PLANT GROWTH PROMOTING AND BIOCONTROL ACTIVITIES OF *Streptomyces* sp. STRAIN BT21

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Abstract. Endophytic actinobacteria play vital roles in plant health and biocontrol, yet their potential in Algerian medicinal plants remains understudied. This research reports the isolation and characterization of *Streptomyces* sp. strain BT21 from the root of *Artemisia herba-alba* Asso collected in Batna Province, Algeria. Using morphological, biochemical, physiological, and molecular approaches, strain BT21 was identified and evaluated for its potential as a bioactive agent. Sequence analysis of the 16S rRNA gene (1,454 bp) revealed 99.24% similarity between strain BT21 and *Streptomyces specialis* GW41-1564^T, supporting its morphological identification. Strain BT21 also demonstrated halotolerance and growth over a wide range of temperatures and pH, indicating strong environmental adaptability. This strain also demonstrated plant growth-promoting (PGP) traits, including positive ammonia production and atmospheric nitrogen fixation, although it lacked phosphate solubilization activity. Additionally, the strain exhibited broad-spectrum antibacterial and antifungal activities, with inhibition zones reaching up to 28 mm against *Micrococcus luteus* ATCC 9314 on SMG and SMA media. Furthermore, the antifungal activity of strain BT21 was observed against all the tested fungi on various media, including SMG, SML, SMA, SSMG, and SSML, with inhibition zones ranging from 13 to 25 mm. Notably, the antimicrobial activities were significantly influenced by culture media composition. The strain produced key extracellular enzymes, including amylases, cellulases, esterases, proteases and chitinases, which are important for nutrient cycling and pathogen suppression. The combined biocontrol and PGP properties of strain BT21 suggest promising applications in sustainable agriculture and biotechnology. Future work will focus on genomic analysis and *in vivo* evaluation to unlock the full potential of this endophytic *Streptomyces* strain.

Keywords: *Artemisia herba-alba*; endophyte; lytic enzymes; molecular identification; PGP; secondary metabolites; *Streptomyces*.

INTRODUCTION

Plants associate with diverse microorganisms, including bacteria, actinobacteria, and fungi, engaging in various symbiotic and beneficial interactions. These microbial endophytes significantly contribute to plant growth, health, and resilience in natural environments [67]. Recent studies have increasingly focused on understanding microbial endophytic communities and the mechanisms by which they enhance plant development and stress tolerance [25, 27].

In Algeria, research on fungal and bacterial endophytes remains limited, with notable studies documenting fungal endophytes in species such as *Argania spinosa* L. [50], *Peganum harmala* L. and *Schinus molle* L. [55] as well as *Anabasis prostrata* Pomel., *Avena fatua* L., *Lolium rigidum* Gaudin, and *Salsola oppositifolia* Desf. [11], and *Artemisia herba alba* [13]. Bacterial endophytes have been reported in several Algerian plants, including weeds [44], *Genista cinerea* [17], *Retama monosperma* [16], *Stipa tenacissima* L. [18], and *Atriplex halimus* L., *Lygeum spartum* L. [19], as well as *Artemisia herba-alba* Asso [10, 24]. Actinobacteria are a well-characterized and important group of bacteria that are frequently found as endophytes in plants [3, 20]. They have been isolated from diverse host plant species, including wheat [15], rice [65], *Aquilaria crassna* Pierre ex Lec. [49], *Dracaena cochinchinensis* Lour. [57], *Thymus roseus* [48], as well as from various medicinal plants [6, 10, 24, 37].

Among these, the genus *Streptomyces* represents the most prevalent actinobacterial endophyte. *Streptomyces* species are well known for their biocontrol potential, owing to their production of diverse antifungal and antibacterial metabolites [23]. Additionally, *Streptomyces* species secrete a broad spectrum of lytic enzymes capable of degrading complex organic polymers such as chitin and cellulose, facilitating nutrient cycling and pathogen suppression [70]. Moreover, actinobacterial endophytes are recognized not only for their antimicrobial metabolites but also for their ability to promote plant growth. Several studies have documented that actinobacterial strains enhance plant growth by producing phytohormones such as indole-3-acetic acid (IAA), solubilizing phosphate, fixing atmospheric nitrogen, and producing siderophores that improve nutrient uptake [2, 18, 21, 24, 52, 70]. These plant growth-promoting (PGP) abilities make actinobacteria valuable candidates for sustainable agriculture and biotechnological applications aimed at improving crop productivity and stress tolerance.

This study aims to identify an actinobacterial endophyte from *Artemisia herba-alba* Asso (*Asteraceae*), locally known as Chih, and to evaluate its biologically active secondary metabolites. We focus particularly on its antibacterial, antifungal, enzymatic,

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and PGP activities, highlighting its potential for agricultural and biotechnological applications.

MATERIALS AND METHODS

Plant sampling

The strain *Streptomyces* sp. BT21 was isolated from the roots of *A. herba-alba* Asso, which were sourced from Ghasrou, Batna province (Algeria) (GPS coordinates: 35°35′47.8″N, 5°54′1.55″E). The root system was first dug out and shaken vigorously to remove any large particles of soil attached to it. Then, it was placed in pre-labelled sterile bags to be transported the same day in an icebox to the laboratory. Without delay, we processed the samples for the isolation step within 48 h of collection, as indicated in Sharma *et al*. [59].

