BIOLOGICAL ACTIVITIES OF *Streptomyces* sp. BTS40 ISOLATED FROM THE RHIZOSPHERE OF *Artemisia herba-alba* Asso

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Abstract. Actinobacteria isolated from the rhizosphere of plants are of interest as they produce a diverse range of molecules, such as antibiotics and enzymes. This study investigates the antibacterial activity, plant growth-promoting (PGP) abilities as well as the production of extracellular enzymes by the actinobacterial strain BTS40. This strain was isolated from the rhizospheric soil of the medicinal plant *Artemisia herba-alba* Asso that was naturally grown in a semi-arid environment. Morphological characteristics showed that the strain BTS40 belongs to the genus *Streptomyces*. Analysis of BTS40's 16S rRNA gene sequence showed 99.45% similarity to *Streptomyces alboniger* NRRL B-1832^T, in the EzTaxon database. This actinobacterium showed only antibacterial activity against Gram-positive bacteria. The strain also showed potential multiple traits for plant growth promotion and hydrolysis of enzymes. Hence, this study reveals that strain BTS40 has multiple PGP traits and produces many extracellular hydrolytic enzymes.

Key words: *Artemisia herba-alba*; rhizosphere; *Streptomyces*; taxonomy; PGP; enzymes; semi-arid area; Algeria.

INTRODUCTION

The plant rhizosphere represents a rich reservoir for organisms including bacteria, fungi, oomycetes, nematodes, protozoa, algae, viruses, archaea, arthropods and actinobacteria [38]. Microorganisms present in the rhizosphere are critical for host plant nutrient acquisition, development, tolerance to diverse abiotic and biotic stresses [35].

Actinobacteria are Gram-positive, filamentous and spore-forming microorganisms that participate actively in many biological processes like recycling biomolecules, the biogeochemical cycles, bioremediation and producing diverse compounds that have agricultural and pharmaceutical applications [48]. They are known as plant growth-promoting rhizobacteria (PGPR) that produce diverse and efficient natural bioactive metabolites including plant growth-promoting substances that directly modulate plant hormone levels or help in nutrient uptake (phosphorus, potassium, etc.) or indirectly by protecting plants from pathogens in the forms of biocontrol agents or production of cell wall degrading enzymes [2, 17, 38].

One of the major producers of biologically important molecules is members of the genus *Streptomyces* which can be found in different environments, including soil [8, 14, 32], sediments [52], plants [30], algae [15], animal feces [25] and rhizosphere soil [6, 20]. It was reported that the abundance of *Streptomyces* was much higher in the rhizosphere than in the bulk soil [28, 34]. In addition, more than half of the antibiotics used nowadays are produced by *Streptomyces* species [36, 45]. The genus *Streptomyces*, which was first proposed by Waksman and Henrici (1943) [54], has 1105 species with validly published names found in the List of Procaryotic names with Standing Nomenclature (http://lpsn.dsmz.de/genus/streptomyces). Research on *Streptomyces* species is of current interest as they are producers of pharmaceutically bioactive compounds like antibiotics, antitumor and immunosuppressive agents as well as several important enzymes [49].

The Algerian untapped habitats represent different ecosystems that can harbor an actinobacterial diversity with biological activities that are worthy of being explored. One of the plants that to our knowledge have not been explored for their culturable actinobacteria associated with its rhizosphere is *Artemisia herba-alba* Asso from the *Asteraceae* family. Its a greenish-silver perennial medicinal plant that is commonly known as white wormwood or desert wormwood in English [44]. *A. herba-alba* Asso grows in arid areas of the Mediterranean region spreading into Middle-East, North-Western Himalayas and India, while it is widely distributed in the arid areas, steppes and Sahara of Algeria [11, 53]. This plant is used in both traditional as well as in contemporary medicine for its biological activities including analgesic, antibacterial, antispasmodic, hemostatic and others [39].

Therefore in this study, we isolated from the rhizosphere of *A. herba-alba* Asso the strain BTS40 that was screened for certain biological activities. According to 16S rRNA sequence analysis, combined with morphological, cultural, physiological and biochemical characteristics, the taxonomic status of the strains was determined. We investigated the *in-vitro*

antagonistic activity against some Gram-positive and Gram-negative bacteria, plant growth-promoting traits as well as hydrolytic enzymes production by strain BTS40.

MATERIALS AND METHODS

Site selection and sampling

Strain *Streptomyces* sp. BTS40 was isolated from rhizospheric soil attached to the roots of *A. herba-alba* Asso that was collected from a natural site in Ghasrou, Batna province (Algeria) (GPS coordinates: 35°35′47.8″N, 5°74′1.55″E). In addition, 1 kg of bulk soil was randomly collected from a 50 m^2 area at a depth of 5 to 20 cm after removing the topsoil. The soil was placed in a sterile plastic bag and then used for determining physico-chemical properties. The samples were transported in an icebox to Laboratoire de Biologie des Systèmes Microbiens (LBSM) at the Ecole Normale Supérieure, Kouba (Algiers, Algeria) where they have been stored at 4°C pending analysis.

