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The Emerging Potential of PGPRs as Biofertilizers for Growth Enhancement of Spinach

[a] **Sadia Bashir*** [a] **Hifza Marium,** [a] **Adeela Haroon**
a Department of Botany, The Women University Multan 66000, Pakistan.

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ABSTRACT

The quest for enhancing the production of leafy vegetables due to their high nutritional status has led to the inevitable use of hazardous agrochemicals leading to chief agricultural losses. Plant growth-promoting rhizobacteria (PGPR) being harmless biofertilizers can serve as suitable candidates for attaining the increasing demands of global agriculture. With the perspective to explore the bio efficiency of *Spinach oleracea* rhizobacteria, the study was designed utilizing rhizospheric bacteria of spinach. Initially, seventeen rhizobacteria (ST1, ST2, ST4, ST5, ST7, ST8, ST11, ST12, ST14, ST15, ST16, ST17, ST18, ST19, ST20, ST23, ST25) were isolated which were identified morphologically. Subsequent biochemical testing revealed their identification *as Bacillus spp, Clostridium spp, Corynebacterium spp, Lactobacillus spp* and *Mycobacterium spp*. The Screening of these isolates for multiple PGP traits exposed their potentialities. However, employment of most potential PGPR at two varieties (Desi palak and Lahori Palak) of spinach further confirmed their putative role in the growth enhancement of spinach. Therefore, current results suggest the significant application of spinach-associated rhizoflora as a safer and effective biofertilizer for extendable agricultural land.

Corresponding Author: Sadia Bashir
Email: sadia.6026@wum.edu.pk

INTRODUCTION

Spinach (*Spinacia oleracea*) is one of the internationally standard nutritional leafy vegetables belonging to the family Amaranthaceae (Naseem *et al*., 2023) with its subfamily of Chenopodioideae. Pakistan occupies 10th position worldwide for the production of spinach (Shah *et al.,* 2015; GoP, 2024). It has been considered as commonly cultivated vegetable producing total yield of 12588 kg per hectare in the country (Shaheen *et al.,* 2017; GoP, 2024). High nutritional status and great economic importance are the key features for the popularity of spinach in the nation. As it contains a high quantity of essential minerals and nutrients including vitamins, flavonoids, and folic acid (Liu *et al.,* 2015). In spinach, a high amount of vitamin K and calcium is also present which are used to strengthen the bones (Rejeki *et al*., 2023). Manganese present in spinach is essential for the regulation of blood sugar, calcium assimilation, and metabolism. Cooked spinach has a high amount of minerals, for example, iron, magnesium, copper, calcium, and potassium which get easily absorbed through the body (Patricia *et al.,* 2014; Miano, 2016). The green leaves of spinach have pharmacological and medicinal properties that are excellent for the health of humans. It not only improves the nutritional status in humans but also decreases the risk of many diseases such as human diabetes, among which the prominent ones are hepatotoxicity, and cancer (Fornaciari *et al.,* 2015). The use of spinach also decreases the threat of cardiovascular disease, strokes, hypertension (Ngo *et al.,* 2005; Rahati *et al.,* 2016). It is also helpful in the cure for tooth and respiratory disorders, gastrointestinal disorders, asthma (Aziz *et al.,* 2016; Khan *et al.,* 2018), acidosis, anaemia, constipation (Ugulu, 2012), pregnancy and urinary disorders (Shah *et al.,* 2015). One of the facts concerning the production of vegetables is that organically grown vegetables do not satisfy the appropriate demand for food. However, the employment of chemical fertilizers for the improved production of dietary vegetables has been a common practice on a worldwide scale (Stewart *et al*., 2005; Pan *et al.*, 2024). The frequent application of synthetic fertilizers not only increases the production rate but also influences the health and nutritional status of essentially required crops (Singh *et al*., 2001; Raman *et al*., 2022). The current scenario demands an alternate source of toxic chemical fertilizers, for safer production of crops. However, a large number of soil-borne microflora of the crops can serve a suitable purpose offering satisfactory nutritional requirements (Singh *et al*., 2022).

These soil-borne microflora include multifarious microbes either around the plant's root as rhizobacteria or exist inside the plant's tissues as called endophytic bacteria. These endophytic bacteria act as plant growth promoters (PGPB) imparting their role in growth promotion by adopting both direct and indirect mechanisms (Kalam *et al.,* 2017; Bashir *et al*., 2024). While implementing the direct means of growth improvement, these endophytes may produce multiple hormones such as auxin (Qi *et al.,* 2018; Khoso *et al*., 2024), cytokinin, gibberellins, ACC deaminase, and also contribute to nitrogen fixation, phosphorus solubilization, and siderophore production (Ji *et al.,* 2014; Tahir *et al.,* 2017; Qi *et al.,* 2018). However, the indirect ways of bacterial flora constitute the prevention of plant pathogenic microorganisms (Zhou *et al.,* 2016; Kalam *et al.,* 2017) using the secretion of cell wall degrading enzymes, antimicrobial components as well as through HCN production (Frampton *et al.,* 2012; Olanrewaju *et al.,* 2017).

Moreover, these PGPRs are comprised of such bacterial communities that multiply faster around the plant roots enhancing the growth of the host plant and raising the crop yield, minimizing the chances of infection and diminishing the stress (abiotic and biotic) of plants. These growth promoters also promote the detoxification of heavy metals in plants' vicinity by the production of effective volatile organic compounds into the interconnected environment (VOCs) (García-Fraile *et al.,* 2015; Gouda *et al.,* 2018). *Acinetobacter, Algaligenes, Arthrobacter, Azospirillium, Azotobacter, Bacillus, Beijerinckia, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Rhizobium,* and *Serratia* genera are the plant growth-promoting rhizobacterial strains (Han and Lee, 2005; Erturk *et al.,* 2011). Thus, the act of plant growth promotion may define the roles of these microbes as bio-stimulants, bio-fertilizers, and bio-protectants. The function of biofertilizers can be expanded to decrease the requirement of inorganic fertilizers and to minimize the unfavourable effect on the environment (Bashir *et al*., 2021).

To investigate alternate sources of hazardous chemicals required for the propagation of vegetables, the present research includes the isolation of rhizospheric bacteria from the rhizosphere of spinach, their preliminary identification and characterization and their evaluation as biofertilizers for the development of two most cultivated varieties of *Spinacia oleracea* (Desi palak and Lahori palak).

MATERIALS AND METHODS

Isolation of rhizobacteria

The soil was randomly collected at Botanical garden of The Women University Multan from the spinach (raw vegetable) rhizosphere (about 2-3 inch depth) for the isolation of rhizospheric bacteria in plastic bags and was serially diluted in distilled autoclaved water. Following the spreading method on nutrient agar (nutrient agar 28g/L with addition 100ml/L of nystatin as antifungal agent) plates at 28°C for 3-4 days, bacterial colonies were marked and further proceeded by quadrant streaking for three consecutive days of incubation at 37˚C. Accomplishing the incubation period, the purified colonies of rhizobacteria were stored in the form of nutrient agar slants at 4˚C refrigeration (Tsegaye *et al.,* 2019a).

Morphological characterization

Morphological features (colour, shape, margins, elevation, size, colony number, texture) of bacterial colonies were recorded after their growth on nutrient agar medium along with the determination of colony forming unit per ml (CFU/ml) (Islam *et al.,* 2016; Bashir *et al*., 2020).