Isolation and maintenance of the endophytic actinobacterium

Isolation of actinobacteria from the endophytic compartment of *A. herba-alba* roots was performed on surface-sterilized roots following Hallmann *et al*. [33]. One gram (1 g) of sterilized roots was aseptically ground in 9 mL of sterile distilled water to prepare a 10^{-1} dilution. This suspension was spread onto Tap Water Yeast Extract (TWYE) medium [38], supplemented with nalidixic acid (50 mg/L) to inhibit Gram-negative bacteria and cycloheximide (80 mg/L) to suppress fungi. After incubation for two weeks at 30°C, colonies resembling actinobacteria were observed under a Zeiss light microscope (40×). The isolate BT21 was purified on International *Streptomyces* Project (ISP) medium 2 [60], and maintained at 4°C.

Polyphasic identification of the endophytic actinobacterium

Isolate BT21 was cultivated on several media for macro- and micromorphological characterization, including tryptone-yeast extract broth (ISP1), agar yeast-malt extract (ISP2), agar starch and inorganic salts (ISP4), Nutrient Agar (NA), and Bennett medium (BM) [60]. After 14 days of incubation at 28 ± 2°C, the strain was identified based on morphological features, including growth rate, aerial mycelium (AM) color, substrate mycelium (SM) color, diffusible pigment production, and microscopic features. Colors were determined using the "Color Name Chart Illustrated with Centroid Color: ISCC-NBS [41]. The growth of strain BT21 on ISP2 was further tested over 10 days across a temperature range of 20–50°C, pH levels of 4–12, and NaCl concentrations of 0–12% (w/v) [2]. Biochemical characteristics and carbohydrate acid production were assessed using Analytical Profile Index 20 Non-Enterobacteriaceae (API 20NE) and Analytical Profile Index 50 Carbohydrate (API 50CH) systems (Biomérieux, France) following the manufacturer's recommendations. Molecular identification was based on 16S rRNA gene sequencing performed by Genwiz Inc. (UK). PCR amplification used primers 10-30F (5′-GAG TTT GAT CCT GGC TCA-3′) and 1500R (5′-AGA AAG GAG GTG ATC CAG CC-3′) [54], with a 50 µL reaction mixture containing 50 ng genomic DNA, 0.5 µM primers, 1X PCR buffer, 10 µM dNTPs, and 1 U Taq DNA polymerase. PCR conditions were: initial denaturation at 98°C for 3 min; 30 cycles of 94°C for 1 min, 52°C annealing for 1 min, 72°C extension for 2 min; final elongation at 72°C for 10 min. PCR product was verified by Nanodrop 2000 spectrophotometer and agarose gel electrophoresis. Sequencing was also performed using the same primers. Sequence alignment and identification of nearest phylogenetic neighbors were conducted using the EzTaxon database (http://eztaxon-e.ezbiocloud.net/) [42]. Phylogenetic analysis was performed using the neighbor-joining method [56] in MEGA version 11 [64], with evolutionary distances calculated by the Jukes-Cantor method [39] and a bootstrap analysis of 1000 replications. The 16S rRNA sequence was submitted to GenBank under accession PQ231190.

PGP traits assessment

The PGP abilities of the actinobacterial strain BT21 were evaluated through standard assays for atmospheric nitrogen fixation, ammonia production, and phosphorus solubilization.

Atmospheric nitrogen fixation was assessed qualitatively using nitrogen-free bromothymol blue (Nfb) semi-solid medium, following the protocol described in [21] with slight modifications. Strain BT21 was inoculated into Nfb semi-solid medium and incubated at 28 °C for 7 days. Nitrogen-fixing activity was inferred from the formation of a subsurface pellicle along the medium.

Ammonia production was determined by culturing the strain in peptone water broth incubated at 28°C for 10 days. After incubation, Nessler's reagent was added to the culture supernatant, and the development of a yellow to brown color indicated positive ammonia production [22].

Phosphate solubilization potential was evaluated on Pikovskaya's agar plates supplemented with tricalcium phosphate. Strain BT21 was spot-inoculated and incubated at 28°C for 7 days. Clear halos around colonies indicated phosphate solubilization [24].

