ANTIMICROBIAL POTENTIAL AND PLANT GROWTH-PROMOTING FEATURES OF *Streptomyces* sp. STP-X ISOLATED FROM ALGERIAN SOIL

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Abstract. In this study, a strain of actinobacteria designed Stp-x was isolated from a soil sample obtained from the Tassala region of the Sidi Bel Abbès province (northern Algeria). The strain was characterized morphologically and genetically. According to morphology and 16S rRNA gene analysis, it was classified as *Streptomyces* and has shared high sequence similarity (98.82%) with *Streptomyces pilosus* NBRC 12807^T. Then, the strain Stp-x was subjected to *in vitro* screening for antimicrobial activity, plant growth promoting (PGP) traits, and hydrolytic enzymes production. The antibacterial activity was tested against *Staphylococcus aureus* ATCC 44300, *Bacillus subtilis* ATCC 6633 and *Pseudomonas aeruginosa* CIP A22 and the highest activity was obtained against *S. aureus*, resulting in an inhibition zone of 45 mm. Also, moderate activity was observed toward target fungi: *Aspergillus niger* OT304, *A. flavus* NRRL 3251, *A. carbonarius* M333, *Fusarium culmorum* NRRL1829 and *Umbelopsis ramanniana* NRRL 1829. Whereas, no activity was detected against the yeast *Candida albicans* IPA 200. Regarding PGP characteristics, the strain was positive for IAA, siderophores and ammonia production, phosphate solubilization and nitrogen fixation. Whereas, it was also able to produce extracellular enzymes: cellulase, amylase, protease, gelatinase, chitinase and lipase. This study showed the importance of the strain Stp-x in antimicrobial molecules production and plant growth promotion.

Key words: 16S rRNA; antimicrobial activity; hydrolytic enzymes; PGP traits; *Streptomyces*.

INTRODUCTION

Actinobacteria is a large phylum within the bacterial domain, widely distributed in both terrestrial and aquatic ecosystems. These Gram-positive bacteria are distinguished by having a high G+C ratio in their genomes [5]. Most of them are aerobic. Actinobacteria are a numerous and extensively dispersed group of soil bacteria, representing between 10 and 50% of the soil microflora population and the source of around two-thirds of naturally occurring antibiotics [24]. The most dominant genus of the actinobacteria phylum is *Streptomyces*, which belongs to the family *Streptomycetaceae* and the order *Streptomycetales* [11].

Streptomyces is an aerobic filamentous bacteri with a GC content of 69-78% and displays a wide range of morphological features [25]. *Streptomyces* is one of the most relevant genera in biotechnology, in which this genus alone accounts for approximately 75% of all bioactive compounds, possessing antiviral, antifungal, anticancer, immunosuppressive and antibiotic properties [11, 33]. The diversity of their metabolism has given them the ability to colonize different habitats such as soils, composts, plants, rivers, lakes and marine ecosystems [11].

Another characteristic of this genus is the released of numerous enzymes that break down insoluble organic polymers, including chitin, cellulose and xylan into substitute sugars that may be absorbed and used as sources of carbon and energy [34]. Additionally, a plethora of studies have emphasized the many advantages of *Streptomyces* species as a biocontrol agent by suppressing plant disease and promoting plant growth (PGPS) [1, 30, 34].

Disease suppression occurs when pathogens and antagonistic microorganisms struggle for resources and space within and around the host plant [26]. The antogonist bacteria use a variety of strategies to inhibit the pathogens: directly through antibiotics and cell wall-degrading enzymes production, and parasitism; or indirectly by induction of host resistance. Plant growth promotion occurs through a variety of mechanisms, including nitrogen fixation, solubilization of mineral phosphate, reduction of stress by production of the enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) deaminases and synthese of siderophores, ammonia, hydrocyanic acid and phytohormones [1, 30, 34].

The main objectives of the present study were to isolate and identify an isolate of actinobacteria from Algerian soil, then to assess its antimicrobial potential and its plant growth promoting properties (PGP) *in vitro*.

MATERIALS AND METHODS

Sampling and strain isolation

Strain Stp-x was isolated from a soil sample obtained from the Tassala region of the Sidi Bel Abbès province, in northern Algeria (35°14′20.98″N, 0°47′57.02″O). The sample was placed in sterile polyethylene bags, closed tightly and stored at 4°C until use.

The soil was then serially diluted up to 10^{-4} and 0.1 mL of this dilutions was applied to the chitin-vitamins-B agar medium (CH–V) surface [15], supplemented with nalidixic acid (10 µg/mL) and cycloheximide (50 µg/mL) to prevent the growth of unwanted bacteria and fungus, respectively.

