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RESEARCH ARTICLE

Impact of *Lysinibacillus macroides*, a potential plant growth promoting rhizobacteria on growth, yield and nutritional value of tomato plant (*Solanum lycopersicum* L. F1 hybrid Sachriya)

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ABSTRACT

Plant growth promoting bacteria enhance the growth in plants by solubilizing insoluble minerals, producing phytohormones and by secreting enzymes that resist pathogen attack. The present study was aimed at identifying the potential of *Lysinibacillus macroides* isolated from pea plant possessing rich microbial rhizobiome diversity in promoting the growth of tomato plant (*Solanum lycopersicum* L.). Potential of *L. macroides* in the promotion of *S. lycopersicum* L. growth by increased shoot length, terminal leaf length and breadth was assessed. Anatomical sectioning of stem and root revealed no varied cellular pattern indicating that the supplemented bioculture is not toxic to *S. lycopersicum*. Plantlets treated with *L. macroides* along with organic compost showed an increased total phenol content (17.58±0.4 mg/gm) compared to control samples (12.44±0.41 mg/g). Carbohydrate content was noticed to be around 1.3 folds higher in the *L. macroides* plus compost mixture supplemented slots compared to control sample. Significant increase in shoot length was evident in the *L. macroides* plus compost supplied slots (23.4±2.7 cm). Plant growth promoting properties might be due to the nitrogen fixing activity of the bacteria which enrich the soil composition along with the nutrients supplied by the organic compost. Rich microbial rhizobiome diversity in pea plant and the usage of *L. macroides* from a non-conventional source improves the diversity of the available PGPR for agricultural practices. Further research is needed to detect the mechanism of growth promotion and to explore the plant microbe interaction pathway.

Introduction

With the expectancy of the global population rising to 9 billion by 2040, there is a major threat faced by the developing countries to address the problems associated with crop production (1). Meeting the demand for high yielding crop varieties with enhanced nutritional content is the need of the hr for agriculture agencies. Better quality and improved productivity of crop plants is possible by replacing the lost nutrients in the cultivable land (2). *Solanum lycopersicum*, one of the most cultivated fruits worldwide for its nutrients such as lycopene, β-carotene, flavonoids and vitamin C are known to have high market value for their antioxidant properties (3). It is used in various cuisines of the world and is also used as a model plant for fleshy fruited dicots (4). Farming practices using chemical fertilizers for long tenure decreases the soil fertility and agricultural productivity thereby affecting the natural process of nitrogen fixation in the soil (5, 6). As an alternative, organic fertilizers and biofertilizers provide an eco-friendly approach by improving nutrient uptake, water absorption, water retainability, atmospheric nitrogen fixation, phosphate solubilisation (7, 8). Plant benefiting microorganisms grouped as Plant Growth Promoting Rhizobacteria (PGPR) offer a wide range of benefits by colonising the root and promote plant growth by improving the accessibility to bionutrients, solubilisation of inorganic phosphate and by limiting plant pathogens (3). PGPR confers growth benefits to plants either directly or indirectly (9). PGPR act directly by aiding nitrogen fixation, enhancing iron uptake, secreting organic acids, solubilizing insoluble

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phosphate and by producing phytohormones (10-12). Indirect mode of action is by producing protease kind of enzymes that resist fungal attack and chemicals like hydrogen cyanide (HCN) that inhibit pathogen growth (12, 13, 14). Several diverse genera of microbes are employed as PGPR including *Bacillus*, *Pseudomonas*, *Arthrobacter*, *Paenibacillus* and *Lysinibacillus* (15, 16). *Bacillus cereus* and *Klebsiella variicola* isolated from the rhizospheric region of tomato capable of secreting phytohormones such as GA3, IAA and kinetin enhanced mineral uptake and upregulated chlorophyll synthesis (17). *Lysinibacillus* reported for its ability to tolerate metal has been tested for its efficacy to promote plant growth promotion in polluted environment (18). *L. macrolides*, supplemented individually or as a consortium culture conferred antimicrobial activity against plant pathogen *Xanthomonas campestris*, causative bacterium that is known to cause black rot of cabbage (19). Total nitrogen content in the soil can be enhanced by *L. macroides* by its ability to fix atmospheric nitrogen and decompose organic matter (20). Nitrfying bacteria convert the atmospheric nitrogen (N_2) into organic nitrogen which can be utilized by plants. Certain bacteria like *Providencia* spp. enhance plant growth by secreting phytohormones such as indole acetic acid (21, 22). Microbe and its secretory products are widely employed in disease inhibition and shelf life extension. Artificial introduction of microbial antagonists found to be more efficient in disease control and exhibited limited pathogen attack (23, 24). A combination of technological advances in agricultural farming comprising both biofertilizers and postharvest treatment will ensure better yield in agriculture (25). Unexplored microbial community from the pea plant cultivated in the agricultural ecosystem result in identification of novel diverse rhizosphere microbiomes for agricultural practices. Our study is focused on identifying a novel plant growth promoting bacteria from the rhizosphere of pea plant and their efficacy in enhancing the growth of *S. lycopersicum* L. was evaluated.

