Identification of antagonistic *Streptomyces* strains isolated from Algerian Saharan soils and their plant growth promoting properties

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Abstract. Aouar L, Boukelloul I, Benadjila A. 2020. Identification of antagonistic *Streptomyces* strains isolated from Algerian Saharan soils and their plant growth promoting properties. Biodiversitas 21: 5672-5683. To produce new bioactive substances of agricultural interest, extreme ecosystems can be a source of unexplored microorganisms. Accordingly, in this study, twenty-two actinobacteria strains were obtained from rhizospheric arid soils of palm groves collected from Biskra and El Oued in the Algerian Sahara. All isolates were examined for the in vitro antifungal potential towards phytopathogenic fungi: *Aspergillus flavus*, *Verticillium dahlia*, *Rhizoctonia solani*, *Botrytis cinerea* and *Fusarium oxysporum* as well as for their antibacterial property toward phytopathogenic bacteria: *Streptomyces scabiei*, *Pectobacterium carotovorum* and *Agrobacterium tumefaciens*. The three isolates (13%) that inhibited at least five pathogens were then selected, identified and assessed for their attributes to produce indole-3-acetic acid (IAA) and siderophores, to solubilize phosphate, and to antagonize *Streptomyces scabiei* in vivo. According to phylogenetic analysis performed with 16S rDNA sequence, chemotaxonomy and phenotypic characteristics, the strain SO1, which inhibited all tested pathogens, was assigned to *Streptomyces flavus*. While, strains SO2 and SB1 were affiliated to *Streptomyces enissoaeulis* and *Streptomyces albidoflavus*, respectively. All strains produced IAA but only SO1 and SB1 were able to elaborate siderophores catecholate-type. Two strains SO1 and SO2 exhibited a capacity to solubilize phosphate and SO1 was able to suppress the pathogenic effect of *Streptomyces scabiei* on radish seedlings. The findings indicate that SO1 strain may reveal the potential for use as a biocontrol agent and plant growth promoter.

Keywords: Antagonism, arid soil, indol-3-acetic acid, phosphate, siderophores, *Streptomyces*

INTRODUCTION

The emergence of resistance in fungal plant pathogen strains poses serious public health and environmental menace all over the world (Sharma and Manhas 2020). Currently, crop-destroying fungi cause yield losses of about 20% worldwide, with an additional 10% loss postharvest (Fisher et al. 2018). In addition to fungal diseases, there are those engendered by bacteria, such as common scab, which is characterized by dark lesions that develop especially on tubers, and the main causal agent is *Streptomyces scabiei* (Khalil et al. 2019). Hence, the utilization of beneficial microorganisms as biological control agents is considered an environmental friendly alternative to combat crop diseases and pesticide resistance. Among microorganisms, actinobacteria especially *Streptomyces* are recognized for their ability to generate various active compounds with agricultural applications (Liu et al. 2019).

Actinobacteria are Gram-positive bacteria that occur naturally in soils. Recently, they have attracted great interest because these bacteria can be applied in agriculture and also in the industry for the synthesis of bioactive metabolites and other natural bioactive compounds. In the rhizospheric soil, actinobacteria constitute a major part of the bacterial community and can exercise an antagonistic and competitive action on the microbial populations. Members of this group produce active compounds such as antibacterial and antifungal antibiotics, plant growth regulators, and siderophores (Khamma et al. 2010).

To promote plant growth, actinobacteria possess multifunctional plant growth-promoting (PGP) attributes and enhance plant growth directly by the production of phytohormones such as indole-3-acetic acid (IAA), solubilization of minerals like phosphate, and siderophores secretion. The latest are low molecular weight compounds, they sequester iron from the soil and deliver it to the plant. Bacteria generally synthesize two types of siderophores: the catechol type and the hydroxamate type. IAA is an indole derivative that has several functions to enhance plant growth, such as root formation, stimulation of cell division, extension, and differentiation (Hider and Kong 2010; Yadav et al. 2018).

The majority of rhizospheric actinobacteria are *Streptomyces* (66%) (Apsari et al. 2019). They are characterized by their complex life cycle and high GC content (more than 70%) (Lee et al. 2020). *Streptomyces* sp. is the most important producer of secondary metabolites (39% of the total metabolites produced by microorganisms) (Subramaniam et al. 2020). Moreover, 60% of antibiotics, insecticides, and herbicides developed for agriculture use are isolated from *Streptomyces* (Suwan et al. 2012; Vurukonda et al. 2018). Unfortunately, many previous screening studies for new compounds have focused on new actinobacteria from the usual natural environment,
resulting in a re-isolation of already existing bioactive compounds. Thus, research has been oriented towards new, extreme, or under-explored habitats in the hope of discovering promising new species capable of producing new bioactive compounds (Mohamed et al. 2017).

