

ENZYMATIC AND ANTIFUNGAL ACTIVITIES OF ENDOPHYTIC *Streptomyces* sp. BK79 ISOLATED FROM THE ROOT SYSTEM OF *Artemisia herba-alba* Asso

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Abstract. Biomolecules from microorganisms, such as Actinobacteria (Phylum *Actinomycetota*), have formed the foundation for numerous medical, agricultural, and biotechnological advancements. This study examined the enzymatic and antifungal activities of the endophytic actinobacterial strain BK79, isolated from the root system of *Artemisia herba-alba* Asso collected from an arid environment in Biskra, Algeria. Morphological traits and 16S rRNA gene sequencing identified BK79 as belonging to the genus *Streptomyces*, with 100% similarity to *Streptomyces mutabilis* NRRL 12800¹ and *Streptomyces rochei* NRRL B-2410¹. The 16S rRNA gene sequence of strain BK79 has been deposited in GenBank under accession number PQ814213. *Streptomyces* sp. BK79 displayed a characteristic hydrolytic enzyme profile, exhibiting detectable activities for protease (1.46 ± 0.07), amylase (1.58 ± 0.08), and pectinase (1.13 ± 0.02), while cellulase activity was not observed. The strain also tested positive for esterase and chitinase activity in qualitative assays, indicating potential for the degradation of diverse biopolymers, including fungal cell wall components. Antifungal activity, assessed by the agar plug diffusion method, was notable in synthetic medium supplemented with dextrin, with a maximum inhibition zone of 30.5 ± 0.87 mm against *Aspergillus carbonarius*. These findings suggest that strain BK79 is a promising source of extracellular hydrolytic enzymes and antifungal compounds, with potential applications in biotechnology and agriculture.

Key words: antifungal activity; carbon source; hydrolytic enzymes; *Streptomyces*; taxonomy.

INTRODUCTION

The *Actinomycetota* phylum, whose members are commonly known as Actinobacteria, is one of the largest and most diverse bacterial groups, with species distributed across a wide range of terrestrial and aquatic environments [2, 44]. Actinobacteria are renowned for their ability to produce a vast array of biologically active compounds, including antibiotics, antifungals, antioxidants, antiparasitics, anticancer agents, immunostimulants, enzymes, and pigments, which are widely used in medicine, agriculture, and biotechnology [4, 52]. Actinobacteria can inhabit the rhizosphere and endosphere of plants, thereby protecting them against both biotic and abiotic stresses [16].

Endophytic Actinobacteria, particularly those associated with medicinal plants, remain underexplored despite their significant potential to contribute to host vitality and survival [11].

Artemisia herba-alba Asso, a medicinal plant native to Algerian ecosystems, is known for its pharmacological properties and traditional uses [14]. Previous studies, including our own, have demonstrated that this plant can harbour endophytic Actinobacteria with plant growth-promoting and antimicrobial properties [13-15]. Its unique ecological niche and medicinal relevance make it an excellent candidate for the discovery of novel bioactive microorganisms.

Within Actinobacteria, the genus *Streptomyces* is particularly notable for its ecological diversity and prolific production of bioactive metabolites. *Streptomyces* species comprise over 759 validly

published and correct name species in the List of Prokaryotic names with Standing in Nomenclature (LPSN) database [45] (accession date July 6, 2025), are found in soils, sediments, plants, and marine environments, and are classified using a multidisciplinary approach [26, 33]. In addition to producing a wide range of medically important antibiotics, such as chloramphenicol, fosfomycin, clavulanic acid, and avermectin [15, 53]. *Streptomyces* species synthesize various extracellular enzymes, including proteases, cellulases, and amylases, which are valuable in industry and biotechnology [16, 48]. The production of these bioactive compounds is influenced by various factors, including culture medium composition, inoculum, nutrient sources, and environmental conditions [51].

Although endophytic *Streptomyces* are widely recognized for their biotechnological promise, their enzymatic and antifungal activities, particularly those associated with *Artemisia herba-alba* Asso in arid environments, remain poorly studied. Thus, this study addresses this gap by isolating and characterizing a *Streptomyces* strain from the root system of *Artemisia herba-alba* Asso, with a particular emphasis on its capacity to produce extracellular enzymes and exhibit antifungal activity. Through this work, we provide new insights into the biotechnological potential of endophytic Actinobacteria from medicinal plants. The goals of the present study were (1) to isolate and identify a potential endophytic actinobacterium from the root system of *Artemisia herba-alba* Asso; (2) to assess its capacity to produce antifungal compounds; and (3) to characterize its *in vitro* ability to produce extracellular enzymes.

MATERIALS AND METHODS

Isolation, characterization and identification of strain BK79

The endophytic isolate BK79 was obtained from *Artemisia herba-alba* Asso root system collected from Djemorah region (GPS: 35°07'02"N, 05°50'22"E), Biskra Province (Algeria). The collection site is characterized by an arid climate, a sandy loam soil, and a pH of 8.35 [14].

The isolation technique outlined by Hayakawa and Nonumura [29] was carried out using Chitin-Vitamins-B agar medium (CH-V) [30] supplemented with nalidixic acid (50 µg/mL) and cycloheximide (80 µg/mL) to inhibit the proliferation of Gram-negative bacteria and fungi, respectively [15, 30]. The isolate BK79 was purified on International *Streptomyces* Project 2 (ISP2) medium and then maintained at 4°C on agar slants [54]. Further, the isolate was identified using cultural and morphological properties that were determined after growth at 28±2°C for 14 days on various media: Bennett agar [31], ISP1 (agar tryptone yeast extract), ISP2 (agar yeast-malt extract), ISP4 (agar starch and inorganic salts), tryptic soy agar (TSA) and nutrient agar (NA) media [54].

The color of the substrate and aerial mycelia, along with any soluble pigments released, were checked. The micromorphology of the strain was examined using a light microscope (B1 Series; Motic). The arrangement and morphology of spore chains were observed, with particular attention given to the type of spore chain (e.g., straight, flexuous, or spiral) and the surface ornamentation of the spores (e.g., smooth or warty). All observations were documented to ensure accurate characterization.

Several physiological and biochemical tests were used to characterize the actinobacterial isolate. The utilization of carbohydrates and decarboxylation of organic acids were studied 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. The growth of BK79 in ISP2 medium at different temperatures, pH values and NaCl concentrations was determined [14].

