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Actinobacteria as alternative agents for sustainable agriculture

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ABSTRACT

Four hundred and seventy-one actinobacteria were isolated from the soil sample and subsequently screened for indole acetic acid (IAA) production. IAA producers (15 only) were tested for phosphate solubilization, hydrogen cyanide (HCN) production, and antifungal activities against several phytopathogens such as *Fusarium proliferatum, Fusarium oxysporum,* and *Alternaria solani.* Isolates C4, W59, P97, and P112 were selected for performing other plant growth-promoting (PGP) activities such as ammonia production, potassium solubilization, nitrogen fixation, and hydrolytic enzyme production including proteolytic, cellulolytic, and chitinolytic enzymes. The four selected actinobacteria were applied to wheat and clover plants in greenhouse and field experiments. *Streptomyces coelicoflavus* strain H-112 and *Streptomyces tricolor* strain H-97 showed significant increases in all growth parameters during seed germination, plant growth, and yield production. *Streptomyces tricolor* strain H-97 was the most isolate-inhibited damping-off disease caused by *Fusarium proliferatum* on wheat plants since the treatment with this strain produced P97 produced 46 plants from 50 infected seeds. Therefore, the actinobacteria could be used as plant growth-promoting agents instead of chemical fertilizers and fungicides.

1. Introduction

The world population is expected to increase by more than nine billion according to Food and Agriculture Organization (FAO) in 2050, therefore it is necessary to increase the production of agriculture in a short period of time [1]. The availability of fertile soil and environmental conditions are the major factors for meeting this goal [2].

Recently, chemical fertilizers such as nitrogen, phosphorous, potassium (NPK), and other nutrients, have been used to supplement plants. Although billion tons of NPK chemical fertilizers are used every year to supply the required nutrients for increasing plant growth and yield performance, small portions of chemical fertilizers are taken by plant and other large portions of fertilizer form complexes with metal cations in soil.

Residue of chemical fertilizers in soil could disrupt the natural ecosystem balance destroying agricultural productivity and causing several health issues [3]. In the last few years, plant growth-promoting bacteria (PGPB) have been used instead of chemicals since they enhance the growth and development of plants through the development of specific communication pathways with plants and may influence plant physiology [4]. Plant growth-promoting bacteria enhance plant growth through direct and indirect mechanisms [5]. The direct mechanisms by which PGPB directly promote plant growth and productivity include phytohormone production, nitrogen fixation, ammonia production, 1-aminocyclopropane-1-carboxylate (ACC) deaminase production, and solubilization of minerals such as phosphorous and potassium while the indirect mechanisms by which PGPB inhibit one or more plant phytopathogens include lytic enzymes production, siderophore production, hydrogen cyanide (HCN) production, and antibiosis [6].

2. Materials and Methods

2.1. Actinobacterial isolates

The soil sample was collected on January 14, 2021, from a cultivated clover field at a depth of five cm in Sindanhour village, Qalyubia governorate. Serial dilution of the sample was performed according to previous method [7]. Starch casein agar (SCA) was used as a selective medium for the isolation of actinobacteria [8]. The plates were incubated at 30℃ for 2 weeks. The actinobacterial isolates were purified onto SCA slants.

2.2. Screening for IAA production

The qualitative indole acetic acid (IAA) produced by actinobacterial isolates was determined according to previous method. All actinobacteria were grown in starch casein broth supplemented with 500 µg/ml tryptophan for 7 days at 30℃. After the incubation period, two milliliters of supernatant were mixed with four milliliters of Salkowski reagent. The development of the pink color indicated the IAA production ability.

2.3. Phosphate solubilization

2.3.1. Qualitative estimation

IAA-producing actinobacteria were tested for phosphate solubilization [9]. The appearance of a clear zone around the culture indicated a positive result.

2.3.2. Quantitative estimation

Phosphate solubilizing actinobacteria were tested for the release of free soluble phosphate. The concentration was determined by the molybdenum blue method [10].

2.4. Hydrogen cyanide (HCN) production

IAA-producing actinobacteria were tested for HCN production by inoculating them in King's B medium supplemented with glycine at 4.4 g/l [11]. The change in color of the filter paper from yellow to orange and finally to brownish orange indicated the production of HCN.