In vitro antimicrobial activity

The ability of strain BT21 to inhibit the growth of different microorganisms was assessed using the agar diffusion method. The method involved inoculating the actinobacterial isolate on different media (synthetic medium (SM) supplemented with starch (S), glucose (G), glycerol (L), dextrine (D) and saccharose (A), semi-synthetic medium (SSM) supplemented with the previously cited carbon sources) via tight streaks and then placing them in an incubator at a temperature of 28 ± 2°C. After a period of ten days, agar cylinders of 10 mm in diameter were extracted from the actinobacterial cultures. These cylinders were then put onto the surface of a semi-solid ISP2 medium (12 g/L) that had been previously inoculated with one of the bacteria (*Vibrio cholerae* ATCC 14035, *Pseudomonas*

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aeroginosa ATCC 9027, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 43300, *Listeria monocytogenes* ATCC 13932, *Escherichia coli* ATCC 8739, *Micrococcus luteus* ATCC 9314 or on semi-solid PDA for fungi (*Aspergillus carbonarius* M333, *Aspergillus brasiliensis* ATCC 16404, *Aspergillus niger*, *Aspergillus alliaceus*, *Aspergillus flavus* NRRL 3251, *Penicillium expansum*, *Umbelopsis ramanniana* NRRL 1829, *Fusarium oxysporum* f. sp. *lycopersici* and *Fusarium oxysporum* f. sp. *albedinis*) being tested at an approximate concentration of 10^7 UFC/mL. A positive result was obtained when an inhibitory zone was seen after 48 and 72 h of incubation at 28 ± 2°C for bacteria and at 25 ± 2°C for fungi, respectively [7].

Screening for extracellular enzymes

The study focused on the hydrolytic enzyme production capability of strain BT21, which was grown for ten days on ISP2 medium at a temperature of 28±2°C. Amylase activity was detected by spot-inoculating BT21 onto 2% starch agar and visualizing medium clearing with an iodine solution after ten days of incubation at 28±2°C [52]. The presence of proteases was checked in the colorless areas surrounding the colonies, which appeared after ten days of incubation at 28±2°C on skim milk agar medium following spot surface inoculation of strain BT21 [28]. Esterase activity was determined by the appearance of deposits around colonies that were sport-inoculated by strain BT21 and incubated for ten days at 28±2°C [28]. In the cellulase activity test, the actinobacterial strain BT21 was spot inoculated on carboxymethyl cellulose (CMC) medium and maintained at a temperature of 28±2°C for 10 days. Next, the Petri plates were immersed in a solution of Congo red (0.1%, weight/volume) for a duration of 15 min, followed by treatment with a sodium chloride solution (1M) to remove any remaining color. A clear halo surrounding the colonies indicates positive cellulase production [53]. To check for chitinase production, isolate BT21 was spot inoculated and grown on chitin agar plates that used colloidal chitin as the main ingredient. The distinct halo surrounding the colony indicates the presence of chitinases after 10 days of incubation at 28±2°C [47]. Pectinase activity was tested on tryptic soy agar containing 1% pectin. The Petri plate was spot-inoculated with strain BT21 and then incubated at 28±2°C for ten days. Upon the addition of 10 mL of Lugol solution to the Petri dish, a positive response is indicated by the presence of halos around the colonies [61].

RESULTS

Identification and characteristics of the strain BT21

The actinobacterium BT21 was morphologically characterized on five different media commonly used for this purpose (Table 1). The colonies exhibited varied growth rates, moderate growth on ISP1, ISP4, and NA, and good growth on ISP2 and Bennett (BM) media. Macroscopically, BT21 colonies showed differences in aerial mycelium (AM) and substrate mycelium (SM) coloration depending on the medium. For instance, a beige color was observed for AM on ISP1 and BM, while beige-grey was noted for ISP2 and NA. SM ranged from brown (ISP1 and NA) to beige shades on other media. Only four media (ISP1, ISP2, ISP4 and NA) promoted the production of diffusible pigments ranging from brown to yellow-greyish (Fig. 1).

Under light microscopy at 40X magnification, strain *Streptomyces* BT21 exhibited well-developed, branched hyphae forming a complex mycelial network. Distinct chains of oval to cylindrical spores were observed along the aerial hyphae, a characteristic of the genus. The dense SM and distinct filamentous structure with segmentation and sporulation patterns are consistent with those of *Streptomyces* species (Fig. 2).

Table 1. Morphological characteristics of *Streptomyces* sp. strain BT21 on different culture media after 14 days of incubation at 28°C

Medium	Growth rate	Aerial mycelium (AM)	Substrate mycelium (SM)	Pigmentation
ISP1	Moderate	Beige	Light brown to medium	Brown
ISP2	Good	Beige greyish	Beige	Dark brown
ISP4	Moderate	Pale green-greyish	Beige-greyish	Yellow-greyish
NA	Moderate	Beige- greyish	Medium brown to dark brown	Brown
BM	Good	Light beige	Light beige	-

ISP1: tryptone-yeast extract broth, ISP2: agar yeast-malt extract, ISP4: agar starch and inorganic salts, NA: nutrient agar, BM: Bennett medium, -: no production.

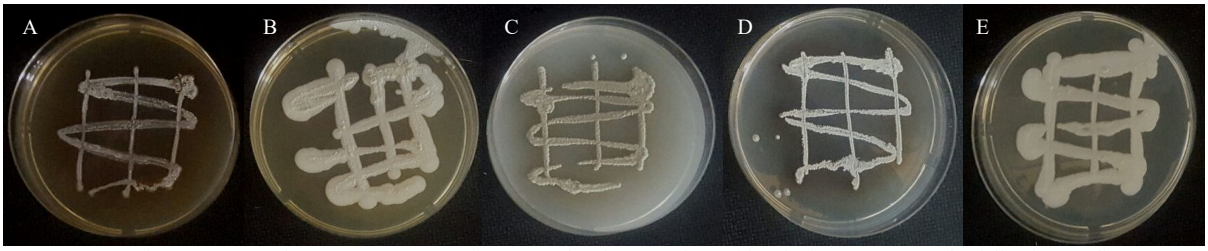

Figure 1. Macroscopic appearance of *Streptomyces* sp. strain BT21 after fourteen days of incubation at 28°C on the following media: (A) ISP1, (B) ISP2, (C) ISP4, (D) Nutrien agar (NA), (E) Bennett agar (BM).