Biochemical identification

Different biochemical tests were performed including gram staining (Khan *et al*., 2018), catalase (Jonit *et al*., 2016), starch hydrolysis (Kaur *et al*., 2012), methyl red (Kumar *et al*., 2015), citrate (Abiola and Oyetayo, 2016), mannitol (Kenny *et al*., 2013), urease, nitrate reduction (Deb *et al*., 2015), potassium hydroxide (Jonit *et al*., 2016) and glucose fermentation test (Abiola and Oyetayo, 2016) for identification of isolated rhizobacterial strains.

Screening of plant growth-promoting traits

Rhizobacterial isolates were screened for different plant growth-promoting traits such as hydrogen cyanide (HCN) production, phosphate solubilization, ammonia production and siderophore production. For the screening of HCN production, bacterial isolates were tested on nutrient media supplemented with glycine and then the plates were covered with Whatman filter paper (soaked in picric acid solution) following four days of incubation at a temperature of 30°C (Tsegaye *et al*., 2019b). For the determination of phosphate solubilization bacterial isolates were screened on Pikovskaya medium and incubated for 2 days (Malleswari and Bagyanarayana, 2013). For the production of ammonia, peptone broth was inoculated with rhizobacteria and incubated for 2 to 3 days at 30°C before growth. Afterward, Nessler's reagent was mixed in test tubes (Geetha *et al.,* 2014). Siderophore production was checked according to the protocol of Singh *et al*., (2015b) and Tirry *et al*., (2018) by spot inoculation of tested bacteria on chrome azurol S agar with an overnight incubation at 37°C.

Plant growth promotion assay

Preceding the validation of plant growth promotion by spinach-associated rhizobacteria, bacterial cultures were cultivated aerobically in a shaking incubator (160rpm) at 37°C for 72 hours (Borah *et al*., 2019; Arkhipova *et al*., 2019). Before inoculation, seeds of spinach were surface sterilized with 10% NaOCl (sodium hypochlorite) solution for eight minutes (Chitwood *et al*., 2016). Then the seeds were rinsed with double distilled water ten times and allowed to air-dry under a laminar airflow cabinet (Akinrinlola *et al*., 2018). These sterilized seeds were inoculated with bacterial suspensions of 10^8 each for 2 hours of continuous shaking (160rpm) at the shaker and utilized for sowing into the pots filled with autoclaved fertile soil (Singh *et al*., 2015a). In this pot experiment, inoculated and control seeds of 2 varieties of spinach (Lahori palak and Desi palak) were grown for each treatment in triplicate pots on the 21st of September 2019 in a wire net house at The Women University Multan.

Collection of data at vegetative stages

Plant growth in the form of different vegetative parameters [Plant height (cm), Plant fresh and dry weight (gm) were recorded at two vegetative stages {25 days/4th week (seedling stage) and 40 days/6th week (vegetative stage) of seed germination} of two varieties (V1-Desi palak and V2-Lahori palak) of spinach. Data was recorded as a mean of three replicas for each treatment and control. For further validation of data, IBM SPSS software was used in which one way ANOVA (analysis of variance) was applied to mean values of three replicates with LSD (Least Significant Difference) and DMRT (Duncan's Multiple Range Test) to measure the significant difference at $P < 0.05$.

RESULTS

Isolation and morphological characterization of rhizobacteria

A total of 17 rhizobacteria (ST1, ST2, ST4, ST5, ST7, ST8, ST11, ST12, ST14, ST15, ST16, ST17, ST18, ST19, ST20, ST23, ST25) were isolated from spinach rhizosphere which on morphological characterization showed variation in characters such as colour, shape, size, margin, elevation and texture (Table 1). The CFU/mL recorded for each bacterial strain revealed a different number of isolated colonies (Table 1).

Table 1. Morphological Characterization of Rhizobacteria.

Sr. no.	Colonies	Colour	Shape	Margins	Elevation	Texture	CFU/mL
1	ST1	Off white	Complex	Lobate	Raised	Moist	4×10^3
2	ST2	Gray	Round with raised margin	Wooly	Raised	Moist	11×10^3
3	ST4	Pale yellow	Rhizoid	Irregular	Raised	Moist	6×10^3
4	ST5	Yellow	Round	Irregular	Flat	Moist	3×10^3
5	ST7	Pale yellow	Round with raised margin	Lobate	Raised	Viscid	3×10^3

6	ST8	Yellow	Rhizoid	Irregular	Convex	Moist	5×10^3
7	ST11	Gray	Round	Undulate	Convex	Viscid	4×10^3
8	ST12	Off white	Wrinkled	Undulate	Convex	Mucoid	2×10^3
9	ST14	White	Rhizoid	Entire	Flat	Moist	5×10^3
10	ST15	Off white	Round	Entire	Raised	Moist	7×10^3
11	ST16	Almond	Round	Entire	Flat	Moist	3×10^3
12	ST17	White	Rhizoid	Lobate	Raised	Moist	5×10^3
13	ST18	Yellow	Round	Entire	Raised	Viscid	12×10^3
14	ST19	Light orange	Round with raised margin	Lobate	Raised	Moist	4×10^3
15	ST20	Lemon	Round with radiating margin	Lobate	Flat	Mucoid	2×10^3
16	ST23	Transparent	Round	Convex	Entire	Moist	9×10^3
17	ST25	Off white	Rhizoid	Wooly	Flat	Moist	6×10^3

Biochemical identification

Regarding biochemical identification, 100% of strains were of gram-positive type as shown in Fig. 1. While 94% of total strains showed positive results and only, 6% appeared as negative for the catalase test. In the starch hydrolysis test, 47% of strains hydrolyzed the starch and 53% did not hydrolyze the starch. All the strains showed positive results for the methyl red test. Similarly, the Simmon citrate test showed 41% positive results and 59% negative results of isolated strains. Mannitol test was also found to be positive for 100% of strains. In the urease test, all strains had negative response. Adding to these tests, 53% positive and 47% negative results were recorded for the nitrate reduction test. For the KOH test, all the strains showed negative results whereas, for the glucose fermentation test, 94% of the strains showed positive results (Table 2, Figure 2).

Screening of plant growth-promoting traits

Results of PGP traits are shown in Table 2 revealing positive responses by all 17 strains for HCN production. While negative results were observed by all strains for phosphate solubilization. However, 100% of tested stains showed ammonia production. On the other hand, 7 out of 17 were found to be siderophore producers and 10 out of 17 strains appeared as non-producers of siderophores. (Table 3, Figure 3).

