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SAUDI BIOLOGICAL SOCIETY

Original article

Role of formulated bacterial consortia in biofortifying tomato fruits with nutrients: A nutritional, genomic and metagenomic analysis

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ARTICLE INFO

Keywords:
Plant growth promoting bacteria
Biofortification
Tomato
Gene expression
Metagenomics

ABSTRACT

Nutrient deficiencies are a major problem that is prone to affect millions of people around the globe. Biofortification, a process of enriching nutrients in staple food crops is an effective method to tackle this malnutrition-associated disorder. Tomato (*Solanum lycopersicum*) is a globally consumed crop and therefore is a suitable candidate for biofortification. Many plant growth-promoting bacteria are reported to have the ability to enhance nutrient content in plants. In the present study, we have investigated the ability of two bacterial consortia (consortia-1 –co-culturing *Lysinibacillus* sp. strain VITKC-5 and *Acinetobacter* Sp. strain VITKC_6; and consortia-2 –co-culturing *Lysinibacillus* sp. strain VITKC-5 and *Enterobacter* sp. strain VITVLC-4) in the nutrient enrichment of tomato fruits. The results were then correlated with the elevated expression of nutrient transporter genes. Furthermore, the effect of these bacterial formulations on the indigenous microbiome has also been evaluated through metagenomic analysis. The application of bacterial formulations significantly improved the nutrient content when compared to the control (untreated) group. These findings advocate that PGPB-assisted biofortification has the potential to alleviate nutrient deficiency in humans.

1. Introduction

Tomatoes are part of many food products such as sauces, salads, soups, and pastes (Ali et al., 2021). Tomatoes are reported to be a source of vitamins, minerals, fiber, protein, essential amino acids, fatty acids, carotenoids, and phytosterols (Abdullahi et al., 2016; Chaudhary et al., 2018; Elbadrawy and Sello, 2016; Ramos-Bueno et al., 2017). These nutrients benefit human body functions such as prevention of constipation, lowering of blood pressure, stimulation of blood flow, preservation of lipid profile and body fluids, removal of body toxins, and maintenance of the bone structure and strength (Campestrini et al., 2019; Cheng et al., 2017; Salehi et al., 2019). All these facts could have influenced the widespread cultivation of tomatoes around the world. In 2013 a huge rise in tomato cultivation was observed to about 163 million tonnes (Arah et al., 2016). According to the report of Agricultural and Processed Food Products Export Development Authority (APEDA) India is one of the top ten tomato producers with a production of 16,089 million tonnes in 2021 (Sahasa et al., 2023). Hence, as a widely consumed crop, tomatoes could emerge as an ideal choice for biofortification.

Biofortification, the process of enhancing the nutrient content of crops, has gained significant attention in recent years as a promising approach to combat global malnutrition (Kiran et al., 2022). The cultivation of nutrient-rich crops holds immense potential to address dietary deficiencies, particularly in resource-limited regions where access to diverse and nutritious food is limited. Among the biofortification targets, tomato (*Solanum lycopersicum*) has garnered considerable interest due to its widespread consumption and versatile culinary applications (Kiferle et al., 2013; Zhu et al., 2013).

There are several methods used for biofortifying plants, which include the agronomic approach (ferti-fortification), breeding approach, transgenic approach, and microbial approach (Dhaliwal et al., 2022;

Abbreviations: PGPR, Plant Growth Promoting Rhizobacteria; PT-1, Phosphate Transporter-1; NRT, 2.3, Nitrate transporter-2.3; AMT-1, Ammonium transporter-1; NCBI, National Center for Biotechnology Information; SRA, Sequence Read Archive; KC-5, Kanyakumari Culture-5; KC-6, Kanyakumari Culture –6; VLC-4, Vellore Lake Culture –4; BFC, BioFertilizer Control.

Peer review under responsibility of King Saud University.

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https://doi.org/10.1016/j.sjbs.2023.103851
Received 14 July 2023; Received in revised form 18 October 2023; Accepted 27 October 2023
Available online 30 October 2023

Dhuldhaj and Pandya, 2017). Among these, the microbial approach is the environment-friendly approach to biofortification. Furthermore, it is also economical when compared to the other approaches. Various microbial interventions take part in improving plant growth and development. Several plant growth-promoting traits has been characterized in microbes over the years (Kaur et al., 2020).