Algerian Sahara is characterized by a hot arid climate and presents a particular ecosystem. Recently, there has been a new interest in the resources of actinobacteria from Algerian arid zones, considering that high temperatures can induce metabolic adaptations which could result in the production of interesting metabolites (Djinni et al. 2019). Effectively, many studies have contributed to the finding of new species of actinobacteria in poorly explored arid environments, notably through the isolation of rare genera: *Mzabiumyces algeriensis* (Saker et al. 2014), *Actinopolyspora algeriensis* (Meklat et al. 2012), and *Planomonospora algeriensis* (Chaabane-Chaouch et al. 2017). As a result, from 2002 to 2019, one new genus and twenty-nine new species were obtained, mainly from palm groves and Algerian Saharan soils. Several of these strains were proved capable of producing antibacterial and antifungal metabolites, including 17 new molecules among the 50 structures reported (Djinni et al. 2019; Lahoum et al. 2019). Actinobacteria isolated from arid and other extreme ecosystems remain the inexhaustible source of new antimicrobials, offering an opportunity to get new bioactive metabolites (Djinni et al. 2019).

The objective of the current study is to isolate rhizospheric actinobacteria from Algerian arid soils and to screen isolates exhibiting antagonistic activity against plant pathogens, fungi, and bacteria. The selected isolates are subjected to polyphasic identification: chemotaxonomic, morphological, physiological, and biochemical characteristics, as well as 16S rRNA gene sequencing. Selected isolates are also screened for their aptitude to solubilize inorganic phosphate, to produce IAA and siderophores, and to antagonize *S. scabiei* in vivo.

**MATERIALS AND METHODS**

**Isolation and screening of antagonistic activity**

The Saharan soils were sampled in January 2016, from the rhizosphere of two palm groves, the first is located on the site (33°09’36”N,6°34’09”E) in Draa Lahmar-Mlhouans, Province of El Oued, and the second is located on the site (34°42’52”N,5°22’16”E) in M’touira-Tolga, Province of Biskra. Based on Köppen climate classification both M’touira-Tolga and Draa Lahmar-Mlhouans are characterized by BW/h (hot desert climate). But according to Emberger’s rainfall quotient, which specifies the bioclimatic stages, Draa Lahmar-Mlhouans region is characterized by the Saharan climate (Q2= 6.75); while M’touira-Tolga region is characterized by the arid climate (Q2= 13.33). Decimal dilutions (from 10⁻¹ to 10⁻⁷) were spread on isolation medium ISP2 (International *Streptomyces* Project-2) amended by cycloheximide (100 µg mL⁻¹), then incubated for 21 days at 28°C (Khamna et al. 2010). Typical actinobacteria colonies were picked, purified, and maintained in glycerol (20% v/v) (Ser et al. 2018; Abbasi et al. 2019).

Antibacterial activity was determined against three phytopathogenic bacteria: *P. carotovorum*, *A. tumefaciens*, and *S. scabiei* using the agar cylinder technique (Rizvi et al. 2014). The inhibition zones were measured within 24 h. The screening of isolates exhibiting antifungal activity was carried out using methanol extracts obtained from cultures on solid medium (glycerol yeast extract peptone agar), according to a modified procedure of Lee et al. (1995). Antifungal property is evaluated by testing the methanolic culture extracts of the isolates against 5 phytopathogenic fungi, which are isolated and identified locally: *B. cinerea* SOL-1, *F. oxysporum* CIT-4, *V. dahlia* OLE-2, *R. solani* SOL-5, and *A. flavus* TRI-4. The disc of the fungal culture (8 mm) was deposited in the middle of the Petri dish containing PDA amended with the methanolic extract. The fungal growth was recorded when control colonies reach 8 cm. The percentage of inhibition was calculated as follows:

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\% \text{ inhibition of mycelial growth} = 1 - \frac{\text{Diameter of mycelial growth with methanolic extract (treated plate)}}{\text{Diameter of mycelial growth on untreated plates}} \times 100
\]

**Phenotypic and chemotaxonomic characteristics**

Morphological traits and melanoid pigments were examined on ISP media (ISP2, ISP3, ISP4, ISP5, ISP6, and ISP7) as recommended by Shirling and Gottlieb (1966). Plates were incubated at 28 °C for 14 days, and then the colony color was determined using CCIS-NBS color maps. The cover-slip technique was performed in order to determine spore chain arrangement as described by Williams et al. (1983). Cover-slips were examined after 14 days of incubation on ISP4 medium at 28 °C. To observe spore-surface ornamentation, cultures were fixed with 1% (w/v) osmium tetroxide then coated with platinum in a sputter coater and observed under scanning electron (Aouar et al. 2012). The isolates were evaluated for 50 biochemical and physiological characteristics (Table 1). The ability to assimilate different sources of carbon was performed on the basic medium ISP 9 (Shirling and Gottlieb, 1966) amended with 1% (w/v) for sugars (D-glucose, L-arabinose, D-fructose, D-lactose, mannitol, D-maltose, raffinose, sucrose, D-mannose, L-rhamnose, D-xylitol, sorbose, Myo-inositol, glycerol, xylitol, adonitol, sorbitol, melezitose, and melibiose) and 0.1% (w/v) for organic acids (citrate and propionate). The use of nitrogen sources on Bennett medium (supplemented at 0.1%, w/v) was assessed as described by Williams et al. (1983). For both carbon and nitrogen sources, growth was noted after 14 days by comparing the test plates with the negative and positive control plates. Enzymatic and degradation activities were evaluated according to the procedures of Williams et al. (1983). Growth inhibition assays were conducted on MBA (modified Bennett’s agar), which was added by the following inhibitors: phenol (0.1%, w/v), sodium azide (0.01%, w/v), crystal violet (0.001%, w/v), thallous acetate (0.01%, w/v) and potassium tellurite (0.01%, w/v). Tolerance sensitivity to lysozyme (10, 50, and 100 µg mL⁻¹) was tested as described by Williams et al.
NaCl tolerance was evaluated in tryptic soybean broth (TSB) with concentrations starting from 0 to 13% (w/v). For all these tests, estimation of growth was noted after 7 and 14 days. Growth at different temperatures was tested within a range of 4 to 45 °C. Tolerance to pH was tested on tryptic soybean broth medium (TSB) with pH values ranging from 4 to 10. Chemical composition of the cell wall and the whole-cell hydrolysate were analyzed according to the procedure of Becker et al. (1964) and Lechevalier and Lechevalier (1980).