Materials and Methods

Isolation and Identification of PGPR from Pisum sativum

Rhizospheric soil sample was collected from *P. sativum* propagated in an agricultural ecosystem near IIHR (Indian Institute of Horticultural Sciences), Bengaluru, India. The sample (1 gm) was suspended in 10 ml of sterile water and was serially diluted. The diluted sample was spread plated onto yeast extract mannitol agar (YEMA) and incubated for 24 hrs at 37 °C (22). The isolates obtained were screened for its nitrogen fixing, phosphate and zinc solubilizing ability (16, 26, 27). Culture was biochemically characterised using Gram's staining, IMViC tests and screened for its ability to produce ammonia, hydrogen cyanide and siderophores (28, 29). The 16S rDNA gene from the isolated genomic DNA was amplified using 27F: 5'-AGAGTTTGATCCTGGCTCAG-3' and 1492R: 5'-GGTTACCTTGTTACGACTT-3' primers. The resulting amplicon was purified and sequenced using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer and the obtained sequence was compared with the homology search tool BLAST in NCBI genbank database for species identification. A phylogenetic tree was constructed using Mega-X version 10.2.4 using the maximum likelihood method based on the Kimura 2-parameter model (30-32).

Application of the isolated PGPR for growth enhancement

Experimental design

A total of five treatment groups along with a control were designed for the application study (Table. 1). *Pseudomonas fluorescens* procured from the culture collection center, GKVK Bangalore, India served as a positive control. *P. fluorescens* is a PGPR well known for its ability to fix atmospheric nitrogen, production of plant growth promoting compounds (IAA) and production of antimicrobial agents such as bacteriocins, antibiotics, and siderophores. Compost was prepared by packing multiple layers of food and vegetable waste over a layer of crushed dried leaves in a compost pit dug to one feet depth. The leaves and waste were laid in 1:1 ratio and the pit was covered with soil and charcoal to facilitate composting. After 30 days, the contents from the pit were mixed with dry cow dung and were utilized as compost.

Seed germination

Seeds of tomato plant (*Solanum lycopersicum* F1 hybrid Sachriya) were incubated for 1 hr in a broth culture of *L. macroides* (8 Log CFU/ml) and *P. fluorescens* (8 Log CFU/ml) separately. Treated and control seeds were planted in a coco peat for germination. After 7 days, plantlets were transferred to pots (33). Inoculation of bioculture was initiated based on the concentration mentioned in Table 1. The study was conducted for a period of thirty days. The bioculture (1ml) was supplemented to the plantlets at every two days interval and was maintained in a controlled environment in a polyhouse.

Table 1. Experimental combinations tested for plant growth promoting properties

Sl. no.	Experimental setup	Concentration
1.	*Lysinibacillus macrolides*	(8 Log CFU/ml)
2	*Pseudomonas fluorescens*	(8 Log CFU/ml)
3.	Compost	2%
4.	Group supplemented with compost and *Lysinibacillus macrolides*	2% compost and (8 Log CFU/ml) culture
5.	Group supplemented with compost and *Pseudomonas fluorescens*	2% compost and (8 Log CFU/ml) culture

Growth and anatomy assessment

The effects of the bioculture treatment on plant growth was analyzed for a period of 4 weeks by measuring the shoot length, leaf number, terminal leaf length and breadth every week (34). To check the effect of the PGPR on the vascular bundles and to determine whether the PGPR invades the root and stem, anatomical variations in control and the treatment group was carried out by analysing the transverse section of stem and root. Thin sections in

triplicates were obtained and stained with safranin. The sections were observed under 150x magnification using a light microscope.