Soil physico-chemical analysis

The physico-chemical analyses of the soil sample that was collected from the sampling sites was carried out at the Laboratory of the National Bureau of Studies for Rural Development (BNEDER), Bouchaoui (Algiers, Algeria). The soil pH and electrical conductivity were determined with a 1:5 mixture (soil: water) by potentiometry using a calibrated pH meter (ISO 10390, 2005) and a calibrated electrical conductivity meter, respectively (AFNOR NF ISO 10-970). The soil organic carbon content was determined using sulfochromic oxidation colorimetry (ISO 14235, 1998). The total carbon [AFNOR NF ISO 10-694], Cation Exchange Capacity (CEC) [AFNOR NF ISO 23-470] and particle size distribution (soil texture) (AFNOR NF X 31-107) were also determined. All the physic-chemical analyses were performed in triplicate and the results are given in Table 1.

Isolation and maintenance of the actinobacterium

The isolation of the actinobacterium from the rhizospheric soil sample of *A. herba-alba* Asso was carried out as recommended by Hayakawa and Nonumura on the surface of Petri dishes containing Chitin-Vitamins-B agar medium [22] supplemented with nalidixic acid (50 µg/mL) and cycloheximide (80 µg/mL). The samples were diluted ten-fold in sterile distilled water and 100 µL from the 10^{-3} dilution was spread onto the medium (two plates per dilution). After an incubation period of 21 days at 28 ± 2°C, one colony that was observed under the light microscope (Zeiss) (X40) showed an Actinobacteria-like morphology was named BTS40. The isolate was picked up, purified on International Streptomyces Project (ISP) medium 2 [47] and then was maintained at 4°C.

Morphological, cultural and physiological analysis

The macro and micro-morphological characteristics were studied after growth of the actinobacterium at 28 ± 2°C for 21 days on five culture media (Agar tryptone yeast extract (ISP1), Agar yeast-malt extract (ISP2), Agar starch and inorganic salts (ISP4), Tryptic soy Agar (TSA) and Potato Dextrose Agar (PDA) [47]. The characteristics evaluated were; growth rate, the formation and color of aerial spore mass and substrate mycelia with the ISCC-NBS color chart used to determine the colony color [27].

A 15 days culture of strain BTS40 at 28 ± 2°C was grown on ISP2 medium was used to cut 1 cm^2 fragments that were dried in the oven for 24h at 45°C. Then, the samples were observed under a Scanning Electron Microscope (SEM Quanta 400 FEI - providing 30KV of acceleration).

The growth of BTS40 on ISP2 medium was monitored for 10 days at different temperatures (20, 30, 40 and 50°C; pH 7), a pH range of 4 to 12, and different NaCl concentrations (0, 1, 2, 3, 4, 5, 6, 7, 8 and 9 % w/v; pH 7) [3].

The conducted biochemical tests and evaluation of acid production from carbohydrates were assessed as described by Gordon et al. (1974) [19] and Cappuccino and Sherman (1998) [10].

Identification by 16S rRNA sequence analysis

The actinobacterium was identified based on 16S rRNA gene sequencing conducted at Macrogen Ltd. (Netherlands). Identification of the nearest phylogenetic neighbors was carried out using the EzTaxon database (http://eztaxon-e.ezbiocloud.net/) [29]. The aligned sequences were used to reconstruct the phylogenetic tree using the neighbor-joining method by MEGA version 7 [46]. The evolutionary distance matrix was generated as described by Jukes and Cantor (1969) [26] and bootstrap analysis was carried out with 1000 replications.

In-vitro antibacterial activity

The *in-vitro* antagonistic ability of strain BTS40 against five tests microorganisms that were obtained from the LBSM bacterial collection, namely; *Staphylococcus aureus* (MRSA 639c), *S. aureus* (ATCC 43300), *S. aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), *Listeria monocytogenes* (ATCC 13932), *Pseudomonas aeruginosa* (ATCC 7029) and *Escherichia coli* (E52) was tested using the agar diffusion method. It consists of inoculating the actinobacterial isolates by tight streaks on the surface of the ISP2 medium and incubating at 28 ± 2°C. After ten days, agar cylinders of 10 mm in diameter were taken from the culture of actinobacteria and were placed on the surface of a semi-solid ISP2 medium (12 g/L) which was previously seeded with one of the tested bacteria (10^7 UFC/mL). The appearance of an inhibition zone after 48 h of incubation was recorded as a positive result [7].

Screening for PGP traits and the production of extracellular enzymes

The strain BTS40 was screened qualitatively for some PGP traits like ACC deaminase activity, inorganic phosphorus and potassium solubilization, ammonia, siderophores and hydrogen cyanide HCN production as described by Boubekri et al. (2021) [9].

In addition, the production of extracellular enzymes, ie: cellulase, protease and amylase was evaluated. The actinobacterium was spot inoculated on agar plates amended with the respective substrates: carboxymethyl cellulose, casein and starch and was incubated for 10 days at 28 ± 2°C [37].

The enzymatic activity was detected by measuring hydrolysis halos and the diameter of colonies in two directions. The solubilization index (SI) was determined by measuring the halo (clear zone) diameter and the colony diameter, using the following formula:

SI = Colony diameter + Halo zone diameter/Colony diameter [16].

Data analysis

The data were presented as Mean ± Standard Deviation (SD) of triplicate experiments.