Molecular identification and phylogenetic analysis
Genomic DNA was extracted and purified as described by Franco-Correa et al. (2010). The amplification primers were F27 [5′AGAGTTTGATCCTGGCTCAG] and R1492 [5′TACGGCTACCTTGTTACGACTT3′] (Aouar et al. 2012; Sadeghi et al. 2020). PCR conditions were 5 min of pre-denaturation at 95 °C; and then 35 cycles including 1 min of denaturation at 95 °C, 1 min of annealing at 50 °C, and 2 min of extension at 72 °C. The PCR amplified samples were immediately sequenced. The obtained sequences were submitted to GenBank under accession numbers KX822695, KX822695, and KX822695 for SO1, SO2, and SB1 isolates, respectively. The rDNA sequences were analyzed by BLAST. The alignment of sequences with their representative related type strains retrieved from the GenBank databases was performed by Clustal W2 software. A phylogenetic tree was built using Mega 6 software (Tamura et al. 2013), according to the Neighbor-Joining (NJ) method supported by the bootstrap test of 1000 replications and the Kimura two-parameter model.

Siderophore production
Siderophore production was detected as stated by Sadeghi et al. (2012) and Gangwar et al. (2014). Discs (6 mm) were collected from isolates cultures (SO1, SO2, and SB1), placed on Chrom Azurol S (CAS) plates, and then incubated for 7 days at 30 °C. The development of an orange zone is considered positive for siderophore production. The positive isolates with the CAS test were maintained and examined for the siderophore type tests. The rhodocystide-type of siderophores was detected by the method of (Atkin et al. 1970). Their presence in the supernatant was revealed by the iron perchlorate reagent. Red color development revealed a positive reaction. The catechol-type of siderophores was assessed using Arnow’s reagent as described by Lee et al. (2012). Isolates were inoculated in a modified Fiss glucose medium. The supernatant was collected, while the biomass was dried and expressed in mg on a dry weight basis. Equal volumes (1 mL) of supernatant, HCl (0.5 N) and NO₂-Mo aqueous solution (10% NaNO₃ and 10% NaMo, wt/vol) were mixed. Then, 1 mL of NaOH (1 N) was added. The change of color to pink-red signifies a positive reaction. To study the kinetics of catechols production, isolates SO1 and SB1 were grown in Fiss glucose. During incubation, catechols were extracted every 12 h with ethyl acetate. The obtained extracts were examined by HPLC using C18 reverse-phase column (Zorbax SB-C18, Agilent Technologies) as described for IAA (Aouar et al. 2016).

Phosphate solubilization
The phosphate solubilization test is performed on Pikovskaya medium containing 5 g L⁻¹ of tricalcium phosphate (Ca₃(PO₄)₂) (TCP) as described by (Franco-Correa et al. 2010, Mohandas et al. 2013). The formation of a clear area surrounding the colonies reveals phosphate solubilization. Halo diameters are determined by subtracting the colony diameter from the total diameter.

In vivo antagonism against Streptomyces scabiei
This test was conducted on radish seedlings (Raphanus sativus L. var sativus) in growth bags as previously described by Legault et al. (2011). Three types of tests were performed positive control (inoculated with S. scabiei) negative control (no inoculated) and treatments (co-inoculated with S. scabiei and the antagonist isolate). Pouches were incubated for 6 days at 21 °C, then scanned and the obtained pictures were processed by Ez-Rhizo 1.0 software to measure root length.

Statistical analysis
The tests were performed in quadruplicate and all results were presented as a mean ± standard deviation (SD). Analysis of the data was performed using an analysis of variance (ANOVA) with the statistical software SPSS Statistics 23.0, consolidated by the least significant difference (LSD) test. The difference was estimated to be statistically significant when p < 0.05.
RESULTS AND DISCUSSION

