Original Research Article

Genetic and Functional Diversity of Bacterial Strains Associated with *Prunus persica* (L.)

ABSTRACT

Aims: This study aimed to evaluate the genetic and functional diversity of bacteria associated with the surfaces of *Prunus persica* (L) Batsch.
Methodology: A total of 15 peach samples from various areas of Lahore city were collected and processed according to standard microbiological protocols. The final taxonomic status of bacterial strains was confirmed by 16S rRNA gene sequencing. Bacterial strains were screened for plant growth-promoting attributes that included auxin production, phosphate, and zinc solubilization, and hydrogen cyanide (HCN) production. Antimicrobial activity of strains was also evaluated. In pot trials, strains were evaluated for their role to enhance plant growth.
Results: Analysis showed that bacterial isolates belonged to *Bacillus, Staphylococcus, Klebsiella, Enterobacter,* and *Providencia* genera. A strain KS 22 of *Staphylococcus sciuri* showed the highest production of auxin. In terms of antibiotic susceptibility, *B. aerius* KS 8 exhibited a pattern of sensitivity toward norfloxacin, amoxicillin, and clavulanic acid. For pot trials with cauliflower under laboratory conditions, *S. sciuri* KS 22 showed 3.1 folds increase in shoot length. In the case of tomato, *P. rettgeri* KS 23 recorded around 1.8 folds increase in shoot length over water-treated control. On the other hand, for cauliflower, around 1.3 folds increase in root length was exhibited by *B. aerius* KS 8, *P. rettgeri* KS 23, and *E. cloacae* KS 29. For tomato, *B. aerius* KS 8 showed 1.6 folds increase in root length in comparison to control. Under natural conditions, for cauliflower, the highest increase in shoot length was exhibited by *P. rettgeri* KS 23 and *E. cloacae* KS 29.
Conclusion: Finally, it has been concluded that bacterial strains *B. aerius* KS 8, *S. haemolyticus* KS 9, *S. sciuri* KS 22, *P. rettgeri* KS 23, and *E. cloacae* KS 29 exhibited plant growth promotion in pot trials.

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Keywords: Antimicrobial resistance, vegetable bacteria, indole-3-acetic acid, fruit bacteria, auxin

1. INTRODUCTION

Nature has created an enormous number of edible fruits and vegetables that constitute an important part of the diet possessing different tastes, flavors, and nutritional values. Fruits not only strengthen our immunity but also develop resistance against many chronic diseases. The presence of dietary fiber helps in reducing constipation and can help in the risk reduction of bowel cancer [1]. Fruits during their growth period can be colonized by many microorganisms. The fruit's normal flora may include both pathogenic and non-pathogenic microorganisms [2,3,4]. The normal flora of fruits can possess antagonistic effects on pathogenic bacteria. For example, some bacteriocin-producing *Enterococcus* and *Pediococcus* bacterial genera have demonstrated the decelerated growth of *Listeria monocytogenes* on mung bean sprouts [5,6].

Foodborne-associated disease outbreaks have continuously been increasing over the last few decades [7]. The contaminants present on the surface of fresh produce may have neutral, positive, or negative effects on human beings. Immunocompromised humans may be exposed to these bacteria

which can lead to serious food-borne illnesses. The fruit-associated bacteria can sometimes cause respiratory tract allergies as well [8]. Several factors predispose to adherence of contaminants on fruits and vegetables from pre-and post-harvesting steps. It included farm locations, storage duration, temperature, and transport conditions [9,10]. The microbes have adopted multiple ways for their colonization on fresh produce. For instance, use of pili, biofilm formation, capsular O antigen, production of cellulose, use of flagella, and type III secretion system [4,11].

One of the major food safety concerns with fresh fruits is the development of antimicrobial resistance in the colonized microorganisms. Especially, the resistance to antimicrobial agents by gram-negative bacteria is a matter of serious consideration. Plant-associated bacterial communities may be involved in horizontal gene transfer for drug resistance. Thus fruits' surfaces can be the carrier and reservoir of antimicrobial-resistant bacteria [12]. The continuous use of pesticides, antimicrobial agents, irrigation wastewater, and use of maneuvers in farming practices as fertilizers are among the key factors for causing the horizontal spread of antimicrobial resistance to microorganisms [13].

Numerous produce-associated bacteria have widely been reported in the literature. Scientists have reported the presence of γ-proteobacteria, and Firmicutes on the surfaces of berries. Similarly, the prevalence of *Photobacterium spp.* on *Malus domestica* has also been reported [3]. In another study, *Lactuca sativa* (lettuce) plants have been shown to harbor *Xanthomonas spp., Pantoea spp., Pectobacterium spp., Leuconostoc spp., Janthinobacterium spp*, *Escherichia coli* O157:H7, and *Vibrio cholerae* [13]. Moreover, the prevalence of *Buchnera aphidicola, Bacillus spp., Pantoea spp.*, *Microbacterium spp., Bacillus spp.,* and *Gluconacetobacter* have been shown to colonize the surfaces of different fruits [2,3]. The bacterial agents that are responsible for food spoilage include *Erwinia carotovora*, *Pseudomonas spp.*, *Corynebacterium diphtheriae*, *Xanthomonas campestris*, and lactic acid bacteria [13]. Amongst fungi, *Botrytis cinerea*, *Aspergillus spp., Cladosporium spp., Colletotrichum spp., Phomopsis spp., Fusarium spp., Penicillium spp., Phoma spp., Phytophthora spp., Pythium spp., Rhizopus spp.*, *Ceratocystis fimbriata*, *Rhizoctonia solani, Sclerotinia sclerotiorum*, and some mildew cause fruits spoilage [14].

In addition to pathogenic bacteria, several beneficial microorganisms may colonize the fruit surfaces. They may contribute beneficial metabolites for plant growth and development [15]. For instance, microbial phytohormones play a vital role in carrying out various physiological and metabolic processes for the development of roots and shoots of a plant [16]. However, the excessive production of these phytohormones can also lead to plant pathogenicity. For example, *Burkholderia solanacearum* raises the amount of indole-3-acetic acid (IAA) to one hundred times causing hernia, vascular wilting, and gall formation [17]. Amongst *Enterobacteriacae* family, *Klebsiella pneumoniae* [18], *K. variicola* [19], *Enterobacter cloacae* [20], and *Pseudomonas spp.* [21] have been reported to be significant in various attributes of plant growth.

IAA has been among the most studied phytohormones because it mediates multidimensional developmental processes in plants [22,23]. IAA is not only produced by plants, but also by a variety of plant-associated bacteria. The effects of plant growth-promoting bacteria *Pseudomonas putida* on *Brassica napus* roots through the construction of a control and test strain with and without IAA

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production ability have been demonstrated by [24]. Keeping given the above scenario, this study aimed to determine the bacterial genetic diversity and functional diversity associated with the fruit surfaces of *Prunus Persica* (L.). Bacterial strains were screened *in vitro* to produce indole-3-acetic acid (IAA) and other plant growth-promoting traits. Finally, non-pathogenic bacteria were used to elucidate their role in plant growth promotion.

