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CHARACTERIZATION OF PLANT GROWTH PROMOTING RHIZOSPHERE BACTERIA FROM HUANGLONGBING (HLB) ESCAPE CITRUS TREES IN PAKISTAN

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Abstract

Huanglongbing (HLB), also known as citrus greening disease, caused by the bacterium *Candidatus* Liberibacter spp (*C*Las) is one of the most devastating diseases affecting citrus plants worldwide. *C*Las is a well-studied bacterium, but the role of microbiome's in HLB development and progression remains unexplored in Pakistani citrus orchards. This study aimed to investigate and profile the microbiomes associated with HLB escape in Pakistani citrus rhizosphere. Ten different bacterial strains were isolated from HLB free rhizosphere by biochemical screening. Bacterial isolates belonged to the class of Firmicutes and Proteobacteria (gamma Proteobacteria) including *Pseudomonas, Enterobacter, Proteus, Citrobacter* and *Serratia*. Analysis of the bacterial diversity abundance revealed that *Pseudomonas* (82%), *Bacillus* (75%) and *Serratia* (69%) were the most abundant bacteria in citrus rhizosphere. The isolated strains were studied for plant growth promoting activities including indole acetic acid (IAA) production, sidophore production, and phosphate solubilization. On the basis of these PGP activities the isolated strains showed positive results highest activities were shown by *Bacillus safensis, Bacillus subtilis Pseudomonas putida, Serratia plymutica*. Our findings reveal that all these PGPR bacteria are beneficial help to reduce the population of viable Ca. L. asiaticus in HLB diseased tree if used as a biofertilizer. This research work provides a foundation for understanding the interaction between the PGPR and Huanglongbing (HLB) in citrus in Pakistan, paving the way for novel disease management strategies and also improving citrus production in the region.

Keywords: Citrus, Huanglongbing (HLB), Rhizobacteria, PGPRs, Diseaese.

INTRODUCTION

Citrus is a major fruit among all fruit varieties grown in Pakistan. It accounts approximately 30% of the country's total fruit production (Shah et al., 2022). About 95% of citrus is produced in Punjab province (Siddique & Garnevska, 2018), with over 90% cultivated in the Sargodha region (TDAP, 2022). In Pakistan huanglongbing (HLB) tops the list among all the bacterial and viral diseases of citrus (Cuenca et al., 2019). The Asiatic form of HLB (*C*Las) is widespread in Pakistan and cause a major risk for citrus industry. Hence, there is a dire need to overcome this issue and control this disease with an acceptable solution (Li et al., 2020). In recent years, HLB has become uncontrollable and is has emerged as the most devastating diseases of citrus crop. Different management and treatment strategies have been adopted so far to overcome this disease in major citrus growing areas, but still, an effective long-term treatment remains elusive (Gottwald, 2017).

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Microbiome of plant has a significant effect on plant productivity and health (van der Heijden & Schlaeppi, 2015). It is also well known that the plant microbiome stimulates or act as a first line of defense against a variety of diseases and insects. The plant microbiome is crucial to the development of soils that prevent disease (Riseh & Vazvani, 2024). Manipulation of microbiomes from citrus tree helps to improve plant health and production (Xu et al., 2023). It is thought that manipulating plant microbiomes could prevent the spread of plant diseases, increase the plant productivity, and reduce the need for chemical inputs, resulting in more environment friendly farming practices (Ray et al., 2020).

To enhance the crop production without the use of fertilizers, researchers have explored the soil dwelling bacteria (Glick, 2012; Lwin et al., 2012; Majeed et al., 2015). Diverse Plant growth-promoting bacteria are present naturally in soil habitat of different plants, that promotes the plant health and develop various mechanism to prevent plant from diseases (Bashan et al., 1993; Kloepper & Schroth, 1981). Rhizobacteria PGPR promote plant growth through a variety of direct and indirect mechanisms (Backer et al., 2018; Rahman et al., 2010). Production of ammonia, solubilization of mineral phosphates, IAA production and siderophore production are the direct mechanism of rhizobacteria to increase plant growth (Asha et al., 2015; Glick, 2012; Majeed et al., 2015).