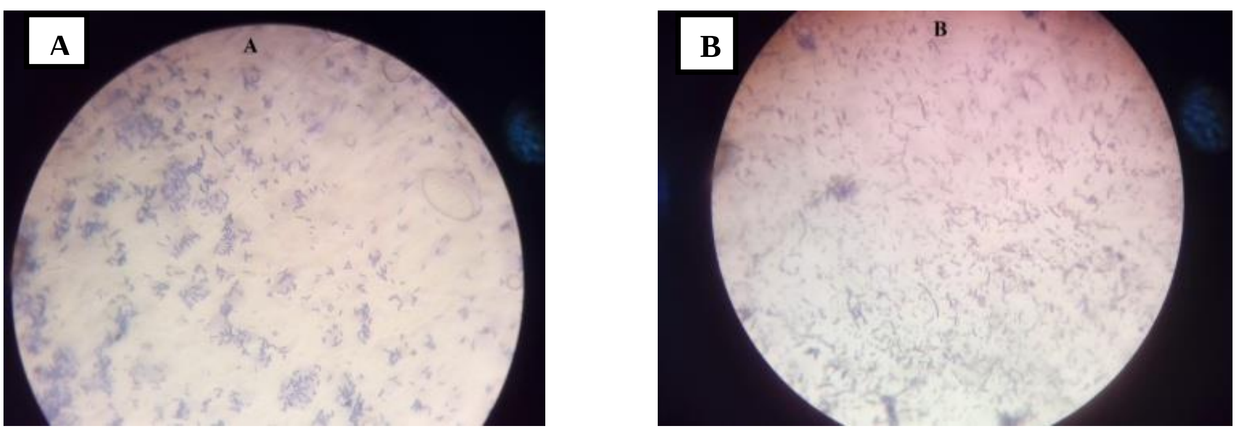

Figure 1. Microscopic slides showing gram staining.
Where A: Gram-positive (rod) B: Gram-Positive (cocci).

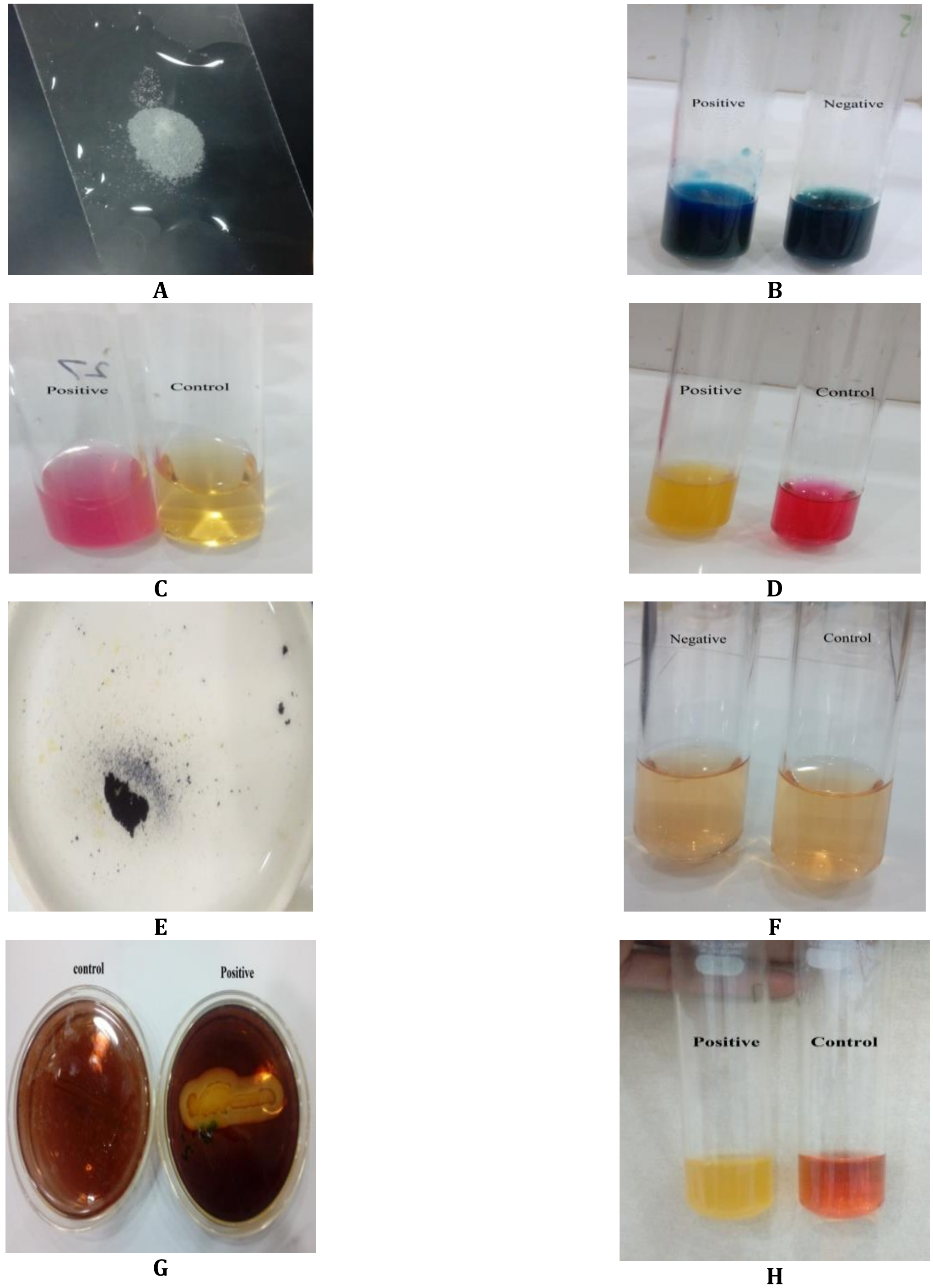

Figure 2. Biochemical characterization A: Catalase Test B: Citrate Test C: Methyl Red Test D: Mannitol Test E: Nitrate Test F: Urease Test G: Starch Hydrolysis Test H: Glucose Fermentation Test.

Table 2. Biochemical Characterization of Rhizobacteria.

Sr no.	Bacterial strains	Biochemical characterization									
		Gram staining	Catalase Test	Citrat Test	Mannitol Ttest	Methyl red test	Urease Test	Nitrate reduction test	Starch test	KOH Test	Glucose Fermentation Test
1	ST1	+ rod	+	+	+	+	-	-	+	-	A
2	ST2	+ rod	+	-	+	+	-	-	-	-	A
3	ST4	+ rod	+	-	+	+	-	+	-	-	A
4	ST5	+ rod	+	-	+	+	-	-	-	-	A
5	ST7	+ rod	+	-	+	+	-	+	+	-	A
6	ST8	+ rod	+	-	+	+	-	-	+	-	A
7	ST11	+ rod	+	+	+	+	-	-	+	-	A
8	ST12	+ cocci	-	-	+	+	-	+	+	-	A
9	ST14	+ rod	+	-	+	+	-	+		-	-
10	ST15	+ rod	+	-	+	+	-	+	-	-	A
11	ST16	+ rod	+	+	+	+	-	-	-	-	A
12	ST17	+ rod	+	+	+	+	-	-	-	-	A
13	ST18	+ rod	+	+	+	+	-	+	-	-	A
14	ST19	+ rod	+	-	+	+	-	+	-	-	A
15	ST20	+ rod	+	+	+	+	-	+	+	-	A
16	ST23	+ rod	+	+	+	+	-	-	+	-	A
17	ST25	+ rod	+	-	+	+	-	+	+	-	A

Where the (+) sign shows a positive result and the (–) sign shows a negative result. (A) sign shows the presence of acid.

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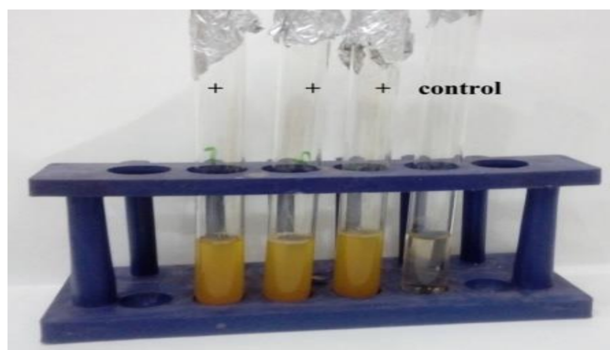
Table 3. Screening of Plant Growth Promoting Trait.