RESULTS

Physico-chemical characteristics of soil

The physical and chemical parameters of the soil were performed and the results were presented in Table 1. The pH of the soil was almost neutral (7.59), the color of the soil was recorded as brown and the texture was loamy clay. The organic carbon and the organic matter contents of the soil were recorded as 1.08% and 1.85%, respectively. This soil had a 0.27 mmhos/cm EC which indicates its non-salinity and a medium Cation Exchange Capacity (CEC) of 14.10 meq/100g.

Identification and characteristics of the strain BTS40

The strain BTS40 had medium brown colonies (substrate mycelium) with white spores (aerial mycelium) on ISP2 agar medium. Very good growth was observed on all used culture media with the results are presented in Figure 1 and Table 2. Strain BTS40 produced diffusible pigments of different colors depending on the used culture media.

As presented in Table 3, different biochemical, physiological and cultural characteristics are observed for strain BTS40. Strain BTS40 was capable to degrade gelatin and the test of urease was negative. It did not produce H_2S but was capable of nitrate reduction and the test of catalase was positive. Among the compounds tested in this study, strain BTS40 used D-glucose, D-fructose, L-arabinose, D-mannose, mannitol, maltose, inositol, sorbitol, L-rhamnose, D-saccharose, glycerol, D-ribose, D-xylose, melbiose, amygdaline, dulcitol and D-cellobiose as carbon sources. The studied strain is halotolerant with growth on ISP2 supplemented with NaCl at a concentration range of 0 to 6% and growth occurred at 10 to 40°C (optimum at 28°C) and a pH 4.0-10.0 (optimum at pH 7.2) (Table 3).

Table 1. Physico-chemical properties of the analyzed soil

Batna Province	Site name	Soil characteristics					
		pH (1/5)	Carbon (%)	Organic matter (%)	Cation Exchange Capacity (meq/100g)	Electrical conductivity (mmhos/cm)	Texture[a]
	Ghasrou	7.59 ± 0.03	1.08 ± 0.01	1.85 ± 0.01	14.10 ± 0.18	0.27 ± 0.02	LC

[a] Texture according to United States Department of Agriculture (USDA): C: clay; L = loam; S = sandy; SL = sandy loam; LC: loamy clay. Results are presented as mean values ± SD.

Table 2. Morphological characteristics of strain BTS40 on different culture media after 14 days of incubation at 28°C

	Agar medium	Growth rate	Aerial mycelium	Substrate mycelium	Pigmentation	Possible genus identification
BTS40	ISP1	Very good	white	Brown	Light orange	*Streptomyces*
	ISP2	Very good	White	Medium brown with green color	Dark brown	
	ISP4	Very good	Beige	Yellow	Orange	
	TSA	Very good	Beige	Light beige yellow	Brown	
	PDA	Very good	Yellow	Dark yellow	Brown	

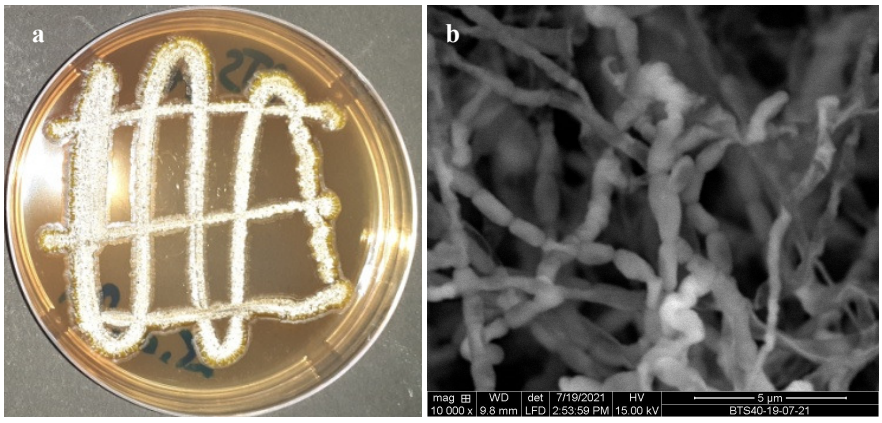

Figure 1. Macroscopic appearance (a) and scanning electron microphotograph (b) of strain BTS40 after cultivation on ISP2 agar medium at 28°C for 15 days of incubation with the photograph of the spores was taken by SEM. Bar 5 µm.

The alignment of the 16S rRNA gene sequence (1448 nucleotides) of strain BTS40, deposited in GenBank under the accession number OK255502, with those of *Streptomyces* reference species available in the EzTaxon-e server can be seen in the neighbor-joining dendrogram (Figure 2). The phylogenetic analysis revealed that strain BTS40 is 99.45% similar to *Streptomyces alboniger* NRRL B-1832^T [43].

Antibacterial activity

The strain BTS40 showed no antibacterial activity against the tested strains of Gram-negative bacteria and low antibacterial activity against the Gram-positive bacteria tested in this study using the disc diffusion method on ISP2 medium (Table 4).

Screening for PGP traits and extracellular enzymes production

It was found that the strain BTS40 was positive for ACC deaminase activity; it solubilized P and K as well as produced ammonia, siderophores and HCN (Table 5). Strain BTS40 was tested for its ability to produce cell wall-degrading enzymes shown in Table 5 and Figure 3. The obtained results showed a positive production of cellulase, protease and amylase.