The ability of plant to endure biotic and abiotic stresses by developing plant resistant mechanism are the primary function of soil associated bacteria that release the adequate quantities of auxin into the soil (Costa-Gutierrez et al., 2020; Glick, 2012; Spaepen et al., 2007). Indole-3-acetic acid (IAA) produced from L-tryptophan is the primary pathway for bacterial IAA synthesis in the rhizosphere. Many soil microorganisms are now known to convert small amounts of plant-derived L-tryptophan into IAA (Zhao, 2010)). Therefore, IAA is known not only as primary hormone in plants but also researchers used IAA production as an important criterion for the selection of PGP rhizobacteria.

Phosphorus (P) deficiency causes adverse effect and hinders the plant growth, hence, it is a vital nutrient to promote plants productivity. Majority of the fertilizer's phosphorus are immobile in the soil while plant can absorb only a small amount of it. PGPR play a significant role in mobilizing both the fixed inorganic phosphate and the organic phosphate pools (Gaind & Gaur, 1989; Khan et al., 2010; Taher et al., 2019), thus decreasing the need for phosphatic fertilizers in crop production. These bacteria utilize various mechanisms, such as the production of organic acids and phosphatase enzymes, to solubilize phosphate.

In addition to IAA production and phosphate solubilization, the synthesis of siderophores by plant growth-promoting rhizobacteria (PGPR) is a critical trait. This is due to the significant role that the interaction between the rhizosphere and microbial communities plays in iron-deficient soils, where the secretion of iron-chelating compounds, such as siderophores, becomes essential (Dertz et al., 2006). While plant roots do secrete siderophores to regulate iron levels necessary for their metabolic and physiological processes in iron-stressed, degraded soils, this mechanism alone often falls short of meeting optimal iron requirements (Herlihy et al., 2020). Conversely, siderophore-

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producing microbes (SPM) are capable of generating a variety of iron-chelating compounds, thereby mitigating plant stress in iron-deficient conditions. Moreover, the nitrogen-fixing ability of PGPR is another crucial mechanism for enhancing plant growth and is commonly employed as a key criterion in the selection of these beneficial microbes (Alam, 2014).

Therefore, these PGP trait assay plays an important role for the identification of beneficial and pathogenic bacteria by targeting different crop plants. It is hypothesized that the use of plant microbe as a biological control might be helpful for the suppression of plant diseases. Culture media screening, biochemical testing and molecular techniques are the standard approaches used for detection of broad range of microbiomes (Franco-Duarte et al., 2019). Rhizosphere of every plant species are the hub of plant growth promoting and pathogenic bacteria. Therefore, it is essential to first identify the appropriate PGPR species for the rhizosphere of the particular plant. The present study was conduct for the isolation, identification and characterization of soil associated PGP bacteria from HLB free citrus cultivars in Pakistan.

MATERIAL AND METHODS

Morphological identification of HLB in citrus cultivars

Morphologically characterization of citrus cultivar was done by visual symptoms of citrus greening on tree, fruit and leaves. On the basis of symptomology of disease healthy and HLB free trees were selected. Healthy plants showed vigorous appearance with defined symmetrical canopy having no sign of HLB abnormal discolored fruit and leaves.

Sample collection

Samples were collected from citrus trees from the “Citrus Research Institute, Sargodha, Pakistan”. Fifteen samples were collected from different cultivars covering mandarin, sweet oranges and rootstock group of citrus. With the help of sterilized spatula, 50g of soil samples (along with root extensions) were collected within the depth of 10 to 15 cm by dividing the area in quadrants. Samples were collected in sterile polythene bags to avoid any contamination and placed in an ice box to prevent any degradation of microbes (Al-Saadi et al., 2018; Rettke, 2019).