In this study, we have evaluated the ability of two prepared bacterial consortia in biofortifying tomato fruits with various nutrients. A metagenomic analysis characterized the soil microbiome of the plant at the time of yielding. In addition, a gene expression analysis of various tomato nutrient genes was conducted to correlate the nutrient richness in the fruit.

2. Materials and methods

2.1. Bacterial culture preparation and plant treatment

The three bacterial isolates used in the present study was isolated from a lake bank in Vellore- Tamil Nadu (12°58′07.6″N 79°09′32.7″ E) and Kanyakumari seashore, Tamilnadu (8°07′30.4″N 77°33′57.1″ E). They were further screened for Plant growth-promoting traits and compatibility (not shown in this article). Two bacterial consortia were formulated; consortia-1 (KC-5 + KC-6)- co-culturing ***Lysinibacillus* sp.** strain VITKC-5 and ***Acinetobacter* Sp.** strain VITKC_6 (NCBI Accession ID: OP080714); and consortia-2 (KC-5 + VLC-4)- co-culturing ***Lysinibacillus* sp.** strain VITKC-5 (NCBI Accession ID: OP070953) and ***Enterobacter* sp.** strain VITVLC-4 (NCBI Accession ID: OP050456). A negative control group (untreated) and a commercially available *Pseudomonas* sp. based biofertilizer (https://www.farmersbiofertilizers.com/) as positive control group were also used in this study. The experimental design of the study is depicted in the Fig. 1.

The individual and cocultures of bacterial strains were proliferated at 30 °C for 72 h. The resulted turbid cells are pelleted and washed thrice in 0.85 % NaCl (saline). The cells were further dissolved in the same solution appropriating 0.6 OD at 600 nm. The prepared bacterial suspensions were then applied on tomato seedlings (Helal et al., 2022).

One month old tomato seedlings (PKM-1 variety; SUMASHI seeds-https://sumashiseeds.com/) were used to conduct further experiment. These seedlings were plucked from the seedling tray and root soil was removed by washing with water. The roots of the 6 seedlings were then soaked in 50 mL of prepared bacterial suspensions for 30 min (Helal et al., 2022). After treatment the seedlings were transplanted to the grow bags and were kept in completely randomized design with 6 replications for each bacterial preparation. The plants were again treated with the bacterial suspension 15 days post the transplantation. Plants were watered regularly, and monitored for any pest attack. No additional growth promoters or fertilizers were provided to the plants until the end of the experiment.

2.2. Soil characterization

Before transplanting the plant in to the grow bags, the soil used in for the study was analysed for its chemical properties and nutrient profile. The presence of macro and micro nutrients of the soil were characterized by the National Agro Foundations (NAF, Chennai) following the protocols of Tandon (1995).

2.3. Evaluation of fruit physiology

The ripened fruit from each plant was harvested regularly. Length, breadth fresh weight, and dry weight of the fruit was recorded (Dursun et al., 2019; Katsenios et al., 2021). The average of each measurement was calculated and incorporated in the table.

2.4. Nutrient profiling of fruits

Fruits from three plants of each bacterial treatment group were harvested dried and powdered. The powdered samples were analysed for its macro and micro nutrient contents by the National Agro Foundations (NAF, Chennai) following the protocols of Tandon (1995).

2.5. Gene expression analysis of nutrient transporter genes

Quantitative realtime PCR was performed to analyse the expression of nutrient transporter genes in *Solanum lycopersicum*. The gene expression analysis was performed at the reproductive stage of the plant.