Screening of antagonistic isolates

Twenty-two isolates were obtained from soils sampled from the two rhizospheric sites: Biskra 15 (68%) and El Oued 7 (31%). All the isolates were maintained and analyzed for antifungal and antibacterial potentials. Putrie et al. (2020) found that the major microorganisms in the rhizospheric soil are actinobacteria. Also, Khamna et al. (2009) demonstrated that microorganisms from the rhizosphere are most ideal for controlling plant pathogens that’s why we have explored rhizospheric soil. According to this study, the soil of Biskra provided the greatest number of isolates compared to that of El Oued. These results are compatible with those of Boudemagh et al. (2005). They reported the abundance of actinobacteria in the soil of Biskra compared to that of El Oued. This is possibly due to the climate, which is more arid in El Oued. Out of the 22 isolates, 12 (54%) presented antagonistic activity towards at least one microorganism-test, 11 (50%) isolates exhibited antifungal activity and 6 (27%) isolates showed antibacterial antagonism. Our results corroborate with those obtained by Singh et al. (2019), where they found that 23% of the isolates had antibacterial activity, while 47% were able to inhibit fungi. However, Boudmagh et al. (2005) reported that, among the 27 isolates of arid soil, only 7% showed antifungal activity. The highest rate of active isolates was recorded against V. dahlia 10 (45%), followed by A. flavus and B. cinerea 9 (40%) and F. oxysporum 7 (31%), while the lowest rate was displayed toward R. solani 4 (18%). Further experiments were conducted on isolates that inhibited the growth of at least five pathogens (SO1, SO2, and SB1). Results of antagonistic activities of the selected isolates are presented in Table 1.

As shown in Table 1, SO1 exhibited antagonistic activity against all pathogens. Isolate SO2 exhibited the highest activity toward P. carotovorum (26.33 mm) (Figure 1). Boudjeko et al. (2017) have also found that S. cameroonensis, isolated from the rhizosphere, was able to inhibit in vitro the growth of S. scabiei and Agrobacterium tumefaciens. SO2 displayed the highest V. dahlia inhibition (60.0%) followed by SO1 (51.25%). However, R. solani was the most resistant. Antagonism of actinobacteria against pathogenic fungi has been assessed for a large variety of fungi. In this view, several researches have been done showing the ability of actinobacteria isolates to antagonize phytopathogenic fungi (Aouar et al. 2012; Goudjal et al 2016; Abbasi et al 2019).

Inhibition rates obtained in our study are not unexpected because, with antagonistic actinobacteria, Goreedema et al. (2020) recorded an inhibition rate of 55% against A. flavus and F. oxysporum. Also, Aouar et al. (2012) reported that 54% of rhizospheric actinobacteria isolates exhibited antagonism against F. oxysporum, and 33% were capable of inhibiting the spread of V. dahliae. The proportion of active isolates against V. dahlia, recorded in this study, was higher than that obtained by Xue et al. (2013). Also, recent studies (Sharma and Manhas 2020; Sudiana et al. 2020) have found that F. oxysporum was also sensitive to antagonistic actinobacteria isolated from soil.

Table 1. Results of antibacterial and antifungal activities of the selected isolates.

| Isolates | Inhibition zones (mm) | % inhibition of mycelial growth |
|----------|-----------------------|--------------------------------|
|          | S. scabiei | P. carotovorum | A. tumefaciens | F. oxysporum | B. cinerea | R. solani | V. dahliae | A. flavus |
| SO1      | 23.66 ± 1.15 | 14.67 ± 1.15 | 17.33 ± 1.52 | 25.00 | 30.25 | 12.5 | 51.25 | 37.5 |
| SO2      | n.i.       | 26.33 ± 2.08 | n.i.       | 10.0 | 18.75 | 6.25 | 60.0 | 43.75 |
| SB1      | n.i.       | n.i.       | n.i.       | 22.5 | 28.75 | 13.75 | 41.25 | 35.00 |

Note: n.i.: no inhibition

Figure 1A. Antibacterial activity against Pectobacterium carotovorum; B. Fusarium oxysporum growth on PDA without SO1 extract; C. F. oxysporum growth with SO1 extract
Genera associated with the selected isolates

Morphological and physiological traits are summarized in Table 2. The results show that all strains are fast-growing. They are differentiated by the color of their spore mass, which is grey, white, and yellow for SO1, SO2, and SB1, respectively. It has been observed that spore chains forms are rectiflexibles for SB1, and spiral for both SO2 and SO1. The scan electron micrographs show the cylindrical form and smooth surface of the spores (Figure 2). For all strains, melanin and colored diffusible pigments are not formed. Selected isolates develop colonies after 48 hours of incubation, which characterizes fast-growing actinobacteria. These rapid-growing strains lack mycolic acids in their walls and can therefore be placed either with Streptomyces and related genera or with Thermomonospora and related genera. For all isolates, the cell wall hydrolysed revealed levo isomer of dianimopimelic acid (LL-DAP) as the major constituent of peptidoglycan in addition to the glycin, and no characteristic sugars (arabinose, xylose, manhrose) have been detected in the whole-cell hydrolysate. Based on Williams et al. (1989) recommendations, the detection of LL-DAP isomer, and the absence of characteristic sugars indicates the presence of the parietal chemotype I, typical of Streptomyces and related genera.

Barka et al. (2016) reported that the principal characteristics employed for determining the genus level of actinobacteria are macroscopic and microscopic morphology in conjunction with chemotaxonomy. For example, substrate mycelium bearing spores, or formation of sporangia, can differentiate between several genera of actinobacteria. By combining chemotaxonomic and morphological data, the three isolates characterized by parietal type I can be assigned to the genus Streptomyces rather than the related genera such as Streptovercillium, Kitasatoa, and Chainia. The latest, are recognized by particular structures such as verticils, sporangia, and sclerotia (Williams et al. 1989). Scan electron micrographs reveal long chains either straight for SB1 or spiral for SO1 and SO2 with smooth spore ornamentation and the absence of specific structures (verticils, sclerotia, synnema, and sporangia). These observations strongly suggest the affiliation of SO1, SO2, and SB1 to the genus Streptomyces.