Table 3. Biochemical, physiological and cultural characteristics of strain BTS40

Properties	*Streptomyces* sp. BTS40	Properties	*Streptomyces* sp. BTS40
Biochemical tests		**Effect of temperature (°C) on ISP2**	
Gelatin hydrolysis	+	10	+
Urease test	-	20	++
Nitrate reduction	+	30	+++
H_2S production	-	40	++
Catalase	+	50	-
Carbon-source utilization		**Effect of pH on ISP2**	
D-Glucose	+	4	+
D-Galactose	-	5	++
D-Fructose	-	6	+++
L-Arabinose	+	7	+++
D-Mannose	+	8	++
Mannitol	+	9	++
Maltose	-	10	+
Inositol	+	11	-
Sorbitol	+	12	-
L-Rhamnose	-	**Effect of NaCl (%) on ISP2**	
D-Saccharose	+	0	+++
Glycerol	+	1	+++
D-Ribose	+	2	++
D-Xylose	-	3	++
Melbiose	-	4	+
Amygdaline	-	5	+
Dulcitol	+	6	+
D-Cellubiose	+	7	-

+, Positive reaction; −, negative reaction. For effects of T°, pH and NaCl: + + +, good growth; ++, moderate growth; +, poor growth; -, no growth

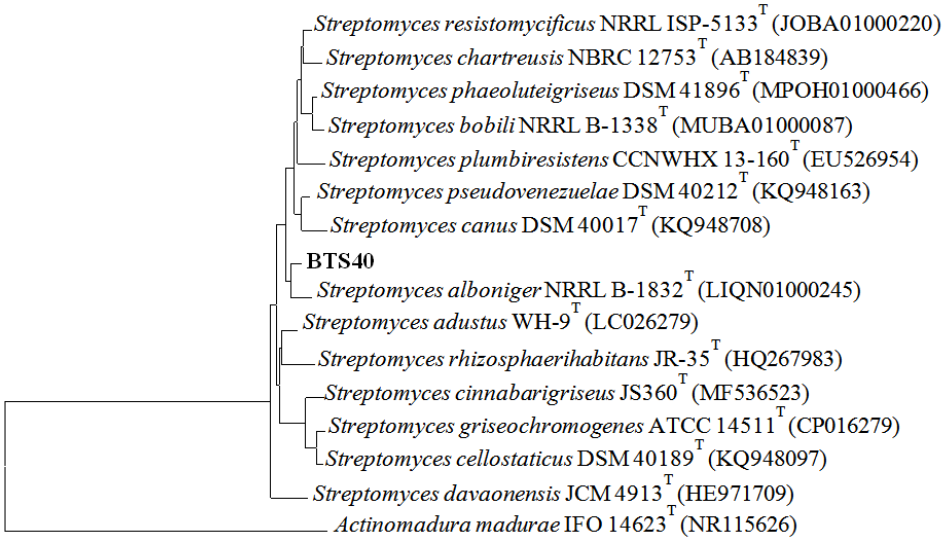

Figure 2. Phylogenetic tree of the strain *Streptomyces* sp. BTS40 and the most related type strains species based on 16S rRNA gene sequences. The evolutionary history was inferred using the Neighbor-Joining (1987) method [46]. The optimal tree with the sum of branch length = 0.16512605 is shown. The evolutionary distances were computed using the Maximum Composite Likelihood (2004) method [51] and are in the units of the number of base substitutions per site. The analysis involved 16 nucleotide sequences. Codon positions included were 1st +2nd + 3rd + Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 1405 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 [31].

DISCUSSION

The rhizosphere which is the narrow zone of soil that surrounds and is influenced by plant roots is inhabitant by an important number of microorganisms [42]. In Algeria, previous studies of different host plants reported the presence of actinobacteria in their rhizosphere [4-5, 13, 40]. Like many plants, the rhizosphere of *A. herba-alba* Asso is colonized by actinobacteria that is unexplored for microbial natural product discovery. In this study, the antibacterial activity and the PGP traits of strain BTS40 that was isolated from the rhizospheric soil of *A. herba-alba* Asso a medicinal plant from the semi-arid and arid environments of Algeria were investigated. The results illustrated that the studied actinobacterium is an important microbial source of multiple beneficial traits worthy of exploration.

The *Streptomyces* genus continues to be a focus in a systematic research point of view as it encompasses a large number of isolates that are closely related and can be difficult to distinguish between them [21]. It constitutes a major clade of the phylum *Actinobacteria* that have remarkable diversity in morphology, genomic size, G + C DNA content and is an important source of bioactive natural product synthesis [34].

The 16S rRNA sequence of strain BTS40 was found to be closely related to *Streptomyces alboniger* NRRL B-1832^T [43] with a sequence identity of 99.45%. Similar carbon-utilization profile to *Streptomyces alboniger* NRRL B-1832^T, as both strains were able to use glucose, arabinose, inositol and mannose whereas they could not use xylose, fructose and rhamnose (https://bacdive.dsmz.de/strain/14940).