After incubation at 30°C for up to two weeks, an isolated colony named Stp-x was picked up and

purified on International *Streptomyces* Project (ISP) medium 2 [32] to obtain a pure culture and then preserved at 4°C.

Morphological, cultural and physiological properties

Cultural and micro-morphological characteristics of the strain Stp-x were explored on ISP2 and ISP4 media after 10 days of incubation at 28°C, based on substrate and aerial mycelium color and pigmentation [32]. Using the same culture media, spores and mycelia were studied under light microscopy (B1 series; Motic). Carbohydrate utilization was determined according to the methods of Gordon *et al*. [14].

Phylogeny based on 16S rRNA gene

For a phylogeny analysis of the strain Stp-x based on the 16S rRNA gene, actinobacterial genomic DNA was extracted according to the method of Liu *et al*. [20]. Then, the 16S rRNA gene was amplified from total DNA by PCR amplification using a Eurogentec kit and primer pair 10-30F (5′-GAGTTTGATCCTGGCTCA-3′) and 1500R (5′-AGAAAGGAGGTGATCCAGCC-3′) [19].

Parameters for thermal cycling were as follows: initial denaturation of the target DNA at 95°C for 5 min, followed by 37 cycles of 95°C for 1 min, annealing at 55°C for 2 min, and extension at 72°C for 3 min, and final extension at 72°C for 10 min.

Purified PCR products were sequenced and the obtained sequence was blast compared against the available sequences of validly described taxa available in the EzBioCloud server (https://eztaxon-e.ezbiocloud.net/) [35]. Phylogenetic analysis was conducted using MEGA 7.0 software [18]. The phylogenetic trees were constructed using the neighbor-joining method and the Kimura 2-parameter model using 1000 replicates.

***In vitro* antimicrobial activity assay**

The agar plug diffusion technique was used to assess the strain Stp-x's potential antimicrobial activity toward a range of pathogenic microorganisms. The pathogenic microorganisms used were obtained from LBSM (Laboratoire de Biologie des Systèmes Microbiens, Algiers), including Gram-positive bacteria (*Staphylococcus aureus* ATCC 44300 and *Bacillus subtilis* ATCC 6633), Gram-negative bacteria (*Escherichia coli* 10536, *Pseudomonas aeruginosa* CIP A22 and *Listeria monocytogenes* CIP 82110), yeast (*Candida albicans* IPA 200) and filamentous fungi (*Aspergillus niger* OT304, *Aspergillus flavus* NRRL 3251, *Aspergillus carbonarius* M333, *Fusarium culmorum* NRRL 1829 and *Umbelopsis ramanniana* NRRL 1829).

Initially, the strain Stp-x was cultured on ISP2 medium for 10 days and incubated at 30°C. Then, 8 mm diameter of actinobacterial agar cylinders were placed on ISP2 or PDA medium (12 g/L) seeded in prior with target pathogenic bacteria or fungi, respectively.

Finally, the antimicrobial activity was checked by measuring the diameter of the clear zone of growth inhibition after incubation at 30°C for 24 h and 48 h for bacteria and fungi, respectively.

Determination of plant growth promoting (PGP) traits

Indole Acetic Acid (IAA) production

IAA production by the strain was done by culturing the strain in yeast extract–tryptone broth (YT) containing 1 mg/mL of L-tryptophan, for five days at 30°C and 250 rpm on a rotary shaker. After centrifuging the culture for 15 minutes at 4000 *g*, IAA was extracted using ethyl acetate (v/v) from the culture supernatant. After evaporation of the extracted fractions dissolved in 1 mL of methanol, it was analyzed by Agilent 1260 HPLC (UV detection at 280 nm). The formation of IAA was emphasized using standard IAA properties (peak retention duration and UV spectrum) [19].

Siderophore production

To assess the capacity of the actinobacterial strain to produce siderophores, a plug of the strain culture (5 mm in diameter, cultivated on ISP2 medium at 30°C for 10 days) was inoculated on Chrome Azurol S (CAS) medium. After five days at 30°C, siderophore activity was evident by the formation of a yellow to orange halo encircling the actinobacteria colonies [2].

Phosphate Solubilization

The actinobacterial strain's ability to solubilize a phosphate was evaluated by incubating a culture plug of actinobacteria on Pikovskaya agar plates (PVK) with 5 g/L of $Ca_3(PO_4)_2$ as an insoluble phosphate source. After 10 days at 30°C, a distinct halo was visible around the plug, which was regarded as positive for solubilizing [2].