Effect of L. macroides on the biochemical profile of S. lycopersicum

Total chlorophyll estimation

Leaf sample (500 mg) obtained from each treatment group was homogenised using 10 ml of 80% acetone and centrifuged at 10000 rpm for 10 min. at 4 °C. The supernatant was collected and the absorbance was measured at 645 nm and 663 nm and chlorophyll content was estimated (35).

Protein estimation

Protein content was estimated using the Bradford assay (36). Leaf samples (500 mg) from each treatment group were homogenised in 10 ml (0.05 M) phosphate buffer and centrifuged at 10000 rpm for 10 min. at 4 ˚C. 0.1 ml of supernatant was made up to 1ml using distilled water and 5 ml of Bradford reagent was added to the test tubes including the blank (1ml of d.H_2O) and the absorbance was measured at 595 nm. A standard calibration curve was prepared using Bovine serum albumin (BSA).

Carbohydrate estimation

Carbohydrate was estimated using phenol-sulphuric acid method (37). Leaf sample (100 mg) was homogenised with 5 ml of 2.5N HCl and was kept in a boiling water bath for duration of 3 hrs. After cooling, sample was neutralized by adding solid sodium carbonate powder till the effervescence ceased and centrifuged at 10000 rpm for 10 min. 200 µl of the supernatant was made up to 1 ml with distilled water. 5 ml of 96% sulphuric acid and 2% phenol were added in a cold water bath and the absorbance was measured at 490 nm. A standard calibration curve was prepared using D-glucose.

Determination of Antioxidant activity

Antioxidant activity was estimated using DPPH scavenging assay (38). 0.1 gm of powdered leaf samples were weighed and ground with 2 ml methanol using mortar and pestle. The solution was transferred to eppendorf tubes and centrifuged at 10000 rpm for 20 min. 20 µl (0.46 mg) of the extract was made up to 3 ml with methanol. 1 ml of DPPH [0.004% (w/v)] was added and incubated for 30 min. in dark condition. Absorbance was measured at 513 nm and the antioxidant activity was calculated (38).

Estimation of phytochemicals

Total phenolic content and tannin estimation (TPC)

The total phenolic content (TPC) and tannin estimation was performed using the Folin-Ciocalteu assay (39). For TPC, 0.1 gm of the powdered leaf sample was weighed and ground with 2 ml of methanol in mortar and pestle. 50 µl of the supernatant was made upto 3 ml with distilled water. To the mixture, 0.5 ml of the Folin-Ciocalteu reagent (1:1) was added followed by mixing with 2 ml of 20% sodium carbonate solution. Absorbance was measured at 638 nm after 3-5 min. incubation in a dark environment. Similarly for tannin estimation, 0.1 gm of the powdered leaf sample was weighed and ground with 2 ml of methanol in mortar and pestle. 50 µl of the supernatant was made upto 1 ml with distilled water. 0.5 ml of the Folin-Ciocalteu reagent (1:9) was added followed by addition of 2 ml of 20 % sodium carbonate solution and the absorbance was recorded at 725 nm after 40 min. incubation in dark. The calibration curves for TPC and tannin were prepared using catechol and tannic acid respectively.

Flavonoid estimation

Flavonoid content was measured by modified aluminium chloride colorimetric method (40). 0.1 gm of the powdered leaf sample was weighed and ground with 2 ml of methanol in mortar and pestle. 50 µl of the supernatant was mixed with 0.1 ml of 10% aluminium chloride and 0.1 ml of 1M potassium acetate. Absorbance at 415 nm was recorded after 40 min. incubation. A standard calibration curve was prepared using quercetin.

Note: Drying of leaves was carried out carefully without exposing the samples to direct sunlight in order to prevent loss of secondary metabolites. Absorbance for all values was measured using the Shimadzu UV-160A spectrophotometer.

