *et al*., 2020; Ali *et al*., 2021). In our investigation, all isolates demonstrate the ability to produce IAA in medium amended with L-Trp, which is certainly synthesized via L-Trp-dependent pathway. The measured IAA concentrations are greater than those obtained by Ashwini *et al*. (2018) and Djebaili *et al*. (2020) that ranged between 6.88-20.22 μg mL^{-1} and 7.44-21.4 μg mL^{-1}, respectively. All tested isolates were positive for IAA production; similar high percentage (100%) has also been obtained in other studies (Sreevidya *et al*., 2016; Aouar *et al*., 2020). However, Djebaili *et al*. (2020) have scored a lower rate of 64%. Hence, it seems that IAA production is an attribute widely encountered among rhizospheric actinomycetes.

Phosphorus is an extremely important element for plant growth. All over the world, soils are enriched with inorganic P in the form of chemical fertilizers to assist crops production. Nerveless, its overuse deteriorates soil quality. Thus, the employment of phosphate-solubilizing microorganisms enhances the soil fertility by converting insoluble P to soluble P (Qin *et al*., 2015). It has been reported that actinomycetes phosphate-solubilizing ability was less explored compared to the other PGPR traits (Sudiana *et al*., 2020). Phosphate solubilization assess revealed that among the six tested isolates, four (67%) were able to dissolve P and exhibit clear zones surrounding the colonies. The obtained results are compatible with those of Djebaili *et al*. (2020) which recorded 79% of positive strains.

Current research has focused on siderophores production by actinomycetes. The detection of siderophores production by three isolates was expected for such stains originating from arid soils. It has been shown that *Streptomyces* sp. from several crop rhizospheric soils have this capacity (Khenaka *et al*., 2019; Aouar *et al*., 2020; Warrad *et al*., 2020). The ability of biocontrol agents to secrete siderophores in appropriate quantities may limit the Fe^{3+} accessibility to the pathogen and may induce plant resistance (Rana *et al*., 2019). Our result revealed the siderophores elaboration by three isolates. These findings are of interest, because they have established a positive correlation between plant development and siderophores secretion, and as a result this factor must be included in the selection process of PGP agents (Sreevidya *et al*., 2016; Ebrahimi-Zarandi *et al*., 2021; Dif *et al*., 2022).

Among actinomycetes genera, *Streptomyces* is recognized as saprophytic and the most important. Species associated to this genus are recognized for their capacity to produce a large number of different secondary metabolites. Nevertheless, some species are phytopathogenic and engender plant disease; *S. scabies* is the type of strains of this group. It provokes common scab, which is a root necrosis of plants such as potato and radish due to the inhibition of cellulose synthesis (Beaudoin *et al*., 2021). In this investigation, we tested the ability of the six isolates to oppose *in vivo* against *S. scabies* and to prevent root necrosis. Results showed that the comparison of the root growth of the negative and the positive controls, revealed a significant decrease ($p < 0.05$) which indicated a root necrosis induced by *S. scabies*. Such findings were reported by previous studies that have carried out on radish seedlings by Legault *et al*. (2011) and Aouar *et al*. (2020) and Aouar *et al*. (2021). However, the co-inoculation of antagonistic isolates Act18, Act24, Act16, and Act17 with the pathogen contributes to the re-appearance of the roots and the disappearance of the symptoms of common scab (more or less significantly). Interestingly, the results revealed that Act18 that had antagonized *S. scabies in vitro*, affected significantly and positively the root surface. The findings were not expected for Act16, Act17 and Act24 since they did not show antagonistic activity *in vitro*, it is possible that they acted by other mechanisms rather than antibiosis, especially that Act16 and Act24 were capable to produce siderophores and solubilize phosphate or it could involve other mechanisms. Laboratory experiments conducted by Suárez-Moreno *et al*. (2019) revealed that *Streptomyces* strains carry several important features for biocontrol, which have also been related to plant-growth promotion. Moreover, according to Parasuraman *et al*. (2022) *in vivo* antagonism may

also involve the induction of the plant defence, through direct and indirect mechanisms. These mechanisms include solubilization of plant nutrients, such as iron and phosphorus, nitrogen fixation and production of various plant hormones such as auxins, cytokines, and ethylene or reducing the harmful effects of plant pathogens by producing antibiotics and siderophores.

Conclusions

The investigation that assesses the potential of six *Streptomyces* sp. strains isolated from the rhizosphere soil of *Atriplex halimus* L. 'Guettaf' for the *in vitro* antagonism against some phytopathogenic fungi and bacteria and the exhibition of some PGPR attributes, leads to the screening of two interesting strains: *Streptomyces* sp. Act18 and *Streptomces* sp. Act24, showing efficient growth under physiological conditions of high temperature, pH and NaCl. Moreover, they revealed a broad spectrum of the *in vitro* antagonistic activity when facing to plant pathogens. In addition to their potential to counteract *S. scabies in vivo*, by preventing common scab and enhancing roots development of the radish seedlings. These abilities probably involved one or several mechanisms, especially since *Streptmyces* are known to produce substrates of agrochemical interest. Interestingly, these two strains displayed some PGPR traits: IAA, siderophores production and phosphate solubilization. Based on the attributes mentioned above, the use of such strains in arid and saline soils may represent a sustainable solution to protect crops and enhance agriculture rather than agrochemical products. However, they need more advanced evaluation under field conditions to be proposed as biocontrol agents.

Authors' Contributions

Conceptualization: LA; Funding acquisition: YN, AZ; Resources: MCB, MD; Software: LA, IB, AZ, MCB and MD; Supervision: LA; Validation: IB, LA, AZ; Writing: IB and LA; Review and editing LA, IB.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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