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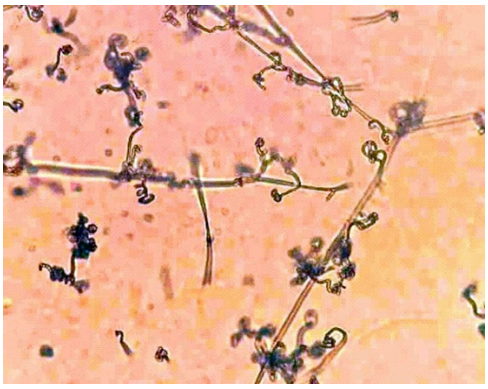

Figure 2. Microscopic observation of *Streptomyces* sp. strain BT21 on ISP2 medium at 40X magnification.

The different biochemical parameters of strain BT21 are presented in Table 2. The isolate BT21 exhibited negative reactions for catalase, H_2S, and nitrate production, as well as gelatinase, while it showed positive reactions for urease and esculin hydrolysis. Isolate BT21 was capable of using different carbon sources such as D-glucose, D-mannitol, L-arabinose, D-ribose, D-xylose, A-adonitol, methyl-βD-xylopyraoside, D-galactose, D-fructose, and D-mannose.

On the other hand, the isolate BT21 was halotolerant, exhibiting growth on ISP2 supplemented with NaCl at concentrations ranging from 0% to 10%. Growth occurred at temperatures between 20 °C and 45°C (optimum at 30°C) and at pH levels of 5.0-11.0 (optimum at pH 7) (Fig. 3).

According to the phylogenetic tree, the sequence alignment (1,454 bp) of the 16S rRNA gene of strain BT21 showed a similarity percentage of 99.24% with *S. specialis* GW41-1564^T (LN929789) (Fig. 4). These results confirm the morphological identification of this study.

Table 2. Biochemical characteristics of *Streptomyces* sp. strain BT21

Biochemical tests	**Result**	**Biochemical tests**	**Result**
Catalase	-	Gelatin hydrolysis	-
H_2S production	-	Esculin hydrolysis	+
Nitrate reduction	-	Urease test	+
Carbon source utlization	**Result**	**Carbon source utlization**	**Result**
Glycerol	-	Salicine	+
Erythritol	-	D-cellobiose	+
D-arabinose	-	D-maltose	+
L-arabinose	+	D-lactose	+
D-ribose	+	D-melibiose	+
D-xylose	+	D-saccharose	+
L-xylose	-	D-trehalose	+
A-adonitol	+	Inuline	+
Methyl-βD-Xylopyraoside	+	D-mélézitose	+
D-galactose	+	D-rafinose	+
D-glucose	+	Amidon	-
D-fructose	+	Glycogène	+
D-mannose	+	Xylitol	+
L-sorbose	-	Gentiobiose	-
L-ramnose	-	D-turanose	-
Dulcitol	-	D-lyxose	-
Inositol	-	D-tagatose	-
D-mannitol	+	D-fucose	-
D-sorbitol	+	L-fucose	-
Méthyl-αD-mannopyranoside	-	D-arabitol	-
Méthyl-αD--gluopyranoside	-	L-arabitol	-
N-acétylglucosamine	-	Potassium gluconate	-
Amygdaline	+	Potassium 2-Cétogluconate	-
Arbutine	+	Potassium 5-Cétogluconate	-
Esculine citrate de fer	+	-	-

