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RESEARCH ARTICLE

An endophyte *Paenibacillus dendritiformis* strain APL3 promotes *Amaranthus polygonoides* L. sprout growth and their extract inhibits food-borne pathogens

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ABSTRACT

Green leafy vegetables are rich sources of antioxidants and minerals, which prevent food-borne pathogen infections during our diet. This study was aimed to isolate and identify the plant growth-promoting endophytic bacterium from several plant species to enhance the growth of *Amaranthus polygonoides* L. and their antimicrobial potential against food-borne pathogens. Seven endophytic bacterial isolates were tested on two *Amaranthus* species to identify the suitable beneficial bacterium. The antioxidants capacity and antimicrobial activity of bacterial isolate (APL3) treated plants were analyzed. The bacterial isolate, APL3 showed a significantly higher growth of *A. polygonoides* L. than other isolates. It was identified as *Paenibacillus dendritiformis* strain APL3 by 16S rRNA gene sequencing and phylogenetic analysis. The endophyte (APL3) treated *A. polygonoides* L. sprouts had higher antioxidants potentials and significantly inhibited the growth of *Escherichia coli*, *Salmonella* sp., *Staphylococcus* sp. and *Pseudomonas* sp. The results of the present study suggest that utilization of *P. dendritiformis* strain APL3 triggers the growth of *A. polygonoides* L. and induces metabolic changes in plants to improve their antimicrobial properties to prevent foodborne pathogens.

Introduction

The food contains a lot of nutrients and microorganisms, and those microorganisms are friends or foes to humans and other organisms (1). The consumption of raw vegetables, half-cooked food, and non-hygienic preparation of food may have the chance to generate diseases in consumers. The anthropogenic activities including sewage disposal to water bodies and improper maintenance of water tanks are the sources of water-borne diseases (2). Food-borne diseases are a major health issue worldwide and reduce national economies (3). The microbial contaminated food and drinking water cause several diseases including diarrhea, typhoid, cholera, salmonellosis and hepatitis A. *Escherichia coli, Salmonella* spp., *Staphylococcus* spp. and *Pseudomonas* are common food-borne pathogens that infect the human populations. The microbiological safety of foods is prime research in the current era. The physicians are suggesting antibiotic drugs to cure the disease. The misuse or overuse of those drugs will not be effective to control the pathogen growth due to antibiotic resistance (4). The antimicrobial potential of plant extracts would be an alternative to synthetic drugs to prevent diseases and defeat antibiotic resistance (5).

Several plants were identified to suppress the growth of food-borne microorganisms. Spinach (*Amaranthus* spp.), is a leafy vegetable contains a rich amount of antioxidants, vitamins and minerals (6) and control pathogen infections. Minerals are essential nutritional elements for living organisms to perform several biochemical reactions by activating enzymes (7). The enhancement of the nutritional value of spinach is one of the major areas of worldwide spinach researchers (8). Spinach cultivation is affected by diseases, insect infestation, soil and other climatic factors. The usage of plant growth-promoting microorganisms in sustainable agriculture is well established to enhance plant growth, nutritional

values, tolerate abiotic stresses and resistance to diseases (9-11). Plant growth-promoting bacteria trigger the growth and yield of crop plants. The plant growth promotion of endophytic bacteria has been an emerging study to improve the crop response against several environmental stresses. Recently, an endophytic bacterium *Bacillus methylotrophicus* KE2 isolated from Kimchi leaves showed plant growth-promoting activities in lettuce, a green leafy vegetable and also enhanced their food values (12). In addition, the association of endophytic bacteria with plants helps to obtain mutual benefits during adverse environmental conditions. Some of the drought, salt, heavy metal and biocontrol microorganisms were identified and successfully applied to plants to enhance resistance or tolerance against unfavorable conditions (13). The drought-tolerant and phosphate solubilizing microorganisms (*Pseudomonas libanensis*, *Streptomyces laurentii*, *Acinetobacter calcoaceticus*, and *Penicillium sp*.) enhance the plant growth under drought stress conditions (14, 15). The bacterial cell-free extracts containing the plant growth-promoting substances enhance the plant growth and increase the macro and micronutrients (16).