2. MATERIALS AND METHODS

2.1. Sample collection and isolation of microorganisms:

All the fruit samples of *Prunus persica* (L.) were collected from various shops and street sellers in Lahore, Pakistan. The city was divided into four sections East, West, North, and South. Unwashed and intact peaches were weighed and peeled off using a sterile fruit peeler and mixed with 200 mL of saline water. The samples were then serially diluted using saline water ranging from 10-1 to10-7. Dilutions from each sample were plated on nutrient agar. The isolated colonies were purified by multiple rounds of quadrant streaking. Dilutions were also plated respectively on Mannitol salt agar and MacConkey agar for the selective isolation of *Staphylococcus aureus* and gram-negative bacteria bacilli. The isolates were then characterized using biochemical tests i.e., catalase test, citrate test, urease tests, triple sugar iron (TSI) test, and sulfide indole motility (SIM) test. The terminal biochemical identification was performed using bioMérieux's Analytical Profile Index 20E (API) which contained a strip for 20 dehydrated biochemicals.

2.2. Antimicrobial susceptibility testing:

The antimicrobial susceptibility pattern of the bacterial isolates was evaluated on Mueller-Hinton (MH) agar. In total, 5 antibiotics discs (Thermo Scientific™ Oxoid™) were dispensed onto each MH agar plate at equal distances. The antibiotic discs for gram-positive bacteria were amikacin (AK-30 µg) amoxicillin/clavulanic acid (AMC-10 µg), chloramphenicol (C-30 µg), ciprofloxacin (CIP-30 µg), and fosfomycin (F-300 µg). Whereas for gram-negative bacteria, ceftazidime (CAZ-30 µg), norfloxacin (N-30 µg), cefoperazone + sulbactam (SCF-100 µg), piperacillin + tazobactam (TZP-100 µg), imipenem (IPM-10 µg) and ciprofloxacin (CIP-5 µg) were used. Zones of the clearing were measured according to CLSI guidelines 2020 (Clinical and Laboratory Standard Institute).

2.3. 16S rRNA gene sequencing:

For genomic DNA isolation, a purified bacterial colony was inoculated in an L-broth medium and incubated on a shaker overnight at 30℃. Bacterial growth was collected from a liquid medium by centrifugation. The genomic DNA was extracted using FavorPrepTM Tissue Genomic DNA Extraction Mini Kit (Favorgen Biotech Corporation, Vienna, Austria). The extracted DNA was subjected to amplification of 16S rRNA gene by using 27f forward (5'-AGAGTTTGATCCTGGCTCAG-3') and 1522r reverse primer (3'-ACGCC(AG)ACCTAGTGGAGGAA-5'). PCR amplification was accomplished by using Thermo Scientific DreamTaq™ Green PCR Master Mix. The reaction mixtures were incubated in a thermocycler Primus 96 (PeQLab, Erlangen, Germany) at 94℃ for 5 min. Then passed through

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30 cycles that included denaturation for 20s at 94℃, primer annealing for 20s at 50℃, and extension at 72℃ for 2 min. Moreover, the final extension was carried out at 72℃ for 5 min. PCR products were purified by using the FavorPrepTM Gel Purification kit (Favorgen Biotech Corporation, Austria) (Figure 1). For sequencing, purified fragments of 16S DNA were sent to First Base Sequencing Agency (Singapore). After obtaining the results, the sequences were analyzed by NCBI tool BLAST to find the similarity with already submitted sequences. Finally, sequences were submitted to NCBI to obtain the accession numbers for the identified bacterial strain.

2.4. Phylogenetic analysis:

All the confirmed sequences were subjected to alignment using a multi-sequences alignment program called Crustal W using MEGA X software. The evolutionary history was inferred using the Neighbor-Joining method [25] (Figure 2).

2.5. Phosphate and zinc solubilizing:

The phosphate-solubilizing test was determined by using the Pikovaskaya agar medium (Mehta and Nautiyal, 2001). The test strains were streaked on agar plates and incubated for 7 days at 28℃. The clear zones around the bacterial colonies were an indication of positive phosphate solubilizing test. For zinc solubilization, Pikovskaya agar medium was also supplemented with zinc carbonate and plates were incubated as mentioned above to record the zone of clearance [26].

2.6. Hydrogen cyanide test:

The activity of bacterial strains to produce hydrogen cyanide (HCN) was evaluated by using the protocol of [27]. The bacterial strains were swabbed thoroughly on tryptose soy yeast extract agar (TSY) medium amended with glycine 94.4g/l and impregnated with soaked filter paper disks having 0.5% picric acid prepared in 2% sodium carbonate. Plates were incubated for 4 days at 30℃. The change in color of filter paper disks from dark yellow to brown indicated a strong positive reaction.

2.7. Bacterial auxin quantification:

Bacterial auxin production was quantified by using Salkowski's reagent as described by [28]. Bacterial culture was inoculated in respective flasks that contained L-broth supplemented with 0, 300, and 600 µg/mL L-tryptophan. The bacterial cultures were incubated at 30℃ for 72 hours. After centrifugation, the supernatant was mixed with Salkowski's reagent and incubated in dark for 30 min for the development of a pink to red color. The optical densities of all samples were noted at 535 nm with a spectrophotometer (CECIL CE 7200). Bacterial auxin was quantified by constructing a standard curve of authentic IAA.

2.8. In vitro bioassays:

After thorough washing, the tomato and cauliflower seeds were placed in the solution of 0.1% mercuric chloride for 1 min. Then seeds were thoroughly washed with autoclaved distilled water. In the next step, seeds were treated with respective bacterial suspensions for 20 min. Finally, five bacteria-treated seeds were placed in Petri plates lined with moistened filter paper. For each strain,

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three plates were used, and the experiment was repeated twice. After 10 days of seed germination, shoot and root lengths were recorded.

2.9. Pot trials under controlled and natural conditions:

The controlled experiment (lab trial) was performed for assessment of the growth parameters that included the measurement of root and shoot length upon sprouting after 21 days. The experiment included 18 pots and 54 seeds during January and February with the strategy to use a triplicate of each seed on a triplicate of pots. The ratio of sand to soil was 70:30. The seeds were bathed with the suspension of five bacterial strains i.e., *B. aerius* KS 8, *S. haemolyticus*, KS 9, *S. sciuri* KS 22, *P. rettgerii* KS 23, *E. cloacae* KS 29. Five pots for each triplicate of seeds were sown with each bacterial suspension and 3 seeds were sown in each pot. The 6th triplicate pot contained a triplicate of control seeds. The experiment trials under natural conditions (wirehouse) were performed using the same seed and pot count as lab-controlled trials on the same bacteria, however, along with root and shoot length; the fresh and dry weight of the grown plants was also measured.

2.10. Statistical analysis:

For bacterial auxin production and plant growth parameters, data were subjected to analysis of variance (ANOVA), and mean values were separated by using Duncan's multiple range test (*P≤0.05*).