+: positive test, -: negative test

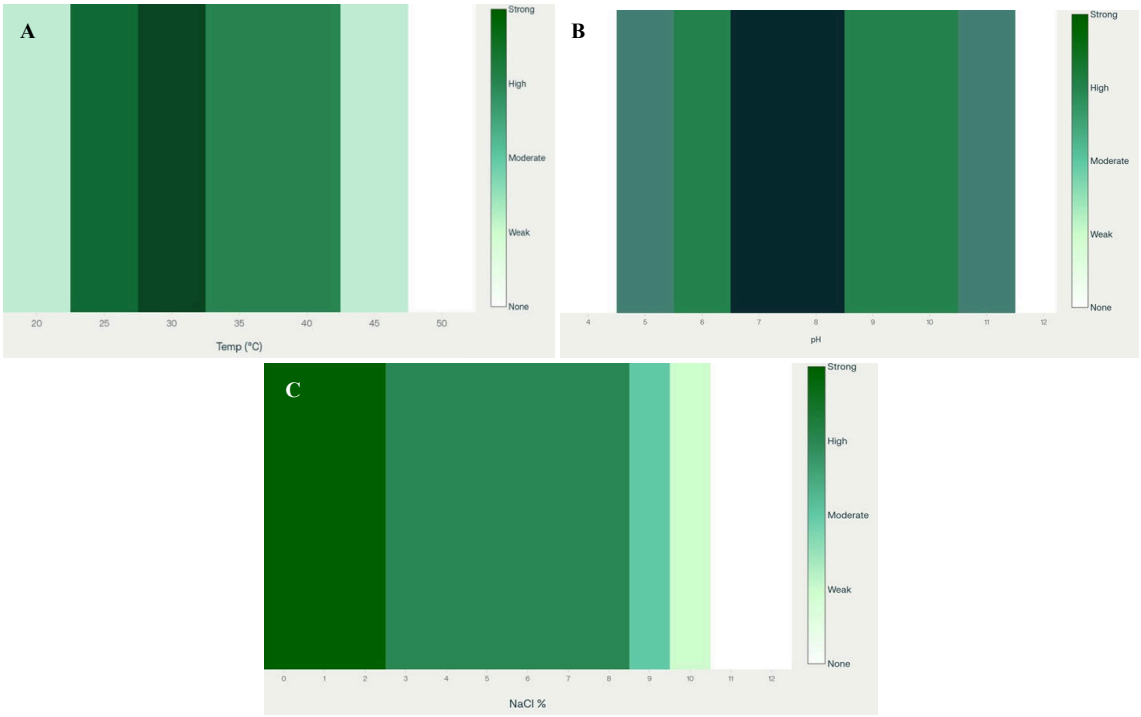

Figure 3. Heat maps illustrating the effects of temperature (A), pH (B), and NaCl concentrations (C) on the growth of *Streptomyces* sp. strain BT21.

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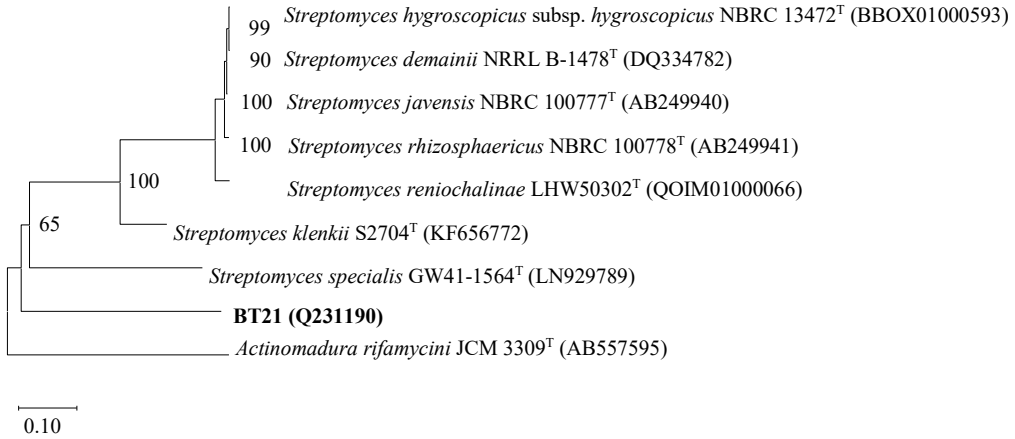

Figure 4. Phylogenetic tree of the strain *Streptomyces* sp. BT21 and the most related type strain species based on 16S rRNA gene sequences. The evolutionary history was inferred using the Neighbor-Joining method [56]. Evolutionary analyses were conducted in MEGA11 [65].

PGP traits

The results of the screening of PGP traits in *Streptomyces* sp. strain BT21 is summarized in Table 3. Strain BT21 tested positive for ammonia production and demonstrated the ability to fix atmospheric nitrogen. These results suggest the strain's potential to enhance nitrogen availability in the plant rhizosphere or endosphere. Conversely, BT21 showed a negative result for phosphate solubilization, as no clear zones indicating phosphate solubilization were observed on Pikovskaya's agar medium.

In vitro antimicrobial activity

The results of the *in vitro* antibacterial and antifungal activities of strain BT21 using different culture media are shown in Tables 4 and 5, respectively.

On the media SMD, SMS, and SSMS, no antibacterial activity was noticed against all the tested bacterial strains. The highest antibacterial activity (28 mm) was given when BT21 was cultured on SMG and SMA media against *Micrococcus luteus* ATCC 9314. On the other hand, six of the seven tested bacteria were inhibited by strain BT21 cultured on SMG medium.

The lowest number of sensitive test strains was obtained with the culture medium SMD, while the highest number of sensitive strains was obtained when BT21 was cultured on SSMG and SSML media. The biggest inhibition zone was obtained when we tested BT21 cultivated on SMA and SMG media with 25 mm each against Fol and Foa strains.

Screening for extracellular enzymes

Extracellular enzymatic activity results for strain *Streptomyces* BT21 are shown in Table 6. This strain was able to produce various hydrolytic enzymes, including amylases, cellulases, esterases, proteases, and chitinases, but was unable to produce pectinases.