Sr. no.	Bacterial isolates	HCN production	Phosphate solubilization	Ammonia production	Siderophore production
1	ST1	+++	-	+	-
2	ST2	++	-	+	-
3	ST4	+	-	+	-
4	ST5	+	-	+	+
5	ST7	+	-	+	-
6	ST8	+	-	+	+
7	ST11	+	-	+	-
8	ST12	++	-	+	+
9	ST14	++	-	+	-
10	ST15	+	-	+	-
11	ST16	+	-	+	-
12	ST17	+	-	+	+
13	ST18	+	-	+	-
14	ST19	+	-	+	+
15	ST20	+	-	+	+
16	ST23	+	-	+	+
17	ST25	+	-	+	-

Where (the +) sign shows a positive result and the (–) sign shows a negative result. (A) sign shows the presence of acid.

A



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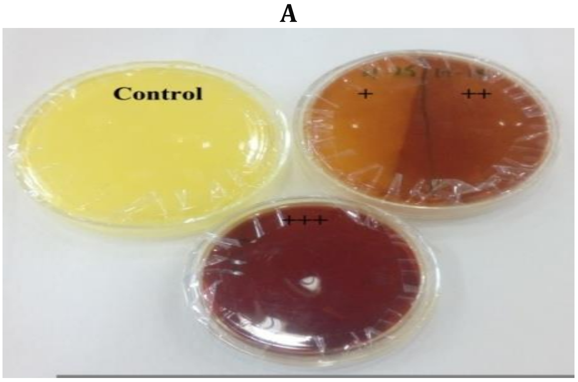

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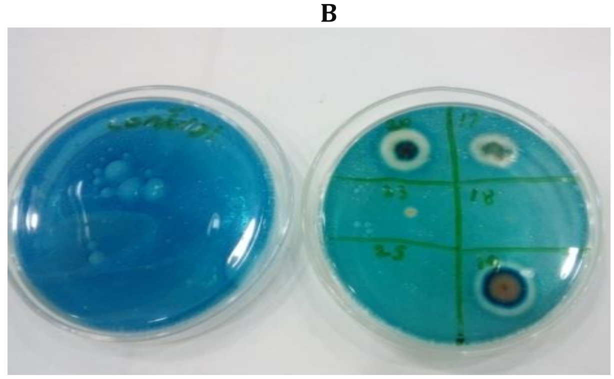
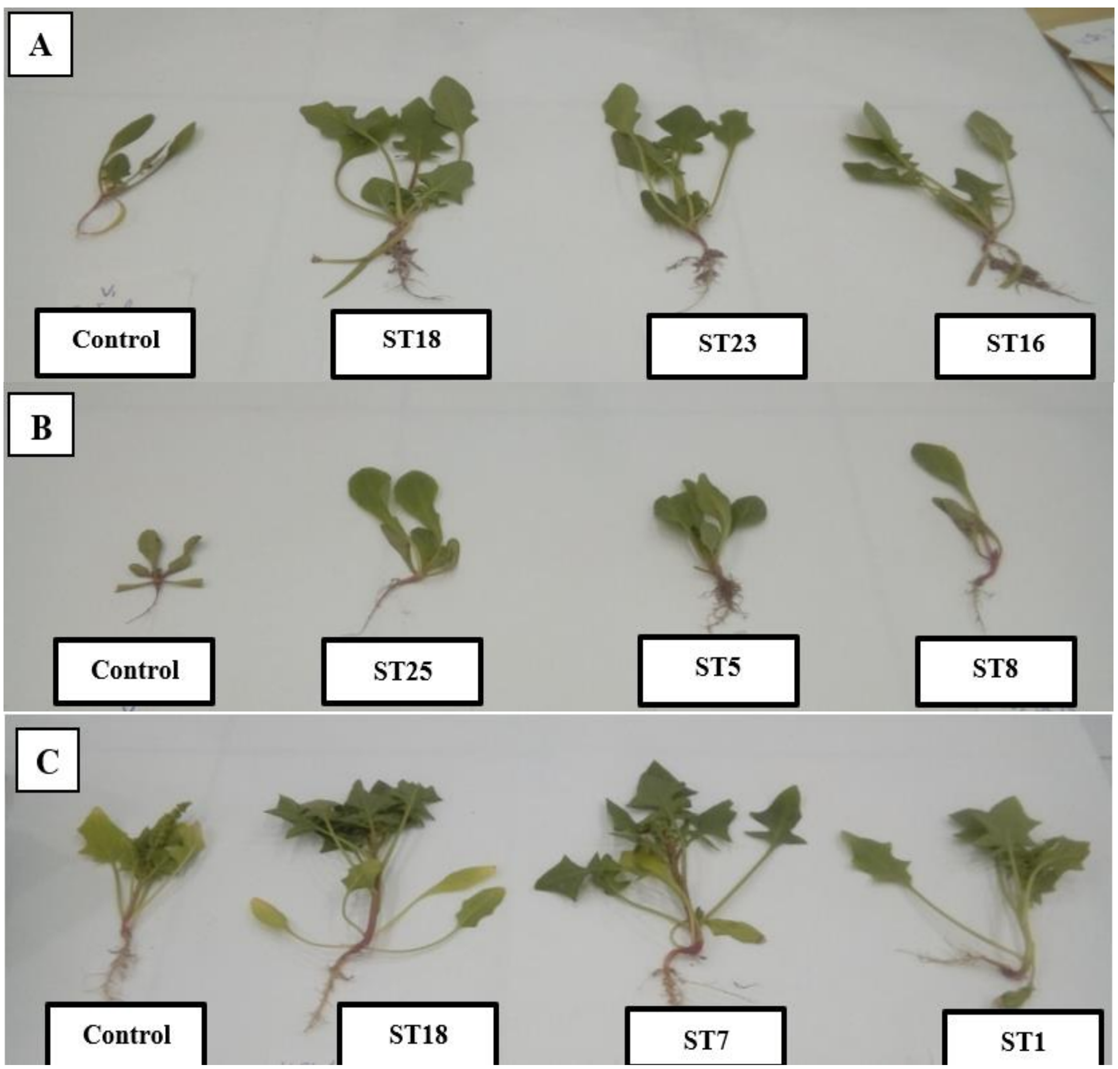

D

Figure 3. Screening of Plant Growth Promoting Traits A: Phosphate Solubilization Test B: Ammonia Production Test C: Screening of HCN D: Siderophore Production.

Collection of data at the vegetative stages

The effect of rhizobacterial inoculations versus control being recorded at two vegetative stages (25 days and 40 days of seed germination) of two varieties (V1-Desi palak and V2-Lahori palak) of spinach are presented in Fig 4 A, B, C, D and Figs 5,6 A, B, C. At vegetative stage I, the most effective bacterial strains were ST18 and ST23 which improved the plant height of V1 from 21cm-19.2 cm respectively compared with control (11cm). Similarly, an increment in plant height of V2 was recorded by ST25 (15.5cm) and ST8 (13.8cm) inoculations upon comparison with control plant height (6.4cm). Likewise, fresh and dry weights of V1 had been improved from 0.20-0.43gm and 0.02–0.06 gm respectively by different rhizobacteria when compared with the control. Bacterial inoculations (ST25 and ST15) also caused a marked increase in fresh (0.25gm) and dry (0.04gm) weights of V2 plants versus their respective controls (0.06 and 0.009 gms).