Statistical analysis

All statistical analysis were carried out using online freeware ASTATSA and advanced tool pak VBA. One way Analysis of variance (ANOVA) was performed with Tukey HSD, Scheffé, Bonferroni and Holm comparisons and a p-value <0.05 was considered significant.

Results and Discussion

PGPR plays a crucial role in enriching the soil nutrients for better crop productivity. Our isolate, *Lysinibacillus macroides* exhibited ameliorative effects on growth and development of *Solanum lycopersicum.* Plant growth promoting bacteria exhibit positive effect in plants by enhancing nutrient availability and nutrient uptake. Few strains confer antagonistic effects by providing protection against plant pathogens and by tolerating abiotic stress factors. Various microorganisms namely *Pseudomonas* sp (41), *Burkholderia ambifaria* (42), *Mesorhizobium sp*. (43), *Bacillus amyloliquefaciens* (44), *Pseudomonas pseudoalcaligenes* and *Bacillus pumilus* (45) are utilised as PGPR for plant growth and for abiotic stress management. A study (42) demonstrated the utility of *Herbaspirillum seropedicae*, *Gluconacetobacter diazotrophicus, Azospirillum brasilense* and *Burkholderia ambifaria* for the growth of *S. lycopersicum.* The study not only assessed the role of PGPR in tomato plant growth but also revealed the host resistance to pathogens and their efficacy in nitrogen fixation (42). Results of this study analysed the effect of *L. macroides* infused compost made of vegetable and food waste on growth and yield of *S. lycopersicum.*

Isolation, screening and identification of PGPR

The isolate obtained was biochemically characterised (Table. 2) and was identified as *L. macroides* (GENBANK accession number; MK517553) after performing a BLAST search with a maximum score of 1967 on a 99.36% query cover. A molecular phylogenetic tree was constructed using MEGA-X Version 10.2.4 by maximum likelihood method based on Kimura 2 parameter (Fig. 1).

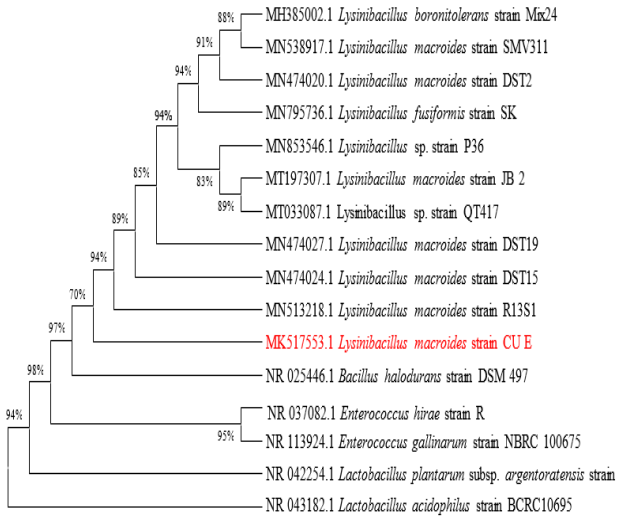

Fig. 1. Molecular phylogenetic analysis of PGPR based on the 16S rRNA partial sequences. The optimal tree with the sum of branch length = 0.34754584 is shown and was constructed using neighbor joining method. The evolutionary distances were computed using the Maximum Composite Likelihood method.There were a total of 1552 positions in the final dataset.*Enterococcus* sp. and *Lactobacillus* sp. were used as outgroups.

Growth Assessment and anatomical examination of S. lycopersicum

Growth enhancement in plants is attributed to good soil conditions that can be further enhanced by the application of organic, biofertilizers and PGPR (46). Compost supplemented with biofertilizer acts as an efficient soil amendment factor that facilitates water and mineral (nitrogen and phosphorus) uptake. Beneficial plant nutrients obtained from compost blended with nitrogen fixing bacteria and improve the availability of nitrogen sources for amino acid synthesis (47). In this study, growth measurements of *Solanum lycopersicum* was assessed for a span of thirty days and the study identified maximum shoot length and terminal leaf length in groups supplemented with *L. macroides* ($p<0.05$) (Fig. 2, Fig. 3 respectively). Increased stomatal conductance in PGPR supplied plants confers better water-use efficiency in PGPR supplemented plants (48). Terminal leaf breadth was highest in the group supplemented with compost only ($p<0.05$) (Fig. 4). Growth enhancement in compost and *L. macroides* supplemented slots can be attributed for better nutrient enrichment in soil conditions. The effect of PGPR *Pseudomonas fluorescens* supplemented along with organic manure containing composted material of vegetable and fruit sources enhanced the growth and yield of maize plant (46).