Additionally, genomic DNA was extracted using the method of Liu *et al.* [40]. PCR amplification of the 16S rRNA gene was performed using universal bacterial primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3'), as described by Rainey *et al.* [49]. The amplified products were sequenced, and the resulting sequences were compared with those in the EzTaxon database, a web-based tool for identifying prokaryotes based on 16S rRNA gene sequences from type strains [35]. The 16S rRNA gene sequences were aligned using the ClustalW algorithm as implemented in MEGA11 software, developed by Tamura *et al.* [57]. A phylogenetic tree was reconstructed using the maximum likelihood method

[19]. Tree topology was evaluated by the bootstrap resampling method with 1000 replicates, and bootstrap values were indicated at the corresponding nodes [20].

Screening for enzymatic activities

The detection of enzymatic activities, including cellulases, proteases, amylases, esterases, pectinases and chitinases was investigated. In all subsequent tests, a loopful of cells from a single colony of a ten-day-old culture of the tested BK79 strain grown on ISP2 medium at 28±2°C was inoculated onto different prepared media to test the enzymatic activities. For comparison, a reference strain named BTS40 [13], known for its positive hydrolytic enzymes production, was included in the tests under identical conditions. The diameter of the clear halos (in mm) around each colony was measured and the solubilization index was calculated as in Djemouai *et al.* [13].

Cellulases production

Cellulase activity was tested on 1% Carboxymethyl Cellulose (CMC) agar medium. After incubation at 28±2°C for ten days, a solution of Congo red was added to the surface of the Petri dish for 15 min, after which excess dye was removed. A 1M NaCl solution was added for 15 min. The presence of cellulase activity was shown by a clear halo around the colonies [59].

Proteases production

Proteolytic activity was tested using the spot plating method on skim milk agar medium [21]. The Petri dish was incubated at 28±2°C for ten days. Protein degradation was detected by a clear halo around the colonies [24].

Amylases production

NA medium supplemented with 2% starch was used to detect the amylolytic activity. The strain was spot-inoculated on the surface of the Petri dish and incubated at 28±2°C for ten days. Lugol's solution was then added to the surface and left to act for 5 min. The presence of a clear halo indicated the action of α -amylase enzyme and starch degradation [50].

Esterases production

To detect esterase activity, the strain BK79 was spot-inoculated on NA medium supplemented with Tween 20, 40 and 80. The Petri dishes were incubated at 28±2°C for ten days. This activity was determined by visualizing deposits around the colonies [24].

Pectinases production

To assess the pectinolytic activity, 1% pectin agar was used. The strain was inoculated by spot and incubated at 28±2°C. On the tenth day of incubation, 10 mL of Lugol's solution was added to the surface of the Petri dish. The appearance of a clear halo around the colonies indicated the presence of pectinases [56].

Chitinase production

Chitinase enzyme production was assayed on chitin agar medium. Spot-inoculated plates were incubated at 28 ± 2°C for ten days. The appearance of a clear halo around colonies indicates a positive reaction [41].

In-vitro antifungal activity

The antagonistic characteristics of strain BK79 were determined using the agar plug diffusion method against eight different fungal strains namely, *Fusarium oxysporum* f. sp. *lini* (Fol), *Umbelopsis ramanniana* (UR), *Aspergillus alliaceous* (AA), *Aspergillus flavus* (AF), *Aspergillus niger* (AN), *Aspergillus carbonarius* (AC), *Aspergillus brasiliensis* (AB), and *Penicillium expansum* (PE). All the strains were obtained from the LBSM fungal collection (Algiers, Algeria). The strain BK79 was grown at $28 \pm 2^\circ\text{C}$ for ten days on different media, including synthetic medium (SM) supplemented with starch (S), glucose (G), glycerol (L), dextrin (D) and saccharose (A), and semi-synthetic medium (SSM) supplemented with the previously cited carbon sources. Then, 10 mm in diameter agar cylinders were removed from the culture of strain BK79 and transferred to plates of Potato Dextrose Agar (PDA) medium inoculated with 10^7 CFU/mL of the test fungus. The plates were subsequently incubated at 25°C for 48 h, and the zones of inhibition surrounding the strain BK79 were measured.

Data analysis

The results were expressed as Mean $\pm$ Standard Deviation (SD) from triple trials.

RESULTS

Identification and characteristics of the strain BK79

The characteristics of strain BK79 are presented in Table 1. Morphological characteristics were recorded

on ISP1, ISP2, ISP4, Bennett agar, TSA and NA media.

The strain BK79 exhibited moderate to very good growth on all the used culture media. The color of aerial mycelium was medium gray on ISP1 and ISP4, beige greyish to light beige gray on ISP2 and Bennett agar and whitish to light gray medium for TSA and NA media. Meanwhile, the substrate mycelium was beige to light beige on ISP1, ISP2, ISP4 and Bennett agar media and medium orange and light to medium gray on TSA and NA media (Figure 1).

As shown in Table 2, a range of biochemical and physiological tests was carried out for the actinobacterial strain BK79. Among the thirty-eight carbon sources tested, strain BK79 was able to assimilate sucrose, sorbitol, maltose, lactose, glucose, D-fructose, mannitol, D-galactose, mannose, adonitol, methyl- α D-glucopyranoside, salicin and D-cellobiose, whereas it did not use the other carbon sources tested in this study. Strain BK79 could hydrolyze gelatin, could reduce nitrate, and the urease test was positive. Furthermore, the strain BK79 grew within the pH range of 5 to 12, between 10 and 40°C (optimum at 28°C) and in the presence of 0 to 6% NaCl.

The amplification product of the 16S rRNA gene, approximately 1484 bp, was sequenced and aligned with other strains of *Streptomyces* reference species from the EzTaxon-e server. The phylogenetic tree obtained by the maximum likelihood method is presented in Figure 2. The phylogenetic analysis revealed that the BK79 strain showed 100% similarity

Table 1. Morphological characteristics of *Streptomyces* sp. BK79 strain on different culture media after ten days of incubation at 28°C

	Agar medium	Growth rate	Aerial mycelium	Substrate mycelium	Pigmentation
BK79	ISP1	++++	Medium grey	Beige	-
	ISP2	++++	Beige greyish	Beige	-
	ISP4	++++	Medium grey	Beige	-
	Bennett agar	+	Light beige gray	Light beige	-
	TSA	+++	Whitish grey	Medium orange	-
	NA	+++	Light to medium grey	Light to medium grey	-

-: pigmentation, +: poor growth, ++: moderate growth, +++: good growth, ++++: very good growth

