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Diversity and Bioactivity of Endophytic Actinobacteria Associated with the Roots of *Artemisia herba-alba* Asso from Algeria

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Abstract

The isolation of endophytic actinobacteria from the roots of wild populations of *Artemisia herba-alba* Asso, a medicinal plant collected from the arid lands of Algeria, is reported for the first time. Forty-five actinobacterial isolates were identified by molecular analysis and in vitro evaluated for antimicrobial activity and plant growth-promoting (PGP) abilities (1-Aminocyclopropane-1-carboxylic acid (ACC) deaminase activity, nitrogen fixation, phosphate and potassium solubilization, ammonia, and siderophores production). The phylogenetic relationships based on 16S rRNA gene sequences show that the genus *Nocardioides* ($n=23$) was dominant in the sampled localities. The remaining actinobacterial isolates were identified as *Promicromonospora* ($n=11$), *Streptomyces* ($n=6$), *Micromonopora* ($n=3$), and *Saccharothrix* ($n=2$). Only six (13.33%) strains (five *Streptomyces* and one *Saccharothrix* species) were antagonistic in vitro against at least one or more indicator microorganisms. The antimicrobial activity of actinobacterial strains targeted mainly Gram-positive bacteria. The results demonstrate that more than 73% of the isolated strains had ACC deaminase activity, could fix atmospheric nitrogen and were producers of ammonia and siderophores. However, only one (2.22%) strain named *Saccharothrix* sp. BT79 could solubilize phosphorus and potassium. Overall, many strains exhibited a broad spectrum of PGP abilities. Thus, *A. herba-alba* provides a source of endophytic actinobacteria that should be explored for their potential biological activities.

Introduction

The diversity and distribution of endophytic fungal and bacterial communities related to numerous plant species have been extensively described in numerous regions across the globe [1, 2]. In Algeria, only one study has recently been published on the fungal endophytes of *Artemisia herba-alba* [3] but not on bacteria from the same plant. Literature and scientific communications have reported the occurrence of actinobacteria, an important and well-studied group of bacteria that has been discovered in a variety of medicinal plants [4–7]. Most of the reported actinobacterial endophytes that inhabit the plant tissues belong to *Streptomyces* as the dominant genus, as well as other genera like *Actinopolyspora*, *Microbacterium*, *Micromonospora*, *Microbispora*, *Micrococcus*, *Nocardia*, *Nonomuraea*, *Nocardiopsis*, *Oerskovia*, *Promicromonospora*, *Rhodococcus*, *Sacchromonospora*, *Saccharopolyspora*, *Spirillospora*, *Streptoverticillium,* and *Streptosporangium* [1, 2, 4].

Several scientific investigations have evidenced the biocontrol abilities against several harmful pathogens (bacteria, fungi, and viruses) of several actinobacterial endophytes,

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including those from medicinal plants [5, 8]. Moreover, many researchers have demonstrated that endophytic actinobacteria can generate various bioactive molecules that promote plant growth [9]. The promotion of growth is owed to the plant growth-promoting (PGP) abilities via several indirect and direct plant biosynthesis processes, including inorganic phosphate solubilization, synthesis of chelating substances like siderophores as well as phytohormones, suppression of plant pathogens, and reduction of diverse abiotic stressors [7, 9].

Artemisia herba-alba Asso is a medicinal plant known as white wormwood or Chih in Arabic that belongs to the Asteraceae family [10]. This plant is native to arid regions, where it has spread to India, the Middle East, and the North-Western Himalayas, and it is extensively grown in Algeria's steppes, arid regions, and desert areas. Since ancient times, numerous cultures have employed this plant to treat many ailments [10–12].

Researchers have demonstrated the diversity of actinobacterial endophytes associated with *A. herba-alba* in the world. Two studies by El-Shatoury et al. [13] described the larvicidal activity of *Streptomyces* sp. strains that were obtained from *A. herba-alba* and El-Shatoury et al. [14], who reported the bioactivities of eighteen strains from *A. herba-alba* in Wadi Shrayj (Egypt). Many published works have shown novel endophytic actinobacteria that were isolated from different *Artemisia* species, including *Pseudonocardia artemisiae* [5] and *Rhodococcus artemisiae* [6], all of which were isolated from *Artemisia annua* L. In addition, the effect of endophytes of *A. annua* from Egypt on plant growth promotion was recently published by Husseiny et al. [7].

On the other hand, two papers on the rhizospheric actinobacteria from *A. herba-alba* were published with dominance in *Streptomyces* species that had various biological activities, such as antioxidant and antifungal properties along with PGP traits [11, 12]. The present study describes the occurrence and diversity of actinobacterial endophytes obtained from the roots of *A. herba-alba* and further characterizes their potential biocontrol activity and in vitro PGP features.

Materials and Methods

Collection of Samples

Roots of three plants of *A. herba-alba* were collected from six naturally growing sites of the Algerian steppes, with three sites in Batna Province (Thniet El Abed; 35.25004° N, 6.18484° E, Tazoult; 35.49023° N, 6.28454° E and Ghasrou; 35.35478° N, 5.74155° E), two sites in Biskra Province (El Kantara; 35.23456° N, 5.69911° E and Djemorah; 35.07870° N, 5.75226° E) and one site in Ouled Djellal Province (Ech Chaïba; 34.84485° N, 4.89772° E). The samples were delivered to the Laboratory of Microbial Systems (LBSM) in sterile polyethylene bags in an icebox and processed immediately within 24-h sampling.

Surface Sterilization of Roots and Verification of Sterilization

Plant roots were cleaned with tap water and after sonication for 15 min at 160 W, one gram (1 g) of the roots was immersed first for 1 min in ethanol (75°), then for 8 min in sodium hypochlorite solution (1%), and finally washed five times with sterile distilled water [15]. To verify that the surface sterilization was adequate, 0.2 mL from the last washing solutions of each sample was inoculated on chitin-vitamins B-agar (CH-V), sodium propionate medium (SPM), and tap water yeast extract (TWYE) medium in triplicate and then incubated at 28 ± 2 °C for 15 days [16]. The Petri dishes were checked daily throughout the incubation period for bacterial growth.

Isolation and Maintenance of Culturable Actinobacteria

Actinobacterial isolation was done following Li et al. [16] protocol. One gram (1 g) of fragmented *A. herba-alba* roots was ground in a commercial mixer and then in a pre-disinfected mortar. Nine milliliters (9 mL) of sterile distilled water were added to each sample, constituting the 10^{-1} mother solution, and decimal dilutions were prepared (10^{-2} and 10^{-3}). Of each solution, 100 µL were spread inoculated in duplicate on three culture media, which are widely used for the isolation of actinobacteria: CH-V, SPM and TWYE. To inhibit the development of fungi and Gram-negative bacteria, respectively, cycloheximide (80 µg/mL) and nalidixic acid (50 µg/mL) were added to each medium. Upon incubation at 28 ± 2 °C for three weeks, the suspected actinobacterial colonies were observed under the light microscope (Zeiss), and representative isolates from each morphological group were picked up and purified on International *Streptomyces* Project 2 (ISP2) medium. Then, all the obtained actinobacteria were inoculated on tubes of ISP2 medium incubated at 28 ± 2 °C for two weeks and preserved at 4 °C.