Microbial isolation from soil sample

The soil bacteria were isolated by serial dilution technique on nutrient agar media by using spreading and streaking methods. Almost 1 g of dried soil sample was suspended in 9 ml of sterile saline water and mix thoroughly by vortexing. Bacteria were isolated from soil by centrifugation at 50 rpm for 30 minutes. Spread plate method was used for the isolation of bacteria from soil sample. 0.1ml of soil suspension from the 10^{-4} dilution was spread on nutrient agar plates and incubated at 37°C for 18-24 hours. All the experiment were done in triplicate.

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Morphological and biochemical identification of bacterial isolates

Screening of isolated bacteria was done by using morphological and biochemical techniques. Morphological screening was done by observing different features of isolated bacteria such as, colony shape, color, size, margins and elevation of colonies while biochemical screening was done by recording the biochemical nature of isolates such as, gram staining, spore type, capsule type, motility and other biochemical test.

Assessment of plant growth promotion traits

All the isolated bacteria were tested for plant growth promoting traits which include, phosphate solubilization and indole acidic acid (IAA) production.

Phosphate solubilization

The phosphate solubilizing activity of isolated bacterial strains was assessed using a medium with tricalcium orthophosphate (TCP). The method was followed Nautiyal (1999) to qualitatively evaluate the phosphate solubilization. Inoculated media plates were incubated at 30±1°C for four days to test the bacterial strain's ability to solubilize phosphate. Clear halos around the bacterial colonies after incubation were the indication of positive phosphate solubilization (Amri et al., 2023; Nautiyal, 1999).

IAA production

IAA production by bacterial strains was quantified using a modified method from Patten and Glick (2002). Bacteria were grown overnight in 5 mL of Tryptic Soy Broth, with or without tryptophan (500 µg/mL), and incubated at 37°C for 24 hours. After centrifugation, 1 mL of the supernatant was mixed with 4 mL of Salkowski's reagent (Gang et al., 2019; Gordon & Weber, 1951) and incubated at room temperature for 20 minutes. Absorbance was measured at 535 nm using a UV spectrophotometer, and IAA concentration was determined using a standard curve with pure IAA (Sigma-Aldrich) ranging from 0.01 to 0.4 µg/mL.

Production of Siderophore

Siderophore production by the microbes was detected using Universal Chrome-Azurol S (CAS) agar medium. The isolated bacterial samples were applied to CAS agar plates and incubated for 48 hours. Following incubation, the plates were examined for distinct zone with the appearance of orange color around the bacterial spots indicating the production of siderophore. Orange zone appearance clearly demonstrates siderophore production (Srimathi & Suji, 2018).

RESULTS

Morphological assessment of HLB escape citrus tree

Trees were morphologically categorized as either healthy or HLB symptomatic. Healthy citrus trees showed no signs of citrus greening and were characterized by a robust and vigorous appearance, with a strong central trunk and a well-branched, symmetrical canopy. The leaves were glossy, smooth, uniform in size and shape, and free from

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disease lesions, spots, or abnormal discoloration. Healthy trees exhibited regular, vigorous growth with balanced canopies and no excessive leaning. Fruits of healthy tree bore uniform, well-formed, and evenly distributed fruit. While, HLB symptomatic trees showed stunted growth, reduced canopy, decline in canopy and vigor, twig dieback, yellowing foliage, starting on individual branches or shoots and spreading throughout the canopy (figure 1). The yellowing appeared irregular and blotchy, with patches of yellow interspersed with normal green areas. Leaves were asymmetrical, often showing curling, twisting, or cupping, along with mottling or blotching in irregular light and dark green patterns (figure 2). Infected trees exhibited premature misshapen small, lopsided, poorly colored, and often with an off-flavor fruit (figure 3).