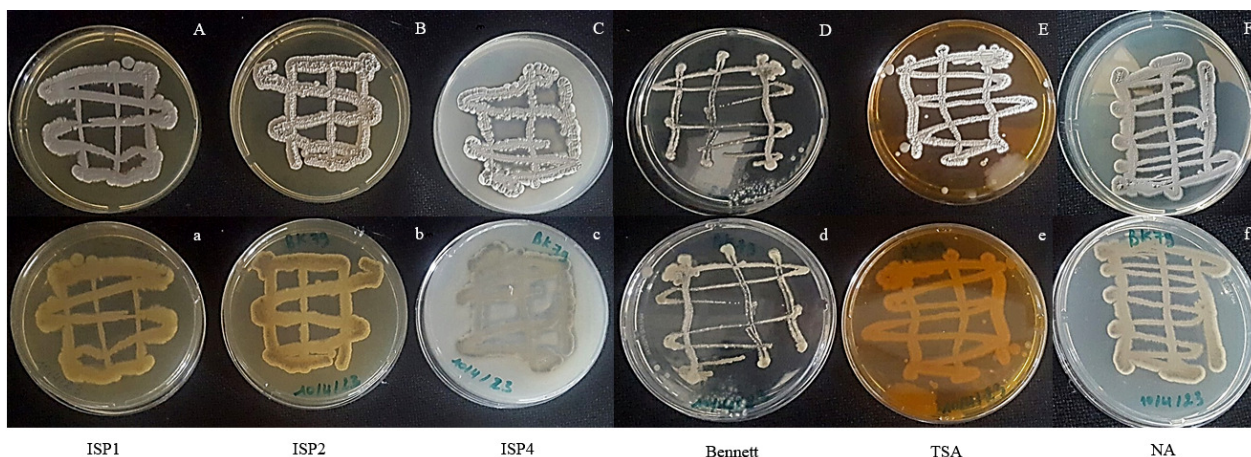


Figure 1. Macroscopic appearance of *Streptomyces* sp. BK79 strain after ten days of incubation at 28°C on the following media: (A, a) ISP1, (B, b) ISP2, (C, c) ISP4, (D, d) Bennett agar, (E, e) tryptic soy agar (TSA), and (F, f) nutrient agar (NA). For each medium, both the obverse (top row) and reverse (bottom row) sides of the Petri dishes are shown.

Table 2. Biochemical and physiological traits of *Streptomyces* sp. BK79 strain

Test	Strain BK79
Catalase	+
Gelatin hydrolysis	+
Nitrate reduction	+
Arginine dihydrolase	+
Indole production	-
Tryptophane desaminase	-
H ₂ S	-
Lysine decarboxylase	+
VP	+
Ornithine	+
Melibiose	-
Sucrose	+
Rhamnose	-
Sorbitol	+
Maltose	+
Lactose	+
Glucose	+
D-Fructose	+
D-Rafinose	-
Mannitol	+
D-Galactose	+
Mannose	+
Adonitol	+
Salicin	+
D-cellobiose	+
Methyl - alpha D - glucopyranoside	-
Urease	+
β-galactosidase	+
NaCl tolerance	0-6%
pH	5-12
Temperature (°C)	20-40

+: Indicates a positive reaction, -: indicates a negative reaction.

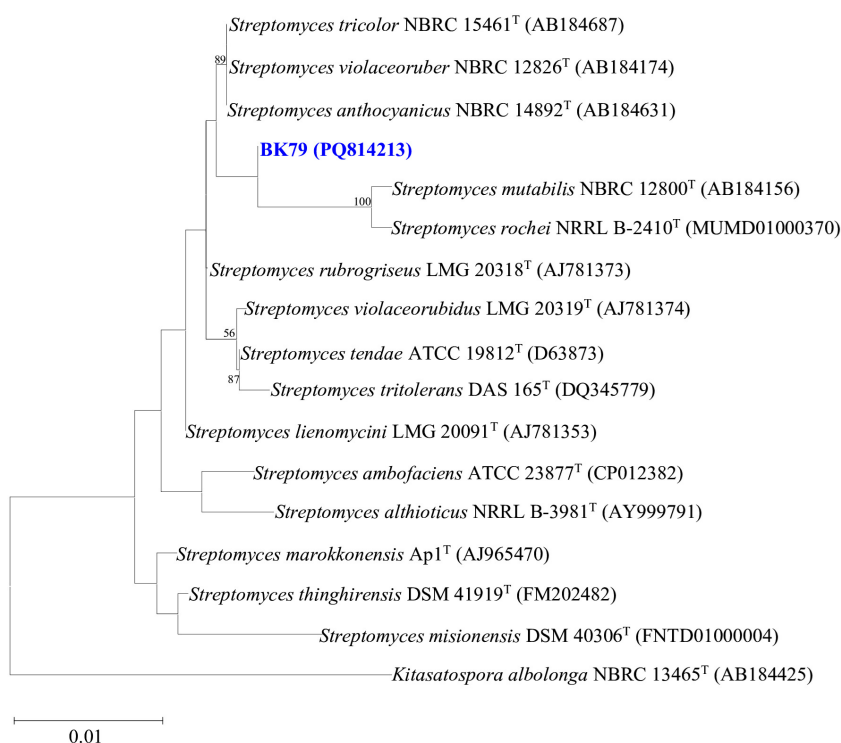


Figure 2. Phylogenetic tree of the *Streptomyces* sp. BK79 strain and its most closely related type strain species, derived from 16S rRNA gene sequences. The evolutionary history was deduced using the maximum likelihood technique [19]. The ideal tree is shown with *Kitasatospora albolonga* NBRC 13465^T used as the outgroup. The proportion of duplicate trees in which the corresponding taxa are grouped during the bootstrap test (1000 repetitions) is shown next to the branches [20]. The tree is accurately scaled, with branch lengths corresponding to the same units as the evolutionary distances used to deduce the phylogenetic tree. The evolutionary distances were calculated using the p-distance technique [43] and are expressed in terms of the number of base changes per site. Included codon locations were the 1st, 2nd, 3rd, and noncoding. All unclear places were eliminated for each sequence pair using the pairwise deletion option. The final dataset included 1487 locations. Evolutionary studies were performed using MEGA11 [57].

with *Streptomyces mutabilis* NRRL 12800^T and *Streptomyces rochei* NRRL B-2410^T. The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain BK79 is PQ814213.

Extracellular enzymes production

The strain BK79 was tested for its ability to produce cell wall-degrading enzymes in comparison to strain BTS40, as shown in Table 3 and Figure 3.

Enzymatic activity profiles of *Streptomyces* sp. BK79 demonstrated notable differences across the tested assays in comparison to the results of *Streptomyces* sp. BTS40 showed substantial cellulase activity (1.34 ± 0.04), which was absent in BK79. For protease production, both strains were positive; however, the level detected in BTS40 (2.32 ± 0.07) significantly exceeded that of BK79 (1.46 ± 0.07).