Identification of Culturable Actinobacteria

All the obtained isolates were grown on ISP2 and ISP4 media. Presumptive identification at the genus level was completed using Bergey's manual [17]. Cultural and morphological characteristics for each isolate (growth rate, color of aerial mycelium (AM) and substrate mycelium (SM), and diffusible pigments) were determined after growth for 15 days at 28 ± 2 °C [4]. Representative strains ($n = 45$) were

selected for the phylogenetic analyses. The total genomic DNA extraction was done using the protocol of Liu et al. [18]. The 16S rRNA gene was PCR-amplified in 50 µL of the reaction mixture using 25 to 50 ng of genomic DNA, 0.5 µM of the forward 10-30F (5′-GAG TTT GAT CCT GGC TCA -3′) and reverse 1500R (5′-AGA AAG GAG GTG ATC CAG CC-3′) primers, 1X PCR buffer, 10-µM deoxynucleoside triphosphate mixture and 1U *Taq* DNA polymerase. The amplification was performed according to the following conditions: initial denaturation at 98 °C for 3 min, 30 cycles of 94 °C for 1 min, primer annealing at 52 °C for 1 min, primer extension at 72 °C for 2 min, and final elongation at 72 °C for 10 min and then cooled to 4 °C. The PCR products were checked for quantity and purity by Nanodrop and electrophoresis on agarose gel by visualizing fluorescence under UV after staining with EZ-Vision. The sequencing reactions were performed by Genwiz Ltd. (Takeley, England) using the same primers described previously. The obtained actinobacterial sequences were compared with the type strains in the EzTaxon-e database (http://eztaxon-e.ezbiocloud.net/) [19] to determine the sequences of closely related species. A phylogenetic tree was generated using MEGA11 [20] with the neighbor-joining method [21] and a bootstrap value of 1000 repeats [22].

Evaluation of Actinobacterial Isolates Tolerance to NaCl and pH and Growth at Different Temperatures

The obtained isolates were scored for their growth on ISP2 supplemented with NaCl at various concentrations (from 0 to 10%, w/v, pH 7.2) and for their growth on the same medium at different pH levels (from 4 to 12) and diverse temperatures (from 10 to 50 °C, pH 7.2). The 10-day-grown cultures were surface inoculated on the solid media and incubated at 28 ± 2 °C for 10 days [23].

Antimicrobial Assay on Solid Media

All the actinobacterial strains were screened for their antimicrobial potential against five bacteria (*Staphylococcus aureus* MRSA 639c, *S. aureus* S, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 8739 and *Pseudomonas aeruginosa* ATCC 9027), five fungi (*Fusarium culmorum* (Fc), *Penicillium expansum* (Pe), *Aspergillus carbonarius* M333, *Fusarium oxysporum* f. sp. *lycopersici* (Fol), and *Umbelopsis ramanniana* NRRL 1829) and one yeast (*Candida albicans* ATCC 1023). The test bacterial strains were cultured on nutrient agar medium at 37 °C overnight, while *C. albicans* was cultured on Sabouraud medium at 28 ± 2 °C for 2 days. In addition, the test fungal strains were cultured on Potato dextrose agar (PDA) medium and were maintained for 4 days at 28 ± 2 °C. The agar plug diffusion method was used to evaluate the antimicrobial activity. After 10 days of actinobacterial isolates growth at 28 ± 2 °C, 10-mm diameter agar cylinders were placed on the surface of Muller–Hinton and PDA media seeded with the tested bacteria (approximately 10^8 CFU/mL) and yeast and fungi (approximately 10^7 UFC/mL), respectively. Controls involved the use of sterile agar plugs. The inhibition zones were measured to determine the antimicrobial activity after 48 h at 28 ± 2 °C for bacteria and 72 h at 25 ± 2 °C for both yeast and fungi [11, 12].

Determination of Plant Growth-Promoting Traits

The in vitro PGP attributes of the tested strains were evaluated using different tests. In all the tests, a loopful of cells from a single colony of 10-day-old cultures of actinobacteria on ISP2 medium at 28 ± 2 °C was prepared and inoculated on the different media. Nitrogen fixation was assessed after spot inoculation and growth on the semi-solid N-free (NFb) medium. After incubation at 28 ± 2 °C for 10 days, colonies of actinobacteria that developed a ring in the shape of a circle beneath the medium's surface were considered positive for atmospheric nitrogen fixation [24].

To assess phosphate and potassium solubilization activities, actinobacterial strains were spot inoculated on the surface of Pikovskaya and Aleksandrov agar media, respectively. After 10 days of culture at 28 ± 2 °C, the visualization of the clear halos surrounding colonies was considered positive for phosphate [9] and potassium solubilization [11].

The production of siderophores was assessed after the actinobacterial spot inoculation and growth on Chrome Azurol S (CAS) agar plates. After 5 days at 28 ± 2 °C, the development of yellow to orange halos surrounding the colonies of actinobacterial strains indicated the production of siderophores [23].

Ammonia production (NH_3) was scored when 10 mL of peptone water broths were inoculated with the actinobacterial strains for 10 days. After the incubation at 28 ± 2 °C, the appearance of a yellow to brown precipitate when using Nessler's reagent (0.5 mL) was considered a favorable indication of NH_3 production [9].

1-Aminocyclopropane-1-carboxylic acid (ACC) deaminase activity was verified by spot inoculating the actinobacterial isolates on the minimal medium of Dworkin and Foster (DF) salts with the addition of ACC [12].

Hydrolytic Enzyme Production

Ten-day-old cultures of actinobacteria on ISP2 medium at 28 ± 2 °C were examined for their hydrolytic enzyme production. To check for amylase production, a loopful of the isolated actinobacteria was spot inoculated on a 2% starch agar medium and media clearing was assessed by an iodine

solution [12]. For the test of cellulase production, the actinobacterial strains were spot inoculated and grown on a Carboxy Methyl Cellulose medium at 28 ± 2 °C for 10 days. Then, the Petri dishes were submerged with Congo red (0.1%, w/v) for 15 min and bleached with NaCl solution (1 M). Cellulase production is positive if a transparent halo is formed around the colonies [9]. The actinobacterial strains were also spot inoculated on skim milk agar medium to check for protease activity. Colorless zones surrounding the colonies indicate the synthesis of proteases after 10 days of incubation at 28 ± 2 °C [25]. Pectinase activity was assessed on tryptic soy agar medium complemented with 1% pectin. The spot-inoculated Petri dishes were incubated at 28 ± 2 °C for 10 days. After adding 10 mL of Lugol solution to the Petri dishes, a positive reaction is evidenced by the halos surrounding the colonies [26]. Esterase activity was determined based on deposits around bacterial colonies after spot inoculation, as described in Ghodsalavi et al. [25].