3. RESULTS

3.1. Morphological and biochemical characterization:

The bacterial isolates streaked on agar plates showed diverse morphological characteristics including color, consistency, shape, size, elevation, and margins. A total of 40 strains were isolated from 15 samples, out of which 24 belonged to gram-negative bacteria. A few strains such as KS 1 and KS 3, KS 13, and KS 23 showed large mucoid pink colonies having entire margins and elevations on MacConkey agar. However, strains KS 12, KS 14, KS 31, and KS 37 recorded small, pale, and raised colonies on Mannitol salt agar. For biochemical profiling, triple sugar iron test, citrate, urease, sulfide, indole, motility, catalase, and DNAse tests were performed. The gram-negative bacteria showed various patterns of reaction in producing blackening, uplifting, and cracking of TSI gel for the production of H_2S and CO_2, respectively. All the bacterial strains showed positive citrate tests except KS 26 and KS 33. For Analytical Profile Index (API), *Klebsiella variicola* showed blue color in citrate test, pink color for urease, purplish-blue color for the reaction of tryptophan deaminase (TDA), a pinkish-red color reaction in indole yellow color for inositol, mannose, and amygdalin. The results of identified strains are given in Table 1.

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Table 1. Biochemical identification of bacterial strains by using API 20E kit

Strain Name	KS 3	KS 13	KS 32	KS 36
CODE	**5215773**	**1215773**	**274301**	**5205773**
Organism Identified	***Klebsiella pneumoniae***	***Klebsiella pneumoniae***	***Klebsiella variicola***	***Enterobacter aerogenes***
ONPG	+	+	-	+
ADH	-	-	-	-
LDC	+	-	-	+
ODC	-	-	-	-
CIT	+	+	+	+
H2S	-	-	-	-
URE	+	+	+	-
TDA	-	-	+	-
IND	-	-	+	-
VP	+	+	-	+
GEL	-	-	-	-
GLU	+	+	+	+
MAN	+	+	+	+
INO	+	+	+	+
SOR	+	+	-	+
RHA	+	+	-	+
SAC	+	+	-	+
MEL	+	+	-	+
AMY	+	+	+	+
ARA	+	+	-	+

Abbreviations: ONPG: o-nitrophenyl-b-D-galactopyranoside, **ADH**: arginine dihydrolase**, LDC**: lysine decarboxylase**, ODC**: ornithine decarboxylase**, CIT**: citrate**, H2S**: hydrogen sulfide**, URE**: Urease**, TDA** Tryptophan deaminase**, IND**: Indole Test**, VP:** Voges-Proskauer test**, GEL**: gelatinase, **GLU**: glucose**, AN:** mannose**, INO:** inositol**, SOR:** sorbitol**, RHA:** rhamnose**, SAC:** sucrose, **MEL:** melibiose**, AMY:** amygdalin**, ARA:** arabinose

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3.2. Antimicrobial susceptibility testing:

Most of the gram-negative bacterial isolates showed resistance against the applied drugs. All isolates showed 81% and 90% resistance against ceftazidime and cefoperazone + sulbactam, respectively. However, most of the organisms showed susceptibility against piperacillin + tazobactam. The strains *Klebsiella oxytoca* KS 12, *K. oxytoca* KS 17, and *K. pneumoniae* KS 31 showed resistance against all the applied antibiotics. The zone of inhibition for *E. coli* KS 1 against ceftazidime, nitrofurantoin, cefoperazone + sulbactam, piperacillin + tazobactam, imipenem, and ciprofloxacin were 15, 11, 16, 8, 18 and 18 mm, respectively. Amongst gram-positive bacteria, a variable range of susceptibility patterns was observed. The strain *S. aureus* KS 7 and *S. hemolyticus* KS 9 showed susceptibility against all the applied antibiotics. All the gram-positive isolates showed the least resistance against amikacin (81%), whereas most of the resistance was shown against ciprofloxacin (61%). The results of the antibiogram for *S. aureus* KS 7 were amikacin (28 mm), amoxicillin-clavulanic acid (17 mm), chloramphenicol (22 mm), ciprofloxacin (38 mm), and fosfomycin (19 mm).

3.3. 16S rRNA gene sequencing:

After comparison with online data submitted to NCBI, strain KS 3 showed 99% similarity with *Klebsiella pneumoniae.* Similarly, strain KS 6 was identified as *Staphylococcus haemolyticus.* On the other hand, strain KS 15 was identified as *Bacillus aerius.* The strains KS 21 and KS 22 showed homology with *S. sciuri.* Accession numbers of all the identified strains are given in Table 2. Figure 2 is showing the phylogenetic relationship among different bacterial isolates. MEGA X software categorized the strains into two major clusters i.e., gram-negative (A) and gram-positive (B). The cluster "A" was divided into two subclusters that represented *P. retgerii* and *Klebsiella spp.* The cluster "B" was divided into two subclusters where KS 8 and KS 15 showed 100% similarity with *B. aerius*. The second subcluster was further subdivided *S. sciuri* and *S. haemolyticus*.

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Table 2. 16S rRNA gene sequencing of bacterial isolates

Sr. No.	Isolates	Identified as	Similarity (%)	GenBank Accessions
1	KS 3	*Klebsiella pneumoniae* KS 3	99.50%	MN967228
2	KS 6	*Staphylococcus haemolyticus* KS 6	99.90%	MN967229
3	KS 8	*Bacillus aerius* KS 8	99.43%	MN967230
4	KS 9	*S. haemolyticus* KS 9	99.90%	MN967231
5	KS 12	*Klebsiella oxytoca* KS12	99.42%	MN967232
6	KS 13	*K. pneumoniae* KS 13	99.89%	MN967233
7	KS 14	*K.oxytoca* KS 14	99.46%	MN967234
8	KS 15	*B. aerius* KS 15	100.00%	MN967235
9	KS 17	*K. oxytoca* KS 17	99.50%	MN967236
10	KS 21	*Staphylococcus sciuri* KS 21	100.00%	MN967237
11	KS 22	*S. sciuri* KS 22	100.00%	MN967238
12	KS 23	*Providencia rettgeri* KS 23	99.50%	MN967239
13	KS 29	*Enterobacter cloacae* KS 29	99.89%	MN967240
14	KS 31	*K. pneumoniae* KS31	99.62%	MN967241
15	KS 32	*Klebsiella variicola* KS 32	99.90%	MN967242
16	KS 36	*Klebsiella aerogenes* KS 36	99.12%	MN967243
17	KS 37	*K. pneumoniae* KS 37	99.60%	MN967244

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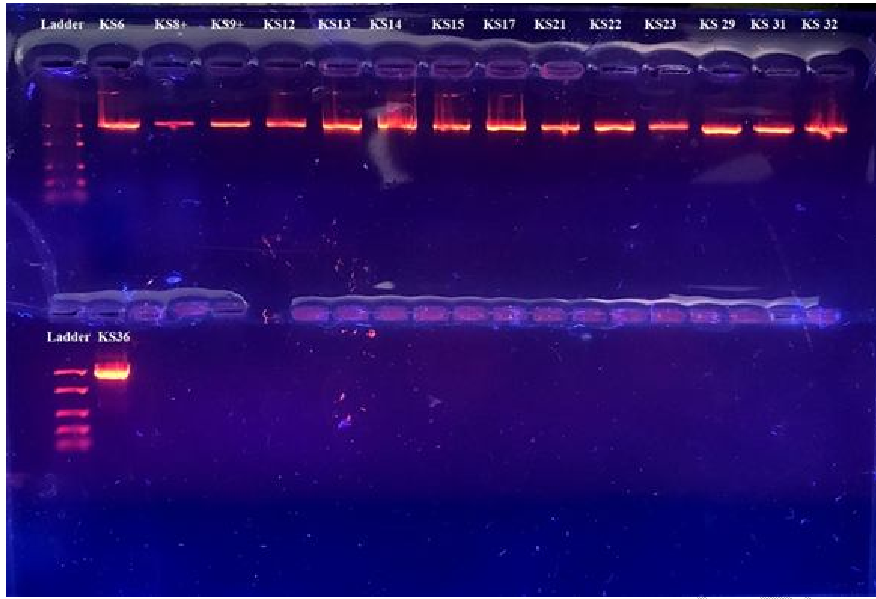