2.5. In vitro biocontrol

The antifungal activity of IAA-producing actinobacteria was estimated against *Fusarium oxysporum* (CCM F-358), *Fusarium proliferatum* (NRRL-2284), and *Alternaria solani* (EMCC-756) according to dual culture technique [12]. All of the fungal strains used in this test were obtained from the microbial center in the Faculty of Agriculture at Ain Shams University. Colony growth inhibition (%) was calculated using the following formula: Inhibition % = (T/C)*100, where T is the growth of the pathogen colony in the dual culture plate and C is the growth of the pathogen colony in the control plate [13].

2.6. Ammonia production

The ability of four selected actinobacteria to produce ammonia was tested according to Cappuccino and Sherman method. The development of a brown-to-yellow color indicated a positive result of ammonia production [14].

2.7. Potassium solubilization

The four actinobacteria were tested for solubilizing potassium by inoculating them in the Aleksandrow medium plate. The development of a clear zone surrounding the growth indicated potassium solubilization activity [15].

2.8. Nitrogen fixation

Nitrogen-free agar medium (NFA) was used to detect the ability of the isolates to fix atmospheric nitrogen. The growth of actinobacteria on NFA indicated a positive result [16].

2.9. Hydrolytic enzymes production

2.9.1. Proteolytic enzyme

The proteolytic activity of the four selected actinobacteria was detected according to the Shirling and Gottlieb method. A colorless zone around the growth indicated a positive result [17].

2.9.2. Cellulolytic enzyme

Carboxy Methyl Cellulose agar medium was used to detect the cellulolytic activity of the isolates. A clear zone around the growth indicated a positive result [18].

2.9.3. Chitinolytic enzyme

The selected actinobacteria were inoculated into a minimal salt agar medium supplemented with 2% moist colloidal chitin to detect chitinolytic activity. A clear zone around the culture indicated the chitinolytic activity of the actinobacterial isolate [19].

2.10. Germination bioassay

Wheat seeds (*Triticum aestivum* L. var. Giza 97, record 448365) and clover seeds (*Medicago sativa* L. var. Aswani 116, record 8536) supplied by the ASUEG Herbarium were used for germination bioassay. All methods were performed in accordance with the relevant guidelines and regulations. The seed surface was sterilized [20]. Seeds were treated with four selected actinobacterial spore suspensions while the control seeds were treated with sterile water in a laminar air flow hood. Seeds were placed in sterile Petri dishes containing two sterile filter papers moistened with 10 ml of sterile water and then incubated at room temperature for 5 days. Shoot length and root length were recorded [21].

2.11. Effect of PGP actinobacteria on clover plants in the greenhouse experiment

Clover seeds (*Medicago sativa* L.) were sterilized and treated with actinobacterial spore suspension according to the previously described method. Seeds were sown to the proper depth in plastic pots filled with soil and then watered. During the growing period, each group of treated plants was treated with broth culture once a month while the control was treated with sterile water. The shoot length, root length, fresh weight, dry weight, leaf number, and leaf size (length and width) were recorded [22]. The total nitrogen and potassium concentrations were estimated for control plants and plants treated with P112 and P97 [23].

2.12. Effect of PGP actinobacteria on wheat plants in the field experiment

Wheat seeds (*Triticum aestivum* L. var. Giza 171) were sterilized and treated with actinobacterial isolates according to the previously described method. Treated seeds were sown in the field and then watered.

During the growing season, each group of treated plants was treated with its culture suspension once a month while the control was treated with water. At the end of the wheat planting season, the average number of seeds in the spike of each treated plant group and control was recorded [22].

2.13. Biocontrol in the greenhouse experiment

Wheat plants (*Triticum aestivum* L. var. Giza 171) were infected with *Fusarium proliferatum* and four selected actinobacteria were used to control the infection. The seed surface was [20]. Sterilized seeds were treated with four selected actinobacterial suspensions separately while the control seeds were treated with sterile water in a laminar flow hood for an hour. The treated seeds were subsequently dried. The soil was sterilized in an autoclave for 60 min at 121℃ then allowed it to cool. The fungal suspension was used to infect sterile soil which was subsequently transferred to plastic pots. Fifty seeds of each treatment were sown in infected soil [24]. The plants were watered with sterile water every two days. The number of plants that produced spikes was recorded.

2.14. Molecular identification

Isolates P97 and P112 were molecularly identified using 16S8FWD (5\`AGAGTTTGATCCTGGCTCAG`3\) and 16S1510RVS (5\`GGTTACCTTGTTACGACTT`3\). Sequence analysis was carried out using a Big TriDye sequencing kit (ABI Applied Biosystems) at Macrogen, Korea. Nucleotide sequences were submitted to GenBank. Phylogenetic analysis of all the isolates was carried out using MEGA version 7.0 via the neighbor-joining method with 1000 bootstrap replications [25].