Statistical Analysis

All experiments were carried out in triplicate. The results represent the mean ± standard deviation (SD). Data were analyzed using GraphPad Prism 9 software. The datasets obtained from the screening of the antimicrobial activity, phosphate and potassium solubilization, and some of the extracellular hydrolytic enzymes production tests were subjected to an analysis of variance (ANOVA) followed by testing the significance of differences using a Tukey LSD test ($p \leq 0.05$). Venn diagram is used for the representation of PGP traits and enzyme production of the tested actinobacterial strains using InteractiVenn, a web-based tool for the analysis of sets (https://www.interactivenn.net/).

Data Availability

The 16S rRNA gene sequences of the endophytic actinobacteria were submitted to NCBI with the GenBank accession numbers PP728057 to PP728101.

Results

Endophytic Actinobacterial Isolation

Selective isolation of actinobacteria was conducted from the roots of *A. herba-alba* collected from the three provinces of Batna, Biskra, and Ouled Djellal using CH-V, SPM, and TWYE media. After incubation, colonies showing the morphological characteristics of mycelial actinobacteria after observation with the naked eye and the light microscope were chosen based on their diversity and dominance, with the selection of forty-five isolates that were sub-cultured on ISP2 medium. On the other hand, no microbial development was observed after incubation at 28 ± 2 °C for 15 days for the last root rinsing water samples. Thus, the absence of microbial growth confirms that the root disinfection protocol was sufficient to eliminate microorganisms from the rhizosphere or rhizoplan.

From the forty-five isolates, twenty-eight (62.22%) were picked up from CH-V medium, ten (22.22%) from SPM medium, and seven (15.56%) from TWYE medium, which means that CH-V medium was by far the favorable medium for actinobacterial growth (Table 1; Table S1, supplementary material). Overall, twenty-nine isolates were obtained from the Batna region. Most were isolated from CH-V medium (heighten), followed by SPM medium (eight isolates) and only three isolates were obtained from the TWYE medium. The other isolates (sixteen) were obtained from Biskra (thirteen isolates, from which ten were isolated from CH-V medium) and from Ouled Djellal (four isolates with three from CH-V medium).

Endophytic Actinobacterial Identification

The morphological study that was carried out consisted of macro- and microscopic observations on ISP2 and ISP4 culture media of the forty-five endophytic isolates (Fig. S1). Interestingly, most of the isolates obtained in this study

Table 1 Distribution and origin of representative endophytic actinobacterial isolates per site and medium

Medium	BTI			BTII			BTIII			BKI			BKII			BKIII		
	M1	M2	M3	M1	M2	M3	M1	M2	M3	M1	M2	M3	M1	M2	M3	M1	M2	M3
Number of isolates	5	2	1	8	1	1	5	5	1	4	2	3	3	0	1	3	0	0
	8			10			11			9			4			3		
Total	29									16								

Batna Province, BTI: Theniet El Abed (35° 25′ 004″ North, 6° 18′ 484″ East), BTII: Tazoult (35° 49′ 023″ North, 6° 28′ 435″ East), BTIII: Ghasrou (35° 35′ 478″ North, 5° 74′ 155″ East)

Ouled Djellal Province, BKII: Ech Chaïba (34° 84′ 485″ North, 4° 89′ 772″ East)

Biskra Province, BKI: El Kantara (35° 23′ 456″ North, 5° 69′ 911″ East) and BKIII: Djemorah (35° 07′ 870″ North, 05° 75′ 226″ East)

M1: Chitin-Vitamins B-agar (CH-V) medium, M2: Sodium Propionate Medium (SPM), M3: Tap Water Yeast Extract (TWYE) medium

showed resembling morphologies. They were grouped into two morphotypes observed according to the presence or absence of AM (twenty-three isolates with AM and eleven isolates without AM) on ISP2 and ISP4 media. Overall, the growth rate was very good on ISP2 medium and average on ISP4 medium. Microscopic examination revealed short to medium sterile filaments in the AM and SM that fragmented excessively in a zig-zag pattern on both media. Also, distinct morphologies were observed for some of the isolated actinobacteria. For example, according to morphological studies, actinobacterial isolates producing sporophorous spore chains on their AM and unfragmented SM are grouped in the *Streptomyces* genus. This was the case for six of the isolated endophytes. Isolates BT1 and BT5 showed similarities in terms of AM and SM, but also in producing diffusible orange pigments only on ISP2 medium. Isolate BT5, on the other hand, showed a different morphology to the other two isolates belonging to this group. In addition, this isolate did not show the production of pigments on the two-culture media used. These isolates had morphologies similar to those of *Micromonospora* strains. Two strains, BT79 and BK37, produced an AM that fragments anarchically into long chains of non-motile (often zig-zag) spores and a fragmented SM, which presumably corresponds to *Saccharothrix* genus.

According to the results of the molecular analyses, the forty-five strains were identified at the genus and species levels with 98.46 to 100% similarity. The comparison of the forty-five isolates 16S rRNA gene sequences with the sequences on the EzBioCloud server revealed their taxonomic position with *Nocardioides* (23 strains) as the dominant genus, followed by *Promicromonospora* (11 strains), *Streptomyces* (six strains), *Micromonospora* (three strains), and *Saccharothrix* (two strains). Furthermore, most of the endophytic strains were affiliated with *Nocardioides* and they were assigned to *Nocardioides albus* with similarity percentages of 99.44 to 99.86% as the dominant species (22 strains) (Fig. 1). This species was found at all sites except Djemorah (Biskra Province). In addition, a strain named BT54 was closely related to *Nocardioides luteus* with 95.51% similarity.

The second group contained (11) strains separated into two distinct clades. The first clade contained the strains BT72, which was related to *Promicromonospora alba* (99.86%) and BT52 and BK78 sharing similarity percentages of 98.70 and 98.99% with *Promicromonospora aerolata,* whereas the second clade contained strains related to different *Promicromonospora* strains, with similarity percentages ranging from 99.24% à 99.51% (Fig. 1). The 16S rRNA gene sequence analyses of BT1, BT17, and BT5 revealed that these strains shared similarity degrees of 99.93%, 99.93%, and 98.46% with *Micromonospora arida*, *Micromonospora phytophila,* and *Micromonospora noduli*, respectively. The strains BT79 and BK37 were affiliated to *Saccharothrix xinjiangensis* (99.72% and 99.65%, respectively) (Fig. 1). Based on the molecular analysis, the six endophytic strains, BT61, BT73, BT76, BT84, BT91, and BK93 were affiliated to five distinct species of *Streptomyces* genus (99.38 to 100% of similarity) (Fig. 2).

Evaluation of Endophytic Actinobacteria Tolerance

All tested strains were found to be halotolerant at different concentrations of NaCl. The majority of the strains tolerated NaCl concentrations up to 7% (71.1%) and could grow at a wide range of pH, from acidic pH (4–6) to alkaline pH (9–12), with the most dominant profile being a pH from 5 to 10 (71.74%). The results show that at 20, 30, and 40 °C, the strains could grow but not at 50 °C, except the strain BT79) (Table S1).