Table 3. PGP traits of *Streptomyces* sp. strain BT21

Trait	Medium	Result	Interpretation
Ammonia production	Peptone water broth medium	+	Ammonia produced; can support plant nitrogen
Nitrogen fixation	Nitrogen-free medium	+	Fixes atmospheric N_2; improves N-availability
Phosphate solubilization	Pikovskaya's agar plate	-	No evidence of phosphate solubilization

+: positive test, -: negative test

Table 4. Antibacterial activity of *Streptomyces* sp. strain BT21

	Inhibition diameter (mm)						
Culture medium	***Vibrio cholerae* (ATCC 14035)**	***Pseudomonas aeroginosa* (ATCC 9027)**	***Escherichia coli* (ATCC 8739)**	***Bacillus subtilis* (ATCC 6633)**	***Staphylococcus aureus* (ATCC 43300)**	***Listeria monocytogenes* (ATCC 13932)**	***Micrococcus luteus* (ATCC 9314)**
SMG	20	18	0	18	20	17	28
SMD	0	0	0	0	0	0	0
SML	25	0	0	22	18	14	26
SMS	0	0	0	0	0	0	0
SMA	18	0	0	16	13	0	28
SSMG	20	0	0	14	0	0	18
SSMD	18	0	0	12	14	0	0
SSML	24	0	0	16	16	0	17
SSMS	0	0	0	0	0	0	0
SSMA	18	16	0	16	0	0	20

SMG: synthetic medium supplemented with glucose; SMD: synthetic medium supplemented with dextrine; SML: synthetic medium supplemented with glycerol; SMS: synthetic medium supplemented with starch; SMA: synthetic medium supplemented with saccharose; SSMG: semi-synthetic medium supplemented with glucose; SSMD: semi-synthetic medium supplemented with dextrine; SSML: semi-synthetic medium supplemented with glycerol; SSMS: semi-synthetic medium supplemented with starch; SSMA: semi-synthetic medium supplemented with saccharose.

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Table 5. Antifungal activity of *Streptomyces* sp. strain BT21

	Inhibition diameter (mm)								
Culture medium	***Fusarium oxysporum* f. sp. *lycopersici* (Fol)**	***Fusarium oxysporum* f. sp. *albedinis* (Foa)**	***Umbelopsis ramanniana* (NRRL 1829)**	***Aspergillus alliaceus* (AA)**	***Aspergillus flavus* (NRRL 3251)**	***Aspergillus niger* (AN)**	***Aspergillus carbonarius* (M333)**	***Aspergillus brasiliensis* (ATCC 16404)**	***Penicillium expansum* (PE)**
SMG	24	25	22	20	24	22	20	22	22
SMD	0	20	0	16	0	0	0	0	0
SML	14	22	18	18	17	20	18	20	18
SMS	14	16	14	14	0	0	0	0	0
SMA	25	18	22	18	20	18	18	24	17
SSMG	20	20	23	16	14	16	16	16	18
SSMD	16	16	14	16	0	0	0	0	14
SSML	18	18	18	16	16	18	14	13	18
SSMS	16	0	0	18	16	0	24	14	15
SSMA	20	16	20	16	18	0	20	17	16

SMG: synthetic medium supplemented with glucose; SMD: synthetic medium supplemented with dextrine; SML: synthetic medium supplemented with glycerol; SMS: synthetic medium supplemented with starch; SMA: synthetic medium supplemented with saccharose; SSMG: semi-synthetic medium supplemented with glucose; SSMD: semi-synthetic medium supplemented with dextrine; SSML: semi-synthetic medium supplemented with glycerol; SSMS: semi-synthetic medium supplemented with starch; SSMA: semi-synthetic medium supplemented with saccharose.

Table 6. Production of extracellular hydrolytic enzymes by *Streptomyces* sp. strain BT21

Production of extracellular hydrolytic enzymes by strain BT21						
Hydrolytic enzymes	Amylases	Cellulases	Esterases	Chitinases	Proteases	Pectinases
Results	+	+	+	+	+	-

+: positive test, -: negative test

DISCUSSION

In this study, we successfully isolated and characterized an endophytic actinobacterium, *Streptomyces* sp. strain BT21, from the roots of wormwood (*Artemisia herba-alba* Asso.). The isolate demonstrated significant potential as a source of antimicrobial compounds and enzymatic activities, highlighting its biotechnological importance.