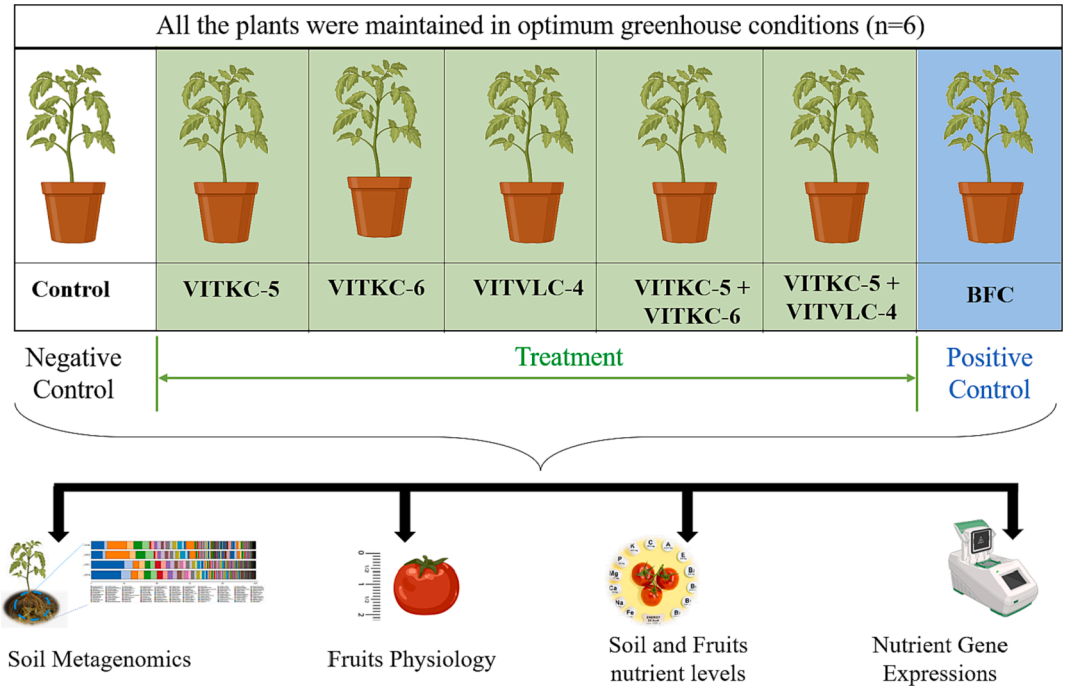

Fig. 1. Experimental design of plants submitted to different bacterial formulations.

Table 1
Characterization of Soil.

Sl no.	Parameter	Unit	Results
1	pH	–	7.19
2	Electric conductivity	mS/cm	0.133
3	Organic matter	%	1.98
4	Nitrate Nitrogen	mg/kg	37.1
5	Available phosphorus	mg/kg	66.34
6	Potassium exchangeable K	mg/kg	143
7	Calcium exchangeable Ca	mg/kg	3743
8	Magnesium exchangeable Mg	mg/kg	245
9	Sodium exchangeable Na	mg/kg	265
10	Sulfur-available S	mg/kg	18.1
11	Zinc-available Zn	mg/kg	2.47
12	Manganese-available Mn	mg/kg	13.46
13	Iron-available Fe	mg/kg	18.17
14	Copper-available Cu	mg/kg	2.42
15	Boron-avaialble B	mg/kg	0.8
16	Cation exchange capacity (by addition)	meq/100 g	22.28
17	K saturation	%	1.65
18	Ca saturation	%	84.02
19	Mg saturation	%	9.17
20	Na Saturation	%	5.17

Young leaves of three plants from each treatment group were clubbed together and subjected for the analysis. RNA from each sample group were isolated using RNA isoplus (Takara, Japan). The isolated RNA was then converted into cDNA using Reverse transcript kit (Takara, Japan). PCR primers were designed targeting the *Solanum lycopersicum* nutrient transporter gene sequences present in NCBI (Table 1). Real-time SYBR PCR amplification was performed in Bio-Rad CFX maestro PCR system under standard conditions of 10 min at 95 °C, 40 cycles of 15 s at 95 °C, and 1 min at 60 °C. All reactions were conducted in duplicates. Based on the $2^{-\Delta Ct}$ comparative method, the resulted cq values were used to analyse the expression pattern of each gene. The actin gene of *Solanum lycopersicum* was used as a reference gene (Saia et al., 2015).

2.6. Soil metagenomic profiling

The tomato rhizosphere soil was collected by gently shaking and mixing the plant root. Three treatment groups (Control, Consortia-1, and Consortia-2) were processed for metagenomic analysis. The crude DNA from each treatment was isolated. The V3-V4 hypervariable regions of 16 s rRNA gene were amplified using 27F (5′ AGAGTTTGATGMTGGCT CAG3′) and 1492R (5′ TTACCGCGGCMGCSGGCAC3′). Following PCR purification of the amplicons, the sequencing was performed using an Illumina Miseq platform. Furthermore, the OTUs were identified and the metagenomic report were generated (Skipper et al., 2022).

2.7. Statistical analysis

The fruit physiology analysis was conducted in multiple replications, and data were statistically analysed. Significant difference among treatments means were calculated by one-way analysis of variance (ANOVA) with Tukey's multiple comparison using GraphPad Prism 8.0.2.