Antimicrobial Activity of Endophytic Actinobacteria

The antimicrobial activity of the forty-five endophytic actinobacterial strains was tested using the agar disk diffusion method. Most of the strains did not show any antimicrobial activity in the experimental conditions used. Only six strains (13.33%) were active against some of the test microorganisms (Table 2). All the active strains were obtained from the root compartment of *A. herba-alba* collected from Batna Province. Five active strains were classified under the genus *Streptomyces*, with three strains showing antibacterial activity and the remaining two strains having antifungal and antibacterial activities. In addition, of the five active strains, the sixth strain which is affiliated to the genus *Saccharothrix* had antimicrobial activity against seven of the microorganisms tested and showed promising results.

PGP Features and Extracellular Enzyme Production

The results of the PGP traits study are summarized in Table 3 and Fig. 3. Of the forty-five actinobacterial strains identified, thirty-seven (82.22%) were positive for ACC deaminase production and could grow on a medium containing ACC as a single nitrogen source. Atmospheric nitrogen fixation and ammonia production were shown by thirty-six (80%) and thirty-eight (84.44%) strains, respectively. Of all the strains, only one (BT79) was positive for phosphorus and potassium solubilization. In addition, siderophores production was shown by thirty-three (73.33%) of the forty-five endophytic strains. Twenty-one strains (46.67%) showed four traits, namely ACC deaminase activity, atmospheric nitrogen fixation, ammonia, and siderophores production (Fig. 3a).

The hydrolytic enzyme production capacity of the forty-five endophytic strains showed that all strains produced

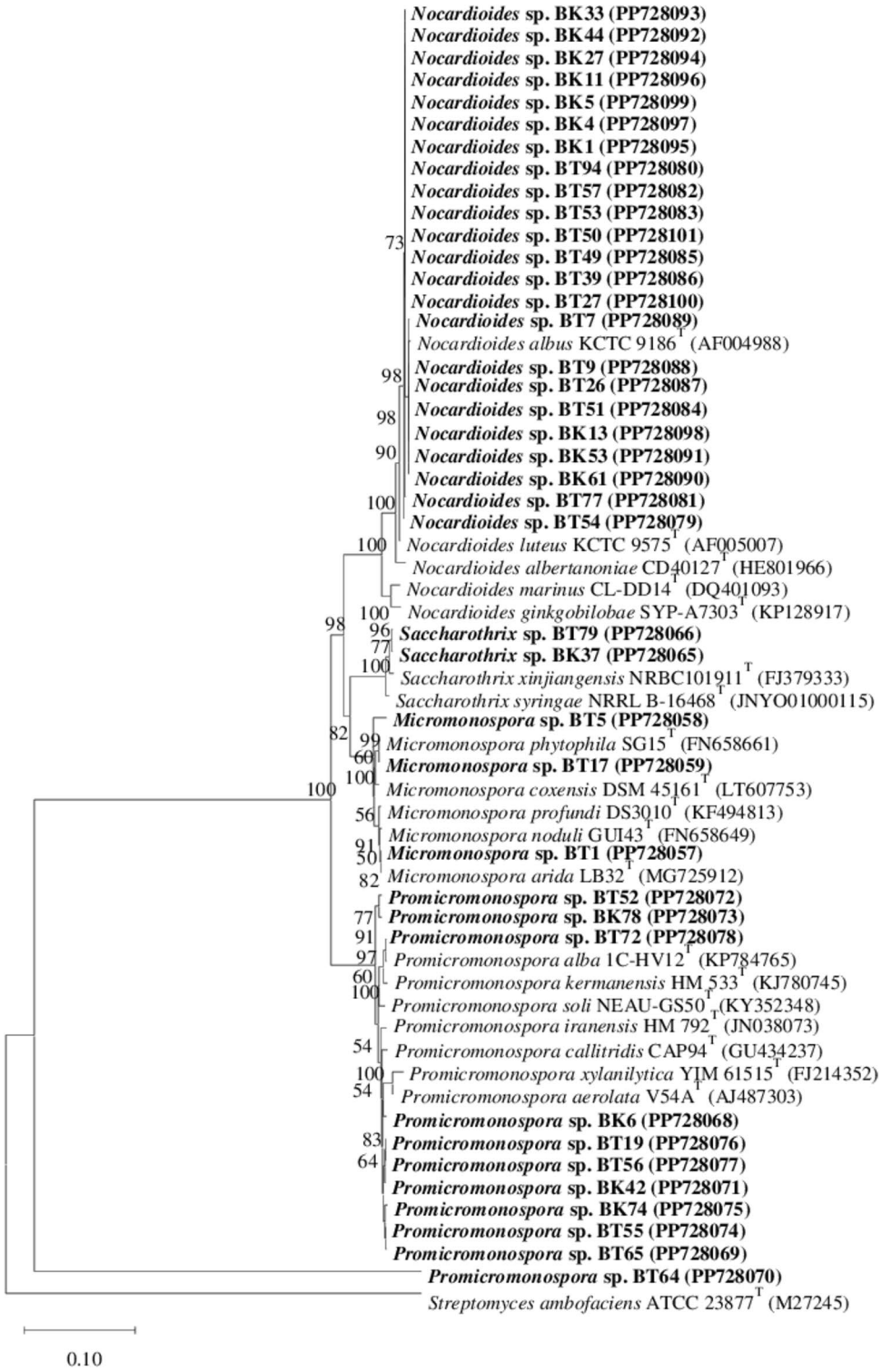

Fig. 1 Phylogenetic tree based on the Neighbor-Joining method [21] of 16S rRNA gene sequences of endophytic actinobacteria isolated from roots of *Artemisia herba-alba* and closely related type strains of the genera *Nocardioides*, *Promicromonospora*, *Micromonospora,* and *Saccharothrix*. The bootstrap consensus tree inferred from 1000 replicates is taken to and branches corresponding to partitions reproduced in less than 50% bootstrap replicates collapsed [22]. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree was rooted in *Streptomyces ambofaciens*. Evolutionary analyses were conducted in MEGA11 [20]. The scale bar represents 0.10 substitutions per nucleotide position

amylases (100%), followed by pectinases (forty-four strains, 97.78%). Proteases (forty-two strains, 88.89%) and esterase producers were also frequent (forty-two strains or 88.89%). In addition, nineteen strains (42.22%) showed cellulolytic activity (Fig. 3b).

Overall, only one strain BT79 showed the five tested PGP traits and was positive for all the extracellular enzyme production. This strain was affiliated with the *Saccharothrix* genus.