3. Results

3.1. Screening of IAA production

Out of 471 actinobacterial isolates isolated from soil, only fifteen were able to produce IAA.

3.2. Qualitative and quantitative phosphate solubilization

Among the tested fifteen isolates, C2, C4, W59, and W51 out of the tested fifteen isolates were able to solubilize phosphate. The concentrations of free phosphate released from C2, C4, W59, and W51 were 2.33, 0.71, 3.32, and 1.06 µg/dl respectively.

3.3. Hydrogen cyanide (HCN) production

All the IAA-producing isolates did not exhibit the ability to produce hydrogen cyanide.

3.4. In vitro biocontrol

All the IAA producers were tested for antifungal activity against Fusarium proliferatum (NRRL-2284), Fusarium oxysporum (CCM F-358), and Alternaria solani (EMCC-756) as shown in Table 1. In case of Fusarium proliferatum (NRRL-2284), twelve (80%) of the tested isolates exhibited antifungal activity. The percentage of fungal growth inhibition ranged from 40:55.5%. P97, C2, and W51 exhibited the highest antifungal activity 55.5, 50, and 50% respectively.

In the case of Fusarium oxysporum (CCM F-358), seven (46.67%) of the tested isolates showed antifungal activity against this phytopathogen. The range of fungal growth inhibition was 26.6:51.5%. M66 and P97 exhibited the highest antifungal activity with growth inhibition percentages of 51.5 and 46.4% respectively. In the final case of Alternaria solani (EMCC-756), eight (53.33%) of the tested isolates exhibited antifungal activity against this phytopathogen. The range of fungal growth inhibition was 15.7:70%. P142 showed the highest antifungal activity with a growth inhibition percentage 70%.3.1.2.

3.5. PGP traits of selected actinobacteria

The P112, P97, W59, and C4 isolates were selected for their PGP activities and subsequently subjected to agricultural bioassays.

3.6. Potassium solubilization

Four selected isolates were streaked on the Aleksandrow medium to detect their ability to solubilize potassium from mica. Only P97 and P112 were able to solubilize the complex phosphate.

3.7. Nitrogen fixation

Isolates P97 and C4 weakly grew substrate mycelia after incubating on nitrogen-free agar media for 21 days which indicated their low ability to fix atmospheric nitrogen.

3.8. Ammonia production and hydrolytic enzymes production

All the selected isolates were tested for their ability to produce ammonia and hydrolytic enzymes including proteolytic enzyme, cellulolytic enzyme, and chitinolytic enzyme. All four abilities were positively correlated with the four isolates

3.9. Germination bioassay

Four selected actinobacteria were used to treat clover and wheat seeds. The treated seeds were allowed to germinate. Shoot and root lengths were recorded.

In the case of clover germination, the treatment with P112 had the maximum increase in shoot length reaching 4.4 cm when compared to the control which reached 3.15 cm while the shoot lengths of the treated clover plants with C4, W59, and P97 reached 3.54, 4.06, and 4.19 cm respectively. On the other hand, the treatment with P97 had the greatest increase in root length, reaching 2.1 cm when compared to the control which reached 1.6 cm, while the root lengths of the treated clover plants with C4, W59, and P112 reached 1.68, 1.71, and 1.14 cm respectively as shown in fig. 1.

In the case of wheat germination, compared with those in the control treatment, the shoot length in P112 treatment reached 5.13 cm, while the shoot length in the C4, W59, and P97 treatment groups reached 4.87, 4.68, and 4.95 cm respectively. Compared with those in the control treatment, the length of the roots in the P97 treatment reached 5.08 cm, while the length of the roots in the W59, C4, and P112 treatment groups reached 4.84, 4.21, and 3.13 cm, respectively as shown in fig. 2.

3.10. Effect of PGP actinobacteria on clover plants in the greenhouse experiment

After 50 days of clover planting in pots, data were recorded for shoot length, root length, fresh weight, dry weight, leaf number and lea size (length and width) as shown in Table 2 and fig.3. Treatment with P112 had the greatest increase in shoot length reaching 28.5 cm, compared to that of the control, which reached 18 cm while the shoot length of clover plants treated with isolates C4, W59, and P97 reached 25.23, 19.44, and 25.53 cm respectively. In term of root length, in comparison to those in the control (15.18 cm), the length of the roots in the P97 treatment reached 18.8 cm while the length of roots in clover plants treated with the C4, W59, and P112 isolates reached 16.84, 15.51, and 12.42 cm respectively.