3. Results

3.1. Evaluation of fruit physiology under the bacterial formulation treatments

Significant variation in fresh weight, dry weight, fruit width and fruit height of fruits have been observed in each of the bacterial treatments when compared to the control group (Fig. 2). The consortia 1 increased the average fresh weight by 67.3 % while the consortia 2 increased it 71.4 % when compared to the control group. The individual cultures along with biofertilizer control (BFC) was also found to influence the fruit weight considerably (Table 2). The inoculation of ***Lysinibacillus* sp.** strain VITKC-5, ***Acinetobacter* Sp.** strain VITKC_6, ***Enterobacter* sp.** strain VITVLC-4 and BFC showed 57.1 %, 48.08 %, 53.9 % and 53.95 % fresh weight respectively. The both the consortia have also shown superior characteristics in fruit dry weight, fruit width and fruit height.

3.2. Evaluation of fruit nutrient content under the influence of each bacterial treatment

The average content of nutrients such as Nitrogen, phosphorous, potassium, calcium, magnesium, sulfur, zinc, iron, manganese, and copper per fruit were quantified. There was significant difference in concentration of each nutrient when compared to the control group (Table 3). The nutrients like nitrogen and potassium almost doubled in both consortia-1 (59 mg and 107.5 mg respectively) and consortia-2 (55.418 mg and 92.44 mg respectively). The rest of the nutrients analysed was also found to be influenced by the bacterial treatments. And in the case of some nutrients the individual cultures like ***Lysinibacillus* sp.** strain VITKC-5 and ***Enterobacter* sp.** strain VITVLC-4 showed better results than that of consortia-2. However, Consortia-1 was found to be positively influencing enrichment of all the analysed nutrients in the fruit.

3.3. Nutrient transporter genes expression analysis

The expression levels of genes such as PT-1, NRT2.3, and AMT-1 have been elucidated. All these genes were found to be upregulated in the plants treated with both the consortia used in this study (Fig. 3). The

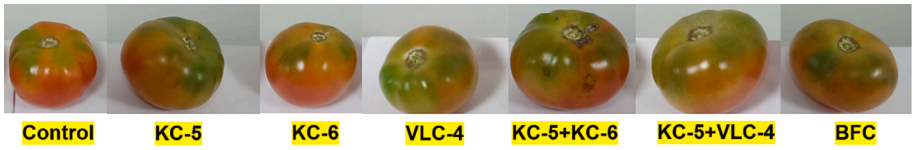

Fig. 2. Physiological comparison of tomato yielded in each treatment group.

Table 2
Physiological changes of tomato fruit on bacterial treatment application.

Parameter	Control	KC-5	KC-6	VLC-4	KC-5 + KC-6	KC-5 + VLC-4	Biofertilizer control
Fresh weight	19.59 ± 5.99	30.79 ± 2.79a****	29.01 ± 3.41a***	30.15 ± 4.34a**	32.78 ± 6.99a****	33.58 ± 5.43a****	30.16 ± 3.38a***
Dry weight	1.16 ± 0.38	2.02 ± 0.40a**	1.94 ± 0.38a**	2.18 ± 0.94a**	2.50 ± 0.93a****	2.42 ± 0.47a****	2.07 ± 0.35a**
Fruit width	11.15 ± 1.17	13.20 ± 0.37a****	12.53 ± 0.72a**	13.38 ± 1.14a***	13.37 ± 1.05a****	13.49 ± 0.71a****	12.87 ± 0.68a***
Fruit height	10.39 ± 1.24	12.22 ± 0.29a****	11.89 ± 0.37a***	12.40 ± 1.11a***	12.39 ± 0.91a****	12.51 ± 0.62a****	11.98 ± 0.58a****

Data in the table represents mean ± SD, and the variable a indicates statistically significant ($p < 0.05$). "a*, a**, a***, a****" implies $p < 0.05$, $p < 0.001$, $p < 0.0002$, $p < 0.0001$ respectively in the control group vs KC-5, KC-6, VLC-4, KC-5 + KC-6, KC-5 + VLC-4, and Biofertilizer control.

Table 3
Influence of bacterial treatment application on fruit biofortification.