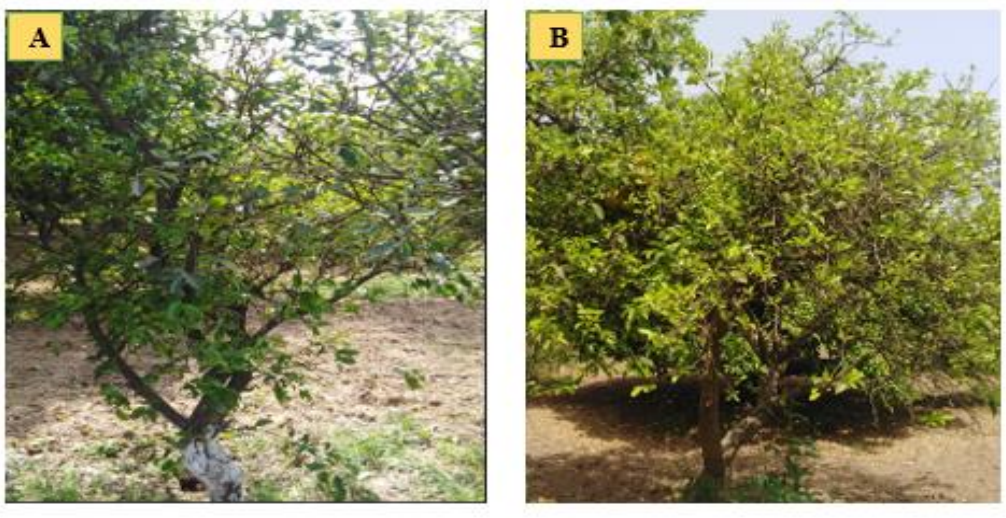

Figure 1: HLB infected citrus tree with yellowing of leaves, reduced tree canopy and stunted growth

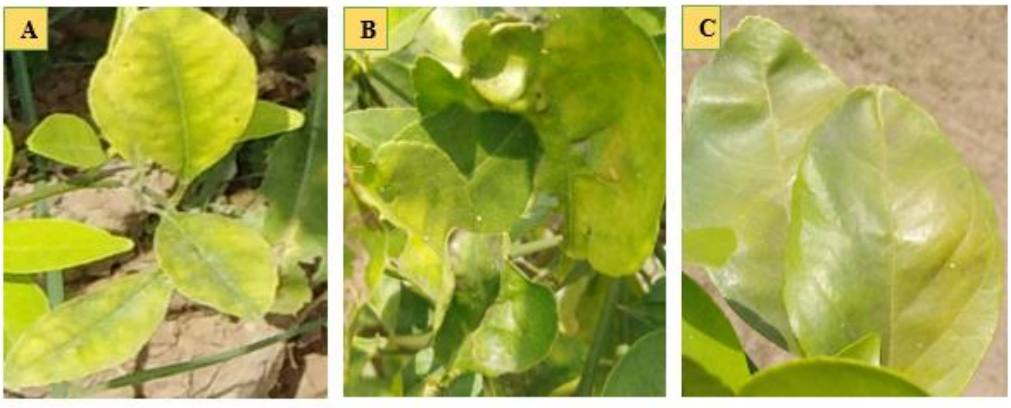

Figure 2: (A) Yellowish midrib (B) asymmetrical blotchy mottling leaves (C) color inversions and yellowing of leaves

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Figure 3: HLB symptomatic misshapen and mature oblong shaped fruits with color inversion

Citrus sampling

After verification of the occurrence of HLB disease in citrus cultivars, 15 samples were included in this study covering major groups of citrus i.e., sweet oranges, rootstock and mandarine. The details of samples are described in Table 1.