Treatment with C4 had the highest increase in fresh weight, reaching 0.64 g when compared to the control which reached 0.4 g while the fresh weights of clover plants treated with isolates W59, P97, and P112 reached 0.38, 0.6, and 0.58 g respectively. In terms of dry weight, treatments with the P112, P97, and C4 isolates increased the dry weight of the treated plants by 0.094, 0.092, and 0.092 g respectively, while treatment with W59 slightly decreased the dry weight reaching 0.068 g compared to that of control which reached 0.072 g.

Out of all the treatments, treatment with W59 increased leaf number yielding 15 leaves compared to the control which produced 14 leaves. Treatment with Streptomyces P112 had the greatest increase in leaf width reaching 0.97 cm compared to that of the control which reached 0.52 cm, while the leaf widths of clover plants treated with Streptomyces C4, W59, and P97 reached 0.54, 0.54, and 0.83 cm respectively. Treatment of Streptomyces P97 had the greatest increase in leaf length reaching 1.97 cm when compared to the control which reached 1.15 cm while the leaf lengths of clover plants treated with Streptomyces C4, W59, and P112 reached 1.66, 1.02, and 1.72 cm respectively.

Clover plants treated with P97, P112, and the control were analyzed for potassium concentration and total nitrogen concentration. The potassium concentrations of clover plants treated with P97 and P112 were 12.58 and 15.38 mg/g fresh weight respectively compared to the control that was 11.97 mg/g fresh weight. For total nitrogen concentration, the total nitrogen concentrations of clover plants treated with P97 and P112 were 6.8 and 4.3 mg/g fresh weight respectively while the control was 3.2 mg/g fresh weight.

3.11. Effect of PGP actinobacteria on wheat plant in a field experiment

Treatment with isolate P112 had the highest number of seeds produced by the formation of 44 seeds in each spike while with the control, thirty-two seeds were produced while each spike of plants treated with isolates C4, W59, and P97 contained 34, 33, and 39 respectively.

3.12. Effect of PGP actinobacteria on the wheat plant in the field experiment

After ending of the wheat planting season, the average number of seeds in a spike of each treated plant group and control were recorded. All treatments showed increasing in number of seeds as compared to the control. Treatment with isolate P112 had the highest number of seeds produced by the formation of 44 seeds in each spike while with the control, thirty-two seeds were produced while each spike of plants treated with isolates C4, W59, and P97 contained 34, 33, and 39 respectively.

3.13.Biocontrol in Greenhouse Experiment

The number of plants that produced spikes were recorded for all the treatments and the control which was infected with the pathogen only. Treatment with P97 inhibited fungal proliferation more than did other actinobacterial treatments and produced more plants than did the other treatments.

Out of 50 seeds, treatment with P97 produced 46 plants whereas the control produced only 36 plants, while treatment with isolates C4 and P112 produced 43 and 44 plants respectively. Treatment with W59 produced 36 plants similar to the control.

3.14. Molecular identification

The nucleotide sequence of the16S rRNA gene from two actinobacterial isolates was subjected to nucleotide BLAST analysis using the NCBI database. Isolate P97 was closely related to *Streptomyces tricolor* with a similarity index of 99.63%, while isolate P112 was closely related to *Streptomyces coelicoflavus* with a similarity index of 99.51%. The sequences of *Streptomyces* P97 and *Streptomyces* P112 were submitted in GenBank as *Streptomyces tricolor* strain H-97 with accession number OL588009.1 and as *Streptomyces coelicoflavus* strain H-112 with accession number OM262199.1 respectively.

Our results were supported by phylogenetic analysis using the neighbor-joining tree method with 1000 bootstrap replications. Phylogenetic analysis indicated that the two isolates were placed in streptomycetes clade. The *Streptomyces tricolor* strain H-97 with accession number OL588009.1 was the most closely related to the *Streptomyces tricolor* strain NBRC15461 with 99% bootstrap support as shown in fig. 4. The *Streptomyces coelicoflavus* strain H-112 with accession number OM262199.1 is the most related to the *Streptomyces coelicoflavus* strain CSSP410 with 100% bootstrap support as shown in fig. 5.

Table 1. Fungal growth inhibition percentage of IAA producing isolates against several phytopathogens including *Fusarium proliferatum* (NRRL-2284), *Fusarium oxysporum* (CCM F-358), and *Alternaria solani* (EMCC-756).