Parameter	Control (mg/ fruit)	KC-5 (mg/ fruit)	KC-6 (mg/ fruit)	VLC-4 (mg/ fruit)	KC-5 + KC-6 (mg/ fruit)	KC-5 + VLC-4 (mg/ fruit)	Biofertilizer control (mg/ fruit)
Nitrogen	31.088	54.54	43.456	57.77	59	55.418	48.438
Phosphorous	7.192	12.524	10.476	12.426	12.75	12.342	11.178
Potassium	53.94	94.536	82.45	96.356	107.5	92.444	84.87
Calcium	5.684	6.666	6.014	8.284	8	7.26	8.073
Magnesium	4.176	6.666	5.626	7.194	7.5	7.018	6.417
Sulfur	3.48	5.454	4.656	5.886	5.25	4.356	5.175
Zinc	0.0450196	0.079244601	0.073293757	0.077934999	0.089625	0.086490808	0.077480098
Iron	0.130012795	0.232199002	0.215865441	0.193998198	0.163775	0.174240017	0.166200296
Manganese	0.029092799	0.0345218	0.025724596	0.0326782	0.033425	0.035695003	0.037694699
Copper	0.023501599	0.0309868	0.035094867	0.026814	0.037475	0.029233603	0.032147099

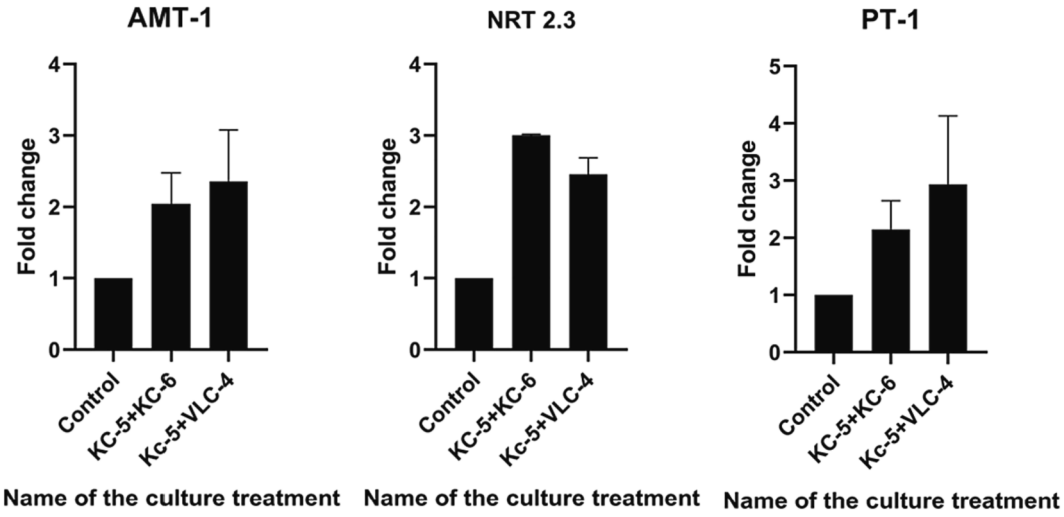

Fig. 3. Differential expression of nutrient transporter genes under the influence of microbial consortia.

consortia-1 improved the expression of the PT-1 gene on an average of 2.395 folds, While the consortia-2 elevated the expression by an average of 2.935 folds. NRT2.3 expression elevated on an average of 3 folds in consortia-1 and 2.45 folds in consortia-2. In the case of the AMT-1 gene, the consortia-1 enhanced the expression 2.04 folds while the consortia-2 enhanced it 2.36 folds.

3.4. Metagenomic profiling of soil microbiome

The microbial diversity in the rhizosphere soil of tomato plants (control, consortia-1 and consortia-2) were elucidated 45 days after the treatments. The metagenomic sequences of the respective sample were then submitted to the NCBI (National Center for Biotechnology Information)-SRA (Sequence Read Archive) database and accession ID for the bio project were retrieved as PRJNA993857. The analysis of soil metagenomics revealed distinct differences between the control soil and the soil treated with PGPB consortia (Fig. 4). In the control soil, the predominant bacterial phyla identified were Proteobacteria (~ 40 %), Actinobacteria (~ 20 %), Firmicutes (~ 20 %), and Bacteroidetes (~ 10 %). This composition represented the baseline microbial community present in the control soil.

However, after the application of PGPR consortia, significant changes in the microbial composition were observed. In the soil treated with consortia-1, the soil microbiome showed a marked increase in Proteobacteria (~ 75 %), while Firmicutes (~ 7 %), Actinobacteria (~ 3 %), and Bacteroidetes (~ 3 %) were also present, although in lower proportions compared to the control soil.