Locci (1989) has reported that most Streptomyces species have smooth spores. Also, according to Ezra et al. (2004), the cylindrical shape of the spores exclusively characterizes Streptomyces. As the spore mass color has been widely used in Streptomyces taxonomy, our isolates should be assigned to grey, white and yellow series of Streptomyces. In addition, the Mol% GC values were found to be high; they are 70.89%, 75.68%, and 73.66%, for SO1, SO2, and SB1 respectively. GC% of the genome of microorganisms is a recommended characteristic for the standard description of microbial genera and species (Gonzalez and Saiz-Jimenez, 2005). These data are in agreement with the GC% range characterizing actinobacteria, which have a GC% greater than 55, and Locci (1989) cited that the GC% of Streptomyces strains ranges from 68 to 78 mol%. Considering all these results, the isolates were assigned to Streptomyces and designated as follows: Streptomyces sp. SO1, Streptomyces sp. SO2 and Streptomyces sp. SB1.

Results obtained in this investigation are not surprising. By comparing them with other studies (Aouar et al. 2012; Xue et al. 2013; Jog et al. 2014; Anwar et al. 2016), it could be said that members of Streptomyces genus are the most common among the rhizosphere isolates. Goudjal et al. (2014), Mouloud et al. (2016), Abdelmoteleb, and Mendoza (2020) have explored rhizospheric Saharan soils. They have isolated actinobacteria, the majority of them were allowed to Streptomyces genus.

Table 2. Morphological and physiological characteristics of the isolates

| Characteristics | SO1 | SO2 | SB1 |
|-----------------|-----|-----|-----|
| Morphological characteristics | Grey | White | Yellowish grey |
| Spore chain morphology | Spiral | Spiral | Rectiflexibles |
| Carbon source utilization | L-Arabinose | + | + | ± |
| | D-Fructose | + | ± | ± |
| | Myo-inositol | + | + | - |
| | D-Lactose | + | + | - |
| | D-Maltose | - | + | - |
| | D-Mannose | + | - | ± |
| | Raffinose | + | - | - |
| | L-Rhamnose | + | ± | - |
| | Sucrose | + | + | - |
| | D-Xylose | - | ± | - |
| Nitrogen source utilization | Cysteine | - | + | + |
| | Tryptophan | + | - | - |
| | Methionine | - | - | - |
| Degradation activities | Tyrosine | - | - | - |
| | Casein | + | + | + |
| | Xanthine | - | + | + |
| Enzyme activity | Nitratre reduction | + | - | - |
| Growth in the presence of inhibitors | Lysozyme (50 μg mL⁻¹) | + | + | + |
| | NaCl (7%) | - | - | + |
| | Phenol (0.1 w/v) | - | - | + |
| | Potassium tellurite (0.001% w/v) | + | - | - |
| | Thallous acetate (0.001% w/v) | - | - | - |
| Environmental characteristics | ± | ± | - |
| 45°C | - | - | + |

Note: ' + ' = positive; ' - ' = negative; ' ± ' = weakly. * All strains were negative for melanoid pigments production and present smooth spore ornamentation. + All strains were positive for assimilation of glucose, mannitol, glycerol, citrate, propionate and negative for assimilation of xylitol, adonitol, sorbitol, sorbose, melizitose and melibiose. All strains were positive for utilization of lysine, phenylalanine, asparagines, KNO3, proline, arginine, and negative for methionine utilization. All strains were positive for degradation of adenine, gelatin, chitin, starch, and negative for insulin degradation. All strains were positive for urease and β-galactosidase activities. + All strains were positive for growth in 4% NaCl, 10 μg mL⁻¹ lysozyme, and negative for growth in sodium azide and crystal violet. + All strains were positive for growth at pH 7, at 25 and 37 °C, and negative for growth at +4 and 10 °C.
Identification of the antagonistic isolates

The isolates showed a variable capacity to use different sources of carbon and nitrogen, enzymatic activities, as well as growth in media containing inhibitors and under different environmental conditions. The results are shown in Table 2. According to Meyer (1989), *Streptomyces* strains can assimilate a vast variety of nutrient sources, except a few compounds such as inulin, xylitol, and methionine, which are infrequently used. All strains grew well between 25 and 37 °C. Exceptionally, *Streptomyces* sp. SO1 and *Streptomyces* sp. SO2 grew at 45 °C. Thus, Meyer (1989) indicated that the majority of *Streptomyces* are mesophilic except a few strains which are capable of growing at +4°C or above 45°C. The isolates grow optimally at pH 7, which characterizes the majority of telluric *Streptomyces*.

A phylogenetic tree gathering the sequences of our isolates as well as those of the type strains, obtained by the BLAST, was constructed by using tree-making algorithms, neighbor-joining (NJ). The NJ methods are from the MEGA 6.0 package. *Streptomyces* sp. SO1, *Streptomyces* sp. SO2 and *Streptomyces* sp SB1 appear only with *Streptomyces* species. Thereby, the phylogenetic tree includes two clades as represented by Figure 3.