At vegetative stage II, remarkable increase in vegetative parameters of V1 {plant height (18.5cm), Plant fresh weight (0.49gm), plant dry weight (0.06gm)} and V2 {plant height (18.2cm), Plant fresh weight (0.38gm), plant dry weight (0.04gm) } was measured as compared to control plants. On the whole, the growth of V1 (Desi palak) was more enhanced as compared to the growth of V2 (Lahori palak) by rhizobacterial inoculation.



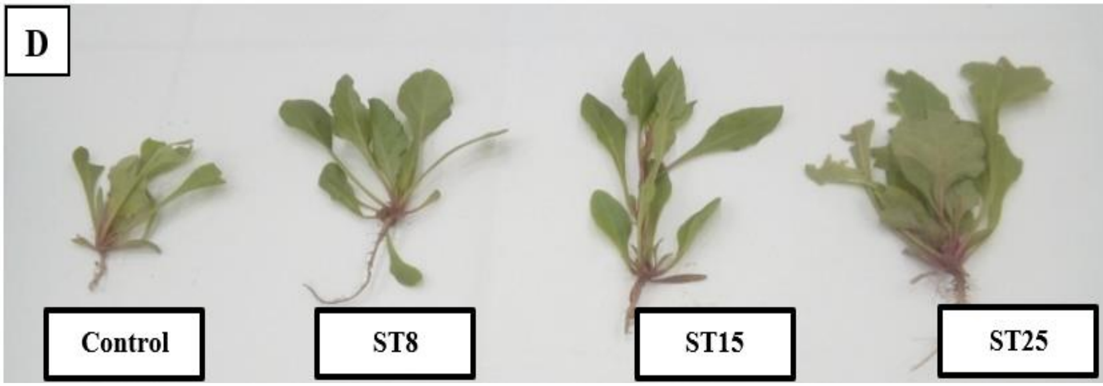
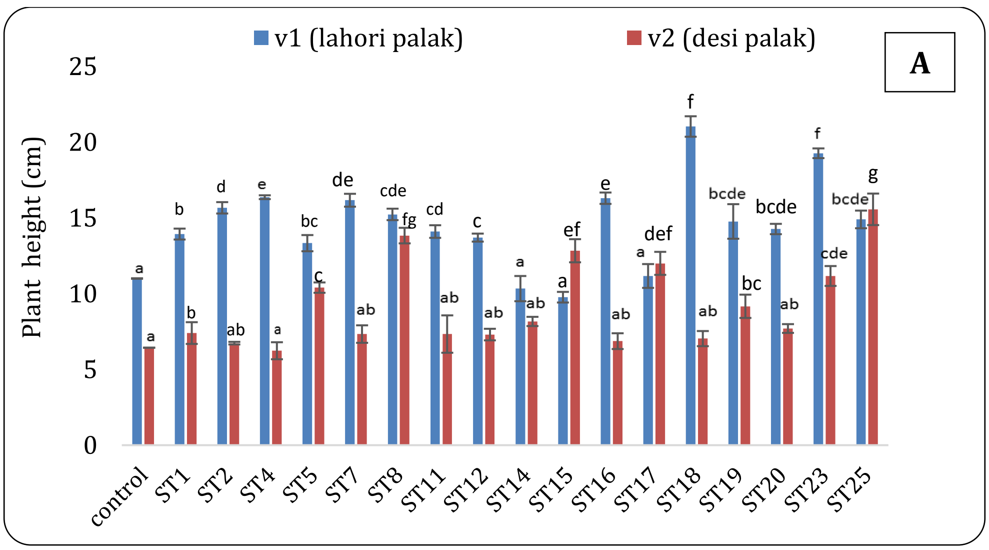
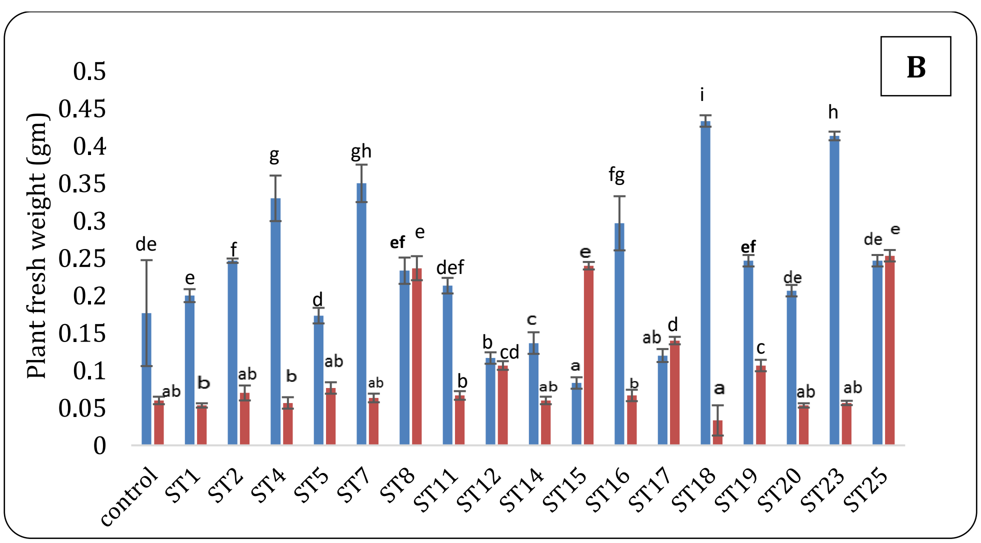


Figure 4. Effect of rhizobacterial inoculation on two varieties of spinach. A: Vegetative stage I of V1 (Desi palak), B: Vegetative stage I of V2 (Lahori palak), C: Vegetative stage II of V1 (Desi palak), D : Vegetative stage II of V2 (Lahori palak).