Similarly, in the soil treated with consortia-2, Proteobacteria exhibited a substantial increase, constituting approximately 85 % of the soil microbiome. Firmicutes (~ 15 %), Actinobacteria (~ 5 %), and Bacteroidetes (~ 2 %) were also detected, albeit in relatively smaller quantities compared to Proteobacteria.

These findings indicate that the application of PGPR consortia led to a significant shift in the microbial composition of the treated soil. The increased abundance of Proteobacteria, in both the consortia treated soil, suggests a potential role of these bacteria in promoting plant growth and nutrient uptake.

4. Discussion

The present study investigated the impact of two prepared bacterial consortiums on the metagenomics of soil and their role in biofortifying tomato fruits with nutrients. The soil characterization showed that the soil was comprised of sufficient nutrients and is suitable to for plant growth (Table 4). The fruit nutrient analysis results revealed that consortia-1 which comprises of *Lysnibacillus* sp. and *Acinetobacter* sp. were slightly superior in biofortifying tomato crops. Both of these bacterial genera have been previously reported to improve nutrient intake in crops (Ali et al., 2022; Jinal et al., 2021). Consortia-2, too showed significant improvement in aiding biofortification of tomato when compared to the control groups. The *Enterobacter* is also a bacterial genus reported that has been previously reported to show biofortification abilities (Khalifa, 2020).

The bacterial aided biofortification of tomatoes is then supported with gene expression analysis. The three genes analysed for differential expression, PT-1, NRT2.3, and AMT-1 were found to be upregulated by the bacterial interventions. The PT-1 (Phosphate Transporter-1) is a well characterized phosphate transporter in tomato plants. As per our results the expression of PT-1 was upregulated in leaves when both the consortia was applied, indicating phosphate sufficiency and mobilization