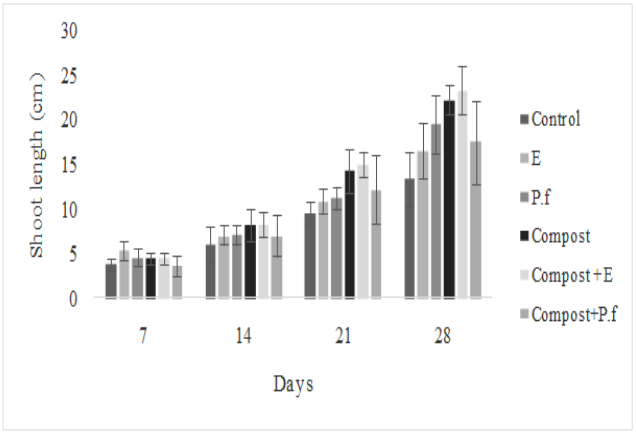

Fig. 2. Effect of culture *Lysinibacillus macroides* (E), *Pseudomonas fluorescens* (Pf), compost, compost + *Lysinibacillus macroides* (E) and compost + *Pseudomonas fluorescens*(Pf) along with control on shoot length.($p<0.05$).*Data represented as standard deviation of triplicate values.

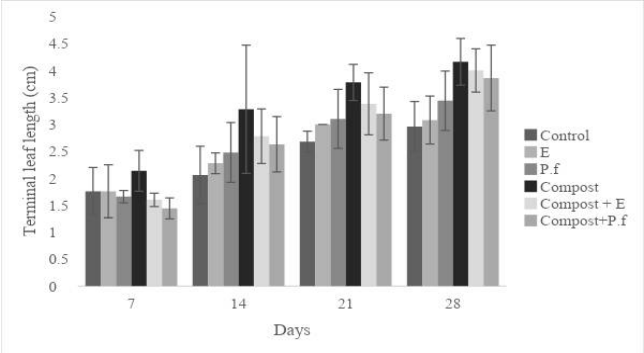

Fig. 3. Effect of culture *Lysinibacillus macroides* (E),*Pseudomonas fluorescens* (Pf), compost, compost + *Lysinibacillus macroides* (E) and compost + *Pseudomonas fluorescens*(Pf) along with control on terminal leaf length ($p<0.05$) *Data represented as standard deviation of triplicate values.

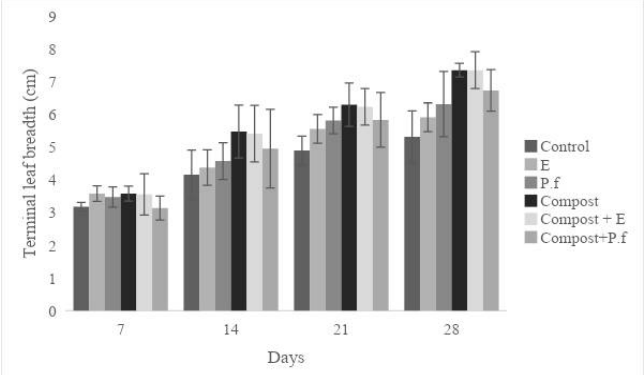

Fig. 4. Effect of culture *Lysinibacillus macroides* (E),*Pseudomonas fluorescens* (Pf), compost, compost + *Lysinibacillus macroides* (E) and compost + *Pseudomonas fluorescens*(Pf) along with control on terminal leaf breadth.($p<0.05$).*Data represented as standard deviation of triplicate values.