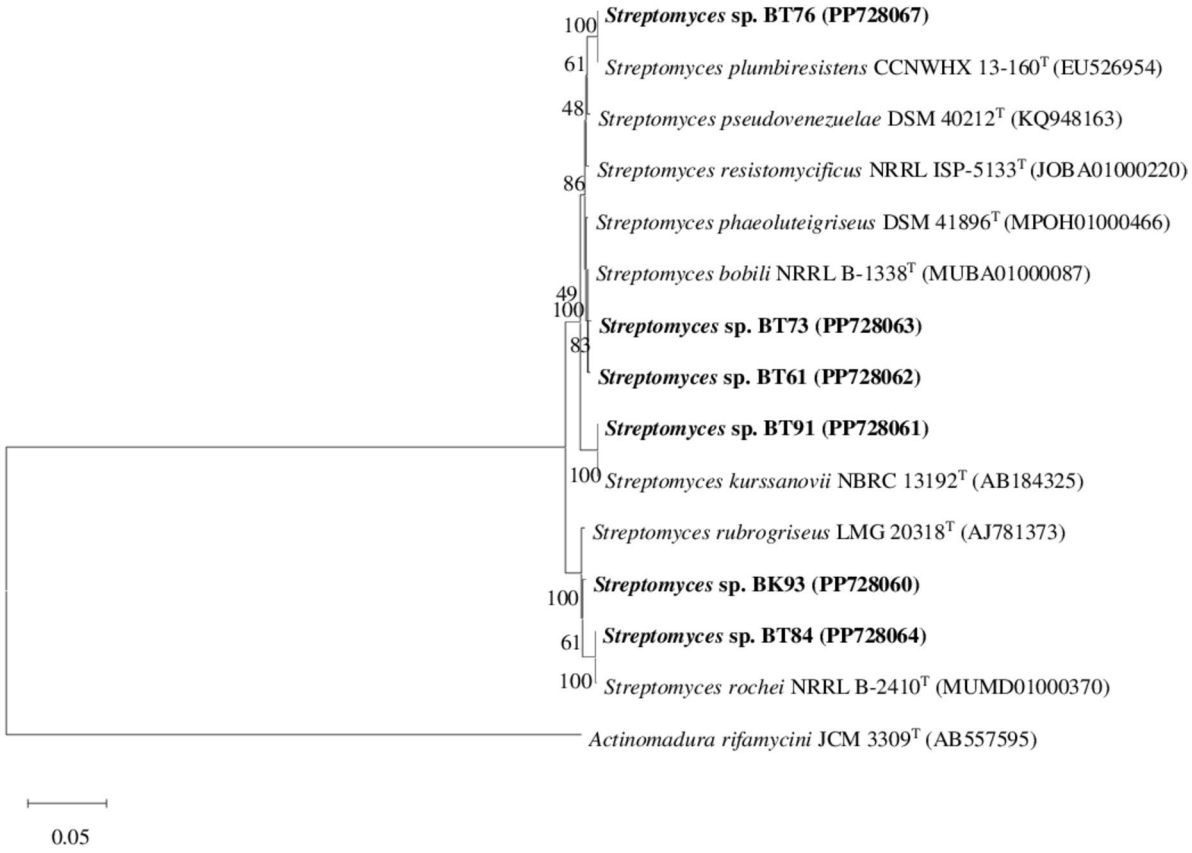

Fig. 2 Phylogenetic tree based on the Neighbor-Joining method [21] of 16S rRNA gene sequences of endophytic actinobacteria isolated from roots of *Artemisia herba-alba* and closely related type strains of the genus *Streptomyces*. The bootstrap consensus tree inferred from 1000 replicates is taken to and branches corresponding to partitions reproduced in less than 50% bootstrap replicates collapsed [22]. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree was rooted in *Actinomadura rifampici*. Evolutionary analyses were conducted in MEGA11 [20]. The scale bar represents 0.05 substitutions per nucleotide position

Discussion

In this study, the biodiversity of the endophytic actinobacterial community isolated from the medicinal plant *A. herba-alba* and their functional attributes were explored. A total of forty-five actinobacterial endophytic isolates were obtained from their host plant using different isolation media. In fact, endophytes are a diverse group of microorganisms residing within their host plants' interior tissues and engaging in a mutually beneficial interaction. Environmental conditions and host plant organs are the main two elements that influence their diversification [27]. Many plant species have been explored to isolate endophytic actinobacteria from crops like rice, wheat, carrots, potatoes, and tomatoes, as well as ferns, mosses, and woody trees [8].

Various publications have documented diverse growth media used to isolate endophytic actinobacteria, including CH-V, TWYE and humic acid vitamin B (HV) agars, and others [16]. The study conducted by Qin et al. [4] showed that eleven selective isolation media were used to isolate actinobacteria. Observations on the isolation frequency indicate that most strains were obtained using simple nutrient media, such as TWYE, SPM, and HV.

Several authors have already stated the occurrence of actinobacteria associated with the genus *Artemisia*, including Li et al. [16] for *Artemisia annua* (China) and El-Shatoury et al. [14] for *A. herba-alba* (Egypt). Morphological and molecular characterization of strains enabled the identification of five different genera in the root endosphere. *Nocardioides* is the most commonly detected genus and the species *N. albus* mostly represents the endophytic community, which contrasts with other studies concerning numerous host plants that show that the genus *Streptomyces* was the most abundant [4, 16]. For instance, Li et al. [16] reported that the genus *Streptomyces* dominated the samples associated with *A. annua* from Yunnan, a southwest China region.

The genus *Nocardioides* (Nocardioidaceae) was originally named to designate the actinobacteria of the nocardioforms group. Members of the genus *Nocardioides* have been isolated from different sources, including soil,

Table 2 Positive in vitro antimicrobial bioassay of endophytic actinobacterial isolates against test microorganisms

No	Strain code	Zone of inhibition (mm)								
		Bacterial test microorganisms				Fungal test microorganisms				
		S. aureus (MRSA 639c)	*S. aureus* S	*B. subtilis* (ATCC 6633)	*Candida albicans* (M3)	*Aspergillus carbonarius* (M333)	*Penicillium expansum* (Pe)	*Umbelopsis ramanniana* (NRRL 1829)	*Fusarium oxysporum* f. sp. *lycopersici* (Fol)	*Fusarium culmorum* (Fc)
1	BT61	18.25 ± 0.35^{a}	–	11.75 ± 0.35^{a}	–	–	14.5 ± 0.71^{a}	–	16.0 ± 0.0	–
2	BT73	21.0 ± 1.41^{a}	18.5 ± 2.12^{a}	17.0 ± 0.0^{b}	13.5 ± 0.71	–	20.5 ± 0.71^{b}	15.5 ± 0.71^{a}	–	15.75 ± 1.06^{a}
3	BT76	13.75 ± 0.18^{b}	12.37 ± 0.27^{b}	11.32 ± 0.23^{a}	–	–	–	–	–	–
4	BT79	21.0 ± 0.0^{a}	18.5 ± 0.71^{a}	18.25 ± 0.35^{b}	–	19.5 ± 0.71	19.75 ± 0.35^{b}	11.0 ± 0.0^{b}	–	17 ± 1.41^{a}
5	BT84	24.5 ± 0.71^{a}	13.0 ± 0.0^{b}	15.5 ± 2.12^{b}	–	–	–	–	–	–
6	BT91	22.5 ± 3.54^{a}	18.5 ± 2.12^{a}	12.25 ± 0.35^{a}	–	–	–	–	–	–

Zones of inhibition values are given as mean values ± SD ($n = 3$) with the value of the agar disk (10 mm) included, results in the same column with different superscript letters are significantly different ($p \leq 0.05$, Tukey test), – no inhibition

sediment, groundwater, seawater, wastewater, plant tissues, and others [28].