The morphological characterization of *Streptomyces* sp. strain BT21 revealed typical features associated with the genus. Macroscopically, the isolate displayed differences in AM and SM coloration across different media. Such variability in morphological traits, including pigment production and mycelium coloration, is a well-known characteristic within *Streptomyces* species [30, 60]. Microscopically, BT21 exhibited highly branched hyphae and spore chains, suggesting its affiliation with the genus *Streptomyces*. These features are consistent with previous descriptions where spore chain arrangement and hyphal branching are critical taxonomic markers [40]. The biochemical characterization further supported the identification of strain BT21. The biochemical traits are frequently observed among endophytic *Streptomyces* species and serve as useful phenotypic markers during preliminary identification [66]. Moreover, BT21 demonstrated the ability to metabolize a wide range of carbon sources. Such metabolic versatility is characteristic of the ecological adaptability of *Streptomyces*, allowing them to thrive in diverse plant-associated environments [30]. Furthermore, the physiological characterization of *Streptomyces* sp. strain BT21 revealed a high degree of environmental adaptability. The strain exhibited halotolerance, growing at NaCl concentrations ranging from 0 to 10%, and was capable of growth over a broad temperature range (20°C to 45°C), as well as a wide pH range. Such physiological flexibility is typical of endophytic and rhizosphere-associated *Streptomyces* species, enabling them to survive in fluctuating environmental conditions, including those with saline and pH-variable soils [29, 34]. This tolerance to abiotic stresses enhances their potential use in biotechnological applications, particularly in saline-affected agricultural soils or under climate variability stressors.

Molecular identification based on 16S rRNA gene sequencing provided further support for the taxonomic placement of *Streptomyces* sp. strain BT21. The sequence alignment revealed a high similarity of 99.24% with *Streptomyces specialis* GW41-1564^T. This high level of sequence similarity is consistent with the morphological and physiological characteristics observed and strongly indicates that strain BT21 belongs to the genus *Streptomyces*. In general, a 16S rRNA gene sequence similarity greater than 98.7% is considered sufficient to assign bacterial isolates to an existing species or closely related taxa [43]. The concordance between morphological, biochemical, physiological, and molecular data highlights the robustness of a polyphasic approach for the reliable identification of actinobacterial isolates, particularly within complex and diverse genera such as *Streptomyces* [31].

PGP activities such as ammonia production and nitrogen fixation are critical mechanisms by which endophytic bacteria enhance plant nutrition and overall health. The positive ammonia production and nitrogen-fixing ability exhibited by *Streptomyces* sp. strain BT21 underscore its potential role in improving nitrogen availability to host plants. Ammonia produced by this actinobacterial strain can serve as a direct and readily assimilable nitrogen source for plants, while

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atmospheric nitrogen fixation converts inert nitrogen gas into bioavailable ammonia or related forms, potentially reducing the need for synthetic nitrogen fertilizers and contributing to sustainable agriculture [2, 16, 68]. However, excessive accumulation of ammonia (NH_3/NH_4^+) may induce phytotoxic effects in plant tissues. High ammonium levels are known to inhibit plant growth and cause physiological and metabolic disturbances, a phenomenon commonly referred to as ammonium toxicity [12, 26]. This toxicity is associated with disruption of intracellular pH, ion homeostasis, carbon–nitrogen metabolism, as well as increased energy demand for ammonium assimilation [46]. Moreover, excessive ammonium can trigger overproduction of reactive oxygen species, leading to oxidative stress, lipid peroxidation, and membrane damage, ultimately compromising cell integrity and function. Therefore, while ammonia production is a beneficial PGP trait, its concentration must remain balanced to avoid detrimental effects on plant growth and cellular stability [32]. Conversely, the absence of phosphate solubilization activity in BT21 indicates that this strain does not facilitate phosphorus mobilization through this particular mechanism. The phosphate solubilization capability among endophytes varies widely and often depends on the secretion of organic acids that chelate or solubilize inorganic phosphates, processes that require specific metabolic pathways [28, 70]. This variability may reflect adaptation to specific ecological niches or limitations in metabolic potential under certain environmental conditions. Notably, many actinobacterial endophytes isolated from the same plant species exhibited similar deficiencies in phosphate solubilization, as previously reported by Djemouai *et al*. [24], suggesting that phosphate solubilization is not a universal trait even among closely related endophytes inhabiting the same host.