Amaranthus polygonoides L. is a herbaceous medicinal plant containing antioxidants, which prevent cancer cell growth and inhibit the growth of disease-causing organisms such as *Staphylococcus aureus, Staphylococcus epidermidis, Micrococcus luteus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Klebseilla pneumonia, Aspergillus niger* and *Aspergillus fumigatus* (17). It is a common green leaves vegetable in southern India. In the present study, we used endophytic bacterium to enhance the growth, increase the antimicrobial and antioxidant properties of *Amaranthus polygonoides* L. The interaction of bacterium, *Paenibacillus dendritiformis* strain APL3 isolated from leaf tissues of *Andrographis paniculata* L. was not reported in *A. polygonoides* L. This study was aimed to promote the *A. polygonoides* L. sprout growth and medicinal values by endophytic bacterial treatment.

Materials and Methods

Collection of plant samples

The leaves or and flowers sample of *Nerium oleander* L., *Hibiscus rosa-sinensis* L., *Plectranthus amboinicus* L., *Ocimum tenuiflorum* L., *Andrographis paniculata* L., *Catharanthus roseus* L., *Mentha arvensis* L. and *Centella asiatica* L. were collected from the south zone of Coimbatore, Tamilnadu, India.

Isolation of endophytic bacteria from different plant samples

The selected plant samples were cleaned in running tap water to remove the dust particles and surface sterilized with 0.1% mercuric chloride for 5 min and 70% ethanol for 45 sec. The chemical deposition from the leaves or flowers was removed after washing with sterile distilled water. The leaves and flowers were separately sliced with a sterile knife and ground in mortar and pestle. The extract of plant materials was serially diluted and spread on the tryptic soy agar (TSA) medium. The inoculated plates were kept at 35 ± 2° C. The different bacterial colonies were observed every day and numbers of bacterial colonies were recorded up to 10 days. The bacterial colonies were differentiated by their morphology, pigmentation and growth rate. The individual bacterial colonies were separated and the pure culture of each isolate was maintained in a nutrient agar medium.

Screening of endophytic bacteria to promote plant growth

Seeds of *Amaranthus polygonoides* L. and *Amaranthus atropurpurea* L. were surface sterilized with 0.1% mercuric chloride and 70% ethanol as per the method described above. The sterilized seeds were three times rinsed with sterile distilled water and transferred to the nutrient broth culture of each bacterial isolate. The seeds were allowed to co-culture with endophytic bacteria for three hours. The seeds were carefully shifted to petri-plates containing sterilized cotton and tissue paper. The sterile water was sprayed on plates to keep the moisture level. The height of seedlings was compared with control (without endophytes treated seedlings) to check the plant growth promotion activity of endophytes.

Identification of endophytic bacterium

The bacterial strain (APL3) was isolated from *Catharanthus roseus* L. leaves and inoculated on plates containing tryptic soy agar medium and incubated for 48 hr at 35° C. The biochemical analysis such as Gram staining, methyl red, Voges Proskauer, citrate utilization, oxidase, catalase, urease, ammonia production and motility test were conducted to identify the bacterium. The bacterial isolate, APL3 was identified based on the partial 16S ribosomal rRNA gene sequence. The 27F primer (50-AGAGTTTGATC(AC)TGGCTCAG-30) and 1492R primer (50-CGG(CT)TACCTTGTTACGACTT-30) were used for PCR amplification of the 16S rRNA gene. The BLAST search program (http://www.ncbi.nlm.nih.gov/BLAST/) was used to find the nucleotide sequence homology of this bacterial isolate. The relatively similar nucleotide sequences were aligned by ClustalW and MEGA (version 5.0) software and the neighbor-joining tree was generated. Bootstrap replication (1000 replications) was used to statistical support for the nodes in the phylogenetic tree.

Antimicrobial activity of endophytes treated plants against food-borne pathogens

The endophytes treated and non-treated sprouts of *A. polygonoids* L. were tried at 40 °C and ground to make powder. The crushed powder was dissolved in 10% dimethyl sulphoxide (DMSO) and applied to well in *E. coli, Salmonella, Staphylococcus* and *Pseudomonas* culture plates. The inhibition of microbial growth was visualized as clear zone formation and recorded by millimeters. The minimum inhibitory concentration was tested with gradients of plant extracts containing DMSO to identify the sub-lethal concentration.

DPPH activity of sprouts

The dried and powdered sprouts were mixed with methanol and DPPH scavenging activity was determined as per Blois (18) method. The methanol

extract was allowed to react with diphenyl-1-picrylhydrozol (DPPH) for 30 min in dark conditions. Hence, the absorbance of the reaction mixture was determined at 517 nm. DPPH scavenging activity (%) was calculated as follows:

DPPH scavenged (%) = (A con- A test) / A con × 100

A con—the absorbance of the control reaction; A test—the absorbance in the presence of the sample of the extracts.