The screening for the antimicrobial activity against some clinical test bacteria showed the activity of strain BTS40 against only the Gram-positive bacteria tested (different strains of *S. aureus*, *B. subtilis* and *L. monocytogenes*). This might be supported by the fact that the extract of *Streptomyces alboniger* was reported to contain pamamycins, a group of polyketides that induce sporulation in *Streptomyces* and inhibit the growth of Gram-positive bacteria, *Mycobacterium tuberculosis* and fungi [41]. Different studies have been carried out in order to screen for antagonistic strains of *Streptomyces*. Barakate et al. (2002) [6] found that most of the isolates (83%) of *Streptomyces* isolated from rhizosphere soils of medicinal plants were active against one or more of the organisms tested in their study.

Plant growth-promoting rhizobacteria (PGPR) are searched by researchers because of their sustainable characteristics in agriculture as their potential use in practice helps in the reduction of the application of polluting fertilizers and pesticides [23]. The studied strain BTS40 showed multiple features of plant growth promotion. It was found to have ACC deaminase activity, one of the major mechanisms of PGPB in

Table 4. *In-vitro* antibacterial activity of strain BTS 40 by the disc diffusion method on ISP2 medium

Isolate code	Antibacterial activity[a]						
	S. aureus (MRSA 639c)	*S. aureus* (ATCC 43300)	*S. aureus* (ATCC 6538)	*B. subtilis* (ATCC 6633)	*L. monocytogenes* (ATCC 13932)	*P. aeruginosa* (ATCC 7029)	*E. coli* (E52)
BTS40	+	+	+	+	+	-	-

[a] Inhibition zone expressed as (+< 10 mm; 20>++>30+++).

Table 5. Plant growth-promoting traits and enzymatic activity of strain BTS40

Isolate code	PGP characteristics						Hydrolytic enzyme production		
	ACC deaminase	P-solubilization	K-solubilization	NH_3 production	Siderophores	HCN	Cellulase	Protease	Amylase
BTS40	+	1.15 ± 0.04	1.12 ± 0.07	+++	++	+	1.34 ± 0.04	2.32 ± 0.07	1.73 ± 0.13

Production of siderophores and ammonia shows the intensity of orange/pink color halo or yellow/brown color, respectively (+ weak, ++ medium and +++ strong) on blue agar medium CAS and peptone water broth, ACC and HCN were classified as positive (+) or negative (-).

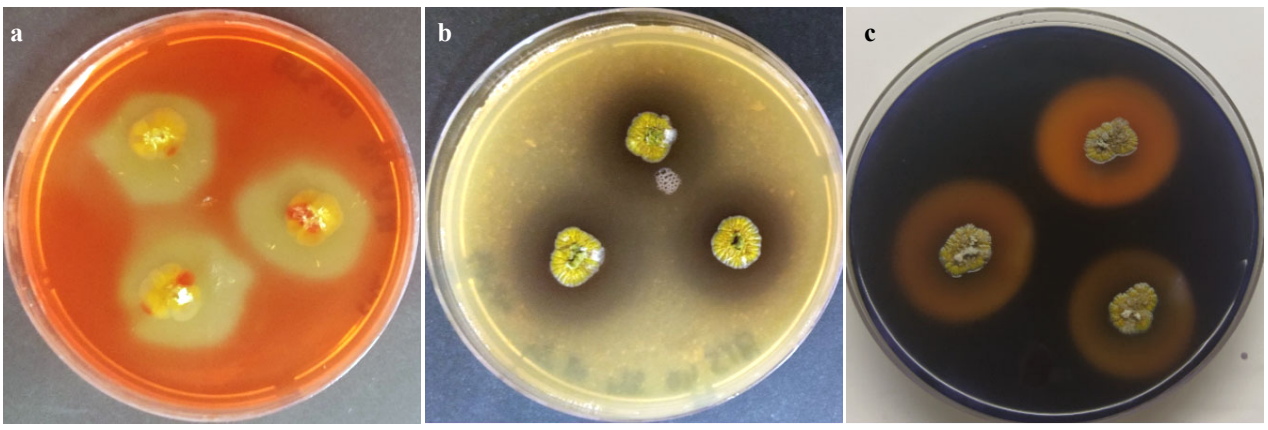

Figure 3. Images showing hydrolysis halos produced by strain BTS40: cellulase (a), protease (b) and amylase (c).

promoting plant growth. It is well known that bacteria that have ACC deaminase activity help in the reduction of the level of stress ethylene for plants [18].

As nutrients are the main factors limiting plant growth, the search for inorganic phosphate and potassium solubilizers is necessary in order to improve the amount of soluble phosphorus and potassium in soil and to meet the requirements of growing plants [1]. These authors reported that all the 10 strains of *Streptomyces* were P and K solubilizers.

The strain BTS40 was able to produce ammonia and therefore supply nitrogen to the host. Also, this strain was positive for the production of siderophore compounds that are potential plant growth promoters and disease suppressors [50]. Strain *Streptomyces* CMU-SK126 isolated from *Curcuma mangga* rhizosphere soil showed a high ability to produce siderophores [28].