![Figure 3. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing relationship between SB1, SO1, and SO2 and their closest relatives. *Bacillus subtilis* ATCC 6633T was used as an outgroup. Bootstrap values based on 1000 replicates. Bar, 0.02 substitutions per nucleotide position](image-url)
The first clade supported by 99% of the bootstrap value, is regrouping the clusters of *Streptomyces* sp SB1 and *Streptomyces* sp. SO2 with their related strains. The second clade is supported by 99% replications of bootstrap, it includes the SO1 isolate and its closely related strains. Regarding previous results, phylogenetic analysis strongly supports those obtained with morphological, physiological, and chemotaxonomic identification. However, to assign them to their respective *Streptomyces* species, a polyphasic approach must be adopted. It involves morphological, cultural, biochemical, and physiological data in conjunction with phylogenetic analysis (Khamma et al. 2009).

The dendrogram illustrated by Figure 3 indicates that *Streptomyces* sp SB1 and *Streptomyces* sp. SO2 strains appear in the same clade supported by 100% bootstrap replicates. The first cluster, supported by 99% bootstrap value, reveals the close phylogenetic correlation between SB1 and its neighbors: *Streptomyces albidoflavus* DSM 40792, *Streptomyces saprophyticus* NBRC 134401, and *Streptomyces argenteolus* CGMCC 41693, in which they occupy the same phyletic line. A comparison of *Streptomyces* sp SB1 characters obtained in this study with data provided by Williams et al. (1989), Islam et al. (2009), and Atta et al. (2011), suggests that this strain belongs to *S. albidoflavus* species. Both show a great morphological similarity with a yellow spor mass and rectifexibles spore chains without melanoid pigments production. Like *S. albidoflavus*; SB1 uses glucose, mannitol, glycerol, cysteine, lysine, asparagine; but does not use xylitol, raffinose, rhamnose, sucrose, xylose, tryptophan, and methionine. In addition, both strains were able to grow at pH 4.3. Nevertheless, the two strains differ in a few characteristics such as assimilation of arabinose, maltose, and gelatin degradation. This close phylogenetic relatedness and similarity of features allowed us to refer to the strain *Streptomyces* sp. SB1 to *S. albidoflavus*.

The second cluster regroups in the same phyletic line, *Streptomyces* sp. SO2 with its related species *Streptomyces plicatus* NBRC 130711, *Streptomyces enissocaesilis* ATCC 43682 and *Streptomyces vinaceudrapus* NRRL 23363 with a bootstrap value of 99%. The comparison with the *S. enissocaesilis* data reported by Sirisha et al. (2014) and Laidi et al. (2007) suggests that the characteristics of *Streptomyces* sp. SO2 matched with those of *S. enissocaesilis*. Their color spor mass is white with spiral spores chain morphology. The two strains assimilate glucose, arabinose, inositol, lactose, maltose, mannitol, glycerol, raffinose, sucrose, lysine, and citrate. Both of them were negative for the assimilation of mannose and tryptophan. Same as *S. enissocaesilis*, *Streptomyces* sp. SO2 degrades casein, starch and gelatin. Both strains do not assimilate fructose, rhamnose, and xylose. They show urease activity but not nitrate reduction. Unlike *S. enissocaesilis*, *Streptomyces* sp. SO2 was able to grow at 45 °C. Probably, our strain is adapted to the high temperatures of the arid climate of the Algerian Sahara. Moreover, it has been found by Sirisha et al. (2014) that *S. enissocaesilis* AUBT-1404 participates a GC% of 76.2%, which is quite near to that of SO2. As a result of these comparisons, the strain SO2 should be assigned to *S. enissocaesilis*.

*Streptomyces* sp. SO1 appears on a distinctive cluster with three representative species: *Streptomyces cavourensis* subsp. *cavourensis* NRRL B-80301, *Streptomyces lavendulae* subsp. *lavendulae* NBRC 123421 and *Streptomyces flavus* NBRC 123451 with a high value of bootstrap (99%) replicates. Data reported by Williams et al. (1989) and Lee et al. (1995) on *S. flavus*, showed a high similarity level with *Streptomyces* sp. SO1. Thus, both strains were positive for the assimilation of glucose, arabinose, fructose, inositol, mannose, mannitol, raffinose, rhamnose, sucrose, citrate, lysine, tryptophan, and phenylalanine. Also, they were able to hydrolyze starch and gelatin, to reduce nitrate, and to grow in the potassium tellurite inhibitor. Unlike *S. flavus*, *Streptomyces* sp. SO1 grew at 45°C, which may be due to its isolation region characterized by high temperatures. Therefore, this polyphasic identification suggests that the strain SO1 belongs to *S. flavus*.