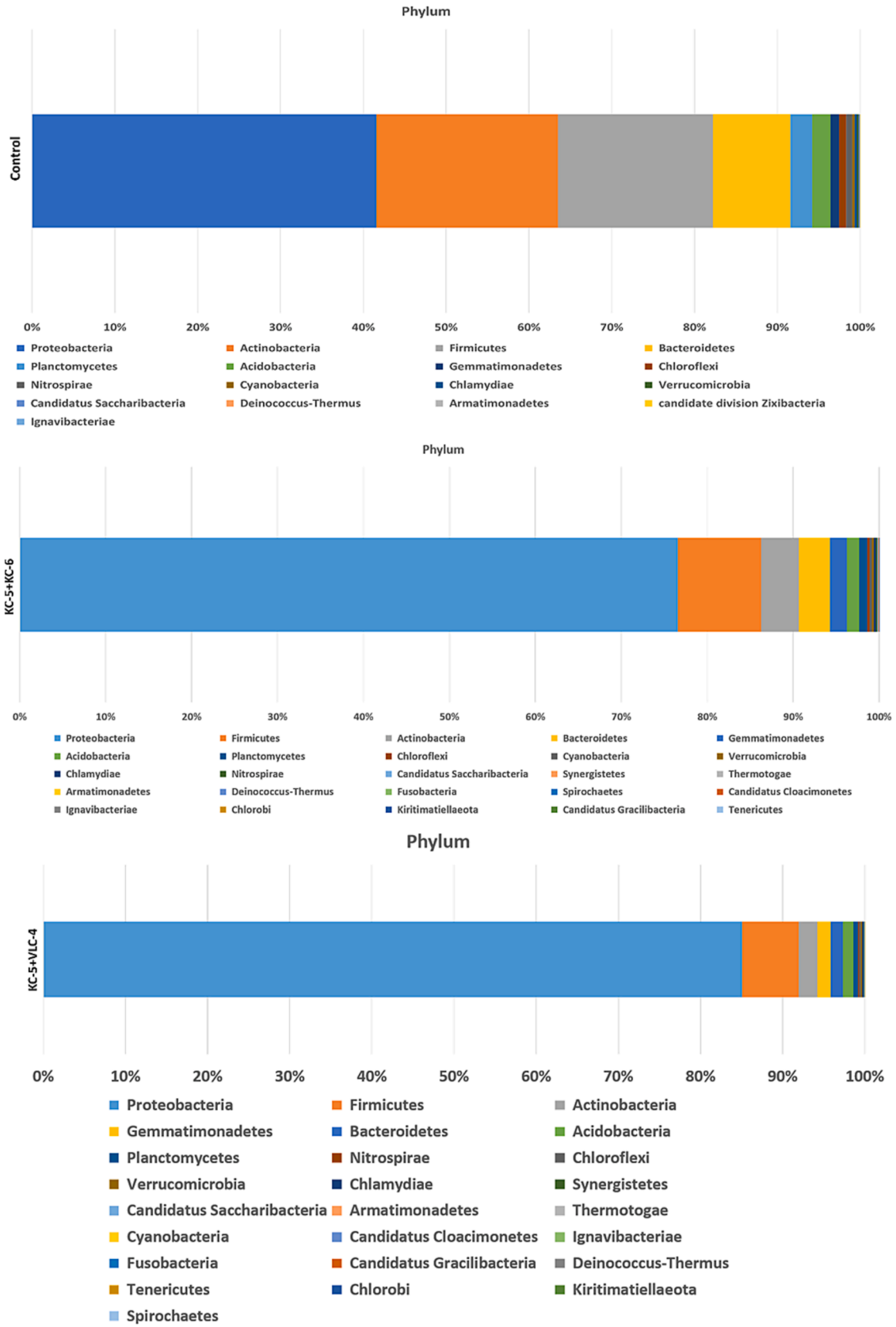

Fig. 4. Phylum level taxonomy of control, KC-5 + KC-6 (Consortia-1) and KC-5 + VLC-4 (Consortia-2) communities based on the average 16S amplicon datasets of each community. The samples were clearly dominated by Proteobacteria after the application of the consortia.

Table 4
Primer sequences used for gene expression analysis.

Gene	Primer Sequence (5′- 3′)	Gene Function
Actin	TGTGCGCGACATGAAAGAGA GGGCATCTGAACCTCTCTGC	House Keeping gene
PT-1	CAGCATTCAAGGGCGCATTC CAGCAGGGATTGCACCAAAC	Indicates phosphate sufficiency, Phosphate Mobilization
AMT-1	GTTGTGGTGCATGGGGGATA TATGCGCCCCGAGTAGTTTC	Transport of Ammonia (nitrogen)
NRT2.3	CCCGTTCACTGCTTGGAGAA GAAGCGAGGTTACCATCAGGT	Transport of Nitrate (nitrogen)

(Chen et al., 2014). Similarly, Tomato NRT2.3 and AMT-1 genes are both associated with the transport of nitrogen across the plant. NRT 2.3 mediates the transport of nitrogen in the form of nitrate which AMT-1 transports as ammonia (Filiz and Akbudak, 2020; Fu et al., 2015). Therefore, an upregulation of these genes could indicate the improved uptake of nitrogen and could be correlated to the PGPB mediated-abundance of nitrogen in the soil.

The metagenomic results revealed significant changes in the microbial composition of the soil following the application of the PGPB consortia. The control soil exhibited a diverse microbial community, with Proteobacteria, Actinobacteria, Firmicutes, and Bacteroidetes being the prominent phyla. These findings align with previous studies that have reported similar compositions in agricultural soils. The presence of these bacterial phyla in the control soil indicates the natural microbial diversity and ecological balance in the agricultural ecosystem.

In contrast, the soil treated with the PGPB consortia showed a significant alteration in microbial composition. The dominance of Proteobacteria, comprising approximately 75 % of the total community, suggests the effectiveness of the bacterial consortia in influencing the soil microbiome. Proteobacteria are known to play crucial roles in nutrient cycling, plant growth promotion, and disease suppression (Ayangbenro and Babalola, 2021). Their increased abundance in the treated soils indicates their potential involvement in enhancing nutrient uptake and bioavailability in tomato plants.

Firmicutes, Actinobacteria, and Bacteroidetes also exhibited similar patterns in both treated soils, maintaining their relative proportions compared to the control soil. This suggests that the introduced bacterial consortia did not disrupt the overall microbial balance of the soil. The preservation of these phyla is important as they contribute to nutrient cycling, soil organic matter decomposition, and the maintenance of soil structure.

The observed changes in the soil microbiome composition following PGPB treatment highlight the potential mechanisms underlying nutrient biofortification in tomato plants. The increased abundance of beneficial bacteria, such as Proteobacteria, may promote nutrient solubilization, facilitate nutrient uptake, and stimulate plant growth. Moreover, the preservation of other phyla, including Firmicutes, Actinobacteria, and Bacteroidetes, may contribute to the overall stability and functionality of the soil microbial community.