Table 1: Samples collection of HLB symptomatic and asymptomatic of citrus varieties

Sr. No	Citrus Groups	Variety Name	Sample Type	Location	Latitude	Longitude
			Soil			
1.		Nova	✓	CRI, Sargodha	32°07'03"N	72°40'37"E
2.		Feuterell's early	✓	CRI, Sargodha	32°07'03"N	72°40'37"E
3.	**Mandarine**	Kinnow	✓	CRI, Sargodha	32°07'03"N	72°40'37"E
4.		Seedless Kinnow	✓	CRI, Sargodha	32°07'03"N	72°40'37"E
5.		Pearl	✓	CRI, Sargodha	32°07'03"N	72°40'37"E
6.		Musambi	✓	CRI, Sargodha	32°07'03"N	72°40'37"E
7.		Washinton Navol	✓	CRI, Sargodha	32°07'03"N	72°40'37"E
8.	**Sweet Oranges**	Pineapple	✓	CRI, Sargodha	32°07'03"N	72°40'37"E
9.		Midsweet	✓	CRI, Sargodha	32°07'03"N	72°40'37"E
10.		Navellina	✓	CRI, Sargodha	32°07'03"N	72°40'37"E
11.		Benton	✓	CRI, Sargodha	32°07'03"N	72°40'37"E
12.		Cleopetra	✓	CRI, Sargodha	32°07'03"N	72°40'37"E
13.	**Root Stock**	Sour Orange	✓	CRI, Sargodha	32°07'03"N	72°40'37"E
14.		Gadadehi	✓	CRI, Sargodha	32°07'03"N	72°40'37"E
15.		Rough Lemon	✓	CRI, Sargodha	32°07'03"N	72°40'37"E

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Morphological identification of isolated Bacterial strains

The isolated colonies were visually examined, and ten distinct bacterial strains were identified based on their morphological characteristics. These characteristics included colony color, texture, shape, opacity, margins, and elevation. The isolates displayed varying features: for example, *Enterobacter aerogenes* typically exhibited white, smooth, shiny, circular, and opaque colonies with entire margins. In contrast, *Serratia fonticola* was characterized by pink to deep red, smooth, round, raised, opaque colonies with irregular margins. The detailed of each bacterial strain was described in table 2 while, pure colonies of these bacteria are shown in figure 4.

Table 2: Showed the colony morphology characters of isolated bacterial strains

Bacterial Isolate	Colony color	Colony texture	Shape	Margins	Elevation	Opacity
Enterobacter aerogenes	White	Smooth and Shiny	Circular	Entire	Convex	Opaque
Citrobacter freundii	Light gray	Smooth and glossy	Round	Entire	convex	Translucent to opaque
Serratia fonticola	Pink to deep red	Smooth	Round	Irregular	Raised	Opaque
Pseudomonas putida	Colorless	Smooth and shiny	Round	Entire	Umbonate	Translucent
Serratia plymuthica	Pink or reddish pigment	Smooth and glistening	Circular	Entire	Raised	opaque
Bacillus safensis	Creamy white to yellow	Mucoid	Circular	Entire	Flat	opaque
Bacillus subtilis	Dirty white	Smooth and shiny	Round	Wavy	Concave	Opaque
Proteus mirabilis	Dirty white	Smooth and shiny	Round	Wavy	concave	Opaque
Enterobacter cloacae	Dirty off white	Smooth	Round	Wavy	Raised	Opaque
Pseudomonas aeruginosa	Dirty off white	Smooth	Round	Wavy	Concave	Opaque

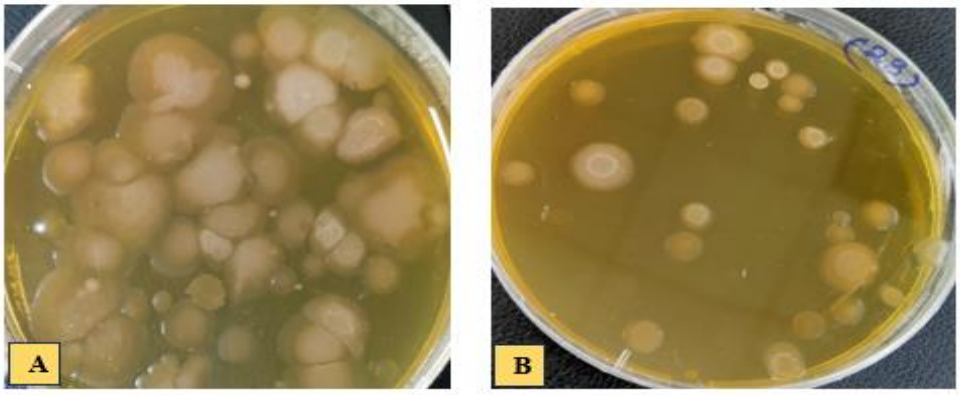

Fig 4: Isolation of bacteria strains from citrus cultivars

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Biochemical characterization of isolated bacterial strains