The *Promicromonospora* genus was also reported in this study. Members of this genus have been isolated from a variety of settings, like soils, marine sediments, and plant tissues, including *A. annua* [29]. Moreover, actinobacteria endophytes isolated from *A. annua* were described in the study by Li et al. [16], where several genera were reported, including those of the genera *Promicromonospora*, with a percentage of 11.4%.

The molecular study based on the 16S rRNA sequencing of the isolated endophytes enabled us to link *Streptomyces* group to five different species: *S. bobili* (BT61 and BT73), *S. plumbiresistens* (BT76), *S. rochei* (BT84), *S. kurssanovii* (BT91), and *S. rubrogriseus* (BK93). *Streptomyces* strains are ubiquitous in soils, the rhizosphere and the endophytic compartment of plants. Several studies have shown that *Streptomyces* species are enriched in the rhizosphere and endosphere of various plants [30]. In addition, several works in Algeria have been published on isolating *Streptomyces* species from several sources [8, 11, 12].

The endophytic strains BT1, BT5 and BT17 were revealed after the taxonomic study to belong to the genus *Micromonospora* and the species *M. arida*, *M. noduli,* and *M. phytophila*, respectively. The growth range of the three *Micromonospora* strains was 20–40 °C, pH 5.0–10.0 (pH 7.0), and 2% NaCl. These results are resembling the study of Carro et al. [31–33] for *M. noduli*, *M. phytophila,* and *M. arida*, respectively. These species were isolated for the first time from hyper-arid soils in the Atacama Desert [33] and from nodules of *Pisum sativum* [31, 32]. *Micromonospora* species have been reported in numerous geographical locations around the world [31–33]. This genus belongs to the *Micromonosporaceae* family and comprises filamentous, spore-producing, aerobic, and mesophilic bacteria. *Micromonospora* colonies are generally pigmented and range in color from orange to red to brown; they do not produce AM and their SM forms isolated spores [17], which was the case for the studied strains.

In this study, two strains were identified by the molecular study as *S. xinjiangensis.* Members of the *Saccharothrix* genus have mainly been isolated from Saharan soils, such as *S. algeriensis* [34] and *S. tamanrassetensis* [35], and from plant roots like *S. yanglingensis* [36]. The two strains grow on ISP medium supplemented with NaCl up to 6% NaCl, with pH values ranging from 5 to 11 and temperatures from 20 to 40 °C. These characteristics align with those of some species of this genus.

The antimicrobial activity of actinobacteria isolated from the root systems of *A. herba-alba* against different target germs is presented. A total of six strains showed activity against the target germs tested and were identified by molecular approaches as *Streptomyces* and *Saccharothrix*

Table 3 Plant growth-promoting traits and production of extracellular hydrolytic enzyme of representative endophytic actinobacterial isolates

No	Strain code	ACC Deaminase activity	Plant growth-promoting traits					Extracellular hydrolytic enzymes production				
			N_2-fixation	NH_3 production	P-solubilization ($Ca_3(PO_4)_2$	K-solubilization	Siderophores production	Amylases	Cellulases	Proteases	Pectinases	Esterases
1	BT1	−	+	+	−	−	−	1.58 ± 0.37^{a}	1.50 ± 0.17^{a}	1.55 ± 0.18^{a}	1.61 ± 0.10^{a}	+
2	BT5	−	+	−	−	−	−	1.56 ± 0.38^{a}	−	−	1.56 ± 0.10^{a}	+
3	BT7	+	+	+	−	−	+	2.64 ± 0.53^{ab}	−	1.56 ± 0.13^{a}	1.38 ± 0.15^{a}	+
4	BT9	+	+	+	−	−	+	2.86 ± 0.34^{b}	−	1.93 ± 0.06^{b}	1.47 ± 0.23^{a}	+
5	BT17	−	−	+	−	−	−	1.44 ± 0.10^{a}	1.56 ± 0.19^{a}	−	1.63 ± 0.12^{a}	+
6	BT19	+	+	+	−	−	+	1.78 ± 0.19^{a}	1.47 ± 0.21^{a}	1.73 ± 0.01^{a}	1.59 ± 0.06^{a}	+
7	BT26	+	+	+	−	−	+	2.65 ± 0.09^{b}	−	1.29 ± 0.07^{a}	1.65 ± 0.13^{a}	−
8	BT27	+	+	+	−	−	+	2.54 ± 0.15^{a}	1.29 ± 0.02^{a}	1.59 ± 0.10^{a}	1.42 ± 0.13^{a}	+
9	BT39	+	+	+++	−	−	+	3.36 ± 0.56^{c}	−	1.47 ± 0.03^{a}	1.62 ± 0.06^{a}	+
10	BT49	+	+	+	−	−	+	1.58 ± 0.13^{a}	−	2.22 ± 0.03^{b}	1.82 ± 0.01^{ab}	+
11	BT50	+	+	+	−	−	+	1.56 ± 0.02^{a}	−	2.08 ± 0.04^{b}	1.83 ± 0.02^{ab}	+
12	BT51	+	−	+	−	−	+	3.32 ± 0.15^{c}	−	1.24 ± 0.01^{a}	1.60 ± 0.11^{a}	+
13	BT52	+	+	+	−	−	+	2.26 ± 0.18^{a}	−	1.26 ± 0.02^{a}	1.53 ± 0.09^{a}	+
14	BT53	+	+	+	−	−	+	3.30 ± 0.62^{c}	−	1.50 ± 0.05^{a}	1.49 ± 0.02^{a}	+
15	BT54	+	+	+	−	−	+	2.58 ± 0.38^{a}	1.50 ± 0.17^{a}	1.26 ± 0.04^{a}	1.62 ± 0.11^{a}	−
16	BT55	+	−	+	−	−	+	2.35 ± 0.11^{a}	−	1.55 ± 0.18^{a}	1.61 ± 0.10^{a}	−
17	BT56	+	+	+	−	−	+	1.97 ± 0.07^{a}	−	1.19 ± 0.31^{c}	1.71 ± 0.10^{a}	+
18	BT57	+	+	+	−	−	+	1.38 ± 0.13^{a}	−	1.66 ± 0.01^{a}	1.96 ± 0.01^{b}	+
19	BT61	+	−	++	−	−	−	1.55 ± 0.01^{a}	1.25 ± 0.01^{ab}	−	−	+
20	BT64	+	−	+	−	−	−	1.67 ± 0.33^{a}	−	1.64 ± 0.02^{a}	1.72 ± 0.03^{ab}	+
21	BT65	+	+	−	−	−	−	2.45 ± 0.03^{a}	1.18 ± 0.02^{b}	1.57 ± 0.09^{a}	1.99 ± 0.14^{b}	+
22	BT72	+	+	−	−	−	+	2.17 ± 0.02^{a}	1.23 ± 0.07^{b}	2.13 ± 0.14^{b}	1.78 ± 0.02^{ab}	+
23	BT73	+	+	+	−	−	−	2.20 ± 0.35^{a}	1.42 ± 0.02^{a}	2.47 ± 0.01^{b}	1.32 ± 0.04^{a}	+
24	BT76	+	+	+	−	−	+	1.58 ± 0.37^{a}	−	1.77 ± 0.02^{a}	1.85 ± 0.01^{ab}	+
25	BT77	+	+	+	−	−	+	1.43 ± 0.02^{a}	−	1.72 ± 0.03^{a}	1.60 ± 0.08^{a}	+
26	BT79	+	+	+	1.11 ± 0.01	11.36 ± 0.05	+	1.69 ± 0.07^{a}	1.41 ± 0.08^{a}	2.11 ± 0.09^{b}	1.65 ± 0.04^{a}	+
27	BT84	+	+	+	−	−	+	1.64 ± 0.02^{a}	1.33 ± 0.01^{a}	1.73 ± 0.07^{a}	1.96 ± 0.21^{b}	+
28	BT91	+	+	+	−	−	−	1.29 ± 0.07^{a}	1.40 ± 0.02^{a}	1.81 ± 0.05^{a}	1.49 ± 0.02^{a}	+
29	BT94	+	+	+	−	−	+	2.40 ± 0.05^{a}	−	1.47 ± 0.01^{a}	1.68 ± 0.04^{a}	+
30	BK1	+	−	−	−	−	+	3.76 ± 0.73^{c}	1.21 ± 0.09^{b}	1.98 ± 0.01^{b}	1.86 ± 0.02^{b}	+
31	BK4	+	+	−	−	−	+	3.28 ± 0.63^{c}	1.25 ± 0.01^{b}	1.41 ± 0.08^{a}	1.42 ± 0.07^{a}	+
32	BK5	+	+	+	−	−	−	3.39 ± 0.19^{c}	1.40 ± 0.02^{a}	1.43 ± 0.06^{a}	1.56 ± 0.11^{a}	+
33	BK6	−	+	+	−	−	+	2.52 ± 0.42^{a}	1.22 ± 0.01^{b}	2.46 ± 0.17^{b}	1.64 ± 0.09^{a}	+