Similarly, while both strains exhibited amylase activity, BTS40 again displayed slightly higher activity (1.73 ± 0.13) than BK79 (1.58 ± 0.08). Pectinase activity distinguished the two strains most markedly; BTS40 recorded considerably higher activity (5.81 ± 0.32) compared to BK79 (1.13 ± 0.02). Both strains tested positive for esterase and chitinase activities, as evidenced by qualitative assays, suggesting their potential for degradation of a broad range of biopolymers, including those present in fungal cell walls.

Antifungal activity

The strain BK79 exhibited different patterns of antifungal activity against the tested fungi in this study, as determined using the agar plug diffusion method on various media (Figure 4).

Table 3. Enzymatic activity of *Streptomyces* sp. BK79 strain compared to *Streptomyces* sp. BTS40 strain

Strain	Hydrolytic enzymes production						Reference
	Proteases	Amylases	Cellulases	Pectinases	Esterases	Chitinases	
BK79	1.46 ± 0.07	1.58 ± 0.08	0.00	1.13 ± 0.02	+	+	This study
BTS40	2.32 ± 0.07	1.73 ± 0.13	1.34 ± 0.04	5.81 ± 0.32	+	+	Djemouai <i>et al.</i> [13]

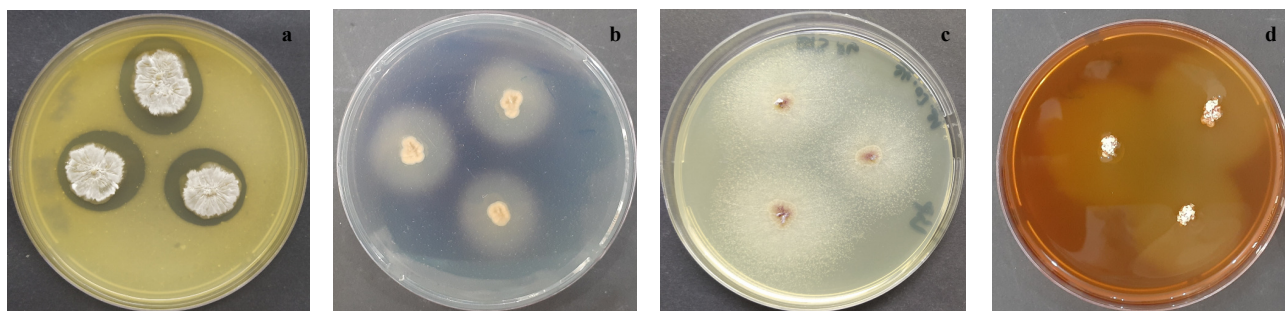


Figure 3. Visual representations depicting positive results of *Streptomyces* sp. BK79 strain for protease (a), amylase (b), esterases (c) and pectinases (d).

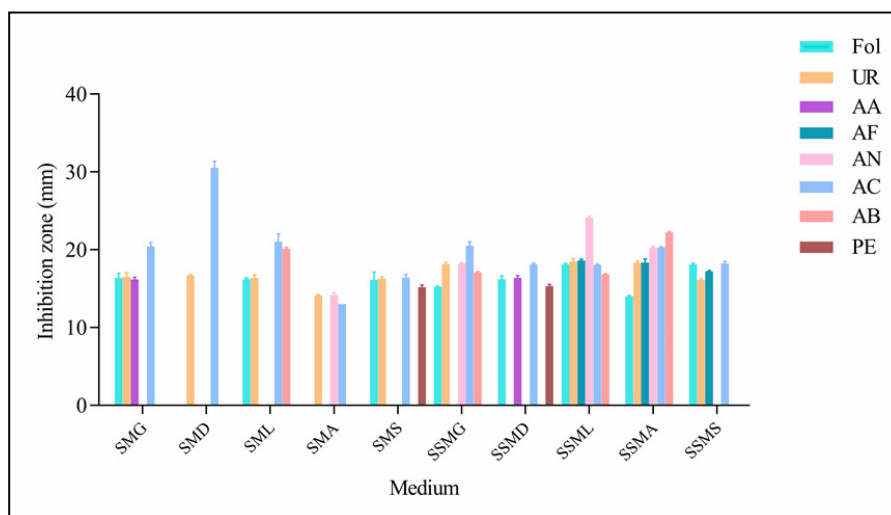


Figure 4. *In-vitro* antifungal activity of *Streptomyces* sp. BK79 strain by the agar plug diffusion method. *Fusarium oxysporum* f. sp. *lini* (Fol), *Umbelopsis ramanniana* (UR), *Aspergillus alliaceous* (AA), *Aspergillus flavus* (AF), *Aspergillus niger* (AN), *Aspergillus carbonarius* (AC), *Aspergillus brasiliensis* (AB) and *Penicillium expansum* (PE). Synthetic medium supplemented with glucose (SMG), synthetic medium supplemented with dextrin (SMD), synthetic medium supplemented with glycerol (SML), synthetic medium supplemented with saccharose (SMA), synthetic medium supplemented with starch (SMS), semi-synthetic medium supplemented with glucose (SSMG), semi-synthetic medium supplemented with dextrin (SSMD), semi-synthetic medium supplemented with glycerol (SSML), semi-synthetic medium supplemented with saccharose (SSMA), semi-synthetic medium supplemented with starch (SSMS).

The antifungal activity of strain BK79 varied significantly across different media. The highest antifungal activity was detected against AC on SMD medium (30.5 ± 0.87 mm diameter). Furthermore, SMD medium showed inhibition of only two fungal strains, followed by SMA medium (three strains). Four media SML, SMS, and SSMD, each demonstrated activity against four strains, while SSMG medium exhibited antifungal activity against five strains. Strain BK79 demonstrated a broad spectrum of low to medium antifungal activity against seven of the eight tested fungal strains on SSML and SSMA media, with no detectable activity against PE. The highest inhibitory effect was observed on SSML medium against AN, producing an inhibition zone of 24.1 ± 0.17 mm.

DISCUSSION

The endosphere of a plant often harbors many different bacterial inhabitants selected by the plant from its surrounding environment. The microorganisms inhabiting the internal tissues of plants are referred to as endophytes [1, 28]. Previous investigations in Algeria have shown the presence of actinobacterial strains in the root system of several host plants [5, 15]. Recent studies by Nafis *et al.* [42], El Moukhtari *et al.* [17], and Djemouai *et al.* [15] have looked into the actinobacterial composition of medicinal plants that might be used for different purposes. This research examined the antifungal activity, and enzyme production characteristics, of strain BK79 isolated from the roots of *Artemisia herba-alba* Asso, a medicinal plant commonly found in Algeria's arid regions. The obtained findings have demonstrated that *Streptomyces* sp. BK79 is an interesting strain exhibiting several advantageous characteristics deserving of investigation.