The hydrogen cyanide was produced by strain BTS40, this compound is reported to have a role in the suppression of plant disease. Husen et al. (2011) [24] reported *Streptomyces* sp. LSW05 strain as a potent HCN producer. Similar results were obtained by Anwar et al. (2016) [3] where they found that from six isolates that were producers of siderophores, *Streptomyces* sp. WA-1 and *S. djakartensis* TB-4 displayed the highest amount of HCN production.

In this study, the activity of the hydrolytic enzymes was investigated for strain BTS40. The results showed that BTS40 was positive for the production of cellulase, protease and amylase. As stated by Dhanasekaran et al. (2008) [12] and Latha et al. (2009) [33], *Streptomyces* species that can produce hydrolytic enzymes can inhibit the growth of soil-borne plant pathogens. PGPR can affect crop growth also indirectly by preventing and reducing the effect of soil-borne plant pathogens through the production of antimicrobial compounds and extracellular enzymes.

The present study revealed important insights into some biological activities of the strain *Streptomyces* sp. BTS40, an actinobacterium that was isolated from the rhizospheric soil of *A. herba-alba* Asso. This strain showed antibacterial activity against Gram-positive bacteria and displayed multiple PGP traits which could be useful for application in further studies on antimicrobial agents or in the application for agricultural practices.

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

REFERENCES

[1] Aallam, Y., Maliki, B.E., Dhiba, D., Lemriss, S., Souiri, A., Haddioui, A., Tarkka, M., Hamdali, H., (2021): Multiple potential plant growth promotion activities of endemic *Streptomyces* spp. from Moroccan sugar beet fields with their inhibitory activities against *Fusarium* spp. Microorganisms, 9: 1429.

[2] Ahemad, M., Kibret, M., (2014): Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. Journal of King Saud University-Science, 26(1): 1-20.

[3] Anwar, S., Ali, B., Sajid, I., (2016): Screening of rhizospheric actinomycetes for various *in vitro* and *in vivo* plant growth promoting (PGP) traits and for agroactive compounds. Frontiers in Microbiology, 7: 1334.

[4] Aouar, L., Boukelloul, I., Benadjila, A., (2021): PGPR traits of rhizospheric *Nocardiopsis* strains isolated from Algerian soils. In: Ksibi, M. et al. (eds.): Recent Advances in Environmental Science from the Euro-Mediterranean and Surrounding Regions (2nd Edition). EMCEI 2019. Environmental Science and Engineering. Springer, Cham.

[5] Aouar, L., Lerat, S., Ouffroukh, A., Boulahrouf, A., Beaulieu, C., (2012): Taxonomic identification of rhizospheric actinobacteria isolated from Algerian semi-arid soil exhibiting antagonistic activities against plant fungal pathogens. Canadian Journal of Plant Pathology, 34: 165-176.

[6] Barakate, M., Ouhdouch, Y., Oufdou, K., Beaulieu, C., (2002): Characterization of rhizospheric soil streptomycetes from Moroccan habitats and their antimicrobial activities. World Journal of Microbiology and Biotechnology, 18: 49-54.

[7] Bauer, A.W., Kirby, W.M., Sherris, J.C., Turck, M., (1966): Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology, 45(4): 493-496.

[8] Bettache, A., Copinet, E., Azzouz, Z., Boucherba, N., Bouiche, C., Hamma, S., Maibeche, R., Duchiron, F., Benallaoua, S., (2020): Purification and characterization of an endoglucanase produced from *Streptomyces* sp. strain bpng23. The Journal of Microbiology, Biotechnology and Food Sciences, 10: 284-288.

[9] Boubekri, K., Soumare, A., Mardad, I., Lyamlouli, K., Hafidi, M., Ouhdouch, Y., Kouisni, L., (2021): The screening of potassium- and phosphate-solubilizing actinobacteria and the assessment of their ability to promote wheat growth parameters. Microorganisms, 9(3): 470.

[10] Cappuccino, J.G., Sherman, N., (1998): Microbiology: a Laboratory Manual. 5th ed. California: Benjamin/Cummings Science Publishing, 477 p.

[11] Dahmani-Hamzaoui, N., Baaliouamer, A., (2010): Chemical composition of Algerian *Artemisia herba-alba* essential oils isolated by microwave and hydrodistillation. Journal of Essential Oil Research, 22(6): 514-517.

[12] Dhanasekaran, D., Thajuddin, N., Panneerseilvam, A., (2008): An antifungal compound: 4′ phenyl-1- napthyl-phenyl acetamide from *Streptomyces* sp. DPTTB16. Facta Universitatis Series: Medicine and Biology, 15: 7-12.

[13] Dif, G., Belaouni, H.A., Goudjal, Y., Yekkour, A., Djemouai, N., Zitouni, A., (2021): Potential for plant growth promotion of *Kocuria arsenatis* Strain ST19 on tomato under salt stress conditions. South African Journal of Botany, 138: 94-104.

[14] Djaballah, C.E., Kitouni, M., Raoult, D., Khelaifia, S., (2018): *Streptomyces massilialgeriensis* sp. nov., a new

bacterial species isolated from an extremely saline soil collected from the dry lake of Ank el Djamel in Algeria. New Microbes New Infect, 21: 18-19.