The antimicrobial activity of *Streptomyces* sp. strain BT21 was strongly influenced by the culture medium used for its growth. No significant antibacterial or antifungal effects were observed when the strain was cultivated on SMD, SMS, and SSMS media, indicating that specific nutritional conditions are necessary to induce the biosynthesis of bioactive secondary metabolites. In contrast, substantial antimicrobial activities were observed when BT21 was grown on SMG and SMA media, demonstrating that the composition of the growth medium plays a crucial role in activating or enhancing the production of antibacterial and antifungal compounds, as previously reported for other *Streptomyces* strains [8, 69]. The main compositional differences among these media lie in their carbon source and base complexity (synthetic *vs*. semi-synthetic). SMG (synthetic medium supplemented with glucose) and SMA (synthetic medium supplemented with sucrose) contain simple, readily assimilated carbon sources that appear to favor the expression of secondary metabolite pathways, whereas SMD (dextrin-supplemented) and SMS (starch-supplemented) are based on complex polysaccharides that did not support detectable antimicrobial activity. Similarly, among the semi-synthetic media, SSMG (glucose) and SSML (glycerol) supported measurable antibacterial and antifungal effects, whereas SSMD (dextrin) and SSMS (starch) yielded little or no inhibition. These observations suggest that simple, easily metabolized carbon sources such as glucose, sucrose, and glycerol act as key inducers of antibiotic production in strain BT21, while polymer-derived substrates such as starch and dextrin favor primary metabolism or fail to trigger the relevant biosynthetic pathways [4, 45]. The antibacterial activity of strain BT21 was particularly notable, with effective inhibition of several bacterial species, highlighting its potential as a source of broad-spectrum antimicrobial agents. This aligns with previous studies showing that *Streptomyces* species are prolific producers of antibiotics, responsible for over two-thirds of naturally derived antibiotics [5]. Furthermore, the variability in the antibacterial spectrum depending on the growth medium suggests that BT21 may produce distinct sets of bioactive molecules under different nutritional conditions. Thus, optimizing culture parameters could be essential for maximizing the yield of specific antimicrobial compounds. Similarly, the antifungal activity of strain BT21 was significantly affected by the growth medium. The lowest antifungal activity was observed when the strain was grown on SMD medium, whereas the highest number of inhibited fungal strains was recorded with cultures grown on SSMG and SSML media. Notably, the largest inhibition zones (25 mm) were obtained when BT21, cultured on SMA and SMG media, was tested against *Fusarium oxysporum* f. sp. *lycopersici* (Fol) and *Fusarium oxysporum* f. sp. *albedinis* (Foa). These findings corroborate previous reports indicating that antifungal metabolite production in *Streptomyces* is highly dependent on medium composition [1, 51]. The strong antifungal effects against *Fusarium* species are particularly significant, considering the devastating plant diseases these pathogens cause worldwide. Many *Streptomyces* isolates produce antifungal compounds such as polyenes, peptides, and azoles that inhibit a wide range of fungal pathogens [63, 68]. The dual antibacterial and antifungal activities of strain BT21 highlight its promising potential as a biocontrol agent in agriculture, offering a sustainable alternative to chemical fungicides and antibiotics for managing plant diseases.

The extracellular enzymatic activity profile of *Streptomyces* sp. strain BT21 further supports its potential as a multifunctional biotechnological agent. As shown in Table 6, BT21 was capable of producing several hydrolytic enzymes, including amylases, cellulases, esterases, chitinases and proteases. The production of such enzymes is a typical feature of many *Streptomyces* species, which play key roles in the degradation of complex organic materials in soil ecosystems [9, 62]. The secretion of amylases and

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cellulases suggests the ability of strain BT21 to break down starch and cellulose, abundant in plant tissues and agricultural residues, potentially contributing to nutrient cycling and organic matter decomposition. Moreover, the production of chitinase is particularly significant for biocontrol applications, as chitin is a major structural component of fungal cell walls. Chitinolytic enzymes have been implicated in the direct antagonism of phytopathogenic fungi by degrading their cell walls and suppressing infection [35]. However, considering the endophytic nature of BT21, the production of cell wall-degrading enzymes may also pose potential risks to host plant tissues. Excessive or unregulated secretion of enzymes such as cellulases and amylases could theoretically lead to partial degradation of plant cell walls or intracellular reserves, thereby affecting cell integrity and function. Similar concerns have been reported for endophytes that can shift from mutualistic to opportunistic behavior under specific environmental or physiological conditions [36, 58]. In particular, the breakdown of structural polysaccharides may compromise root cell stability, while excessive hydrolysis of starch reserves could interfere with plant energy metabolism. Nevertheless, in most plant-endophyte interactions, a fine-tuned balance is maintained, whereby enzymatic activities are tightly regulated and spatially restricted, allowing colonization without causing significant damage to host tissues [14].

The combined production of hydrolytic enzymes and antimicrobial compounds thus reinforces the potential of strain BT21 as a candidate for integrated plant protection strategies. The absence of protease activity, although somewhat unusual, may reflect a more specialized ecological adaptation or a trade-off that allows BT21 to focus its metabolic resources on carbohydrate and lipid degradation rather than protein breakdown.

In this study, the endophytic actinobacterial strain *Streptomyces* sp. BT21, isolated from the roots of wormwood (*Artemisia herba-alba*), was comprehensively characterized using morphological, biochemical, physiological and molecular approaches. Molecular identification based on 16S rRNA gene sequencing confirmed its close phylogenetic relationship with *Streptomyces specialis*, supporting its taxonomic placement within the *Streptomyces* genus. Importantly, BT21 exhibited key plant growth-promoting traits, including the production of ammonia and the ability to fix atmospheric nitrogen, suggesting a beneficial role in enhancing nitrogen availability in the plant rhizosphere or endosphere. However, the strain lacked phosphate solubilization activity, a trait that varies widely among endophytic actinobacteria and may reflect niche-specific adaptations or metabolic constraints. Strain BT21 demonstrated strong antibacterial and antifungal activities, with its bioactivity influenced significantly by the composition of the culture medium. Additionally, the strain produced a suite of extracellular hydrolytic enzymes, including amylases, cellulases, esterases, proteases and chitinases, underscoring its versatile metabolic capabilities. The combination of antimicrobial properties, enzymatic arsenal, and selected PGP activities highlights BT21 as a promising candidate for the development of novel biocontrol agents and biofertilizers. Future research should aim for comprehensive *in vivo* assessments of its biocontrol effectiveness and PGP potential under greenhouse and field conditions, which are crucial steps toward its practical application in agriculture and biotechnology.

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

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