The taxonomic identification of strain BK79 presents some challenges, as 16S rRNA sequencing revealed 100% similarity to both *S. mutabilis* and *S. rochei*. This ambiguity highlights the well-documented limitations of 16S rRNA gene sequencing for accurate species-level identification [12]. Recent studies have demonstrated that 16S rRNA sequences do not map sufficiently well to *Streptomyces* taxonomy, with distinct species often sharing identical full-length 16S sequences [36]. To resolve this classification uncertainty, additional genomic analyses are recommended: (i) Average Nucleotide Identity (ANI) analysis, with a threshold of 95-96.7% for species delineation in *Streptomyces*; (ii) digital DNA-DNA hybridization (dDDH), with a 70% threshold as the species boundary; and (iii) multi-locus sequence analysis (MLSA) using secondary markers such as *rpoB* and *gyrB* genes, which provide higher resolution than 16S rRNA for *Streptomyces* identification [12, 32]. These approaches would provide definitive taxonomic classification and support the description of strain BK79's precise phylogenetic position within the *Streptomyces* genus.

Strain BK79 was evaluated for enzymatic activities, yielding mixed results. While the strain demonstrated positive activity for several hydrolytic enzymes, it was unable to degrade CMC, indicating an absence of cellulase activity. Although *Streptomyces* species are widely recognized for their cellulolytic capabilities, strain BK79 did not exhibit cellulase activity when tested on CMC medium. This negative result, despite the use of a standard inducer substrate, suggests that BK79 either lacks the genetic determinants for cellulase biosynthesis or that expression of these genes is stringently regulated and not induced under the tested conditions. It is well established that cellulase production in *Streptomyces* can vary significantly at the strain level, with some isolates demonstrating robust activity and others showing none, even within the same species [63]. Such variability is often attributed to ecological adaptation, as strains from non-cellulose-rich environments may downregulate or lose cellulase genes [7]. Additionally, while CMC is a common substrate for screening endoglucanase activity, other environmental factors, such as nitrogen source, micronutrient availability, and incubation parameters, may further influence enzyme expression [63]. The absence of CMC degradation by BK79 under our experimental conditions therefore highlights the metabolic diversity within the genus and suggests that this strain may be specialized for alternative ecological functions rather than cellulose decomposition.

On the other hand, our tested strain produces amylases, a group of enzymes used in industry for the hydrolysis of starch molecules into polymers composed of glucose units. These enzymes are produced by a variety of microorganisms; however, amylases of microbial origin are the most widely used in industrial sectors [10]. The presence of amylases in actinobacteria is generally observed in *Nocardia* and *Streptomyces*, *Arthrobacter*, *Brevibacterium* and *Nocardiopsis* [27, 61]. A clear protease activity was also detected in strain BK79. Indeed, several studies have reported the production of proteases from actinobacterial endophytes, especially those obtained from *Artemisia herba-alba* [15, 25]. In addition, alkaline proteases are produced by certain members of the actinobacteria, such as *Streptomyces clavuligerus* [60]. Esterases are enzymes produced by animals, plants and microorganisms, including actinobacteria [22]. These enzymes have applications in various industries: cosmetics, pharmaceuticals, detergents, food, leather, textiles, paper and biodiesel [64]. Our strain was positive for esterases production. In fact, several *Streptomyces* strains have been reported to have esterases production, such as *Streptomyces rimosus* and *Streptomyces lavendulae* [27].

Pectinase activity was also positive for our tested actinobacterial strain. Pectinases are a group of enzymes that play a role in the biodegradation of pectin [9]. This type of enzyme is produced by various organisms, including bacteria, fungi, plants and insects [37]. Actinobacterial pectinases have enormous

industrial potential. They are produced by several *Streptomyces* species, like *Streptomyces lydicus* and *Streptomyces thermocarboxydus* [6, 38]. Chitinase activity was revealed in our strain. Chitinases in microorganisms are crucial for degrading and recycling carbon and nitrogen trapped in insoluble chitin [31] and are widely used to prepare biopesticides and mosquito control [39]. Our results corroborate those of Passari *et al.* [46], who showed that 19 (86.3%) endophytic isolates of actinobacteria were positive for extracellular chitinase production and demonstrated the presence of the chitinase gene. Most *Streptomyces* species possess chitinase genes, enabling them to hydrolyze chitin in nature [8]. The chitinolytic activity of *Streptomyces* species has also been used as a biocontrol agent [15]. Thus, chitinases are potential antifungal agents useful against phytopathogenic fungi [46].

Numerous *Streptomyces* species isolated from arable soil, compost, and compost-amended soil demonstrated antagonistic activity against multiple plant pathogens, including bacterial diseases such as potato ring rot (*Clavibacter michiganensis* subsp. *sepedonicus*), black rot (*Xanthomonas campestris*), fire blight (*Erwinia amylovora*), and bacterial leaf spot (*Pseudomonas syringae*), as well as fungal diseases including grey mould (*Botrytis cinerea*), leaf spots (*Alternaria tenuissima*), early blight (*Alternaria arborescens*), and *Fusarium* head blight (*Fusarium poae*) as reported by Kováčsová *et al.* [37]. Also, Ghadbane *et al.* [23] identified four strains of *Streptomyces* isolated from the rhizosphere of *Ononis angustissima* Lam that exhibited a notable antibacterial activity and served as promising biocontrol agents against chickpea wilt disease.

The antifungal potential observed in strain BK79 is consistent with the biocontrol capabilities reported for other *Streptomyces* isolates. This finding mirrors our previous work, where we documented a broad-spectrum inhibition of six phytopathogenic fungi (inhibition zones of 13-35.33 mm) for *Streptomyces* sp. BKS30 against several phytopathogenic fungi, using similar culture conditions, further underscoring the versatility of actinobacterial strains in plant protection [14]. The research conducted by Peng *et al.* [47] showed that a strain of *Streptomyces* reduced the development of eight tested phytopathogenic fungi, supporting the notion that endophytic *Streptomyces* species, such as our BK79 strain, possess inherent antifungal properties that could be exploited for agricultural biocontrol applications. The analysis of antifungal activity of strain BK79 indicated that the strain exhibited optimal growth and maximum antifungal production against most of the tested fungi in the semi-synthetic medium containing glycerol and saccharose as the sole carbon sources. Other researchers has also reported similar results, reporting that a modification of the carbon source can enhance the antimicrobial activity. For instance, Tata *et al.* [58] demonstrated that starch incorporation significantly

affected the production of new antibacterial molecules on *Streptomyces* PAL 114 strain. The *Streptomyces* sp. HG29 strain demonstrated activity against various bacteria and filamentous fungi [34]. In recent years, numerous studies have focused on the impact of culture medium conditions on the production of antimicrobial molecules from various genera of actinobacteria [51, 58]. In addition, the *Streptomyces* genus is renowned for producing over 35,000 bioactive compounds with activity against various pathogens [8]. Moreover, similar results about the antimicrobial activity of different actinobacterial strains from various sources [13, 14, 58].