Isolate's code	Fungal growth inhibition percentage (%)		
	F.proliferatum	*F.oxysporum*	*A.solani*
C2	50	-	29.40
C4	40	-	-
P83	40	-	-
P97	55.50	46.40	-
P102	43.30	-	-
P112	43.30	-	47.30
P126	48.20	-	20
P142	43.30	-	70
P147	48	26.60	20
P149	44.80	33.30	30
W29	46.60	32.10	-
W51	50	-	15.70
W59	-	40	-
W91	-	36.60	-
M66	-	51.50	17.60

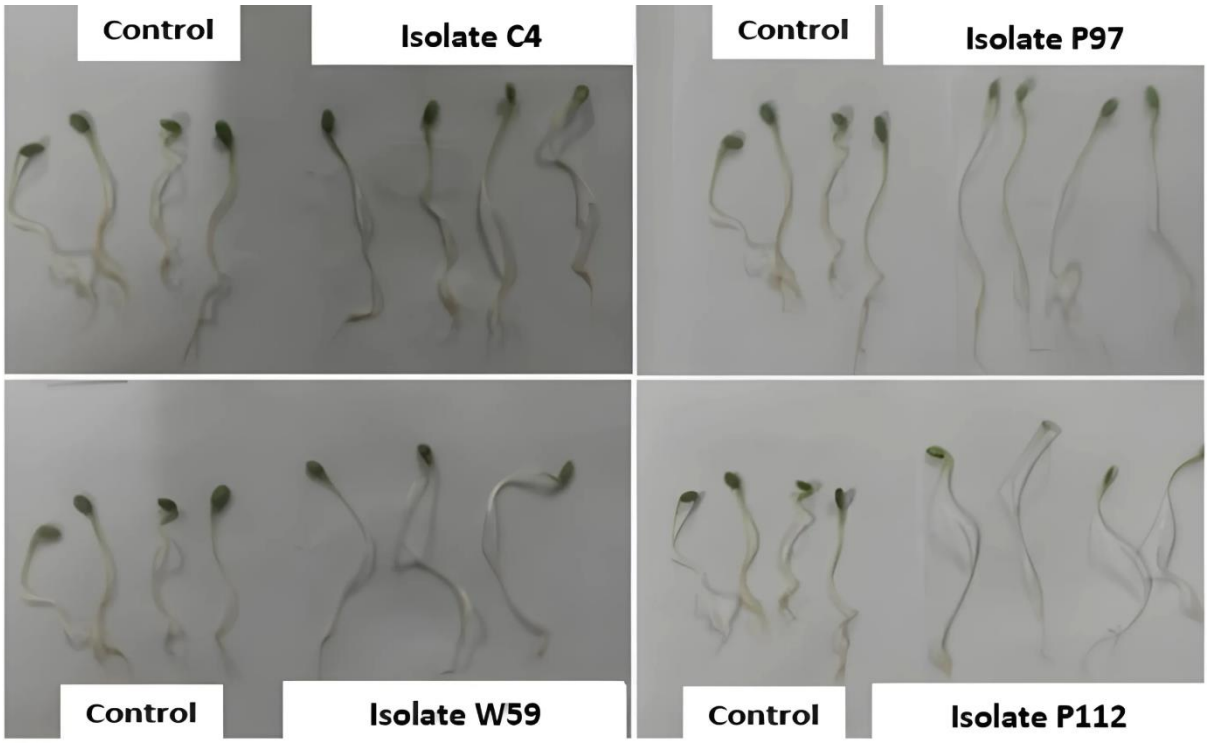

Fig. 1 Effect of four selected actinobacterial isolates on germination of clover seeds.