Statistical analysis

The growth of sprouts treated with endophytes and other analyses were compared with their controls by statistical software, SPSS 11. The calculation of mean ± SE (standard error) and one-way analysis of variance (ANOVA) of each sample were used to find out the significant difference between control and treatment.

Results and Discussion

Plant growth-promoting bacteria alter the physiological changes in plants to enhance plant growth (19). Endophytic bacteria present in plants significantly change the growth pattern and metabolites of host plants. The number of bacteria and fungi isolated from roots, stems, leaves, flowers, fruits and seeds expressed beneficial effects on plant growth and yield (20, 21). Forty-three bacterial isolates were obtained from *Nerium oleander* L., *Hibiscus rosa-sinensis* L., *Plectranthus amboinicus* L., *Ocimum tenuiflorum* L., *Andrographis paniculata* L., *Catharanthus roseus* L., *Mentha arvensis* L. and *Centella asiatica* L. plant tissues in this study (Table 1). The leaves of *Andrographis paniculata* L., *Catharanthus roseus* L., the root of *Mentha arvensis* L. and the stem of *Centella asiatica* L. had four numbers of different bacterial species. Leaf of *Mentha arvensis* L. had less number (one) of an endophytic bacterium.

Table 1. Endophytic bacteria isolated from different plant samples

Sl. No	Plant	Plant part	Total number of Bacterial isolates
1.	*Nerium oleander* L.	Flower	NOF1, NOF2
2.	*Nerium oleander* L.	Leaf	NOL1,NOL2,NL3
3.	*Hibiscus rosa-sinensis* L.	Flower	HRF1,HRF2,HRF3
4.	*Plectranthus amboinicus* L.	Leaf	PAL1,PAL2
5.	*Ocimum tenuiflorum* L.	Flower	OTF1,OTF2,OTF3
6.	*Ocimum tenuiflorum* L.	Leaf	OTL1,OTL2,OTL3
7.	*Andrographis paniculata* L.	Root	APR1,APR2,APR3
8.	*Andrographis paniculata* L.	Leaf	APL1,APL2,APL3,APL4
9.	*Catharanthus roseus* L.	Root	CRR1,CRR2
10.	*Catharanthus roseus* L.	Leaf	CRL1,CRL2,CRL3,CRL4
11.	*Mentha arvensis* L.	Root	MAR1,MAR2,MAR3,MAR4
12.	*Mentha arvensis* L.	Leaf	MAL1
13.	*Centella asiatica* L.	Leaf	CAL1,CAL2,CAL3
14.	*Centella asiatica* L.	Root	CAR1,CAR2,CAR3
15.	*Centella asiatica* L.	Stem	CAS1,CAS2,CAS3,CAS4

The diversity of endophytes present inside the plant tissues was determined by tissues, developmental stage, and plant species (22, 23). The association of endophytes and their composition influences the growth of host plants and stimulates the production of valuable compounds (24). Endophytic bacterial interaction supports plant growth against various environmental stresses including soil salinity. It regulates nutritional and water uptake and balance in plants, stomatal conductance, osmolytes accumulation, photosynthesis, hormones, toxic substances and antioxidants to enhance the stress-affected plant growth (25, 26).

In addition, the plant growth promotion activity of endophytic bacterial isolates in two spinach species such as *A. polygonoides* L. and *A. atropurpurea* L. was observed in this study. The obtained results were recorded in Table 2. Bacterial isolates, APL3, CL3, CR1 and CL showed the best positive response in seedling development of two spinach species than others (Fig. 1). *A. polygonoides* L. growth was significantly higher due to the interaction of APL3 isolate. It was identified as *Paenibacillus dendritiformis* strain APL3 by 16S rRNA sequencing and phylogenetic analysis (Fig. 2). APL3 bacterial inoculation effectively promoted the shoot length of *A. polygonoides* L. (Fig. 3).