Figure 1. PCR purified product of different bacterial strains on agarose gel electrophoresis

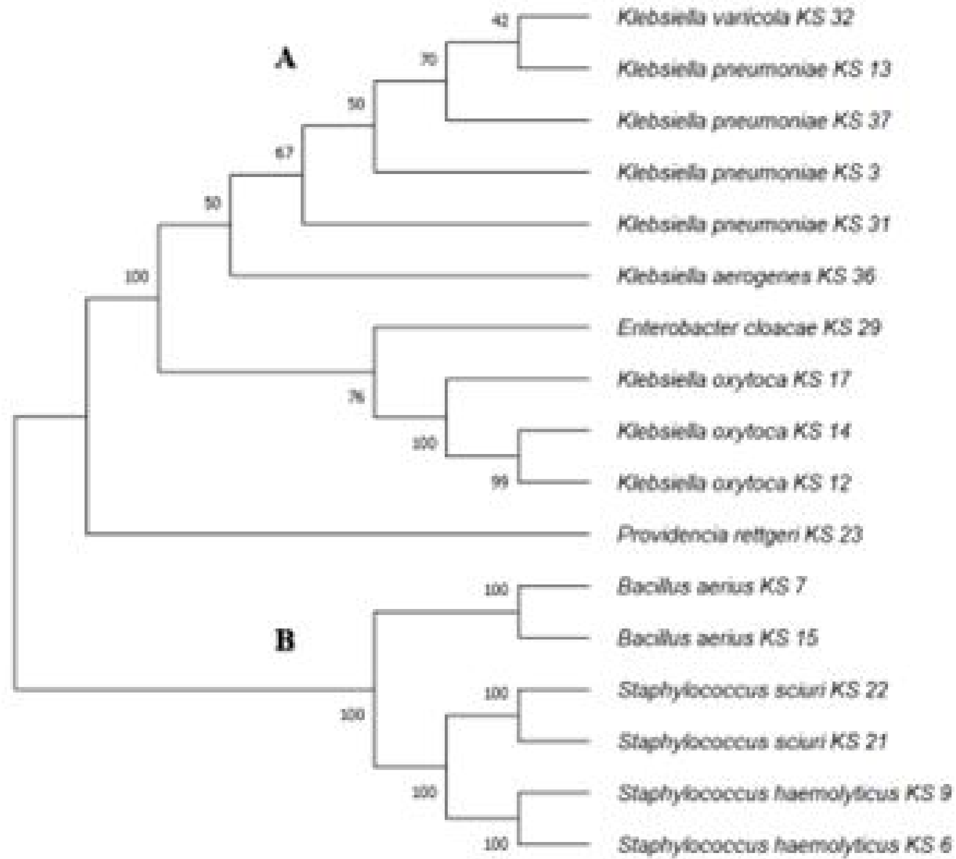

Figure 2. Phylogenetic tree showing relationships among different bacterial isolates. The upper (A) cluster represents gram-negative bacteria and the lower (B) cluster represents gram-positive bacteria

3.4. Multiple plant growth-promoting traits:

Without the addition of L- tryptophan (0µg/mL), the optical density of auxin production for the test strains was: *B. aerius* KS 8 (14.1), *S. haemolyticus* KS 9 (13.1) *S. scuri* KS 22 (5.9), *P.rettgeri* KS 23 (9.3) and *E. cloacae* KS 29 (12.3). With the addition of L-tryptophan at 300 µg/mL; an upsurge was witnessed in the case of *B. aerius* KS 8 (3.2 folds) and (23 folds) in the case of *S. scuri* KS 22. However, at the same concentration of L- tryptophan; a decline in the auxin production was observed in the case of *B. aerius* KS 9 (0.8 fold), and *P.rettgeri* KS 23 (0.2 fold), and *E. cloacae* KS 29 (0.9 folds). All the isolates showed a slight response for auxin production with an increase in L-tryptophan concentration at 600µg/mL, in comparison with (0µg/mL) L- tryptophan; only *S. scuri* KS 22 showed (2.5 folds) increase in auxin production, whereas a low level of auxin production was observed in case of *B. aerius* KS 8 (0.7 folds), *S. haemolyticus* KS 9 (0.6 folds), *P.rettgeri* KS 23 (0.8 folds) and *E. cloacae* KS 29 (0.9 folds). For the construction of the auxin standard curve, we used authentic IAA concentration. The standard curve of auxin production was prepared using a gradually increasing amount of L-tryptophan and observing the increase in concentration.

The test strains exhibited different levels of hydrogen cyanide production however, the experiment was performed qualitatively. *B. aerius* KS 8 showed a weak response in HCN production while *S. sciuri* KS 22*, P. rettgeri* KS 23*,* and *E. cloacae* KS 29 showed a moderate response. *S. haemolyticus* KS 9 showed a strong response and a high formation of hydrogen cyanide occurred. None of the test strains exhibited phosphate solubilization and zinc solubilization.

3.5. Rooting bioassay:

In the case of cauliflower, in comparison to the control, the root length of seeds for *B. aerius* KS 8, *S. haemolyticus* KS 9, *S. sciuri* KS 22, *P. rettgeri* KS 23, and *E. cloacae* KS 29 increased by 4.1, 5.7, 6.6, 7.7 and 2.8 folds respectively. However, the shoot length of cauliflower seeds for *B. aerius* KS 8, *S. haemolyticus* KS 9, *S. sciuri* KS 22, *P. rettgeri* KS 23, and *E. cloacae* KS 29 increased by 4.3. 3.4, 3.8, 2.5, and 1.9 folds respectively over the control. For tomatoes, the root length of tomato seeds for *B. aerius* KS 8, *S. haemolyticus* KS 9, *S. sciuri* KS 22 *P. rettgeri* KS 23, and *E. cloacae* KS 29 increased by 2.8, 2.4, 2.3, 1.5, and 1.1-fold respectively. The shoot length of tomato seeds for *B. aerius* KS 8, *S. haemolyticus* KS 9, *S. sciuri* KS 22, *P. rettgeri* KS 23, and *E. cloacae* KS 29 increased by 8.3,7.4. 6.3, 5.0, and 3.9 folds respectively.