Treatment of tomato plant with phytohormone secreting *Sphingomonas sp* exhibited remarkable growth and physiological characteristics such as stimulated plant cell division, enhanced root formation and shoot elongation (49). In our research, maximum number of leaves was observed in plant groups supplemented with compost plus *L. macroides* by the end of 21 days (Fig. 5). This enhancement could be due to the upregulation of molecules

secreted by the bioculture that mediate phytostimulation. The transverse section of stem and root of control and the most effective treatment group (compost supplemented with *L. macroides*) was assessed. PGPR supplementation to *S. lycopersicum* increases the number and diameter of cortical cells and vascular tissue which could be attributed to the enhanced nutrient and mineral uptake (50, 51). Nutrient deficiency and stress leads to reduced size of vascular bundles affecting the overall plant health (52, 53). An increase in pith diameter was noted in the treatment groups whereas size of the cortical cells and xylem vessels remain the same in all groups. Pathogenic effects such as abnormal vasculature or any visual deformities because of PGPR supplementation affecting the morphology and anatomy of *S. lycopersicum* was not observed throughout the treatment period (Fig. 6).

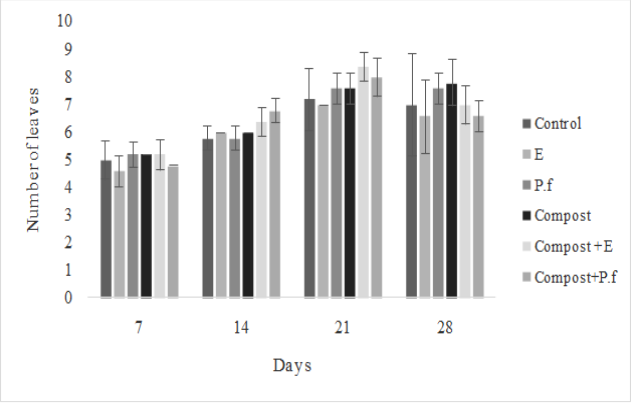

Fig. 5. Effect of culture *Lysinibacillus macroides* (E), *Pseudomonas fluorescens* (Pf), compost, compost + *Lysinibacillus macroides* (E) and compost + *Pseudomonas fluorescens* (Pf) along with control on number of leaves. (p>0.05).*Data represented as standard deviation of triplicate values.

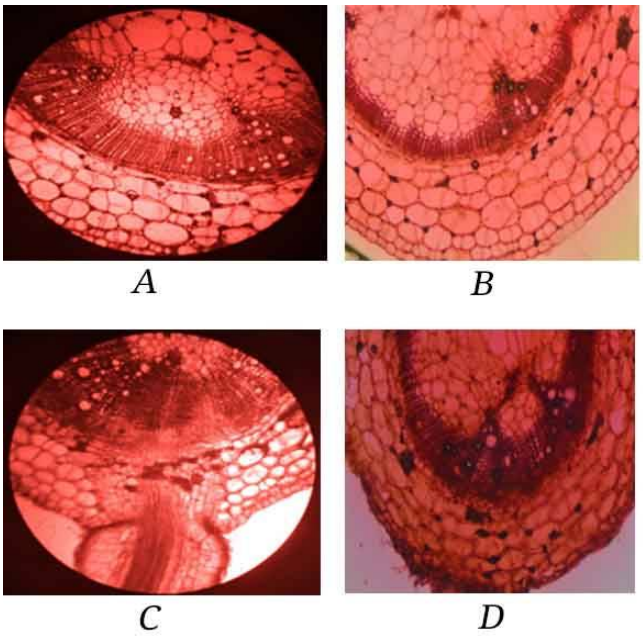

Fig. 6. Showing cross sections of transverse sections of stems and roots of control and the treatment group (Compost supplemented with *Lysinibacillus macroides*) after the 30 day trail. A and B-stem sections of control and treated groups respectively and C, D-root sections of control and treated groups respectively. The specimens were observed at 150X magnification after staining with safranin.

Estimation of biochemical constituents and phytochemicals

Efficacy of *L. macroides* on the synthesis of plant pigment chlorophyll, carbohydrate and protein were assessed upon treatment with biofertilizer and compost (Table. 3). Maximum biochemical constituents were observed in compost and biofertilizer supplemented samples ($p<0.05$). Specific rates of nitrogen supply increase the accumulation of photosynthetic pigments. Higher pigment synthesis could be due to enhanced nitrogen fixation caused by the inoculated PGPR. Nitrogen constitutes the major component of the chlorophyll structure. *Bacillus pumilus* enhances the synthesis of soluble proteins in soybean plants (54). Combined or sole application of organic manures and biofertilizers increases the yield of the phenolic compounds and phyto components that influence the quality attributes in the tested plant samples. Appropriate usage of *L. macroides* along with compost material increased solubility and bioabsorbtion of nutrients in the current study. Similar results were obtained in *Brassica oleracea* treated with *P. fluorescens* and humic acid combinations (55, 56). Co-inoculation of plant growth promoting rhizobacteria along with farm yard compost enhances the bioaccumulation of nutrients in the mungbean by aiding in nutrient fixation, release, uptake and transportation (57).