Streptomyces strains demonstrate exceptional biotechnological potential across multiple industrial sectors due to their remarkable metabolic diversity and bioactive compound production capabilities [15, 16]. In agricultural biocontrol, *Streptomyces* species serve as highly effective biological control agents through their production of chitinases, antifungal metabolites, and multiple hydrolytic enzymes that disrupt plant pathogen cell walls and enhance competitive advantage in the rhizosphere. Commercial success is already evident with products like Actinovate (containing *S. lydicus*) and Mycostop (containing *S. griseoviridis*) for controlling soil-borne diseases, while strains can be formulated as biofertilizers that simultaneously promote plant growth and suppress pathogens [16]. For industrial enzyme production, *Streptomyces* represents a prolific source of commercially valuable enzymes including amylases for starch processing and biofuel production, proteases for detergent and food industries, esterases for biodiesel synthesis, pectinases for fruit juice clarification, and chitinases for waste management applications [62]. In pharmaceutical development, *Streptomyces* continues to be the most important source of bioactive secondary metabolites, producing approximately 70-80% of all natural antibiotics and numerous anticancer, immunosuppressive, and antifungal compounds [3]. The genus's potential for novel drug discovery is further enhanced by genome mining approaches that reveal extensive biosynthetic gene clusters capable of producing structurally diverse compounds with therapeutic applications. The endophytic nature of many strains also provides opportunities for developing plant-based delivery systems and exploring previously untapped metabolic pathways for pharmaceutical innovation [55].

The current work elucidated significant findings about some biological activities of the strain *Streptomyces* sp. BK79, an actinobacterium isolated from the endosphere of *Artemisia herba-alba* Asso. This strain showed notable antifungal activity against a variety of phytopathogenic fungi, depending on the culture medium, and produced several hydrolytic enzymes *in vitro*. These results underscore the strong potential of BK79 for applications in agricultural biocontrol, industrial enzyme production, and the development of novel antimicrobial agents. To fully

realize this potential, future research should include whole-genome sequencing to identify biosynthetic gene clusters responsible for antifungal activity and further evaluation of BK79's efficacy in greenhouse and field trials to validate its practical use as a biocontrol agent.

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REFERENCES

- [1] Adeleke, B.S., Babalola, O.O., (2021): The endosphere microbial communities, a great promise in agriculture. *International Microbiology*, 24(1): 1-17.
- [2] Ait Barka, E.A., Vatsa, P., Sanchez, L., Gaveau-Vaillant, N., Jacquard, C., Klenk, H.P., Klenk, H-K., Clément, C., Ouhdouch, Y., van Wezel, G.P., (2016): Taxonomy, physiology, and natural products of actinobacteria. *Microbiology and Molecular Biology Reviews*, 80(1): 1-43.
- [3] Alam, K., Mazumder, A., Sikdar, S., Zhao, Y.M., Hao, J., Song, C., Wang, Y., Sarkar, R., Islam, S., Zhang, Y., Li, A., (2022): *Streptomyces*: The biofactory of secondary metabolites. *Frontiers in Microbiology*, 13: 968053.
- [4] Amin, D.H., Abdallah, N.A., Abolmaaty, A., Tolba, S., Wellington, E.M., (2020): Microbiological and molecular insights on rare actinobacteria harboring bioactive prospective. *Bulletin of the National Research Centre*, 44: 1-12.
- [5] Baoune, H., El Hadj-Khelil, A.O., Pucci, G., Sineli, P., Loucif, L., Polti, M.A., (2018): Petroleum degradation by endophytic *Streptomyces* spp. isolated from plants grown in contaminated soil of southern Algeria. *Ecotoxicology and Environmental Safety*, 147: 602-609.
- [6] Bharadwaj, P.S., Udupa, P.M., (2019): Isolation, purification and characterization of pectinase enzyme from *Streptomyces thermocarboxydus*. *Journal of Clinical Microbiology and Biochemical Technology*, 5(1): 1-6.
- [7] Book, A.J., Lewin, G.R., McDonald, B.R., Takasuka, T.E., Wendt-Pienkowski, E., Doering, D.T., Suh, S., Raffa, K.F., Fox, B.G., Currie, C.R., (2016): Evolution of high cellulolytic activity in symbiotic *Streptomyces* through selection of expanded gene content and coordinated gene expression. *PLoS Biology*, 14(6): e1002475.
- [8] Chater, K.F., Biró, S., Lee, K.J., Palmer, T., Schrempf, H., (2010): The complex extracellular biology of *Streptomyces*. *FEMS Microbiology Reviews*, 34(2): 171-198.
- [9] Dai, X.Y., Kong, L.M., Wang, X.L., Zhu, Q., Chen, K., Zhou, T., (2018): Preparation, characterization and catalytic behavior of pectinase covalently immobilized onto sodium alginate/graphene oxide composite beads. *Food Chemistry*, 253: 185-193.
- [10] de Souza, P.M., de Oliveira Magalhães, P., (2010): Application of microbial α -amylase in industry - A review. *Brazilian Journal of Microbiology*, 41(4): 850-861.
- [11] Delbari, Y., Mohassel, Y., Kakaei, E., Bahrami, Y., (2023): Identification and anti-bacterial property of endophytic actinobacteria from *Thymes kotschyanus*, *Allium hooshidaryae*, and *Cerasus microcarpa*. *Scientific Reports*, 13(1): 13145.
- [12] Dif, G., Djemouai, N., Bouras, N., Zitouni, A., (2025) : Reclassification of two *Nocardioopsis* species using whole genome analysis. *Antonie van Leeuwenhoek*, 118(1): 28.
- [13] Djemouai, N., Meklat, A., Gaceb-Terrak, R., Oulad Hadj Youcef, K., Nacer, A., Mokrane, S., Bouras, N., Verheeecke-Vaessen, C., (2022): Biological activities of *Streptomyces* sp. BTS40 isolated from the rhizosphere of *Artemisia herba-alba* Asso. *Analele Universității din Oradea, Fascicula Biologie*, 29(1): 7-14.
- [14] Djemouai, N., Meklat, A., Gaceb-Terrak, R., Oulad Hadj Youcef, K., Nacer, A., Saadi, S.A., Saad S., Verheeecke-Vaessen, C., Bouras, N., (2022): *Streptomyces* species from the rhizosphere of the medicinal plant *Artemisia herba-alba* Asso: screening for biological activities. *Biologia*, 77(8): 2281-2299.
- [15] Djemouai, N., Meklat, A., Oulad Hadj Youcef, K., Nacer, A., Saadi, S.A., Verheeecke-Vaessen, C., (2024): Diversity and bioactivity of endophytic actinobacteria associated with the roots of *Artemisia herba-alba* Asso from Algeria. *Current Microbiology*, 81(12): 402.
- [16] Djemouai, N., Meklat, A., Yekkour, A., Verheeecke-Vaessen, C., (2023): Actinobacteria: an underestimated source of potential microbial biocontrol agents against fusarium-related diseases in cultivated crops. *European Journal of Plant Pathology*, 167(4): 477-537.
- [17] El Moukhtari, A., Ziati, I., Lamsaadi, N., Mouradi, M., Farissi, M., El Hassni, M., (2021): Isolation and characterization of endophytic actinomycetes associated with *Thymus saturoides* and *Lavandula multifida* grown in Beni-Mellal region in Morocco. *Journal of Biopesticides*, 14(2): 165-178.
- [18] Fang, Q., Maglangit, F., Wu, L., Ebel, R., Kyremeh, K., Andersen, J.H., Annang, F., Pérez-Moreno, G., Reyes, F., Deng, H., (2020): Signalling and bioactive metabolites from *Streptomyces* sp. RK44. *Molecules*, 25(3): 460.
- [19] Felsenstein, J., (1981): Evolutionary trees from DNA sequences: a maximum likelihood approach. *Journal of Molecular Evolution*, 17(6): 368-376.
- [20] Felsenstein, J., (1985): Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, 39(4): 783-791.
- [21] Frazier, W.C., Rupp, P., (1928): Studies on the proteolytic bacteria of milk III. action of proteolytic bacteria of milk on casein and gelatin. *Journal of Bacteriology*, 16(3): 187-196.
- [22] Genilloud, O., (2017): Chapter 7 - Physiology of actinobacteria. pp. 151-180. In Wink, J., Mohammadipanah, F., Hamed, J., (eds.): *Biology and Biotechnology of Actinobacteria*. Springer.
- [23] Ghabbane, M., Medjekal, S., Belhadj, H., Bounar, R., Khellaf, R., Benderradji, L., Smaili, T., Harzallah, D., (2015): Biocontrol of chickpea wilt disease by *Streptomyces* sp. isolated from the rhizosphere of *Ononis angustissima* Lam. *World Applied Sciences Journal*, 33(9): 1414-1427.
- [24] Ghodsavali, B., Ahmadzadeh, M., Soleimani, M., Madloo, P.B., Taghizad-Farid, R., (2013): Isolation and characterization of rhizobacteria and their effects on root extracts of *Valeriana officinalis*. *Australian Journal of Crop Science*, 7(3): 338-344.