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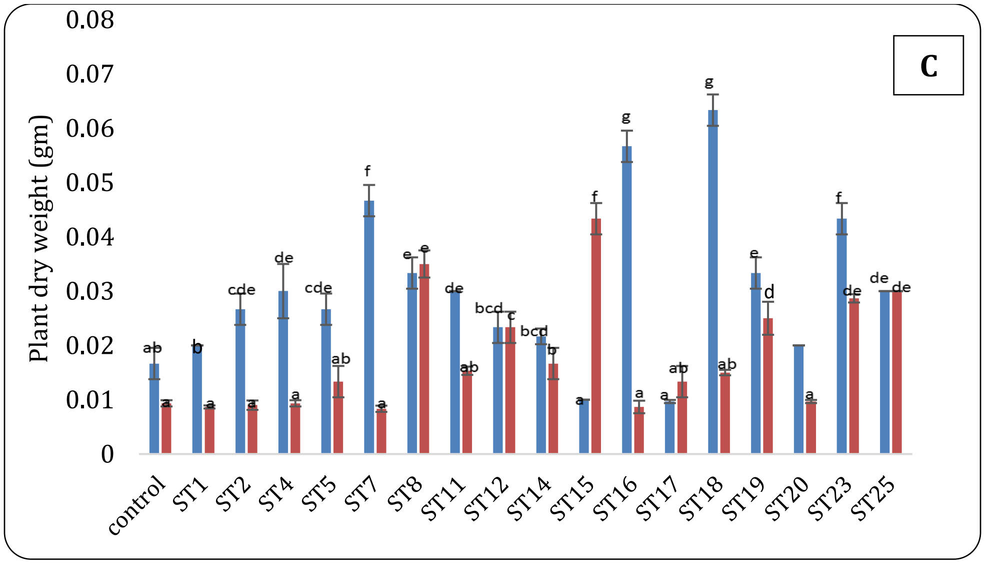
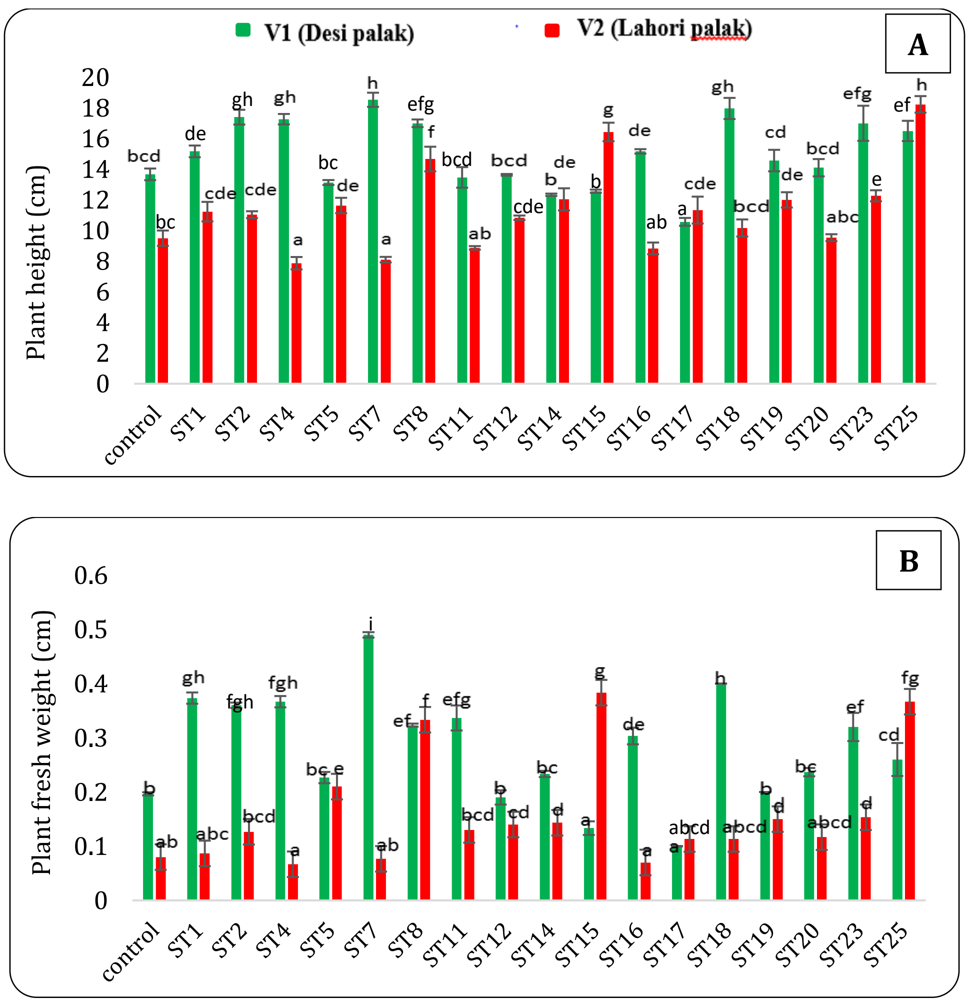


Figure 5. Effect of rhizobacterial inoculations at vegetative stage I of two varieties of spinach. Error bars depict data accuracy among the replicates and least significant differences recorded at P < 0.05 shows significance of mean values. Where A: plant height, B: plant fresh weight, C: plant dry weight.



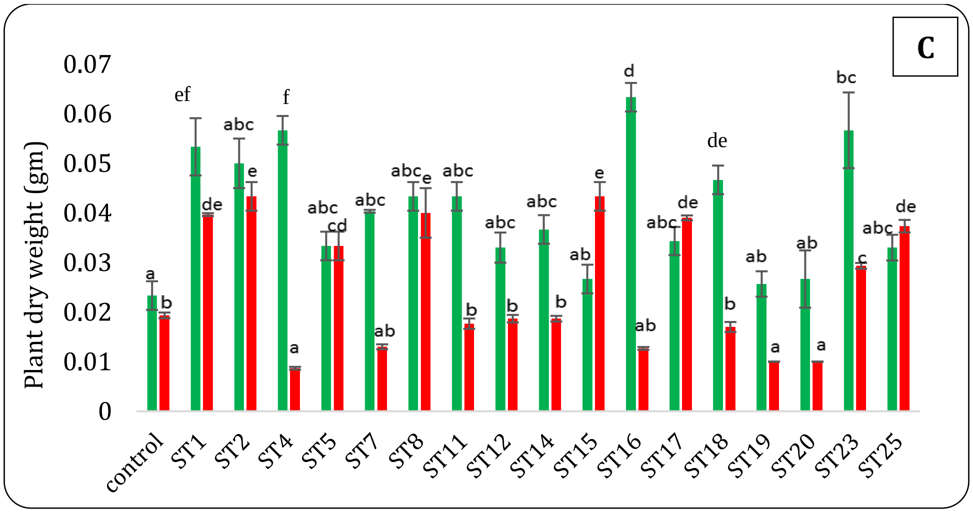


Figure 6. Effect of rhizobacterial inoculations at vegetative stage II of two varieties of spinach.Error bars depict data accuracy among the replicates and least significant differences recorded at P < 0.05 shows significance of mean values. Where A: plant height, B: plant fresh weight, C: plant dry weight.

DISCUSSION

Plant growth-promoting rhizobacteria overcoming the side effects of agrochemicals, not only improves soil fertility but also imparts in plant development (Biswas *et al.,* 2024). Studies have shown that the rhizosphere is a reservoir of promising plant growth-promoting bacteria (Gianelli *et al.,* 2024). With this perspective, the current study is based on isolating and characterising spinach-associated bacteria. Seventeen rhizobacteria were isolated from spinach's rhizospheric soil, which were identified based on morphological features like colony shape, margins and texture. Clear variations in these morphological features (yellow and off-white colored, circular, round and rhizoid in shape, irregular and entire in margins, and viscous and mucus in texture) were observed among the isolates. Variations in morphological features of soil bacteria have also been found earlier and it is reported that these variations could be due to genetic diversity (Emitaro *et al*., 2024). Yulikasari *et al.,* (2024) also documented morphological variability in 10 indigenous bacterial isolates of Indonesia. In the present study, 100% of strains isolated from the rhizospheric soil of spinach were gram-positive and were identified by biochemical tests as *Bacillus sp., Clostridium spp., Corynebacterium sp., Lactobacillus spp. and Mycobacterium spp.* Bashir *et al.,* (2024) also identified the bacterial isolates as gram-positive types belonging to *Bacillus sp.* and highlighted the potential role of different bacillus species in growth enhancement of sunflower crops.