Table 2. Plant growth-promoting ability of endophytic bacteria in two spinach species

Sl. No	Bacterial isolates	*Amaranthus polygonoides* L.	*Amaranthus atropurpurea* L.
1	NOF1	-	+++
2	APL3	++++	-
3	MAR1	-	+++
4	MAL1	-	++
5	CAL3	++	-
6	CRR1	+++	-
7	CRL3	++	+

Some of the species of *Paenibacillus* produce several enzymes and acids to degrade the biological materials. For instance, the *P. polymyxa* CR1 genome contains endoglucanases, cellodextrinases, xylanases, mannanases, arabinofuranosidase, DyP-peroxidase and laccase genes, which are involving in solubilization of the biological macromolecules (27). The glucose degradation (methyl red test), oxidase, catalase, urease and ammonia production characters of APL3 might be a reason for enhancing *A. polygonoides* L. growth. The antioxidants enzymes like oxidase and catalase reduce the oxidative stress in plants (10) and the synthesis of those enzymes in plants by bacteria would be a favor for plant growth improvement. Nitrogen is a major essential nutrient

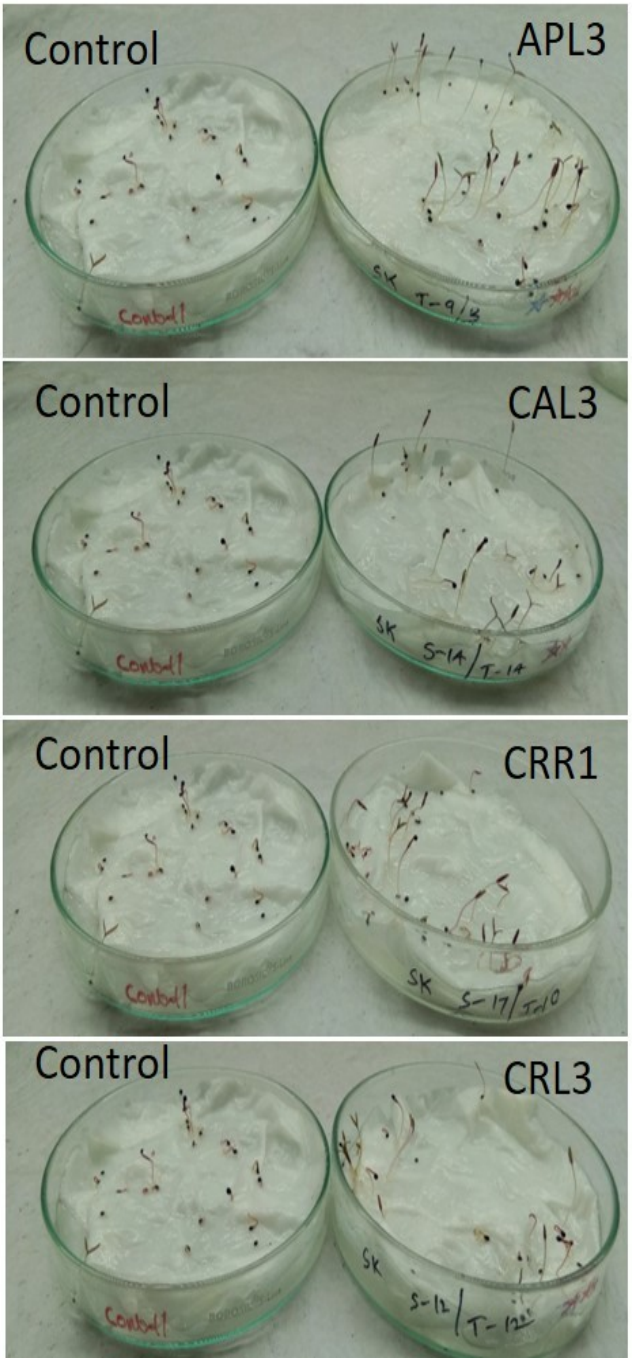

Fig. 1. Effect of endophytic bacteria on *A. polygonoides* L. seedling growth.

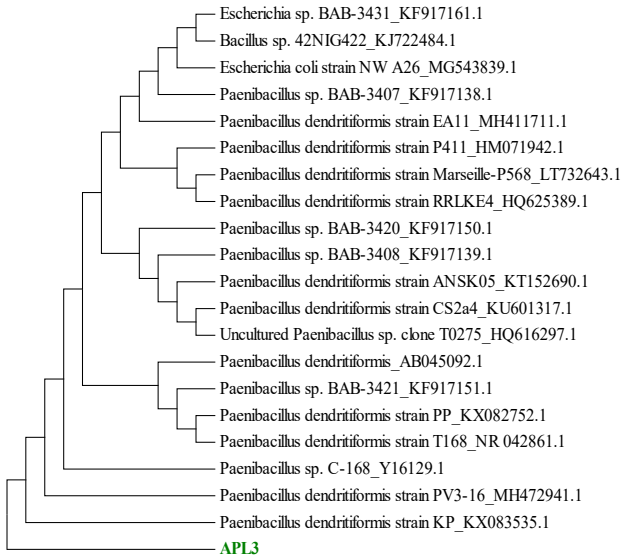

Fig. 2. Phylogenetic tree based on the sequence obtained from 27F and 1492R primers of 16S rRNA gene of endophytic bacteria (APL3) and those of related bacteria.