Several studies on the antagonistic activity of rhizospheric *Streptomyces* have been documented (Khamma et al. 2009; Anwar et al. 2016; Aouar et al. 2016; Sharma and Manhas, 2020) since it is the largest and the active genus among actinobacteria, and 75% of biologically active compounds are produced by members of this genus (Singh et al. 2019). However, to our knowledge, very few publications are available in the literature that explores antagonistic activity of *S. albidoflavus*, *S. enissocaesilis*, and most particularly *S. flavus*. Our findings are in agreement with those obtained by Laidi et al. (2007). They found that *S. enissocaesilis* strain RAF10, showed good antifungal (against *Fusarium*, *Botrytis*, and *Aspergillus*) and antibacterial activities (against both Gram-positive and Gram-negative bacteria). In this study, *S. enissocaesilis* SO2 exhibited the most significant inhibition zone against Gram-negative bacteria, which is in disagreement with that obtained by Sinisha et al. (2014), they found that *S. enissocaesilis* exhibits very low activity against Gram-negative bacteria. The only strain that inhibited all the phytopathogens tested was *S. flavus* SO1. This is supported by another study, showing the inhibition capacity of *Rhiizoctonia solani* by a low concentration of *S. flavus* filtrate (Lee et al. 1995). Antagonistic capacities exhibited by *S. albidoflavus*, corroborate with those obtained by Atta et al. (2011), they reveal the absence of antagonism towards Gram-negative bacteria, but the presence of an interesting activity against *F. oxysporum*, *A. niger*, and *A. flavus*. Also, Brezeziska et al. (2013) confirmed the antifungal potential of *S. albidoflavus*.

Indol-3-acetic acid quantification

The colorimetric assay revealed that all strains are able to produce IAA when the medium is amended with L-tryptophan (L-Trp). The IAA amounts ranged from 41.88 to 107.48 μg mL⁻¹. *S. flavus* SO1 recorded the greatest quantity. As presented in Figure 4, the results suggest strongly that IAA production by *S. flavus* SO1 and *S. enissocaesilis* SO2 was not affected by L-Trp concentration (P >0.05). Nevertheless, the IAA produced by *S. albidoflavus* SB1 was positively and significantly (P < 0.05) correlated with L-Trp concentration. Interestingly, all
our selected isolates produced IAA. It has been observed that aptitude to produce IAA is a frequent trait among antagonistic species of *Streptomyces* (Abbasi et al. 2019).

All isolates require the presence of L-Trp, suggesting that they use a pathway L-Trp-dependent for the IAA synthesis. Among the three strains, IAA production was greater for *S. flaveus* SO1; probably, this strain was already adapted to high concentrations of L-Trp in its rhizosphere, which enhance IAA biosynthesis. These results corroborate with other reported studies (Qin et al. 2015; Legault et al. 2011; Aouar et al. 2016). Conversely, de Oliveira et al. (2010) have reported that some actinobacteria are able to produce IAA without L-Trp, possibly due to the use of another synthesis pathway. Anwar et al. (2016) have found that *S. enissocaesilis* TA-3 produced 69.26 µg mL\(^{-1}\) of IAA, which is equivalent to that obtained by *S. enissocaesilis* SO2.

Several studies have explored the IAA production by *Streptomyces* species. Compared to other studies, our results are comparable to those found by Khamna et al. (2009) (5.5-144 µg mL\(^{-1}\)) and Goudjal et al. (2016) (35.9-117 µg mL\(^{-1}\)); but lower than those obtained by Chaiharn and Lumyong (2011) (2.55-291.97 µg mL\(^{-1}\)). However, they are higher than those reported by Qin et al. (2015) (2.21-9.14 µg mL\(^{-1}\)) and Abbasi et al. (2019) (7.0-40.9 µg mL\(^{-1}\)).

**Siderophore production and kinetics of catechols**

CAS assay showed that only *S. albidoflavus* SB1 and *S. flaveus* SO1 (66%) produced siderophores. According to the specific tests, both strains produced catechol-type of siderophores in a range of 32.01 to 160.63 µg mg bact. DW\(^{-1}\) (Figure 5). Several reports have shown that *Streptomyces* sp. from many crop rhizosphere soils have this ability (Khamna et al. 2009; Aouar et al. 2016; Goudjal et al. 2016; Warrad et al. 2020). However, only a few studies have reported the ability of *S. enissocaesilis* strains to produce siderophores, for example, *S. enissocaesilis* TA-3 (Anwar et al. 2016) and *S. enissocaesilis* IC10 (Abbasi et al. 2019). To our knowledge, the siderophore production by *S. albidoflavus* and *S. flaveus* has not been reported.

Another important characteristic of rhizobacteria that promote plant growth is the production of siderophores, they could antagonize fungi by iron chelation. Moreover, microbial siderophores may be utilized by plants as an iron source. Usually, actinobacteria can elaborate more than one type of siderophores simultaneously (e.g., catechols, hydroxamates, and salicylates). Although, hydroxamates and catechols are the most important. In this study, specific assays demonstrated that both positive strains produced only catechol-type siderophores. Our results are in concordance with those of Gangwar et al. (2012), they found that the catechols producing isolates represent the largest proportion. In contrast, Lee et al. (2012) reported that only 20% of actinobacterial isolates produced catechols while all produced hydroxamates. Results obtained by Lee et al. (2012), suggest that catechols are only produced at times of severe iron starvation and they found that the proportions of actinobacterial isolates producing catechols were only 3%. Production started after 24 h and catechols accumulation increased with time.