The plant-associated bacteria may possess multiple growth-promoting traits (Sritongon *et al*., 2023; Danchin, 2024). As nitrogen is an essential requirement for different life forms, it also acts as the most vital nutrient for plant growth and productivity. However, one of the issues with nitrogen is its unavailability to plants which can be resolved using microflora, converting atmospheric nitrogen to the available form of ammonia (Wei *et al.,* 2024). In the current study, all the isolated strains produced ammonia which coincides with the results of Agunbiade *et al.,* (2024) showing the growth promotion of maize by ammonia-producing bacteria. Some bacteria promote the growth of plants indirectly by releasing various chemicals that are particularly essential to resist multiple plant diseases. HCN is considered an important metabolite that helps to combat various plant diseases by acting as a biocontrol agent (Bashir *et al.,* 2021). In the current study, 100% spinach rhizobacteria were declared positive for hydrogen cyanide production which might indicate their ability to promote plant growth indirectly, by preventing pathogenic attacks. However, the maintenance of plant health using rhizobacterial phosphate solubilization can never be ignored (Qingwei *et al*., 2023; Damo *et al*., 2024). In the current work, isolated bacteria lacked the ability of phosphate solubilization which might indicate their adoption to another growth-promoting potential. Among beneficial mechanisms, siderophore production by rhizobacteria is another striking attribute for the stimulation of plant growth by the formation of iron complexes in the rhizosphere making iron available for plant growth and at the same time causing deficiency of iron for phytopathogens and managing the plant stress (Wang *et al*., 2022). The current results revealed 41% of isolates as siderophore producers. These findings are in agreement with Ehsan (2022) declaring the involvement of bacterially synthesized siderophores in the development of wheat.

Considering the application of biofertilizers as an eco-friendly and suitable strategy for crop improvement, leading to high agricultural productivity (Du *et al*., 2018; Enrico *et al*., 2020; Akhtar *et al*., 2022), all seventeen isolated spinach rhizobacteria were used as a biofertilizer on two varieties (Desi palak and Lahori palak) of spinach at two vegetative stages (25 days and 40 days of seed germination) to explore their plant growth promotion efficacy in the current study. Inoculation response of rhizobacteria in comparison with the uninoculated plant (control), at the vegetative stage I showed remarkable enhancement in plant height (18-90% in V1 and 15-142 % in V2), plant fresh weight (17-152% in V1 and 16-316 % in V2) and plant dry weight (100-530% in V1 and 11-344 % in V2). Likewise, proceeding towards the vegetative stage II, vegetative parameters including plant height (3.6-36% in V1 and 13-89% in V2), plant fresh weight (100-390% in V1 and 37-375% in V2) and plant dry weight (50-200% in V1 and 100-300% in V2) were development more efficiently due to bacterial inoculation as compared with control ones of spinach. Our findings coincide with the results given by (Ghazanfar *et al*., 2024) as they recorded a 58% increase in shoot length, 54% in root length, 67% in root fresh biomass and 76% in root dry biomass on maize using different Bacillus species. Further justifying the current outcomes, Weinand *et al*., in 2023 emphasized the significant interface between bacteria and Rice (*Oryza sativa* L.) cultivars in determining the beneficial effects of Bacillus spp. on plants under biotic or abiotic stresses.

CONCLUSION

The current study includes the isolation of spinach-associated rhizobacteria and the investigation of their putative role as plant growth enhancers. Successfully seventeen bacterial isolates were obtained which on preliminary characterization showed multiple traits of plant growth promotion. These traits were proved to be utilized by isolates for successive development of spinach cultivars in a pot experiment. Therefore, it is inferred from the current study that isolated rhizobacteria of spinach can be suitable candidates not only for the crop productivity of spinach but also reflect the scope of these PGPRs as efficient biofertilizers for future crops, minimizing the chemical toxicity from the agricultural environment. However, future research may suggest their molecular identification and physiological functioning for the advancement of agriculture.

AUTHOR CONTRIBUTIONS

The research was designed and supervised by SB and all experimental work was performed by HM at The Women University Multan, Pakistan. The manuscript was written by HM after discussions with SB and proof read by AH.

FUNDING

There is no source of funding.

CONFLICT OF INTEREST

There is no conflict of interest.

DATA AVAILABILITY STATEMENT

All the data will be available

ETHICS AVAILABILITY STATEMENT

As all research work is plant-based and no work related to animal or human study is involved so ethical availability statement is not applicable.

REFERENCES

Abiola, C. and V. Oyetayo. 2016. Isolation and biochemical characterization of microorganisms associated with the fermentation of Kersting's groundnut (*Macrotyloma geocarpum*). Research Journal of Microbiology, 11, 47-55.

Agunbiade, V. F., A. E. Fadiji, N. A. Agbodjato and O.O. Babalola. 2024. Isolation and Characterization of Plant-Growth-Promoting, Drought-Tolerant Rhizobacteria for Improved Maize Productivity. *Plants*, *13*(10): 1298. https://doi.org/10.3390/plants13101298.

Akhtar, N., N. Ilyas, T.A. Meraj, A. Pour-Aboughadareh, R.Z. Sayyed, Z.U.R. Mashwani and P. Poczai. 2022. Improvement of plant responses by nanobiofertilizer: A step towards sustainable agriculture. Nanomater, 12, 965.

https://doi.org/10.3390/nano12060965.

Akinrinlola, R.J., G.Y. Yuen, R.A. Drijber. and A.O. Adesemoye. 2018. Evaluation of Bacillus strains for plant growth promotion and predictability of efficacy by *In Vitro* physiological traits. International Journal of Microbiology, 2018, 5686874. https://doi.org/10.1155/2018/5686874.

Arkhipova, T.N., L. Galimsyanova, L. Kuzmina, L. Vysotskaya, I. Sidorova, A. Gabbasova, A. Melentiev. and G. Kudoyarova. 2019. Effect of seed bacterization with plant growth-promoting bacteria on wheat productivity and phosphorus mobility in the rhizosphere. Plant and Soil Environment, 65, 313-319. https://doi.org/10.17221/752/2018-PSE.

Aziz, M.A., M. Adnan, A.H. Khan, A.U. Rehman, R. Jan. and J. Khan. 2016. Ethno-medicinal survey of important plants practised by the indigenous community at Ladha subdivision, South Waziristan agency, Pakistan. Journal of Ethnobiology and Ethnomedicine, 12, 53. DOI 10.1186/s13002-016-0126-7.

Bashir, S., A. Iqbal and S. Hasnain. 2024. Exploring the supremacy of Bacillus species among sunflower endophytes as a promising bio stimulating tool. World Journal of Biology and Biotechnology, 9 (3). https://doi.org/10.33865/wjb.009.03.1337.

Bashir, S., A. Iqbal, S. Hasnain. and J.F. White. 2021. Screening of sunflower associated bacteria as biocontrol agents for plant growth promotion. Archives of Microbiology, 203, 4901-4912.

Bashir, S., A. Iqbal. and S. Hasnain. 2020. Comparative analysis of endophytic bacterial diversity between two varieties of sunflower *Helianthus annuus* with their PGP evaluation. Saudi Journal of Biological Sciences, 27, 720-726.