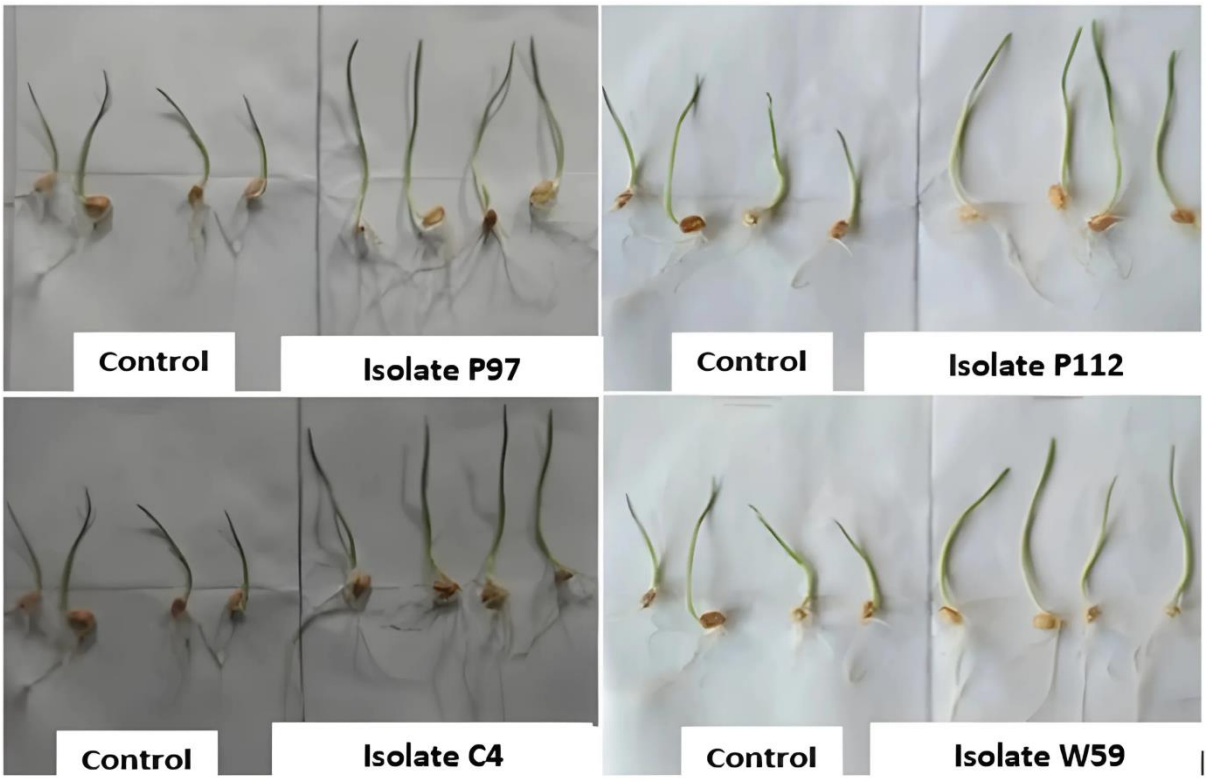

Fig. 2 Effect of four selected actinobacterial isolates on germination of wheat seeds.

Table 2. Statistical analysis result of influence of actinobacterial isolates on shoot length, root length, fresh weight, dry weight, leave number, leaf width, and leaf length of clover plant using ANOVA one-way in SPSS program

Treatment	Control	Isolate C4	isolate W59	isolate P97	isolate P112	LDS
Shoot length (cm)	18±0.868^{c}	25.23±2.503^{b}	19.44±1.157^{c}	25.53±1.86^{b}	28.5±1.096^{a}	0.7608
Root length (cm)	15.18±0.702^{c}	16.84±0.965^{b}	15.51±2.165^{bc}	18.8±1.664^{a}	12.42±1.233^{d}	0.6802
Fresh weight (g)	0.4±0.121^{b}	0.64±0.121^{a}	0.38±0.07^{b}	0.6±0.07^{a}	0.58±.118^{a}	0.0488
Dry weight (g)	0.072±0.014^{b}	0.092±0.024^{a}	0.068±0.013^{b}	0.092±0.012^{a}	0.094±0.027^{a}	0.0092
Leave number	14±1.5^{a}	14±1.5^{a}	15^{a}	14 ±1.5^{a}	14±1.5^{a}	0.6156
Leaf width (cm)	0.52±0.097^{c}	0.54±0.088^{c}	0.54±0.072^{c}	0.83±0.122^{b}	0.97±0.097^{a}	0.0457
Leaf length (cm)	1.15±0.229^{c}	1.66±0.141^{b}	1.02±0.156^{c}	1.97±0.12^{a}	1.72±0.268^{b}	0.0457

Values with a column with the same lowercase letters are not significantly different ($p < 0.05$).

Fig.3 Effect of selected actinobacteria on clover plant in green house experiment.

Fig. 4 Neighbor-joining phylogenetic tree of 16S rRNA gene sequence of *Streptomyces tricolor* strain H-97 and its related species. The numbers on the nods are bootstrap values recovered from 1000 trees. The bar indicates 2% nucleotide substitution. The accession number of isolate was written in bold.