Current findings on the use of plant growth-promoting bacteria (PGPB) to improve the nutritional value of tomatoes show that bacterial application significantly increases tomato nutritional value. Notably, our consortia treatment has the best activity among the treatments studied. There are several studies demonstrating benefits of bacterial consortia for plants from different perspectives. For example, Neha Pandey et al., 2023 showed that bacterial consortia in treatment with *Oryza sativa L* seedlings reduced H_2O_2 levels and maintained antioxidant levels (Pandey et al., 2023). Another study shows under metal-stressed circumstances, plants' highly sensitive antioxidant activities are modulated, leading to the ameliorative action of bacterial consortia against metal oxidative damage in plants (Ghosh et al., 2018).

Although there are several such studies involving antioxidative benefits and growth-promoting effects of consortia, there are very few studies on the use of consortia to improve crop nutrient value. For Instance, Cai et al (2021) studied on the combined inoculation of Bacillus and arbuscular mycorrhizal fungi has demonstrated a very good treatment for plant root diseases (Cai et al., 2021). In addition to reducing tomato fusarium root rot severity by 85.0–93.4 %, the combined inoculation of *G. mosseae* and *Bacillus subtilis* significantly enhanced plant nutrients, leaf colour, total soluble sugar, total soluble protein, and total free amino acid content. Additionally, Kumar et al (2020) suggested that the multifunctional influence of rhizospheric microorganisms, either alone or in combination, on the soil–plant system includes enhanced nutrient use efficiency, increased nutrient uptake, advancement of plant development, nodulation, and plant resistance to abiotic and biotic stress, as well as decreased environmental contamination and increased agrarian sustainability (Kumar et al., 2020). These studies also suggest that some bacteria benefit plants more in combination with other bacteria than when used alone. Therefore, we evaluated the potential of two consortia preparations in biofortifying tomatoes. The mechanism of microbial consortia-based plant biofortification involves several key processes. Firstly, these consortia can fix atmospheric nitrogen, making it available to plants in a form they can absorb. Additionally, they can solubilize phosphorus and other nutrients, increasing their availability in the soil. Moreover, these bacteria can produce growth-promoting substances like phytohormones (for example, Indole acetic acid) that stimulate plant growth and development. Overall, the combined action of these microbes in a consortium leads to improved nutrient availability, and enhanced plant growth, ultimately resulting in better plant health and productivity (Kaur et al., 2021, 2020; Kour et al., 2020).

The findings of this study support the notion that the application of specific bacterial consortia can positively influence soil health and plant nutrition. Biofortification of agricultural crops, such as tomatoes, through the utilization of PGPB consortia, presents a promising approach to address nutrient deficiencies in human diets. By enhancing the nutrient content in crops, this strategy can contribute to improving food security and public health, particularly in regions where malnutrition is prevalent.

Future research should focus on unraveling the functional genes and metabolic pathways associated with the identified bacterial phyla. Understanding the molecular mechanisms underlying nutrient acquisition and utilization by the bacterial consortia and their interaction with plants will provide valuable insights into the biofortification process. Additionally, long-term studies assessing the stability and persistence of the introduced bacterial consortia in the soil and their effects on soil fertility and crop productivity would further validate the potential of this approach in sustainable agriculture.

5. Conclusion

In conclusion, the present study demonstrated that the application of two prepared bacterial consortia significantly influenced the tomato fruit nutrient content and microbial composition of the soil. The dominance of Proteobacteria and the preservation of other phyla in the treated soils suggest their potential role in enhancing nutrient uptake and bioavailability in tomato plants. These findings contribute to our understanding of the complex interactions between soil microbiome, plant health, and nutrient biofortification. Implementing PGPB consortia-based biofortification strategies has the potential to contribute to sustainable agriculture, food security, and improved human nutrition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The authors thank management of the Vellore Institute of Technology for providing 'VIT SEED GRANT(RGEMS)-Sanctioned order NO: SG20230005 for carrying out this work. The authors acknowledge Dr. Sudhakaran R of Vellore Institute of Technology – aquaculture laboratory for his guidance in executing this research work.

Data Availability Statement

The metagenomic datasets generated for this study can be found in the NCBI-SRA repository [https://www.ncbi.nlm.nih.gov/sra].

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