Out of ten isolated bacterial strains, two isolates were gram positive and spore forming while, remaining was gram negative and non-spore forming. According to cell shape all the isolates were rod shaped in nature. The identified bacteria belonged to different genus i.e. *Serratia, Enterobacter, Citrobacter, Bacillus,* and *Pseudomonas.* The detail of biochemical test results was explained in table 3.

Table 3: Showed the biochemical characters of isolated bacterial strains

Bacterial Isolates	Biochemical Test											
	Cell shape	Gram type	Spore type	Capsule stain	Motility test	Indole test	Methyl red test	Citrate utilization test	Hydrogen sulphide test	Nitrate reduction test	Oxidase test	Catalase test
Enterobacter aerogenes	rod	-ve	-ve	+ve	+ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve
Citrobacter freundii	Straight rods	-ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve
Serratia plymuthica	rod	-ve	-ve	-ve	+ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve
Pseudomonas putida	rod	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve
Serratia fonticola	rod	-ve	-ve	-ve	+ve	-ve	+ve	+ve	-ve	+ve	-ve	+ve
Bacillus safensis	rod	+ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve
Pseudomonas aeruginosa	rod	-ve	-ve	-ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve
Proteus mirabilis	rod	-ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve
Bacillus subtilis	rod	+ve	+ve	-ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve
Enterobacter cloacae	rod	-ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve	+ve

Characterization of isolated bacteria based on plant growth promoting (PGP) traits

All the isolated bacterial strains showed encouraging results for plant growth promoting activities. Phosphate solubilization results showed that out of 10 isolates CB-47, CB-14, CB-77 and CB-39 showed the development of clear halo zone of about 5-8mm while CB-2, CB-33, CB-65, CB-17, CB-30 and CB-7 displayed phosphate solubilization activity with zone diameter (2-3mm to 2-5 mm).

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For Indole acetic acid (IAA) production, the bacterial strains showed highest concentration of IAA production in the presence of tryptophan, but the production of IAA was found comparatively low without tryptophan. All the tested strains showed positive results for IAA production but the highest concentration was found in CB-47, CB-77, CB-17, CB-39 and CB-14.

Great variation was observed in the IAA production capacity among tested isolates. All the bacterial strains showed positive results for ammonia production but CB-47 and CB-77 highest ability to produce ammonia.

The tested isolates also showed positive results for siderophore production but the highest zone size (0.5 to 0.15mm) was observed in *Bacillus* and *Pseudomonas* genus. The details of all the tested isolates and PGP trait assay was described in table 4.

Table 4: Plant growth stimulating traits of tested bacterial isolates

Strain ID	Bacterial Isolates	Phosphorus Solubilization	IAA production (µg mL^{-1})		Ammonia Production	Siderophore production
			With tryptophan	Without tryptophan		
C	Control	-ve	1.53	0.26	-ve	-ve
CB-2	*Enterobacter aerogenes*	+ve	15.34	7.13	+ve	+ve
CB-33	*Citrobacter freundii*	+ve	16.81	6.29	+ve	+ve
CB-77	*Serratia plymuthica*	+ve	40.76	10.64	+ve	+ve
CB-47	*Pseudomonas putida*	+ve	39.58	11.10	+ve	+ve
CB-65	*Serratia fonticola*	+ve	18.34	6.82	+ve	+ve
CB-39	*Bacillus safensis*	+ve	31.58	9.01	+ve	+ve
CB-17	*Pseudomonas aeruginosa*	+ve	17.52	7.56	+ve	+ve
CB-14	*Bacillus subtilis*	+ve	35.52	9.46	+ve	+ve
CB-30	*Proteus mirabilis*	+ve	18.52	6.50	+ve	+ve
CB-7	*Enterobacter cloacae*	+ve	17.42	4.43	+ve	+ve