3.6. Pot trials in the laboratory:

The effect of bacterial inoculations on the growth of plants was evaluated in pot trials under laboratory conditions. In the case of cauliflower seeds, the comparison with control showed that the root length of seed for *B. aerius* KS 8, *P. rettgeri* KS 23, and *E. cloacae* KS 29 increased by 1.3 folds and *S. haemolyticus* KS 9, *S. sciuri* KS 22, increased by 1.1-fold each. While on the other hand, the shoot length for *B. aerius* KS 8, *S. haemolyticus* KS 9, *S. sciuri* KS 22, *P. rettgeri* KS 23, and *E. cloacae* KS 29 increased by 2.8, 2.7, 3.1, 2.0 and 1.9 folds respectively over the control.

For tomatoes, bacterial strains showed a significant increase in root length for *B. aerius* KS 8, *S. haemolyticus* KS 9, *S. sciuri* KS 22, *P. rettgeri* KS 23, and *E. cloacae* KS 29 by 1.6, 1.2, 0.9, 1.6 and

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1.0 (folds) respectively. While on the other hand, the increase in shoot length of tomato seeds for *S. haemolyticus* KS 9 and *E. cloacae* KS 29 by 1.2 folds each, and *B. aerius* KS 8, *S. sciuri* KS 22, and *P. rettgeri* KS 23 increased by 1.4, 1.3 and 1.8 folds respectively (Figure 3).

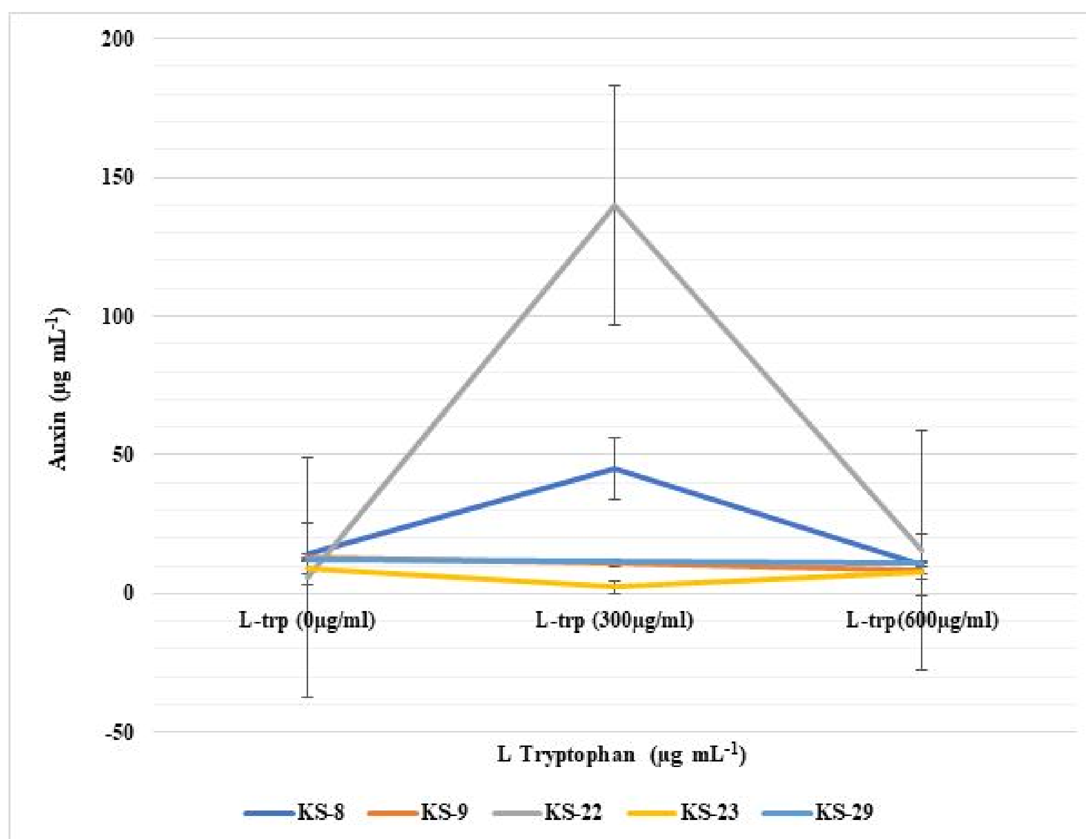

Figure 3. Effect of different L-tryptophan concentrations on auxin biosynthesis by bacterial strains. The bars at different points indicate SE for each treatment

3.7. Pot trials in the wire house:

After laboratory trials, the efficacy of bacterial strains to enhance the growth of cauliflower and tomato was evaluated by conducting pot trials under natural environmental conditions. For cauliflower, the increase in root lengths showed by *B. aerius* KS 8 and *E. cloacae* KS 29 were 1.2 folds, *S. haemolyticus* KS 9, *S. sciuri* KS 22, and *P. rettgeri* KS 23 increased by 1.0, 1.1, 1.3 folds respectively. While on the other hand, the increase in shoot lengths for seeds of suspension with *B. aerius* KS 8 was 1.5 folds, *S. haemolyticus* KS 9 and *S. sciuri* KS 22 were 1.4 folds, *P. rettgeri* KS 23 and *E. cloacae* KS 29 were 1.6 folds. The fresh weight for the cauliflower plants grown in bacterial suspensions of *B. aerius* KS 8, *S. haemolyticus* KS 9, and *E. cloacae* KS 29 increased by 1.2 folds, *S. sciuri* KS 22 by 1.3 folds, and *P. rettgeri* KS 23 by 1.6 folds over the control. In comparison to the dry weight of control, the dry weight of the plants for bacterial suspension of *S. haemolyticus* KS 9 and *S. sciuri* KS 22 increased by 1.3 folds, *B. aerius* KS 8 (1.1 folds), 1.9 and 1.4 folds each for *P. rettgeri* KS 23 and *E. cloacae* KS 29 (Table 3).

Table 3. Effect of bacterial inoculations on vegetative growth parameters of cauliflower under natural conditions

S. No.	Test Strain	Cauliflower			
		Root Length(cm)	Shoot Length(cm)	Fresh Weight(g)	Dry Weight(g)
1	Control	4.06±0.54 (a)	4.76±0.46 (a)	6.8±0.78 (a)	1.73±0.73 (a)
2	KS-8	4.73±0.24 (a)	7.2±0.40 (b)	7.86±0.66 (a-b)	1.84±0.12 (a)
3	KS-9	4.06±0.27 (a)	6.51±0.70 (a)	8.01±1.13 (a-b)	2.26±0.49 (a)
4	KS-22	4.5±0.50 (a)	6.96±0.39 (b)	8.52±0.99 (a-b)	2.17±0.21 (a)
5	KS-23	5.4±0.57 (a)	7.8±0.67 (b)	10.93±1.31 (b)	3.25±0.63 (a)
6	KS-29	4.88±0.40 (a)	7.63±0.53 (b)	8.17±0.95 (a-b)	2.46±0.40 (a)

Mean ± S.E. of 48 plants. Different letters in parenthesis indicate significant differences among treatments using Duncan's multiple range test *P≤0.05*).