Phenolic compounds synthesised by plants contribute for growth and development and involve in various metabolic activities such as plant protection from free radical attack, protection against plant invading pathogens and as signalling messengers (58). In this study, secondary metabolites like polyphenols, flavonoids and tannins were estimated in control and *L. macroides* supplemented plant samples (Table. 4). Supply of *L. macroides* alone and *L. macroides* co-inoculated with compost slot exhibited higher synthesis of flavonoid. No significant differences on flavonoid synthesis were noted between the *Pseudomonas* and *Pseudomonas* plus compost supplemented groups. Difference in the synthesis of flavonoid between the treatment groups could be due to the factor associated with the nutrient breakdown and uptake ability of PGPR amended with the compost. Enhanced phenolic content in *Serratia marcescens* supplemented *Piper belte* L. was noted (59). Parallel results were observed in chickpea by inoculation of PGPR *P. fluorescens* and *P. aeruginosa* (60). Enhanced flavonoid concentration was observed in compost and *Lysinibacillus macroides* co-inoculated compost samples. PGPR stimulates the phenylpropanoid pathway for enhanced polyphenols synthesis thereby contributing to host defense against plant pathogens (61). Induction of polyphenols by compost supplementation along with bacterization using *L. macroides* increased the total phenolic content in the *S. lycopersicum*. Results of the study suggest that the use of organic fertilizers along with PGPR can enhance the production of plant secondary metabolites. Tannin accumulation is one the primary ways the plant system adopts to avoid stress induced changes. Higher synthesis of tannins and other phenolics are reported for radical quenching activity in *Acacia gerrardii* supplemented with *Bacillus*

subtilis and Mycorrhizal fungi (62). The aromatic oxygen substituted derivatives such as tannins and flavonoids contribute for homeostasis and health maintenance (63). PGPRs influence plant growth, nutrient utilization by producing metabolites such as flavonoids, phenols, saponins and alkaloids that benefit plant growth and stimulation (24).

Antioxidant activity

Oxidative stress can reduce the nutritional quality of crops. Environmental factors such as air pollution, extensive herbicide/pesticide application, contamination by heavy metal, drought, salinity, injuries can induce oxidative stress in plants. Use of the PGPR can reduce the negative effects caused due to environmental stress by enhancing the antioxidant state (57). PGPR application aids in plant growth by reducing the effect of cold stress and also by activating the enzymes that reduce Reactive Oxygen Species (ROS) (58). In our study, maximum antioxidant activity was observed in plants supplemented with *P. fluorescens* (76.55±1.45%) compared to other treatments by 4th week (Fig. 7). Enhanced DPPH radical quenching activity was noted in *S. lycopersicum* samples treated with compost infused with plant growth promoting bacteria. Mechanism related to enhanced antioxidant activity could be due to the elevation in the expression of antioxidant genes that confer homeostasis in the plant metabolism (64, 65). *Cucumis sativus* infused with *Promicro monospora, Burkholdera cepacia*, and *Acinetobacter calcoaceticus* showed enhanced phenol production due to upregulation of antioxidant enzymes (66).