- [25] Gopal, A., Swamy, N., Arul, V., Jayashankar, M., (2022): Chapter 79 - Screening of protease from actinobacteria. pp. 533-536. In Dharumadurai, D., (eds): Methods in Actinobacteriology. Springer Protocols Handbooks.
- [26] Gopalakrishnan, S., Srinivas, V., Naresh, N., Pratyusha, S., Ankati, S., Madhuprakash, J., Govindaraj, M., Sharma, R., (2021): Deciphering the antagonistic effect of *Streptomyces* spp. and host-plant resistance induction against charcoal rot of sorghum. *Planta*, 253: 1-12.
- [27] Hamed, J., Poorinmohammad, N., Wink, J., (2017): Chapter 10 - The role of actinobacteria in biotechnology. pp. 269-328. In Wink, J., Mohammadipanah, F., Hamed, J., (eds.): Biology and Biotechnology of Actinobacteria. Springer.
- [28] Hardoim, P.R., van Overbeek, L.S., Berg, G., Pirttilä, A.M., Compant, S., Campisano, A., Döring, M., Sessitsch, A., (2015): The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiology and Molecular Biology Reviews*, 79(3): 293-320.
- [29] 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.
- [30] Hoang, K.C., Lai, T.H., Lin, C.S., Chen, Y.T., Liao, C.Y., (2010): The chitinolytic activities of *Streptomyces* sp. TH-11. *International Journal of Molecular Sciences*, 12(1): 56-65.
- [31] Hsu, S.C., Lockwood, J., (1975): Powdered chitin agar as a selective medium for enumeration of actinomycetes in water and soil. *Applied Microbiology*, 29(3): 422-426.
- [32] Hu, S., Li, K., Zhang, Y., Wang, Y., Fu, L., Xiao, Y., Tang, X., Gao, J., (2022): New insights into the threshold values of multi-locus sequence analysis, average nucleotide identity and digital DNA-DNA hybridization in delineating *Streptomyces* species. *Frontiers in Microbiology*, 13: 910277.
- [33] Karasz, D.C., Weaver, A.I., Buckley, D.H., Wilhelm, R.C., (2022): Conditional filamentation as an adaptive trait of bacteria and its ecological significance in soils. *Environmental Microbiology*, 24(1): 1-17.
- [34] Khebizi, N., Boudjella, H., Bijani, C., Bouras, N., Klenk, H.P., Pont, F., Mathieu, F., Sabaou, N., (2018): Oligomycins A and E, major bioactive secondary metabolites produced by *Streptomyces* sp. strain HG29 isolated from a Saharan soil. *Journal de Mycologie Medicale*, 28(1): 150-160.
- [35] 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.
- [36] Kiepas, A.B., Hoskisson, P.A., Pritchard, L., (2024): 16S rRNA phylogeny and clustering is not a reliable proxy for genome-based taxonomy in *Streptomyces*. *Microbial Genomics*, 10(9): 001287.
- [37] Kováčsová, S., Javoreková, S., Medo, J., Charousová, I., Elbl, J., Plošek, L., (2015): Characteristic of *Streptomyces* species with antimicrobial activity against selected phytopathogenic bacteria and fungi. *The Journal of Microbiology, Biotechnology and Food Sciences*, 5(1): 55-59.
- [38] Kumar Praven, G., Suneetha, V., (2015): Pectinases from actinomycetes: a thorough study. *International Journal of ChemTech Research*, 8(7): 345-350.
- [39] Lacombe-Harvey, M.É., Brzezinski, R., Beaulieu, C., (2018): Chitinolytic functions in actinobacteria: ecology, enzymes, and evolution. *Applied Microbiology and Biotechnology*, 102: 7219-7230.
- [40] Liu, D., Coloe, S., Baird, R., Pedersen, J., (2000): Rapid mini-preparation of fungal DNA for PCR. *Journal of Clinical Microbiology*, 38(1): 471-471.
- [41] 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.
- [42] Nafis, A., Kasrati, A., Azmani, A., Ouhdouch, Y., Hassani, L., (2018): Endophytic actinobacteria of medicinal plant *Aloe vera*: isolation, antimicrobial, antioxidant, cytotoxicity assays and taxonomic study. *Asian Pacific Journal of Tropical Biomedicine*, 8(10): 513-518.
- [43] Nei, M., Kumar, S., (2000): Chapter 4 - Synonymous and nonsynonymous nucleotide substitutions. pp. 51-72. In Nei, M., Kumar, S., (eds.): Molecular evolution and phylogenetics. Oxford University Press.
- [44] Oren, A., Garrity, G.M., (2021): Valid publication of the names of forty-two phyla of prokaryotes. *International Journal of Systematic and Evolutionary Microbiology*, 71(10): 005056.
- [45] Parte, A.C., Sardà Carbasse, J., Meier-Kolthoff, J.P., Reimer, L.C., Göker, M., (2020): List of Prokaryotic names with Standing in Nomenclature (LPSN) moves to the DSMZ. *International Journal of Systematic and Evolutionary Microbiology*, 70(11): 5607-5612.
- [46] Passari, A.K., Mishra, V.K., Gupta, V.K., Yadav, M.K., Saikia, R., Singh, B.P., (2015): *In vitro* and *in vivo* plant growth promoting activities and DNA fingerprinting of antagonistic endophytic actinomycetes associates with medicinal plants. *PLoS One*, 10(9): e0139468.
- [47] Peng, C., Zhuang, X., Gao, C., Wang, Z., Zhao, J., Huang, S.X., Liu, C., Xiang, W., (2021). *Streptomyces typhae* sp. nov., a novel endophytic actinomycete with antifungal activity isolated the root of cattail (*Typha angustifolia* L.). *Antonie van Leeuwenhoek*, 114: 823-833.
- [48] Pizarro, I.F., de Souza, H.F., dos Santos Ferreira, J., Maldonado, R.R., Kamimura, E.S., Aguiar-Oliveira, E., (2022): Chapter 75 - Production of lipase by actinobacteria. pp. 505-512. In Dharumadurai, D., (eds): Methods in Actinobacteriology. Springer Protocols Handbooks.
- [49] Rainey, F.A., Ward-Rainey, N., Kroppenstedt, R.M., Stackebrandt, E., (1996): The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of *Nocardiopsaceae* fam. nov. *International Journal of Systematic and Evolutionary Microbiology*, 46(4): 1088-1092.
- [50] Raj, S.V., Raja, A.K., Vimalanathan, A.B., Tyagi, M.G., Shah, N.H., Justin, N.J.A., Santhosh B.I., Sathiyaseelan, K., (2009): Study of starch degrading bacteria from kitchen waste soil in the production of amylase by using paddy straw. *Recent Research in Science and Technology*, 1(1): 8-13.