Ammonia production

The production of ammonia was measured by introducing an actinobacteria into a tube holding 10 mL of peptone water. The tube was subsequently cultured for 15 days at 30°C. A positive test for NH_3 generation was shown by the formation of a brown to yellow hue in the tube following the addition of 0.5 mL of Nessler's reagent [9].

N_2 fixation

Nitrogen fixation was examined by placing a plug from an actinobacterial culture on a semi-solid N-free (NFb) medium. After 10 days of incubation at 30°C, bacterial growth was seen, which was interpreted as proof of atmospheric nitrogen fixation [28].

Production of lytic enzymes

Cellulase

Cellulase was measured by inoculation of the actinobacterial strain on carboxymethyl cellulose (CMC) agar medium. Following ten days of incubation at 30°C, the plates were flooded with 1% congo red dye (w/v) and stained for fifteen minutes with a

solution of 1 N HCl to confirm the cellulose breakdown. Cellulose hydrolysis was demonstrated by the sight of a transparent halo encircling the bacterial colonies cultured on CMC agar media [27].

Amylase

The actinobacterial strain was inoculated onto 2% soluble starch agar medium to measure the amount of amylase. After 10 days of incubation at 30°C, plates were first flooded with Lugol's iodine for 3-5 min then drained. The colonies on the plate showed signs of a visible halo, indicating positive amylase production [27].

Protease

The actinobacterial strain was inoculated on skim milk agar medium to determine protease production. The existence of distinct zones surrounding the colony on the skim milk agar medium after 10 days of incubation at 30°C indicated protease activity [10].

Gelatinase

By inoculating the actinobacterial strain on nutritional gelatin (15% soluble) agar medium, the amount of gelatinase production was measured. After adding the Frazier solution, the presence of clear zones surrounding the gelatinase-positive colonies showed the hydrolysis of the gelatin [4].

Chitinase

In order to analyze chitinase production, an actinobacterial strain was inoculated into chitin agar media. Actinobacterial growth was seen after 10 days of incubation at 30°C, which was taken as evidence of chitinase production [15].

Lipase

The actinobacterial strain was cultivated on rhodamine olive oil agar medium in order to evaluate the production of lipase. Once an orange fluorescent halo around actinobacterial colonies was observed under UV light as fluorescent, lipase activity was found [16].

RESULTS

Morphological and physiological study

A preliminary investigation based on morphological and physiological studies was done to classify the strain Stp-x at the genus level. This strain grew well on ISP2 medium and formed yellow lemon aerial mycelium. While it grew very low on the ISP4 medium and did not form aerial mycelium on this medium. The substrate mycelium was also yellow lemon on the ISP2 and non colored on the ISP4 medium. No diffusible pigment was produced on the two media tested. The strain formed short to medium zigzag aerial mycelium and fragmented substrate mycelium on ISP2 medium.

The strain Stp-x was able to utilize D-raffinose, D-galactose, D-arabinose, D-sorbitol, maltose, D-xylose, D-tréhalose, D-mannitol, D-arabitol and D-lactose as sole carbon source.

16S rRNA gene sequence analysis

The resultant 16S rRNA gene sequence of the strain has been deposited in the GenBank database under accession number PP275146 and compared against corresponding sequences of the type strains of the most nearly related species retrieved from the EzBiocloud server [35]. Phylogenetic trees were inferred using neighbor-joining algorithm.

According to the phylogenetic analysis, strain Stp-x belonged to the genus *Streptomyces* and had the greatest 16S rRNA gene sequence identity value with *Streptomyces pilosus* NBRC 12807^T with a similarity of 98.82%. The phylogenetic tree (Fig. 1) shows that strain Stp-x formed a distinct phylogenetic line from the cluster containing the type strains of the *Streptomyces* species shown in Table 1.