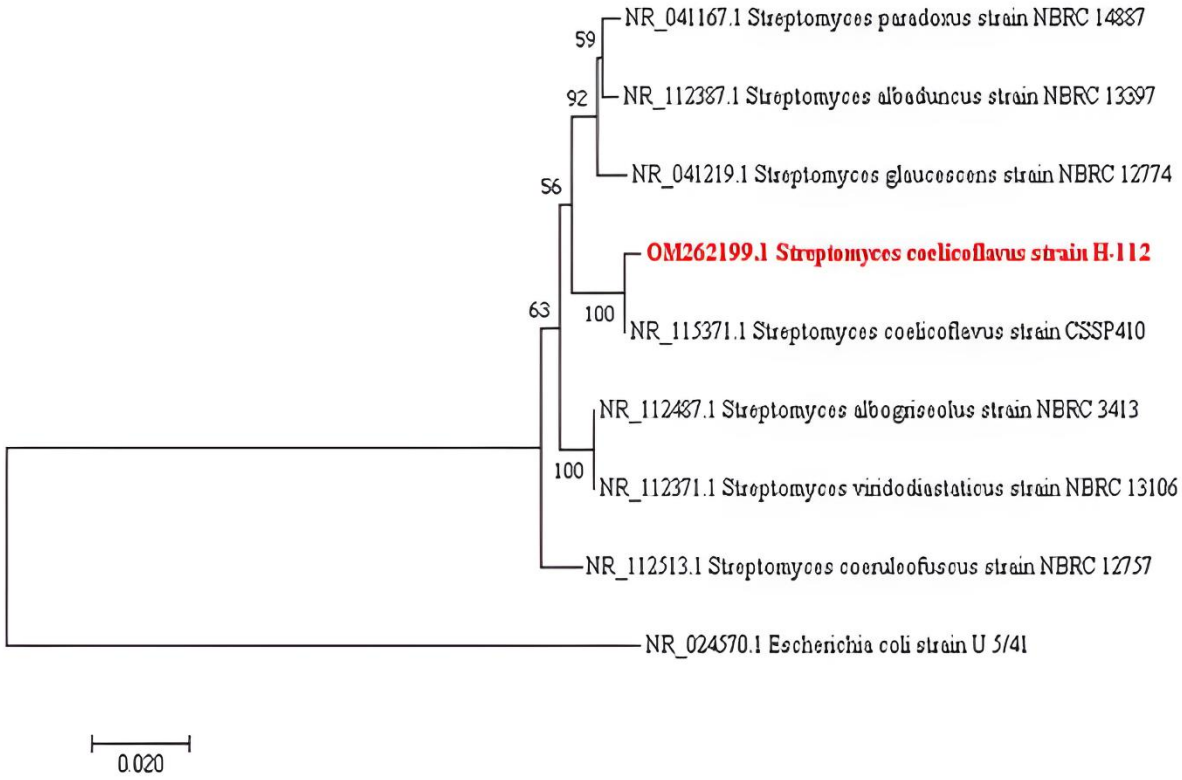

Fig. 5 Neighbor-joining phylogenetic tree of 16S rRNA gene sequence of *Streptomyces coelicoflavus* strain H-112 and its related species. The numbers on the nods are bootstrap values recovered from 1000 trees. The bar indicates 2% nucleotide substitution. The accession number of isolate was written in bold.

4. Discussion

Out of 471 actinobacterial isolates, 15 (3.1%) isolated from different rhizospheric soils could produce IAA which is consistent with the previous findings [26, 27], who reported that several actinobacteria including those in the Streptomycetes group and rare-actinomycetes group isolated from different rhizospheres can produce IAA. The activity of microorganisms in the production of IAA is supported by the root exudates of plants which vary according to plant species [28]. Concerning phosphate solubilization, out of the IAA producers only 4 (26.66%) isolates were able to solubilize tricalcium phosphate. Microbial solubilization might be due to chelation or acidification resulting in increased phosphate solubilization [29].

Only 46.67% of the IAA-producing actinobacterial isolates exhibited antifungal activity against *F.oxysporum.* On the other hand, 53.33% of the isolates exhibited antagonistic effects on *A. solani.* Our results agree with other results [30, 31]. Of the IAA-producing actinobacteria, 80% of the actinobacterial isolates exhibited antifungal activity against *F.proliferatum* which was supported by findings of another study [32].

The four selected isolates showed the ability to produce ammonia where the production of ammonia had a positive impact on plants by supplying plants with nitrogen. Additionally, the overproduction of ammonia acts as a factor promoting the virulence of phytopathogens [33, 34]. Potassium solubilization was detected only by *Streptomyces* P97 and *Streptomyces* P112. Rhizospheric actinobacteria including *Arthrobacter* sp. 4 and 42, *Microbacterium* FS-01, *Nocardiopsis abla* BC-11, *Streptomyces griseorubens* BC-3, and *Streptomyces* KNC-2 have been reported as potassium solubilizer.