The pot trials with tomato seeds exhibited a significant increase in the root length mixed with bacterial suspension of *B. aerius* KS 8 and *S. haemolyticus* KS 9 was 1.5-fold, *S. sciuri* KS 22 and *P. rettgeri* KS 23 by 1.4 folds and *E. cloacae* KS 29 by 1.3 folds respectively over the control. While on the other hand, the increase in shoot length is shown by *B. aerius* KS 8, *S. haemolyticus* KS 9, *S. sciuri* KS 22, *P. rettgeri* KS 23, and *E. cloacae* KS 29 were 2.1, 2.0, 1.8, 1.9 and 1.7 folds respectively. The fresh weight for the tomato plants grown in bacterial suspensions of *B. aerius* KS 8 and *S. haemolyticus* KS 9 increased by 3.5 folds, *S. sciuri* KS 22 and *E. cloacae* KS 29 by 3.0 folds, *P. rettgeri* KS 23 by 3.6 folds over the control. The dry weight for *B. aerius* KS 8 increased by 4.0 folds, 5.2 folds for *S. haemolyticus* KS 9, and 4.2 folds in the case of *S. sciuri* KS 22, *P. rettgeri* KS 23, and *E. cloacae* KS 29 against the control. (Table 4).

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Table 4. Effect of bacterial inoculations on vegetative growth parameters of tomato under natural conditions

S. No.	Test Strain	Tomato			
		Root Length(cm)	Shoot Length(cm)	Fresh Weight(g)	Dry Weight(g)
1	Control	3.63±0.50 (a)	4.66±0.88 (a)	2.66±0.46 (a)	0.56±0.23 (a)
2	KS-8	5.46±0.33 (b)	9.61±0.46 (b)	9.3±0.85 (b)	2.26±0.21 (b)
3	KS-9	5.54±0.49 (b)	9.5±1.04 (b)	9.33±1.38 (b)	2.91±0.40 (b)
4	KS-22	5.16±0.40 (b)	8.6±0.67 (b)	8.06±0.95 (b)	2.37±0.37 (b)
5	KS-23	4.95±0.40 (a-b)	9.01±0.67 (b)	9.51±1.15 (b)	2.34±0.29 (b)
6	KS-29	4.65±0.41 (a-b)	8.1±0.63 (b)	8.1±0.88 (b)	2.37±0.47 (b)

Mean ± S.E. of 48 plants. Different letters in parenthesis indicate significant differences among treatments using Duncan's multiple range test $P \leq 0.05$).

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4. DISCUSSION

From being a seed to a plant, a diversity of bacteria, fungi, archaea, and parasites are gathered in the caulosphere, phylloplane, anthosphere, and carposphere of a plant [29]. Bacterial diversity in agricultural produce has been studied by various scientists across the globe. The present study aimed to study the contaminants associated with the carposphere of *Prunus persica* where the terminal identification by 16S rRNA gene sequencing showed that most of the isolates belonged to the family *Enterobacteriaceae.* It included *Klebsiella pneumoniae*, *K. oxytoca, Providencia rettgeri, Enterobacter cloacae, K. variiocola,* and *K. aerogenes*. The study by [3] reported that the contaminants present in *Ananas comosus* (pineapple), *Citrullus lanatus* (watermelon), *Malus domestica* (apple), *Prunus persica* (peach), and salads belonged to the *Enterobacteriaceae* family. The molecular characterization of *Enterobacteriaceae* isolated from spinach in South Africa showed the prevalence of *Serratia fonticola*, *Escherichia coli,* and *K. pneumoniae* [30]. Similarly, the samples of pineapple, watermelon, and salads were shown to harbor *Bacillus, Staphylococcus aureus*, *Escherichia coli*, *Salmonella spp.*, *Klebsiella spp.*, *Pseudomonas aeruginosa*, *Proteus spp.*, *Micrococci* and *Lactobacilli* species [31,32]. The predominance of *Enterobacteriaceae* on these fruits indicates that these fruits have been largely associated with contaminated water or have been harvested using sewerage water [33].

B. aerius is usually present in higher altitudes, however, in our study; the isolation of *B. aerius* KS 8 in peach samples indicated that these strains were brought to this area from high altitudes where peach was growing. [34] have reported the antifungal role of *B. aerius* in plants; the presence of *B. aerius* KS 8 and KS 15 in the current study could be associated with fungicidal activity for the prevention of

peach fruit. The strains KS 22 and KS 23 of *Staphylococcus sciuri* were reported for the first time by [35] when they collected the samples from animal and human skin. Recent studies reported that the *Staphylococcus sciuri* is used to improve the root growth of plants and other health parameters [36]. The presence of *Staphylococcus haemolyticus* on the test samples could be associated with contamination of the skin's normal flora during packaging and delivery to the terminal consumer.

K. variicola has been reported as an emerging pathogen for cattle & human beings. The endosymbiotic relation of this strain with plants has also been reported by [37]. *K. oxytoca* and *K. pneumoniae* are associated with plants in host-plant association by fixing nitrogen acting as diazotrophs attaching themselves with the root nodules thus increasing agricultural yield [38]. *E. cloacae* are used to enhance the seed germination and growth attributes in rice crops by using a substitute of ammonium sulfate as ACC [39]. *P. rettgeri* and *E. cloacae* are also associated with growth promotion for potatoes, the study carried out by [40] showed that these bacteria can show resistance against potato wilt disease.

In the current study, *in vitro* root and shoot bioassays were performed by inoculating the seeds of tomato and cauliflower onto a culture plate. Results of the *in vitro* root and shoot bioassays showed that all the strains expressed positive effects on both types of seeds and enhanced root and shoot length was witnessed upon application of *B. aerius* KS 8, *S. haemolyticus* KS 9, and *P. rettgeri* KS 22. For instance, *B. aerius* KS 8 expressed thrice the root length and eight times the shoot length as compared to the control of tomato seed. In the case of cauliflower, the root and shoot length on the application of *P. rettgeri* KS 22 was five and four folds respectively. The results are comparable with the study by [21] where bacterization of *Pseudomonas aeruginosa* on peanut seeds on culture plates exhibited enhanced growth of roots and shoots along with biomass production. Similarly, another study from India by [41] reported the enhanced role of *Bacillus sonorensis* NR1 for in-vitro bioassays of *Lycopersicon esculentum* (Tomato).

Auxin is a plant growth-promoting hormone synthesized from L-tryptophan. Under soil stress conditions; the plant growth-promoting bacteria utilize L tryptophan thus producing indole-3-acetic acid by causing an increase in the root growth and biomass induction, hormonal transcriptome changes, defense-related mechanisms, cell wall-related genes, and a decrease in size and density of stomata [42,43,44]. The identified strains i.e., *B. aerius* KS 8, *S. haemolyticus* KS 9, *S. sciuri* KS 22, *P. rettgeri* KS 23, and *E. cloacae* KS 29 were evaluated for in vitro indole-3-acetic acid (IAA) production. In the present study, the auxin production test was applied at three different concentrations of L-tryptophan, where *S. sciuri* KS 22 exhibited an optical density of 140 at 300µg/mL concentration of L-tryptophan; confirming the plant growth-promoting trait. Without the addition of L tryptophan; 1.4 folds auxin production was noted in the case of *B. aerius* KS 8. The findings of the current study are comparable with the study of [22,45,46] where the isolates of *Myroides spp.*, *Pseudomonas putida, Proteus vulgaris, Myroides spp.* and *Providencia spp.* produced a significant amount of IAA. Finally, it can be concluded that the strains *B. aerius* KS 8 and *S. sciuri* KS 22 have a significant capability for *in vitro* auxin production.