Table 3 (continued)

No	Strain code	ACC Deaminase activity	Plant growth-promoting traits					Extracellular hydrolytic enzymes production				
			N_2-fixation	NH_3 production	P-solubilization ($Ca_3(PO_4)_2$	K-solubilization	Siderophores production	Amylases	Cellulases	Proteases	Pectinases	Esterases
34	BK11	+	+	+	−	−	+	3.73 ± 0.66^{c}	1.27 ± 0.09^{ab}	1.57 ± 0.06^{a}	1.83 ± 0.01^{b}	+
35	BK13	−	+	+	−	−	+	2.87 ± 0.56^{b}	−	1.92 ± 0.04^{b}	1.61 ± 0.10^{a}	+
36	BK27	−	+	+	−	−	−	2.55 ± 0.39^{a}	−	1.68 ± 0.20^{a}	1.72 ± 0.10^{b}	+
37	BK33	+	+	+	−	−	+	2.99 ± 0.53^{b}	−	1.66 ± 0.15^{a}	1.78 ± 0.19^{b}	+
38	BK37	+	+	−	−	−	+	1.72 ± 0.02^{a}	1.16 ± 0.07^{b}	1.84 ± 0.08^{a}	1.92 ± 0.14^{b}	+
39	BK42	−	−	+	−	−	−	1.67 ± 0.33^{a}	1.40 ± 0.14^{a}	1.35 ± 0.07^{a}	1.56 ± 0.10^{a}	+
40	BK44	−	+	+	−	−	−	2.37 ± 0.03^{a}	−	1.72 ± 0.05^{a}	1.75 ± 0.08^{b}	+
41	BK53	+	+	+	−	−	+	2.57 ± 0.13^{a}	−	1.99 ± 0.07^{b}	1.68 ± 0.02^{a}	+
42	BK61	+	+	−	−	−	+	3.50 ± 0.15^{c}	−	1.26 ± 0.04^{a}	1.48 ± 0.02^{a}	+
43	BK74	+	−	+	−	−	+	1.77 ± 0.21^{a}	−	1.49 ± 0.08^{a}	1.68 ± 0.14^{a}	+
44	BK78	+	−	+	−	−	+	1.99 ± 0.02^{a}	−	1.96 ± 0.25^{b}	1.71 ± 0.04^{b}	+
45	BK93	+	+	+	−	−	+	1.46 ± 0.04^{a}	−	1.48 ± 0.10^{a}	1.94 ± 0.01^{b}	+

Production of ammonia shows the intensity of yellow/brown color (+ weak, ++ medium and +++ strong) on peptone water broth, ACC, N_2-fixation, siderophores production and esterase activity were classified as positive (+) or negative (−), values are given as mean ± SD ($n = 3$), and values having different superscripts differ significantly ($p \leq 0.05$, Tukey test)

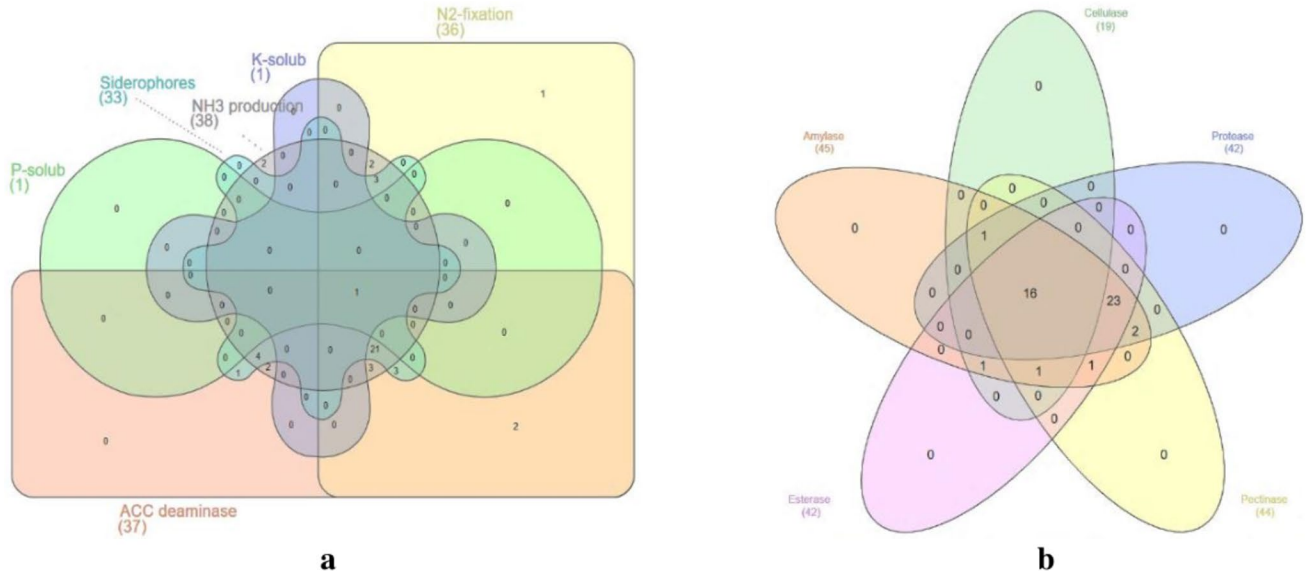

Fig. 3 Venn diagram representation of PGP attributes (**a**) and extracellular hydrolytic enzymes production (**b**) of the tested actinobacterial strains. Numbers falling within a colored part represent that particular character or characters