Potassium solubilization occurs through various mechanisms including acidification, chelation, and polysaccharide production [35]. The isolates P97 and C4 could grow under nitrogen deficiency resulting in weak substrate mycelia growth through fixing atmospheric nitrogen which is supported by [36], who reported that the activity level of nitrogen fixation by actinobacteria is indicated by colony development in media. All the selected *Streptomyces* isolates exhibited the ability to produce hydrolytic enzymes, including protease, cellulase, and chitinase.

The proteolytic, chitinolytic, and cellulolytic activities of soil microbes play vital roles not only in nutrient mineralization and organic matter decomposition, but also in plant protection against fungal phytopathogens which contain cellulose, chitin, and proteins in the fungal cell wall [21].

During clover germination, treatment with isolates W59 and P97 increased both shoot and root length which is consistent with previous findings [21]. Isolate C4 increased shoot length but did not affect root length. It is possible that the amount of phytohormone produced by C4 affects only the shoot system which is supported by a previous finding [37]. Treatment with *Streptomyces* P112 resulted in a maximum increase in shoot length but inhibited the growth of roots compared with those of the control. It is possible that the amount of actinobacterial auxin taken up by plant changes the hormonal pool in roots from a saturated level to a supersaturated level, causing the inhibition of root growth which is consistent with previous studies [38].

With respect to wheat germination, the increase in shoot and root lengths in response to actinobacteria in germinated wheat is in line with the findings of [29], who reported that *Streptomyces nobilis, Streptomyces kunmingensis, Streptomyces mutabilis, Streptomyces enissocaesilis,* and *Streptomyces djakartensis* increased shoot length, root length, and root number. The effect of *Streptomyces* P112 on the germination of wheat seeds is similar to that on clover germination which confirms that the concentration of IAA produced by *Streptomyces* P112 elicits a negative effect on root growth. Similar to clover plants, isolate C4 increases the length of the shoot system and does not increase the root system.

Among all the treatments, C4, P97, and P112 isolates significantly increased all growth parameters in clover plants (except for the root length of plants treated with *Streptomyces* P112), this finding was supported by findings of other studies which reported that the application of actinobacteria to several other crops resulted in significant increase in all the growth parameters such as tomato. rice, and soybean [37].

Biochemical analysis of clover plants treated with *Streptomyces* P112 and *Streptomyces* P97 revealed high increase in total nitrogen and potassium concentrations with respect to those of the control that is consistent with a previous study [39].

The increase in potassium concentration in both treatments compared with that in the control was due to the ability of both actinobacterial isolates to solubilize potassium complexes which were tested before in-vitro by using Aleksandrow medium. The total nitrogen concentration in clover plants treated with *Streptomyces* P97 was greater than that in clover plants treated with *Streptomyces* P112 because of the ability of *Streptomyces* P97 to fix atmospheric nitrogen, which was recorded in-vitro using NF media.

In all the treatments, the number of seeds per spike increased. Several studies have reported that the use of actinobacterial isolates as biofertilizers has a great impact on wheat grain yield and can reduce chemical fertilizers without compromising yield [32, 35]. Treatment with *Streptomyces* P97 inhibited fungal proliferation more than treatment with other actinobacterial agents producing more plants because *Streptomyces* P97 was the most active actinobacterial isolate that inhibited 55.5% of fungal growth in vitro while C4 and P112 inhibited 40% and 43.3% respectively of fungal growth which is consistent with the previous findings [40].

5. Conclusion

Actinobacteria isolated from different rhizospheric soils have several direct and indirect mechanisms for plant growth promotion in-vitro. *Streptomyces coelicoflavus* strain H-112 and *Streptomyces tricolor* strain H-97 are well adapted to clover and wheat rhizosphere environments where they not only promote plant growth, increase productivity, and protect plants from phytopathogens but also enhance the nutritional value of product yield. Therefore, these strains could be used as plant growth-promoting agents instead of chemical fertilizers and fungicides.

6. Abbreviations

IAA: indole acetic acid; PGP: plant growth-promoting; PGPR: Plant growth-promoting rhizobacteria; FAO: Food and Agriculture Organization; PGPB: plant growth-promoting bacteria; ACC: 1-aminocyclopropane-1-carboxylate; HCN: hydrogen cyanide; SCA: Starch casein agar; NFA; Nitrogen free agar

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