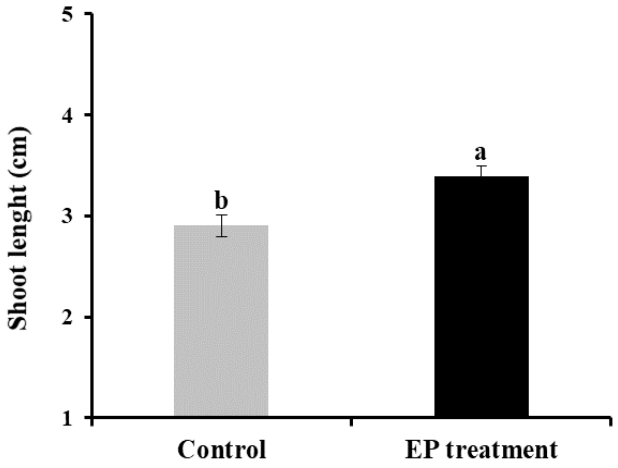

Fig. 3. Influence of *P. dendritiformis* strain APL3 on shoot length of *A. polygonoides* L. seedlings.

required for plant cell development. The urease and ammonia production ability of APL3 stimulated the nitrogen accumulation in plants to promote their growth and health. Recently, it was reported that *P. polymyxa* promoted the growth of maize, cucumber and potato plants by the utilization of atmospheric nitrogen (28). In addition, *Paenibacillus sps*. produce indole 3 acetic acid in *Curcuma longa* L. (29).

The bioactive compounds present in the plants can act as antimicrobial drugs to prevent the infection of several foodborne pathogens (30). The common human pathogens including *Escherichia coli, Salmonella, Staphylococcus aureus* and *Pseudomonaus aeruginosa* infections are prevented by several herbal treatments. The edible green leafy vegetables of *Amaranthus* species contain antimicrobial compounds which were inhibited *E. coli, S. aureus* and *P. aeruginosa* growth (17). In the present study, results revealed that APL3 treated plant extract produced higher antimicrobial activity against *E. coli, S. aureus* and *P. aeruginosa* than control plants (Fig. 4). The different concentrations of the plant extract from 50 µl to 100 µl showed a similar pattern of antimicrobial response against the pathogens, which was confirmed the endophytic bacterial (APL3) treatment can able to promote the antimicrobial contents of the plants. It was suggested that endophytes had a wide range of metabolites which is act as antimicrobial agents (31). The medicinally valuable antimicrobial compounds such as polymyxins and fusaricidins derived from the *Paenibacillus* sp. are useful to cure several diseases (32, 33). In addition, the presence of antioxidants was detected and quantified in both control and endophytes treated plants (Fig. 5). The antioxidants activity of *A. polygonoides* leaves was recorded (17). The significantly higher rate of antioxidant activity