[15] Djinni, I., Djoudi, W., (2021): *Streptomyces* sp. WR1L1S8 a potent endophytic marine strain for heavy metal resistance and copper removal enhanced by RSM modeling. Acta Ecologica Sinica, *In press.*

[16] Edi-Premono, M., Moawad, A.M., Vlek, P.L.G., (1996): Effect of phosphate-solubilization *Pseudomonas putida* on the growth of maize and its survival in the rhizosphere. Indonesian Journal of Crop Science, 11: 13-23.

[17] El-Tarabily, K.A., (2006): Rhizosphere-competent isolates of streptomycete and non-streptomycete actinomycetes capable of producing cell-wall-degrading enzymes to control *Pythium aphanidermatum* damping-off disease of cucumber. Canadian Journal of Botany, 84(2): 211-222.

[18] Glick, B.R., (2014): Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiological Research, 169: 30-39.

[19] Gordon, R., Barnett, D.A., Handerhan, J.E., Pang, C., (1974): *Nocardia coeliaca*, *Nocardia autotrophica*, and the Nocardin Strain. International Journal of Systematic and Evolutionary Microbiology, 24: 54-63.

[20] Guo, Y.H., Tang, X.K., Hu, S.R., Zhou, M.L., Gao, J., (2021): *Streptomyces liangshanensis* sp. nov., a novel actinomycete isolated from rhizosphere soil of *Fagopyrum tataricum*. Archives of Microbiology, 203(6): 3055-3059.

[21] Guo, Y., Zheng, W., Rong, X., Huang, Y., (2008): A multilocus phylogeny of the *Streptomyces griseus* 16S rRNA gene clade: use of multilocus sequence analysis for streptomycete systematics. International Journal of Systematic and Evolutionary Microbiology, 58(Pt 1): 149-159.

[22] Hayakawa, M., Nonomura, H., (1987): Humic acid-vitamin agar, a new medium for the selective isolation of soil actinomycetes. Journal of Fermentation Technology, 65(5): 501-509.

[23] Hu, D., Li, S., Li, Y., Peng, J., Wei, X., Ma, J., Zhang, C., Jia, N., Wang, E., Wang, Z., (2020): *Streptomyces* sp. strain TOR3209: a rhizosphere bacterium promoting growth of tomato by affecting the rhizosphere microbial community. Scientific reports, 10(1): 20132.

[24] Husen, E., Wahyudi, A., Suwanto, T., Giyanto, A., (2011): Growth enhancement and disease reduction of soybean by 1-aminocyclopropane-1-carboxylate deaminase-producing *Pseudomonas*. American Journal of Applied Sciences, 8: 1073-1080.

[25] Jiang, Y., Cao, Y., Han, L., Jin, R., Zheng, D., He, W., Li, Y., Huang, X., (2012): Diversity and bioactivity of culturable actinobacteria from animal feces. Wei sheng wu xue bao = Acta Microbiologica Sinica, 52(10): 1282-1289.

[26] Jukes, T.H., Cantor, C.R., (1969): Evolution of protein molecules. In: Munro, H.N., (Ed.): Mammalian Protein Metabolism. Academic Press, New York, pp. 21-132.

[27] Kelly, K.L., (1964): Inter-society color council- national bureau of standards color name charts illustrated with centroid colors. US government printing office, Washington DC, 196 p.

[28] Khamna, S., Yokota, A. Lumyong, S., (2009): Actinomycetes isolated from medicinal plant rhizosphere soils: diversity and screening of antifungal compounds, indole-3-acetic acid and siderophore production. World Journal of Microbiology and Biotechnology, 25: 649-655.

[29] Kim, O.S., Cho, Y.J., Lee, K., Yoon, S.H., Kim, M., Na, H., Park, S.C., Jeon, Y.S., Lee, J.H., Yi, H., Won, S., Chun, J., (2012): Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. International Journal of Systematic and Evolutionary Microbiology, 62(Pt 3): 716-721.

[30] Klykleung, N., Yuki, M., Kudo, T., Ohkuma, M., Phongsopitanun, W., Inahashi, Y., Matsumoto, A., Tanasupawat, S., (2020): *Streptomyces mimosae* sp. nov., an endophytic actinomycete isolated from the root of *Mimosa pudica* in Thailand. International Journal of Systematic and Evolutionary Microbiology, 70(5): 3316-3322.

[31] Kumar, S., Stecher, G., Tamura, K., (2016): MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution, 33: 1870-1874.

[32] Laassami, A., Yekkour, A., Meklat, A., Djemouai, N., Zitouni, A., Mokrane, S., Lecomte, P., Rey, P., Berraf-Tebbal, A., (2020): Actinobacteria associated with vineyard soils of Algeria: Classification, antifungal potential against grapevine trunk pathogens and plant growth-promoting features. Current Microbiology, 77(10): 2831-2840.

[33] Latha, P., Anand, T., Ragupathi, N., Prakasam, V., Samiyappan, R., (2009): Antimicrobial activity of plant extracts and induction of systemic resistance in tomato plants by mixtures of PGPR strains and *Zimmu* leaf extract against *Alternaria solani*. Biological Control, 50(2): 85-93.

[34] Li, S., Dong, L., Lian, W-H., Lin, Z.L., Lu, C.Y., Xu, L., Li, L., Hozzein, W.N., Li, W.J., (2021): Exploring untapped potential of *Streptomyces* spp. in Gurbantunggut desert by use of highly selective culture strategy. Science of The Total Environment, 790: 148235.