Biodiversity of Plant growth promoting rhizobacteria (PGPR)

These bacterial isolates belonged to the class of Firmicutes and Proteobacteria (gamma Proteobacteria). Mostly predominant genera was Firmicutes including genus *Bacillus* (20%) while, Proteobacteria included genus *Pseudomonas* (20%), *Enterobacter* (20%), *Proteus* (10%), *Citrobacter* (10%) and *Serratia* (20%). Analysis of the bacterial diversity abundance among citrus cultivar at the genus level revealed that *Pseudomonas* (82%), *Bacillus* (75%) and *Serratia* (69%) were the most abundant bacteria in citrus rhizosphere. According to the results, *Enterobacter aerogenes* was only found in seedless kinnow and Midsweet, while *Citrobacter freundii* was found in pearl, Musambi and pineapple cultivar. However, the overall population of *Pseudomonas, Serratia* and *Bacillus* was more in citrus varieties as compared to other bacteria species. Variety wise distribution of bacteria at genus and species level in all the citrus cultivars is shown in Figure 5.

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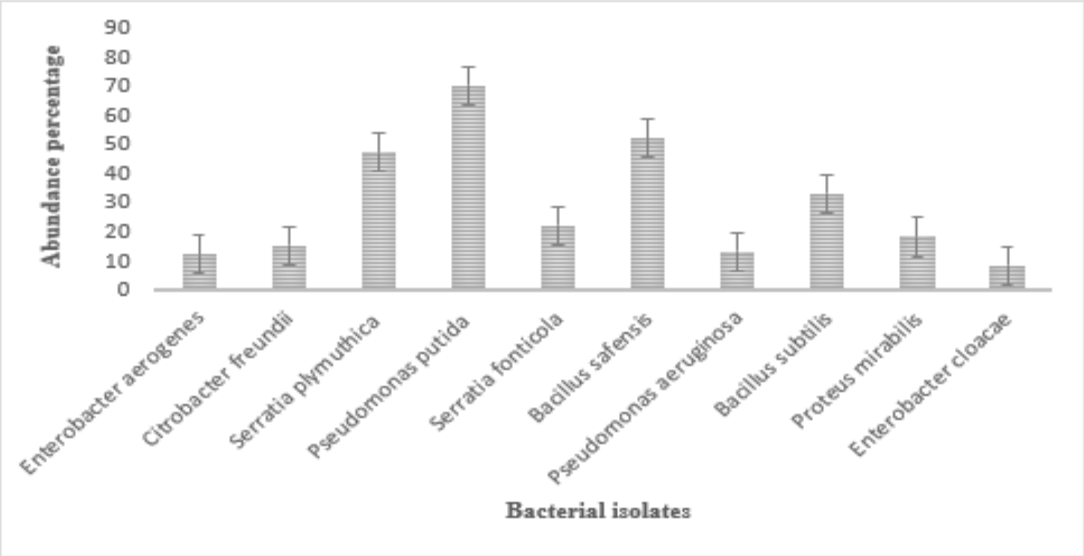

Figure 5: Frequency distribution of bacteria isolated from different citrus cultivars

DISCUSSION

The Pakistani citrus industry is endangered by Huanglongbing (HLB), also known as citrus greening disease, showing severe long-term economic impact and adverse effects on tree health, fruit production, and overall economic returns (Makam et al., 2023). Considering the significance of citrus in Pakistan and the impact of HLB in citrus-growing regions, this study was conducted to isolate, characterize, and identify bacterial strains from the soil of HLB-free citrus plants. The goal was to identify plant growth-promoting bacteria that could potentially be used as biofertilizers to help prevent citrus greening in Pakistan.