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In the laboratory under controlled conditions; The highest root and shoot length were exhibited by tomato seed inoculated with *P. retgerii* KS 23. The results of the current study are comparable with the findings of [47] where the scientists reported the plant growth-promoting traits of *P. retgerii*. However, in the present study, an increase in root and shoot length for cauliflower seeds was shown by *S. sciuri* KS 22. The study of [48] also confirmed the increased root and shoot length of sweet cherry rootstocks when inoculated with the mixture of *S. sciuri*. All strains showed an almost positive growth trend for root & shoot length both for tomato and cauliflower.

Different *Bacillus* species combined with PGPR-characterized strains play role in increasing the crop biomass [49,50]. [51] reported the role of *Bacillus* species in increasing the dry weight of corn. [52] reported that maize plants inoculated with PGPR strain increased about 2-3 folds of maize biomass. The present study is comparable with the findings of literature where the dry weight of test strains increased around 5 times for tomato and on average 1.7 folds for cauliflower.

5. CONCLUSION:

In conclusion, a diverse range of gram-positive and gram-negative bacteria were isolated from the carposphere of *P. persica.* More than half the proportion of the isolates belonged to *Klebsiella* genera which are considered noxious for human health. One-fourth of the bacteria belonged to *Staphylococcus* genera indicating most of them were skin commensals. The plant growth-promoting testing of the isolates exhibited a positive response in terms of auxin production, increasing biomass weight, and root and shoot length. The auxin production test from the bacterial strains is representative of the fact that if the tomato and cauliflower seeds are allowed to mix with the suspension of said strains; they can be used as biofertilizers rather than using synthetic chemical fertilizers.

6. REFERENCES

1. Jideani AI, Silungwe H, Takalani T, Omolola AO, Udeh HO, Anyasi TA. Antioxidant-rich natural fruit and vegetable products and human health. Int. J. Food Prop. 2021;24(1):41-67.
2. Buck JW, Walcott RR, Beuchat LR. Recent trends in microbiological safety of fruits and vegetables. Plant Health Prog. 2003;4(1):25.
3. Leff JW, Fierer N. Bacterial communities associated with the surfaces of fresh fruits and vegetables. PloS one. 2013;8(3):e59310.
4. Mostafidi M, Sanjabi MR, Shirkhan F, Zahedi MT. A review of recent trends in the development of the microbial safety of fruits and vegetables. Trends Food Sci. Technol. 2020;103:321-32.
5. Liu H, Li Z, Zhang X, Liu Y, Hu J, Yang C, Zhao X. The effects of ultrasound on the growth, nutritional quality and microbiological quality of sprouts. Trends Food Sci. Technol. 2021;111:292-300.
6. Rincón VM, Neelam DK. An overview on endophytic bacterial diversity habitat in vegetables and fruits. Folia Microbiol. 2021;66(5):715-25.

UNDER PEER REVIEW

7. Aworh OC. Food safety issues in fresh produce supply chain with particular reference to sub-Saharan Africa. Food Control. 2021;123:107737.
8. Vieths S. Allergens in fruits and vegetables. InHandbook of Plant and Fungal Toxicants. CRC press; 2020 (pp. 157-174).
9. Yeni F, Yavaş S, Alpas HA, Soyer YE. Most common foodborne pathogens and mycotoxins on fresh produce: a review of recent outbreaks. Crit. Rev. Food Sci. Nutr. 2016;56(9):1532-44.
10. Doyle MP, Diez-Gonzalez F, Hill C, editors. Food microbiology: fundamentals and frontiers. John Wiley & Sons; 2020.
11. Holden N. How can plant pathology help in the control of human pathogens associated with edible crop plants?. InPlant diseases and food security in the 21st century. Springer, Cham; 2021 (pp. 259-275).
12. Jiang W, Paudel SK, Amarasekara NR, Zhang Y, Etienne X, Jones L, Li K, Hansen F, Jaczynski J, Shen C. Survey of small local produce growers' perception of antibiotic resistance issues at farmers markets. Food Control. 2021;125:107997.
13. Betbui BT, Masse MT, Bougnom BP. Antibiotic enteric resistant bacteria are abundant on lettuce from urban agriculture in the city of yaounde. Int. J. Trend Res. Dev. 2021;5:708-712.
14. Buchholz F, Kostić T, Sessitsch A, Mitter B. The potential of plant microbiota in reducing postharvest food loss. Microb. Biotechnol. 2018;11(6):971-5.
15. Raheem A, Ali B. The Microphenotron: a novel method for screening plant growth-promoting rhizobacteria. PeerJ. 2022;10:e13438.
16. Gusain P, Bhandari BS. Rhizosphere associated PGPR functioning. J. Pharmacogn. Phytochem. 2019;8(5):1181-91.
17. Kabbara S, Bidon B, Kilani J, Osman M, Hamze M, Stock AM, Papon N. Cytokinin sensing in bacteria. Biomolecules. 2020;10(2):186.
18. Liu W, Wang Q, Hou J, Tu C, Luo Y, Christie P. Whole genome analysis of halotolerant and alkalotolerant plant growth-promoting rhizobacterium Klebsiella sp. D5A. Sci. Rep. 2016;6(1):1-0.
19. Kim AY, Shahzad R, Kang SM, Seo CW, Park YG, Park HJ, Lee IJ. IAA-producing Klebsiella variicola AY13 reprograms soybean growth during flooding stress. J Crop Sci Biotechnol. 2017;20(4):235-42.
20. Khalifa AY, Alsyeeh AM, Almalki MA, Saleh FA. Characterization of the plant growth promoting bacterium, Enterobacter cloacae MSR1, isolated from roots of non-nodulating Medicago sativa. Saudi J. Biol. Sci. 2016;23(1):79-86.
21. Gupta V, Kumar GN, Buch A. Colonization by multi-potential Pseudomonas aeruginosa P4 stimulates peanut (Arachis hypogaea L.) growth, defence physiology and root system functioning to benefit the root-rhizobacterial interface. J. Plant Physiol. 2020;248:153144.