members. The study by Peng et al. [37] on an endophytic *Streptomyces* strain showed growth inhibition for the eight phytopathogenic fungi tested. The low or narrow activity of the strain could be explained by the use of only one medium (ISP2) in the screening of the antagonistic activity, which was not favorable for the production of antimicrobial molecules by the studied strains. In fact, several parameters affect the production of such molecules, such as carbon sources, aeration, and the time of incubation [8]. On the other hand, antimicrobial compounds can be produced by microorganisms in response to phytohormone exposure in the root system [38].

Because they can participate in vital processes, including nutrient mobilization, phytohormone production, and pathogen suppression, most endophytes are important for supporting plant growth and resilience [8]. These facts have motivated the search for the PGP features of the isolated actinobacterial strains. Actinobacterial strains, either alone or in combination, have been shown by several authors to positively impact plant development. Specifically, actinobacteria stimulate plant development directly by producing phytohormones like IAA, ethylene, gibberellic acid, and cytokines [38], supplying nutrients to plants by solubilizing essential elements, such as phosphate [39], producing siderophores, and fixing atmospheric nitrogen [40]. Furthermore, many actinobacterial strains can control plant pathogens through their antifungal, insecticidal, and antibacterial activities [40] and the production of extracellular enzymes and hydrogen cyanide [8].

ACC deaminase activity was positive for thirty-seven of the strains. The plant precursor of ethylene, ACC, is hydrolyzed by ACC deaminase produced by actinobacteria. It plays a part in preventing phytopathogenic agents and is essential in reducing the stress brought on by excessive salinity, drought, heavy metal pollution, and organic pollutants in plants [41].

Atmospheric nitrogen fixation was demonstrated by several strains in the obtained collection (36 strains). Studies on the nitrogen-fixing properties of actinobacterial strains showed that species like *Propionibacteria*, *Corynebacterium*, *Arthrobacter*, *Micromonospora*, *Agromyces*, and *Streptomyces* have nitrogen-fixing ability [42]. Some reports have indicated that members of the *Micromonospora* genus associated with root nodules can fix atmospheric nitrogen [33].

In this study, the ability to produce ammonia in thirty-eight of the endophytic strains was revealed. Since ammonia production is essential to plant growth, it is thought to be a direct process underlying PGP ability [9]. Nimnoi et al. [43] reported ammonia production by *Streptomyces hainanensis* strain S4303, a strain isolated from *Aquilaria crassna*.

Phosphorus and potassium solubilization were rare in the tested strains (only one positive strain). This strain (BT79) was identified as a member of the *Saccharothrix* genus. However, strains of the other identified genera could not solubilize inorganic phosphate and potassium. These results do not agree with the findings of Passari et al. [9], who stated that *Streptomyces* isolates from medicinal plants showed phosphate-solubilizing activity. Several investigations have been carried out in the literature on *Streptomyces* species from different origins that can solubilize phosphate [39, 44]. Potassium solubilization results reported by Boubekri et al.

[44] showed that three strains of *Streptomyces* and one strain of *Nocardiopsis* could solubilize potassium.

Low-molecular weight compounds called siderophores function as scavengers, preventing plant pathogens from accessing iron [39]. Many of the tested strains (thirty-three) were positive for siderophores production. These results align with the findings of Khamna et al. [45], who stated that several actinobacteria-produced siderophores are generally in short supply in the soil and nutrients needed for plant growth, including *Actinomadura*, *Streptomyces*, *Nocardia,* and *Microbispora*.

All the strains in the present study showed interesting enzymatic activities for most of the tested enzymes. Actinobacterial species are known for producing extracellular enzymes that control plant pathogens [8, 11, 12]. All the isolated strains are amylase producers. Various microorganisms produce these enzymes, the most widely used source in the industrial sectors [46]. Amylases are generally detected in actinobacteria, such as *Streptomyces*, *Arthrobacter*, *Brevibacterium*, *Nocardia,* and *Nocardiopsis* [47]. Cellulase activity was revealed in nineteen of the endophytic strains. Actinobacteria, particularly *Streptomyces*, play a crucial part in the breakdown of cellulose [8]. It is believed that cellulase is necessary for rhizobia to infect host roots for the primary symbiotic infection [48]. One example is the endophytic *Promicromonospora* sp. strain isolated from *A. annua*, which has been shown to produce both proteinases and cellulases [16]. Proteases were widely detected in *A. herba-alba* actinobacteria (forty-two strains). This was also reported by Ashwitha Gopal et al. [49]. A significant number of the tested strains (forty-two strains) are esterase producers. Indeed, several strains are reported to have this activity, such as *Streptomyces rimosus*, *S. lavendulae,* and *Rhodococcus erythropolis* [47]. Pectinase activity is common in the collection of actinobacterial strains (forty-four strains). Pectinases are a group of enzymes that hydrolyze pectin and can be produced by insects, plants, fungi, and actinobacteria [50]. Actinobacterial pectinases are produced by several species of *Streptomyces*, like *S. fulvissimus* and *S. fumigatiscleroticus* [50].

Conclusion

This study investigated the actinobacterial composition of the roots of *A. herba-alba* from the arid lands of Algeria. Through selective isolation, significant insights into the strains of actinobacteria that inhabit the root of the studied plant were uncovered. Moreover, the molecular identification of the isolated strains shows resemblances in the six localities sampled. These findings thus, suggest that the actinobacterial composition is not so different depending on the geographical location. Conversely, the promising in vitro potential of the endophytes sourced from *A. herba-alba* native to arid regions in enhancing plant growth via different mechanisms was revealed, while only a few strains showed antimicrobial activity. Therefore, further research is necessary to investigate potential mechanisms of the biocontrol and plant growth-promoting effects of the most interesting strain or as a synthetic community and would be worthy of assessment in a range of agronomic trials to demonstrate their in vivo effectiveness.

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Author Contributions N.D. and A.M. conceived and managed the project. N.D., K.O.H.Y., S.A.S., and A.N. performed the experiments. C.V-V., A.M., and A.N. performed molecular analysis. N.D. drafted the manuscript, which was critically reviewed by all of the authors.

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Data Availability The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request, while 16S rRNA data generated are available in the Genbank repository, please refer to Figs. 1 and 2.

Code Availability Not applicable.

Declarations

Conflict of interest The authors declare that they have no competing interests.

Ethical Approval The authors declare that ethical standards have been followed and that no human participants or animals were involved in this research.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

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