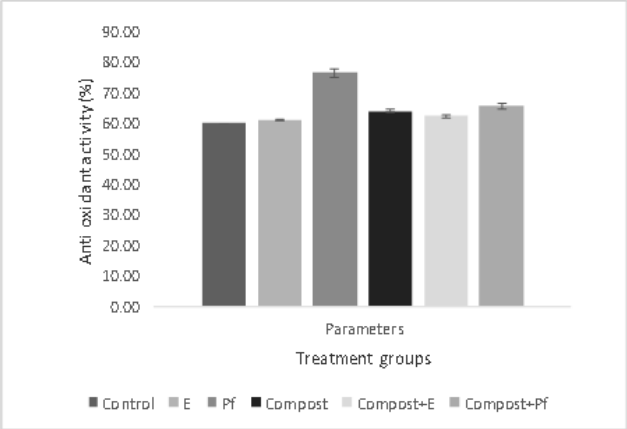

Fig. 7. Antioxidant activity of the culture *Lysinibacillus macroides* (E),*Pseudomonas fluorescens* (Pf), compost, compost + *Lysinibacillus macroides* (E) and compost + *Pseudomonas fluorescens* (Pf) in comparision to the control. (p<0.05). *The error bars indicate standard deviation.

Conclusion

Development of a cost effective, eco-friendly approach for enhancing plant growth is a much needed practice towards developing a productive agricultural ecosystem. Identification and development of functional soil amendments will minimise the usage of chemical fertilisers thereby nourishing the soil with less artificial pollutants. In our study, supplementation of *Lysinibacillus macroides* infused compost exhibited a promising effect in enhancing the overall plant growth. However, molecular mechanisms that govern the interaction of PGPR with host biomolecules need to be further characterized. Identification and utilisation of novel PGPRs with potential applications in agriculture will increase crop productivity and minimize negative impacts of organic waste.

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Table 2. General characteristics of *Lysinibacillus macroides*

Sl. no.	Tests performed	Result
1	Gram's staining	Gram positive
2	Shape	Bacilli
3	Motility (Hanging drop method)	Motile
4.	Indole test	Negative
5.	Methyl red test	Negative
6.	Voges-Proskauer test	Positive
7.	Simmon citrate test	Negative
8.	Catalase test	Positive
9.	Phosphate solubilization	Negative
10.	Ammonia production	Negative
11.	Siderophore production	Negative
12.	Zinc solubilization	Negative
13.	Nitrogen fixation	Positive

Table 3. Effect of treatments, *Lysinibacillus macroides* (E), *Pseudomonas fluorescens* (Pf), compost, compost + *Lysinibacillus macroides* (E) and compost + *Pseudomonas fluorescens* (Pf) along with control on quantitative biochemical parameters chlorophyll, carbohydrate and protein content.

Parameters	Control	E	Pf	Compost	Compost+E	Compost+pf
Chlorophyll *(mg/gm)	1.56± 0.015	1.65± 0.015	1.56± 0.01	1.82± 0.05	1.74± 0.02	1.75± 0.12
Carbohydrate* (mg/gm)	34.33± 0.38	41.58± 1.2	25.33± 2.04	43.08± 0.94	44.25± 0.25	27.25± 1.08
Protein* (mg/gm)	5.68± 0.05	7.61± 0.049	6.42± 0.01	8.63± 0.021	7.98± 0.031	7.88± 0.091

Note: All the parameters are significant at p<0.05 and indicated by *.

Table 4. Effect of treatments, *Lysinibacillus macroides* (E), *Pseudomonas fluorescens* (Pf), compost, compost + *Lysinibacillus macroides* (E) and compost + *Pseudomonas fluorescens* (Pf) along with control on quantitative phytochemical parameters chlorophyll, carbohydrate and protein content. *Data represented as standard deviation of triplicate values.

Parameters	Control	E	Pf	Compost	Compost+E	Compost+pf
Total phenolic content*(mg/gm)	12.44± 0.41	17.43± 0.51	14.48± 0.32	14.87± 0.40	17.58± 0.4	13.03± 0.22
Flavonoids*(mg/gm)	9.40± 0.06	11.43± 0.028	9.54± 0.05	12.86± 0.03	13.04± 0.05	12.54± 0.06
Tannins* (mg/gm)	8.01± 0.01	15.78± 0.07	11.30± 0.03	10.10± 0.03	13.62± 0.04	9.04± 0.13

Note:: All the parameters are significant at p<0.05 and indicated by *.

Authors' contributions

JS was involved in the experimental design, data collection. RA and BN were involved in data analysis and drafted this manuscript. PKA conceptualized the idea, involved in funding and revised this manuscript. All authors read and approved the manuscript.

Conflict of interests

The authors declare no conflicts of interest.

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