- [51] Saadi, S.A., Meklat, A., Mokrane, S., Yaiche Achour, H., Holtz, M.D., Klenk, H.P., Bouras, N., (2021): Isolation and characterization of a new *Saccharothrix* strain AHO23 with antimicrobial activity from an unexploited Algerian Saharan region. *Analele Universității din Oradea, Fascicula Biologie*, 28(1): 71-77.
- [52] Salwan, R., Sharma, V., (2018): Chapter 11 – The role of actinobacteria in the production of industrial enzymes. pp. 165-177. In Singh, B.P., Gupta, V.K., Passari, A.K., (eds): *New and future developments in microbial biotechnology and bioengineering: Actinobacteria: Diversity and Biotechnological Applications*. Elsevier.
- [53] Sharma, S.B., Sayyed, R.Z., Trivedi, M.H., Gobi, T.A., (2013): Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. *SpringerPlus*, 2: 587.
- [54] Shirling, E.B., Gottlieb, D., (1966): Methods for characterization of *Streptomyces* species. *International Journal of Systematic and Evolutionary Microbiology*, 16: 313-340.
- [55] Sivalingam, P., Easwaran, M., Ganapathy, D., Basha, S.F., Poté, J., (2024): Endophytic *Streptomyces*: an underexplored source with potential for novel natural drug discovery and development. *Archives of Microbiology*, 206(11): 442.
- [56] Soares, M.M., Silva, R.D., Gomes, E., (1999): Screening of bacterial strains for pectinolytic activity: characterization of the polygalacturonase produced by *Bacillus* sp. *Revista de Microbiologia*, 30: 299-303.
- [57] Tamura, K., Stecher, G., Kumar, S., (2021): MEGA11: molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, 38(7): 3022-3027.
- [58] Tata, S., Aouiche, A., Bijani, C., Bouras, N., Pont, F., Mathieu, F., Sabaou, N., (2019): Mzabimycins A and B, novel intracellular angucycline antibiotics produced by *Streptomyces* sp. PAL114 in synthetic medium containing L-tryptophan. *Saudi Pharmaceutical Journal*, 27(7): 907-913.
- [59] Teather, R.M., Wood, P.J., (1982): Use of Congo red-polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen. *Applied and Environmental Microbiology*, 43(4): 777-780.
- [60] Thumar, J.T., Singh, S.P., (2009): Organic solvent tolerance of an alkaline protease from salt-tolerant alkaliphilic *Streptomyces clavuligerus* strain Mit-1. *Journal of Industrial Microbiology and Biotechnology*, 36(2): 211.
- [61] Vigal, T., Gil, J.A., Daza, A., Dolores García-González, M., Martín, J.F., (1991): Cloning, characterization and expression of an α -amylase gene from *Streptomyces griseus* IMRU3570. *Molecular and General Genetics*, 225: 278-288.
- [62] Vojnovic, S., Aleksic, I., Ilic-Tomic, T., Stevanovic, M., Nikodinovic-Runic, J., (2024): *Bacillus* and *Streptomyces* spp. as hosts for production of industrially relevant enzymes. *Applied Microbiology and Biotechnology*, 108(1): 185.
- [63] Waheeb, M.S., Elkhatab, W.F., Yassien, M.A., Hassouna, N.A., (2021): Production of cellulase by soil isolated *Streptomyces* sp. *Archives of Pharmaceutical Sciences Ain Shams University*, 5(2): 225-233.
- [64] Welz, P., Swanepoel, G., Weels, S., Le Roes-Hill, M., (2021): Wastewater from the edible oil industry as a potential source of lipase-and surfactant-producing actinobacteria. *Microorganisms*, 9(9): 1987.

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