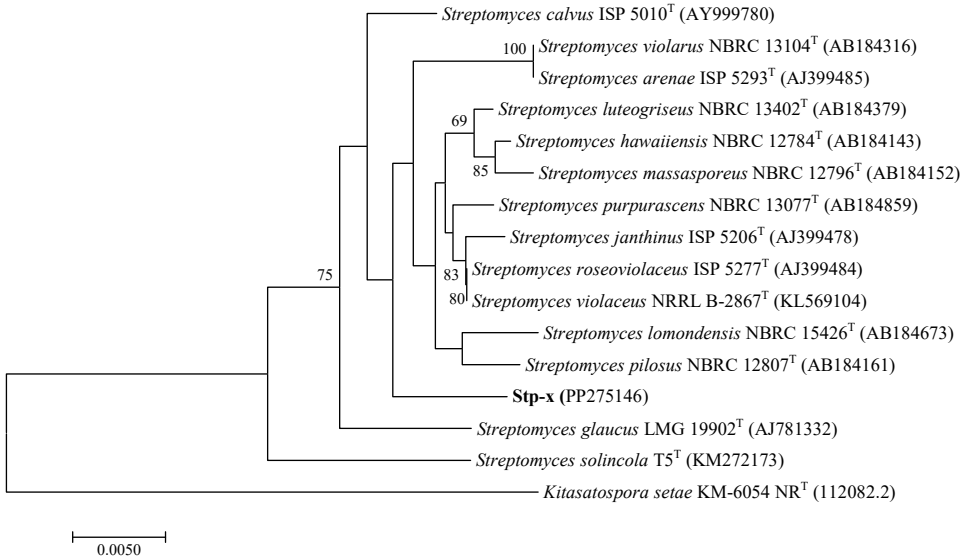

Figure 1. Phylogenetic tree derived from nearly complete 16S rRNA gene sequences, showing relationships between the isolate *Streptomyces* Stp-x and their phylogenetic neighbors. The tree was constructed using the neighbor-joining method. Bootstrap values greater than 50% are indicated at nodes. Bar, 0.005 substitutions per nucleotide position.

Table 1. Levels of 16S rRNA sequence similarities with *Streptomyces* sp. Stp-x by using EzTaxon server.

Closest species	Accession number	Similarity (%) with Stp-x
S. pilosus NBRC 12807^T	AB184161	98.82
S. violaceus NRRL B-2867^T	KL569104	98.68
S. roseoviolaceus ISP 527^T	AJ399484	98.68
S. violarus NBRC 13104^T	AB184316	98.67
S. arenae ISP 5293^T	AJ399485	98.66
S. hawaiiensis NBRC 12784^T	AB184143	98.54

Antimicrobial activity

Antimicrobial activity of actinobacterial strain Stp-x against several pathogenic microorganisms has been realized using the agar plug diffusion method and was indicated by the formation of a hallo zone around the colony. The antibacterial activity test showed that *S. aureus* ATCC 44300, *B. subtilis* ATCC 6633 and *P. aeruginosa* CIP A22 were sensitive to the strain Stp-x, and the highest activity was shown against the Gram-positive bacterium *S. aureus* ATCC 44300 with an inhibition zone of 45 mm. On the other hand, the Gram-negative bacterium *P. aeruginosa* CIP A22 has a smaller inhibition zone of 14 mm. Additionally, the strain Stp-x exhibited moderate antifungal activity against all target fungi, and the inhibition zones were ranged between 11 and 15 mm. However, no activity was detected toward the target yeast, *Candida albicans* IPA 200 (Table 2).

Table 2. Antimicrobial activity of *Streptomyces* sp. Stp-x by the agar plug diffusion method.

Target microorganisms	Inhibition zone (mm)
Bacillus subtilis ATCC 6633	25
Staphylococcus aureus ATCC 44300	45
Pseudomonas aeruginosa CIP A22	14
Aspergillus niger OT304	11
Aspergillus flavus NRRL 3251	15
Aspergillus carbonarius M333	12
Penicillium expansum PE1	13
Fusarium culmorum NRRL1829	14
Umbelopsis ramanniana NRRL 1829	12

Plant Growth Promoting Activities

To investigate the growth-promoting impact of the strain Stp-x, an *in vitro* experiment was performed. The results suggested that this strain was able to produce IAA in YT broth. Siderophore generation by this strain was checked after 10 days of inoculation in Chrome Azurol S agar. The colony developed a noticeable yellow halo, indicating siderophore production. The strain Stp-x was also able to solubilize inorganic phosphate, forming clear halos around colonies on PVK medium. Moreover, it was also qualified to produce ammonia, which has been verified by appearing brown color in peptone water. Furthermore, this strain was able to grow on N-free medium after 10 days, suggesting its nitrogen-fixing activity.

Production lytic enzymes

Screening of different lytic enzyme production by the strain Stp-x was carried out *in vitro*. After incubation of the strain 10 days at 30°C on skim milk agar medium, distinct zones surrounding the colonies were observed, which indicated protease activity. On the other hand, the plates of starch agar medium, CMC agar medium and nutritional gelatin were respectively flooded with Lugol's iodine, Congo red dye and Frazier solution, consequently a visible halo around the colonies appeared that indicated positive amylase, cellulase and gelatinase production. Whereas, lipase production was determined by the observation of an orange fluorescent halo around actinobacterial colonies under UV on rhodamine olive oil agar medium. Finally, chitinase production was confirmed after the actinobacteria grew on chitin agar medium.