![Figure 4. Indole-3-acetic acid production with different concentrations of L-Trp](image)

![Figure 5. Kinetics of catechols production by *Streptomyces albidoflavus* SB1 and *S. flaveus* SO1, as a function of time.](image)
Thus, *S. albidoflavus* SB1 production reached its peak after 60 h and then was decreasing slowly, while *S. flavus* SO1 production continued to increase after 72 h. Kinetics of siderophores studied by Dimkpa, et al. (2009), is in agreement with our findings, they demonstrated their positive detection as early as 24 h. Additionally, similar kinetics of SB1 was reported for the *Streptomyces griseus* Lac1 strain (Aouar et al. 2016).

Phosphate solubilization

It has been established that phosphate is a plant growth-limiting element. Thus, several studies have focused on the capacity of microorganisms to solubilize phosphate (Sezen et al. 2016). Which have shown their efficiency in improving the accessibility of phosphate to plants by laboratory, greenhouse, or field trials (Salcedo et al. 2014). Nevertheless, actinobacteria have been extensively studied for their capacity to produce new antibiotics, but few studies have explored their aptitude to solubilize phosphate (Sudiana et al. 2020). In this study, a qualitative assessment of phosphate solubilization showed the apparition of a clear solubilization zone around colonies of *S. flaveus* SO1 and *S. enissoaesilis* SO2. The recorded diameters were 0.7±0.1mm and 0.9±0.1mm, respectively. Our results corroborate with those of Franco-Correra et al. (2010), they found that phosphate solubilizing ability was widely exhibited by actinobacteria isolates. However, it has been found by Qin et al. (2015) and Aouar et al. (2016) that the greater part of their isolates was unable to solubilize phosphate.

Biocontrol assay

As presented in Figure 6, only *S. flaveus* SO1 was able to inhibit *S. scabiei* in vivo. It demonstrates no significant difference (p < 0.05) with negative control. Also, the results revealed that no significant difference (p < 0.05) was recorded between *S. enissoaesilis* SO2, *S. albidoflavus* SB1, and positive control. Biocontrol trial showed that *S. flavus* SO1 was able to antagonize significantly *S. scabiei* and to suppress symptoms of common scab, especially root necrosis (Figure 7).

Mechanisms of biocontrol include antibiotic production, secretion of hydrolytic enzymes, production of siderophores, and niche exclusion. It is clear that our isolates act by antibiosis, which results in clear lysis zones around bacterial colonies as shown in Figure 1 a. According to the literature, there are already some antibiotics elaborated by *Streptomyces* that are used against bacterial plant diseases such as streptomycin synthesized by *Streptomyces griseus* to control *Xanthomonas oryzae* and *Pseudomonas lachrymans* (Copping and Menn 2000). It is evident that one of the mechanisms used by *S. flaveus* SO1 is antibiosis, as this strain has already shown interesting antagonistic activity in vitro against this pathogen. Furthermore, it has been stated that antibiosis is the main mechanism in biocontrol since isolates that are active in vitro are also effective in soil environment (Goudjal et al. 2014). However, this antibiosis does not accept the involvement of other plant growth-promoting properties, considering that this strain is also capable to produce siderophores and to solubilize phosphate. *Streptomyces flaveus* SO1 isolated from an arid soil collected from a region characterized by a Saharan climate, is a rhizospheric strain which means that it is already adapted to the different influences and interactions characterizing the rhizosphere. Furthermore, this strain presents PGPR characteristics in addition to its antagonism against certain bacteria and phytopathogenic fungi. All these promising properties qualify it to be a good candidate as a biocontrol agent and plant growth promoter, especially in arid regions.

Figure 6. Biocontrol assay against *Streptomyces scabiei*

Figure 7. Effect of SO1 on the development of radish seedlings. A. Radish seedlings no treated (negative control); B. Radish seedlings treated with *S. scabiei* and SO1; C. Radish seedlings treated with *S. scabiei* (positive control)
In the present study, the enhancement of root growth cannot be related to the IAA effect, because the medium has not been amended with L-Trp, especially since these strains require L-Trp as precursor to synthesize IAA. The effect of IAA, produced by actinobacterial strains, on plant growth has already been explored. Therefore, Merzaeva and Shirokikh (2010) found that IAA produced by actinobacteria improves rye seed germination and promotes seedling growth. In addition, Goudjal et al. (2014) have reported that the IAA extracted from Streptomyces sp. exhibited a promoting effect on seeds germination and root growth of tomatoes seedlings.

From the research that has been done, it is possible to conclude that three interesting rhizospheric isolates were selected from Algerian Saharan soils. Their pronounced antagonistic profiles highlight their potential as antibacterial and antifungal agents against plant phytopathogens. Particularly they have been able to degrade chitin, which can also contribute to the antagonistic capacity against fungi. These isolates belong to the genus Streptomyces and have been identified as S. flavus SO1, S. enisocassalis SO2, and S. albidoflavus SB1. Among these isolates, Streptomyces flavus SO1 has Inhibited in vitro all tested pathogens including bacteria and fungi. In addition, it has demonstrated the potential to produce IAA, to elaborate siderophores type catechol, to solubilize phosphate, and to antagonize S. scabiei in vivo. These characteristics confer its potential as a promising candidate to enhance plant growth and to control plant pathogens. Therefore, this preliminary study must be completed; it requires further investigations to evaluate the antagonistic capacity in the field and to elucidate the other processes implicated in plant growth promoting and biocontrol.

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