In this study a total of 10 rhizobacteria were isolated from different citrus cultivars from citrus rhizosphere taking the soil sample. Initial colony morphology identification showed a vast variety of bacteria where almost 10 different types of strains were identified from 15 different cultivars of citrus which were subjected to biochemical characterization. Similar method of colony counting and identification was used by (Ezrari et al., 2021; Trivedi et al., 2011). Predominant genera of bacterial isolates belonged to Firmicutes (20%) including genus *Bacillus* while Proteobacteria (80%) including genus *Pseudomonas, Enterobacter, Proteus, Citrobacter* and *Serratia* were also found.

One of the key objective of current study was to identify the beneficial bacterial strains which could be either plant growth promoting, or otherwise help in HLB escape or can help to reduce the severity of HLB infection or can promote the overall citrus tree health. Phosphate solubilization, indole-3-acetic acid (IAA) production, siderophore and ammonia production are crucial components of the plant growth-promoting rhizobacteria (PGPR) groups (Swarnalakshmi et al., 2020).

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So, in this study plant growth promoting aspects of the bacteria were examined i.e., Indole acetic acid production, phosphate solubilization as well as siderophore production by the selected strains. All isolated bacteria showed positive results for Phosphorus solubilization, siderophore production, higher levels of IAA production (µg mL^{-1}) as compared to control. *Serratia plymuthica* showed the highest level of IAA production (in presence of tryptophan) with a value of 40.76 µg mL^{-1} followed by *Pseudomonas putida* (39.58 µg mL^{-1}) and *Bacillus safensis* (31.58 µg mL^{-1}). Alemneh *et al*., 2021 also did a similar study with 841 rhizobacteria belonging to 15 genera of phosphate solubilizing bacteria (PSB) capable of producing different levels of IAA in presence of tryptophan (Alemneh et al., 2021).

There are many other reports that enhanced IAA production is linked to P-solubilizing ability of rhizobacteria which directly relate to their role as PGPRs (Dutta & Thakur, 2017; Govindasamy et al., 2017). In our study we found the highest level of IAA up to 40.76 µg mL^{-1} whereas some studies have reported it up to 142.5 µg mL^{-1} under invitro conditions (Arruda et al., 2013; Ghosh et al., 2013). The biochemical qualities like phosphate solubilizing, siderophore, IAA production and ammonia production were studied for potential screening of potential PGPRs.

Siderophore-producing bacteria (SPB) produce siderophore and have activities of biofertilizers and bio-control for the plant; thus SPB acts as a signature for sustainable agriculture and is eco-friendly for crop production. Siderophore-producing microbes reduce Fe deficiency and enhance all physiological and biochemical processes of the plant (Sultana et al., 2021). Our research also explains that isolated isolates were also showed positive results for siderophore production but the highest zone size (0.5 to 0.15mm) was observed in *Bacillus, Serratia* and *Pseudomonas* genus which was related to the results of (Srimathi & Suji, 2018). These results showed that all the isolated straits have the ability for PGP bacteria, because these are isolated from those citrus trees that combat HLB disease.

The effectiveness of PGPB in disease management depends on various factors, including the specific strain of bacteria, the plant species, and environmental conditions. Research has shown that the benefits of PGPB can be maximized through careful selection of bacterial strains and application methods which could lead to disease escape in plants. Additionally, the establishment of PGPB in the rhizosphere and their persistence over time are critical for sustained disease management.

In this study we have reported the production of IAA, solubilization of mineral phosphate, and sidophore production from the isolated rhizobacteria, all of which are considered as primary criteria for selecting PGPR. Results have shown these PGPRs helping in HLB escape in citrus trees and improving plant growth parameters. Further, the application of these bacteria with plant growth promoting traits can be used to promote plant growth after evaluation of biofunctionalities under in vitro and in vivo conditions and detailed molecular characterization.

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