DISCUSSION

In the present study, a strain named Stp-x isolated from Algerian soil was obtained, then studied for taxonomic, antagonistic and plant growth promotion properties.

Considering the phylogenetic analysis, this strain was assigned to *Streptomyces pilosus* NBRC 12807^T with a similarity of 98.82%. *Streptomycetes* is the largest genus of actinobacteria and is widely distributed in nature, especially in soils. Since techniques for the isolation of this genus are well understood, it has been frequently isolated [11].

The screening for the antimicrobial activity showed that the strain Stp-x exhibit a hight activity against *Staphyloccocus aureus* and *Bacillus subtilis* and moderate against the remaining target tested microorganisms. Previous studies reported the antimicrobial strength of the actinobacteria isolated from Algerian soil [6, 8, 19, 22]. Also, the *Streptomyces* genus is well known as one of the major sources of bioactive substances because of its capacity to synthesize complex secondary metabolism [25, 29, 31].

The strain Stp-x has been able to produce IAA, which is the most common natural plant hormone. This hormone controls many fundamental cellular mechanisms in plants, such as the development of embryos and fruit, vascular tissue differentiation, organogenesis, tropic growth, apical dominance, and apical hook formation. IAA also enhances root hair formation, which improves plant nutrient absorption capacity from soil [23, 26]. Production of IAA by *Streptomyces* genus has been documented in several literature [12, 13, 21].

Streptomyces sp. Stp-x produced siderophores, which are known as chelating compounds with a high affinity for iron. They could suppress pathogens and protect plants from pathogen infection by forming complexes with iron Fe^{3+} in the rhizosphere, making it unavailable to phytopathogens. In addition, they stimulate plant growth by facilitating plant uptake of iron from the soil [25, 26]. Several actinobacteria have been reported to produce siderophores [12, 13, 21, 25].

Another key characteristic of PGP is the solubilization and mineralization of phosphorus by bacteria. Because the amount of phosphorus (P) in the

soil is typically very low and available in insoluble form, it cannot support plant growth. Therefore, it has been observed that bacteria that promote plant development may transport precipitated phosphorus to plants, suggesting a potential strategy for increasing plant growth in outdoor settings [17, 26]. In our results, the strain Stp-x was able to dissolve tricalcium phosphate to a simpler form potentially available for the plant. This result is supported by similar papers that state phosphate solubilization by *Streptomyces* [2, 17, 24].

Production of ammonia is another mechanism that is correlated with plant root and shoot elongation. In addition, it contributes to the breakdown of organic materials, which increases plant output and resistance to phytoparasites [10]. In our findings, the strain Stp-x was able to produce ammonia, and many authors highlight the capacity of *Streptomyces* strains to produce ammonia [2, 3, 10].

Nitrogen is one of the most important elements for plant growth and accounts for about 78% of the atmosphere, but it is biochemically unavailable to most organisms [7, 23]. Only few prokaryotes, nitrogen-fixing bacteria, convert atmospheric dinitrogen (N_2) into ammonia (NH_3) [26], which can be assimilated by plants for the synthesis of nitrogenous biomolecules and can also play a key role in the promoting activity of biocontrol agents on plant root growth and development [12, 26]. Among these prokaryotes that are well known for nitrogen fixation, various species belong to the actinobacteria group [7, 12, 26]. In the present study, strain Stp-x was found to be positive for nitrogen fixation.

This strain was also positive for protease, amylase, cellulase, gelatinase, lipase and chitinase production. Hydrolytic enzymes generated by actinobacteria can play a critical role in maintaining soil fertility and providing soluble nutrients to plants by converting complex nutrients into simple forms via secreting various enzymes, such as amylase, chitinase, cellulase, invertase, lipase, keratinase, peroxidase, pectinase, protease, phytase, and xylanase [34]. The hydrolytic enzymes have also been shown to protect the plant through inhibiting the growth of several phytopathogens including bacteria, fungi and insect. These enzymes break down fungal and bacterial cell walls, membrane proteins, and extracellular virulence factors. As well, they can degrade the insect cell walls whith chitinases [23, 26].

In this study, the actinobacterial strain Stp-x was isolated and determined to be closely related to *Streptomyces pilosus*. This strain was revealed as potential microbial inoculants because of its potential antimicrobial activity against several pathogenic microorganisms, and its intensified PGP activities. These demonstrated promising potentialities would merit further comprehensive evaluations under various agricultural conditions to valid the practical effectiveness of the strain Stp-x.

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Conflict of interest. There is no actual or potential conflict of interest in relation to this article.

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