22. Khare E, Tyagi S, Patil KS. Language of plant-microbe-microbe interactions in rhizospheric ecosystems. InMolecular aspects of plant beneficial microbes in agriculture. Academic Press; 2020 (pp. 59-76).
23. Kumar M, Kumar P, Das P, Solanki R, Kapur MK. Proactive role of Streptomyces spp. in plant growth stimulation and management of chemical pesticides and fertilizers. Int. J. Sci. Environ. 2021:1-20.
24. Patten CL, Glick BR. Regulation of indoleacetic acid production in Pseudomonas putida GR12-2 by tryptophan and the stationary-phase sigma factor RpoS. Can. J. Microbiol. 2002;48(7):635-42.
25. Kapli P, Yang Z, Telford MJ. Phylogenetic tree building in the genomic age. Nat. Rev. Genet. 2020;21(7):428-44.
26. Mumtaz MZ, Ahmad M, Jamil M, Hussain T. Zinc solubilizing Bacillus spp. potential candidates for biofortification in maize. Microbiol. Res. 2017;202:51-60.
27. Sehrawat A, Sindhu SS, Glick BR. Hydrogen cyanide production by soil bacteria: Biological control of pests and promotion of plant growth in sustainable agriculture. Pedosphere. 2022;32(1):15-38.
28. Cavalcante da Silva MJ, Palmeira SF, Fortes K, Nascimento VX, de Medeiros AS, Cavalcanti da Silva SJ, de Sousa Alves MM, Sant'Ana AE. IAA production of indigenous isolate of plant growth promoting rhizobacteria in the presence of tryptophan. Aust. J. Crop Sci. 2020;14(3):537-44.
29. Yadav AN. Beneficial plant-microbe interactions for agricultural sustainability. J. Appl. Biol. 2021;9(1):i-v.
30. Richter L, Du Plessis EM, Duvenage S, Korsten L. Occurrence, phenotypic and molecular characterization of extended-spectrum-and AmpC-β-Lactamase producing Enterobacteriaceae isolated from selected commercial spinach supply chains in South Africa. Front. Microbiol. 2020;11:638.
31. Cavalcante da Silva MJ, Palmeira SF, Fortes K, Nascimento VX, de Medeiros AS, Cavalcanti da Silva SJ, de Sousa Alves MM, Sant'Ana AE. IAA production of indigenous isolate of plant growth promoting rhizobacteria in the presence of tryptophan. Aust. J. Crop Sci. 2020;14(3):537-44.
32. Idaszkin YL, Polifroni R, Mesa-Marín J. Isolation of Plant Growth Promoting Rhizobacteria from Spartina densiflora and Sarcocornia perennis in San Antonio polluted salt marsh, Patagonian Argentina. Estuar. Coast. Shelf Sci. 2021;260:107488.
33. Anwar S, Ali B, Sajid I. Screening of rhizospheric actinomycetes for various in-vitro and in-vivo plant growth promoting (PGP) traits and for agroactive compounds. Front. Microbiol. 2016;7:1334.
34. Shafi J, Mingshan J, Zhiqiu Q, Xiuwei L, Zumin G, Xinghai L, Yang Z, Peiwen Q, Hongzhe T, Wunan C, Kai W. Optimization of Bacillus aerius strain JS-786 cell dry mass and its antifungal activity against Botrytis cinerea using response surface methodology. Arch. Biol. Sci. 2017;69(3):469-80.
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35. Kloos WE, Schleifer KH, Smith RF. Characterization of Staphylococcus sciuri sp. nov. and its Subspecies1. Int. J. Syst. Evol. 1976 ;26(1):22-37.
36. Dutta A, Ghosh S, Choudhury JD, Mahansaria R, Roy M, Ghosh AK, Roychowdhury T, Mukherjee J. Isolation of indigenous Staphylococcus sciuri from chromium-contaminated paddy field and its application for reduction of Cr (VI) in rice plants cultivated in pots. Bioremediation Journal. 2017;21(1):30-7.
37. Rodríguez-Medina N, Barrios-Camacho H, Duran-Bedolla J, Garza-Ramos U. Klebsiella variicola: an emerging pathogen in humans. Emerg. Microbes & Infect. 2019;8(1):973-88.
38. Berde, C.V., Salvi, S.P., Rawool, P.P., Prathyusha, A.M.V.N. and Berde, V.B. Role of medicinal plants and endophytic bacteria of medicinal plants in inhibition of biofilm formation: interference in quorum sensing. In implication of quorum sensing and biofilm formation in medicine, agriculture and food industry. Springer, Singapore; 2019 (pp. 177-188).
39. Sagar A, Shukla PK, Sayyed RZ, Ramteke PW. stimulation of seed germination and growth parameters of rice var. Sahbhagi by Enterobacter cloacae in the presence of ammonium sulphate as substitute of ACC. InPlant growth promoting rhizobacteria (pgpr): prospects for sustainable agriculture. Springer, Singapore; 2019 (pp. 117-124).
40. Chamedjeu RR, Joel M, Viviene M, Steven R. Potential use of soil bacteria associated with potato rhizosphere as bio-control agents for effective management of bacterial wilt disease. J. Microbiol Res. 2019;9:12-24.
41. Vaikuntapu PR, Dutta S, Samudrala RB, Rao VR, Kalam S, Podile AR. Preferential promotion of Lycopersicon esculentum (Tomato) growth by plant growth promoting bacteria associated with tomato. Indian J. Microbiol. 2014;54(4):403-12.
42. Fu SF, Wei JY, Chen HW, Liu YY, Lu HY, Chou JY. Indole-3-acetic acid: A widespread physiological code in interactions of fungi with other organisms. Plant Signal. Behav. 2015;10(8):e1048052.
43. Raheem A, Shaposhnikov A, Belimov AA, Dodd IC, Ali B. Auxin production by rhizobacteria was associated with improved yield of wheat (Triticum aestivum L.) under drought stress. Arch. Agron. Soil Sci. 2018;64(4):574-87.
44. Bukhat S, Imran A, Javaid S, Shahid M, Majeed A, Naqqash T. Communication of plants with microbial world: Exploring the regulatory networks for PGPR mediated defense signaling. Microbiol Res. 2020;238:126486.
45. Meena M, Swapnil P, Divyanshu K, Kumar S, Tripathi YN, Zehra A, Marwal A, Upadhyay RS. PGPR-mediated induction of systemic resistance and physiochemical alterations in plants against the pathogens: Current perspectives. J. Basic Microbiol. 2020;60(10):828-61.
46. Jabborova D, Enakiev Y, Sulaymanov K, Kadirova D, Ali A, Annapurna K. Plant growth promoting bacteria Bacillus subtilis promote growth and physiological parameters of Zingiber officinale Roscoe. Plant Sci. Today. 2021;8(1):66-71.

47. Gowtham HG, Singh SB, Niranjana SR. Evaluation of plant growth promoting ability of Providencia spp. collected from North Eastern Region of India in Crucifers. International Res. J. Agric. Sci. 2015;5:321-8.
48. Zhou W, Qin S, Lyu D, Zhang P. Soil sterilisation and plant growth-promoting rhizobacteria promote root respiration and growth of sweet cherry rootstocks. Arch. Agron. Soil Sci. 2015;61(3):361-70.
49. Sadiq A, Ali B. Growth and yield enhancement of'Triticum aestivum'L. by rhizobacteria isolated from agronomic plants. Aust. J. Crop Sci. 2013;7(10):1544-50.
50. Korir H, Mungai NW, Thuita M, Hamba Y, Masso C. Co-inoculation effect of rhizobia and plant growth promoting rhizobacteria on common bean growth in a low phosphorus soil. Front. Plant Sci. 2017;8:141.
51. Batista BD, Lacava PT, Ferrari A, Teixeira-Silva NS, Bonatelli ML, Tsui S, Mondin M, Kitajima EW, Pereira JO, Azevedo JL, Quecine MC. Screening of tropically derived, multi-trait plant growth-promoting rhizobacteria and evaluation of corn and soybean colonization ability. Microbiol Res. 2018;206:33-42.
52. Khati P, Chaudhary P, Gangola S, Bhatt P, Sharma A. Nanochitosan supports growth of Zea mays and also maintains soil health following growth. 3 Biotech. 2017;7(1):1-9.

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