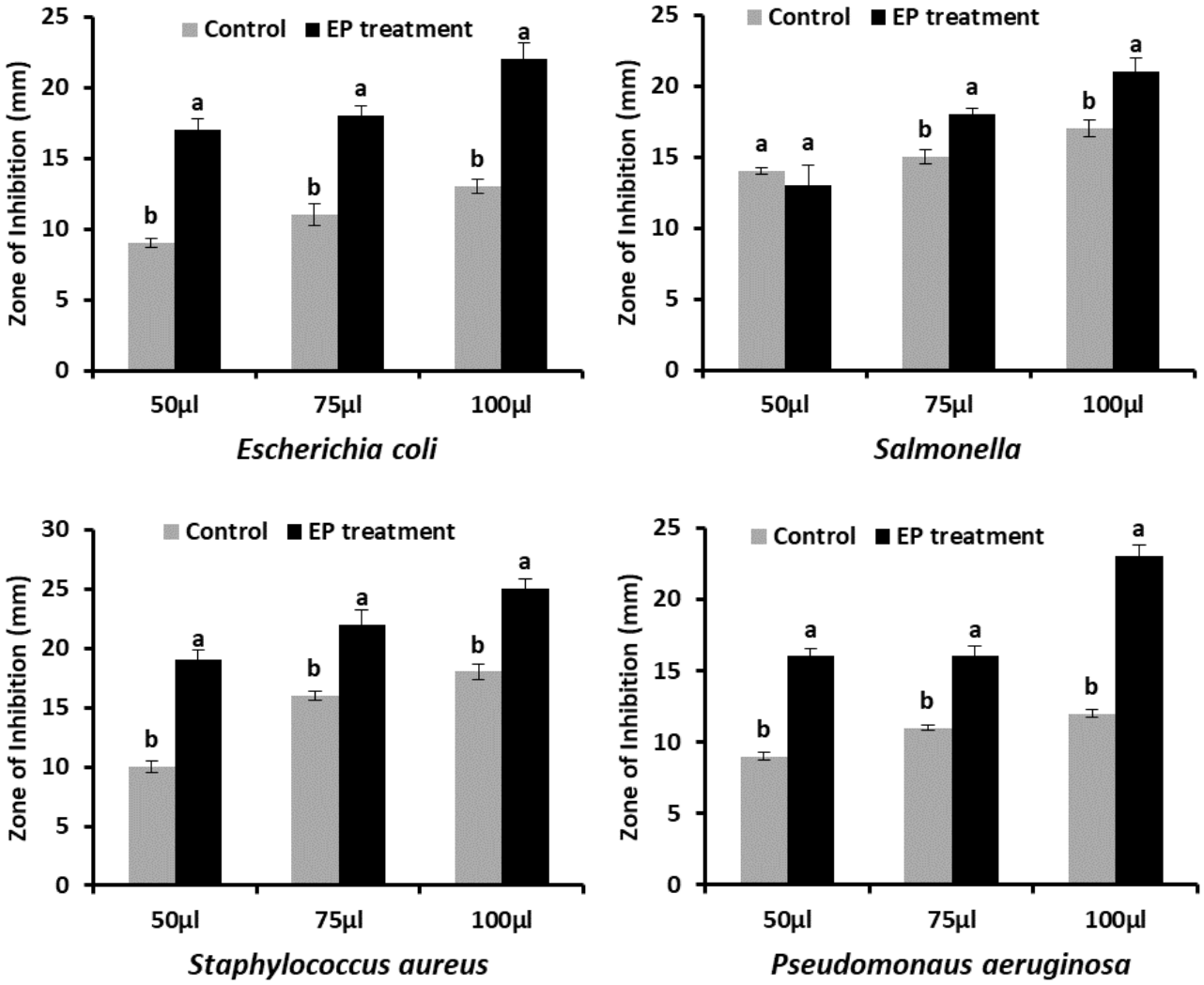

Fig. 4. Antimicrobial activity of *P. dendritiformis* strain APL3 associated *A. polygonoides* L. against food-borne pathogens.

was noticed in plants treated with APL3 while compared to their control, and the application of bacterial culture to plants enhanced the plant growth and their antioxidants activity would be useful for human consumption.

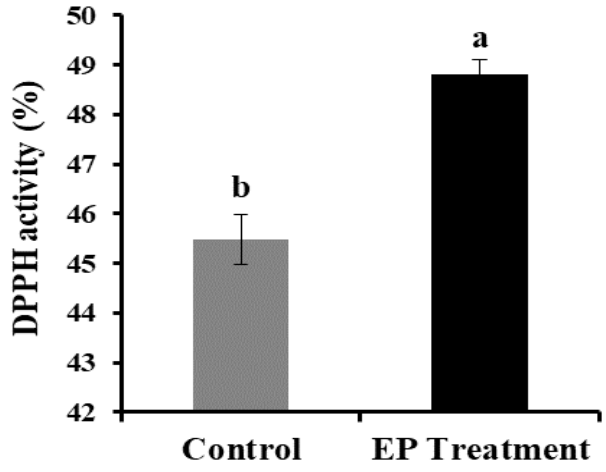

Fig. 5. Effect of *P. dendritiformis* strain APL3 on antioxidant capacity (DPPH activity) of *A. polygonoides* L.

Conclusion

The results of the present study suggest that utilization of *P. dendritiformis* strain APL3 triggers the growth of *A. polygonoides* L. and induces the metabolic changes in plants to improve their antimicrobial properties to reduce the growth of foodborne pathogens. Further study will be focused on the isolation of antimicrobial compounds from endophytes treated plants to know the mode of action for controlling foodborne pathogens.

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Authors' contributions

RR and SUP conceived the study. AP, MA, RS, SS, JC, and PBV. performed the experiments and analyzed the data. SR performed computational analysis. RR, RS, and SUP. Writing – review and editing. All of the authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors do not have any conflict of interest to declare.

Ethical issues: None.

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