[35] Liu, H., Brettell, L.E., Qiu, Z., Singh, B.K., (2020): Microbiome-mediated stress resistance in plants. Trends in Plant Science, 25(8): 733-743.

[36] Lucas, X., Senger, C., Erxleben, A., Grüning, B.A., Döring, K., Mosch, J., Flemming, S., Günther, S., (2013): Streptome DB: a resource for natural compounds isolated from *Streptomyces* species. Nucleic Acids Research, 41(Database issue): D1130-D1136.

[37] Mamangkey, J., Suryanto, D., Munir, E., Mustopa, A.Z., Sibero, M.T., Mendes, L.W., Hartanto, A., Taniwan, S., Ek-Ramos, M.J., Harahap, A., Verma, A., Trihatmoko, E., Putranto, W.S., Pardosi, L., Rudia, L.O., (2021): Isolation and enzyme bioprospection of bacteria associated to *Bruguiera cylindrica*, a mangrove plant of North Sumatra, Indonesia. Biotechnology Reports, 30: e00617.

[38] Mendes, R., Garbeva, P., Raaijmakers, J.M., (2013): The rhizosphere microbiome: Significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiology Reviews, 37(5): 634-663.

[39] Mohamed, A.E.-H.H., El-Sayed, M.A., Hegazy, M.E., Helaly, S.E., Esmail, A.M., Mohamed, N.S., (2010): Chemical constituents and biological activities of *Artemisia herba-alba*. Records of Natural Products, 4: 1-25.

[40] Nabti, E., Bensidhoum, L., Tabli, N., Dahel, D., Weiss, A., Rothballer, M., Schmid, M., Hartmann, A., (2014): Growth stimulation of barley and biocontrol effect on

plant pathogenic fungi by a *Cellulosimicrobium* sp. strain isolated from salt-affected rhizosphere soil in Northwestern Algeria. European Journal of Soil Biology, 61: 20-26.

[41] Natsume, M., Yasui, K., Kondo, S., Marumo, S., (1991): The structures of four new pamamycin homologues isolated from S*treptomyces alboniger*. Tetrahedron Letters, 32(26): 3087-3090.

[42] Philippot, L., Raaijmakers, J.M., Lemanceau, P., van der Putten, W.H., (2013): Going back to the roots: the microbial ecology of the rhizosphere. Nature reviews. Microbiology, 11(11): 789-799.

[43] Porter, J.N., Hewitt, R.I., Hesseltine, C.W., Krupka, G., Lowery, J.A., Wallace, W.S., Bohonos, N., Williams, J.H., (1952): Achromycin: a new antibiotic having trypanocidal properties. Antibiotics and Chemotherapy, 2: 409-410.

[44] Quézel, P. Santa, S., (1962, 1963): Nouvelle Flore d'Algérie et des Régions Désertiques Méridionales. 2 Tomes, Editions CNRS, Paris.

[45] Quinn, G.A., Banat, A.M., Abdelhameed, A.M., Banat, I.M., (2020): *Streptomyces* from traditional medicine: sources of new innovations in antibiotic discovery. Journal of Medical Microbiology, 69(8): 1040-1048.

[46] Saitou, N., Nei, M., (1987): The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution, 4(4): 406-425.

[47] Shirling, E.B., Gottlieb, D., (1966): Methods for characterization of *Streptomyces* species. International Journal of Systematic and Evolutionary Microbiology, 16: 313-340.

[48] Shivlata, L., Satyanarayana, T., (2015): Thermophilic and alkaliphilic actinobacteria: biology and potential applications. Frontiers in Microbiology, 6: 1014.

[49] Sivalingam, P., Hong, K., Pote, J., Prabakar, K., (2019): Extreme environment *Streptomyces*: Potential sources for new antibacterial and anticancer drug leads? International Journal of Microbiology, 2019: 5283948.

[50] Sulochana, M.B., Jayachandra, S.Y., Kumar, S.A., Parameshwar, A.B., Reddy, K.M., Dayanand, A., (2014): Siderophore as a potential plant growth-promoting agent produced by *Pseudomonas aeruginosa* JAS-25. Applied Biochemistry and Biotechnology, 174(1): 297-308.

[51] Tamura, K., Nei, M., Kumar, S., (2004): Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences (USA), 101: 11030-11035.

[52] Tokatli, A., Idil, O., Saygin, H., Sahin, N., (2019): *Streptomyces ureilyticus* sp. nov. and *Streptomyces mesophilus* sp. nov., two novel Actinobacteria with antimicrobial activity isolated from lake sediment. International Journal of Systematic and Evolutionary Microbiology, 71(3): 10.1099/ijsem.0.004738.

[53] Vernin, G., Merad, O., Vernin, G.M., Zamkotsian, R.M., Parkanyi, C.D., (1995): GC-MS analysis of *Artemisia herba-alba* Asso. essential oils from Algeria. Developments in Food Science, 37: 147-205.

[54] Waksman, S.A., Henrici, A.T., (1943): The nomenclature and classification of 490 actinomycetes. Journal of Bacteriology, 46: 337-341.

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