Biswas, D., A. K. Chakraborty, V. Srivastava and Mandal, A. 2024. Plant Growth Promoting Rhizobacteria (PGPR): Reports on Their Colonization, Beneficial Activities, and Use as Bioinoculant. Advances in Agriculture, 2024(1): 8173024. https://doi.org/10.1155/2024/8173024.

Borah, A., R. Das, R. Mazumdar and D. Thakur. 2019. Culturable endophytic bacteria of Camellia species endowed with plant growth promoting characteristics. Journal of Applied Microbiology, 127, 825-844.

Chitwood, J., A. Shi, M. Evans and C. Rom. 2016. Effect of temperature on seed germination in spinach (*Spinacia oleracea*). HortScience, 51(12): 1475-1478.

Damo, J. L. C., M. Pedro and M.L. Sison 2024. Phosphate Solubilization and Plant Growth Promotion by Enterobacter sp. Isolate. Applied Microbiology, 4(3): 1177-1192. https://doi.org/10.3390/applmicrobiol403008.

Danchin, A. 2024. Exploring overlooked growth-promoting mechanisms by plant-associated bacteria. Sustainable Microbiology, qvae011. https://doi.org/10.1093/sumbio/qvae011.

Du, C., J.J. Abdullah, D. Greetham, D. Fu, M. Yu, L. Ren, S. Li. and D. Lu. 2018. Valorization of food waste into biofertiliser and its field application. Journal of Cleaner Production, 187, 273–284.

Ehsan, S., A. Riaz, M.A. Qureshi, A. Ali, I. Saleem, M. Aftab, K. Mehmood, F. Mujeeb, M.A. Ali, H. Javed, F. Ijaz, A. Haq, K.Ul. Rehman and M.U. Saleem. 2022. Isolation, purification and application of siderophore producing bacteria to improve wheat growth. Pakistan Journal of Agricultural Research, 35(2): 449-459.

Emitaro, W. O., F. Kawaka, D.M. Musyimi and A. Adienge. 2024. Diversity of endophytic bacteria isolated from leguminous agroforestry trees in western Kenya. AMB Express, 14(1): 18. https://doi.org/10.1186/s13568-024-01676-6.

Enrico, J.M., C.F. Piccinetti, M.R. Barraco, M.B. Agosti, R.P. Eclesia. and F. Salvagiotti. 2020. Biological nitrogen fixation in field pea and vetch: response to inoculation and residual effect on maize in the Pampean region. European Journal of Agronomy, 115, 126016. https://doi.org/10.1016/j.eja.2020.126016.

Erturk, Y., R. Cakmakci, O. Duyar. and M. Turan. 2011. The effects of plant growth promotion rhizobacteria on vegetative growth and leaf nutrient contents of hazelnut seedlings (Turkish hazelnut cv, Tombul and Sivri). International Journal of Soil Sciences, 6, 188-198.

Fornaciari, S.F., F. Milano, L. Mussi, L. Pinto-Sanchez, A. Forti, Buschini. and L. Arru. 2015. Assessment of antioxidant and antiproliferative properties of spinach plants grown under low oxygen availability. Journal of the Science of Food and Agriculture, 95, 490-496.

Frampton, R.A., A.R. Pitman. and P.C. Fineran. 2012. Advances in bacteriophage-mediated control of plant pathogens. International Journal of Microbiology, 2012, 326452. https://doi.org/10.1155/2012/326452.

García-Fraile, P., E. Menéndez. and R. Rivas. 2015. Role of bacterial biofertilizers in agriculture and forestry. AIMS Bioengineering, 2, 183-205. https://doi.org/10.3934/bioeng.2015.3.183.

Geetha, K.E., A. Venkatesham, Hindumathi. and B. Bhadraiah. 2014. Isolation, screening and characterization of plant growth promoting bacteria and their effect on *Vigna Radita* (L.) R. Wilczek. International Journal of Current Microbiology and Applied Sciences, 3, 799-803.

Ghazanfar, S., A. Hussain, A. Dar, M. Ahmad, H. Anwar, D.A. Al Farraj, M. Rizwan and R. Iqbal 2024. Prospects of iron solubilizing Bacillus species for improving growth and iron in maize (*Zea mays* L.) under axenic conditions. Scientific Reports, 14(1): 26342.| https://doi.org/10.1038/s41598-024-77831-7.

Giannelli, G., L.D. Vecchio, M. Cirlini, M. Gozzi, L. Gazza, G. Galaverna, S. Potestio and G. Visioli. 2024. Exploring the rhizosphere of perennial wheat: potential for plant growth promotion and biocontrol applications. Scientific Reports, 14: 22792. https://doi.org/10.1038/s41598-024-73818-6.

Gouda, S., R.G. Kerry, G. Das, S. Paramithiotis, H.S. Shin and J.K. Patra. 2018. Revitalization of plant growth promoting rhizobacteria for sustainable development in agriculture. Microbiological Research, 206,131-140.

Government of Pakistan. 2024. Fruit, vegetables and condiments statistics of Pakistan. https://mnfsr.gov.pk/SiteImage/Misc/files/draft_fvc_1_0.pdf.

Han, H. and K. Lee. 2005. Plant growth promoting rhizobacteria effect on antioxidant status, photosynthesis, mineral uptake and growth of lettuce under soil salinity. Research Journal of Agriculture and Biological Sciences, 1, 210-215.

Islam. S., A.M. Akanda, A. Prova, M.T. Islam and M.M. Hossain. 2016. Isolation and identification of plant growth promoting rhizobacteria from cucumber rhizosphere and their effect on plant growth promotion and disease suppression. Frontiers in Microbiology, 6, 1360. http://dx.doi.org/10.3389/fmicb.2015.01360.

Ji, S.H., M.A. Gururani. and S.C. Chun. 2014. Isolation and characterization of plant growth promoting endophytic diazotrophic bacteria from Korean rice cultivars. Microbiological Research, 169, 83-98.

Jonit, N.Q., Y.C. Low. and G.H. Tan. 2016. *Xanthomonas oryzae* pv. oryzae, Biochemical Tests, Rice (*Oryza sativa*), Bacterial Leaf Blight (BLB) Disease, Sekinchan. Journal of Pure and Applied Microbiology, 4, 63-69.

Kalam, S., S.N. Das, A. Basu. and A.R. Podile. 2017. Population densities of indigenous Acidobacteria change in the presence of plant growth promoting rhizobacteria (PGPR) in rhizosphere. Journal of Basic Microbiology, 57, 376-385.

Kaur, A., M. Kaur, M.L. Samyal and Z. Ahmed. 2012. Isolation, characterization and identification of bacterial strain producing amylase. Journal of Microbiolog and Biotechnology, 2, 573-579.

Kenny, J.G., J. Moran, S.L. Kolar, A. Ulanov, Z. Li, L.N. Shaw, E. Josefsson. and M.J. Horsburgh. 2013. Mannitol utilisation is required for protection of *Staphylococcus aureus* from human skin antimicrobial fatty acids. PLoS One, 8, e67698. https://doi.org/10.1371/journal.pone.0067698.

Khan, Z.I., I. Ugulu, K. Ahmad, S. Yasmeen, S.R. Noorka, N. Mehmood and M. Sher. 2018. Assessment of trace metal and metalloid accumulation and human health risk from vegetables consumption through spinach and coriander specimens irrigated with wastewater. Bulletin of Environmental Contamination and Toxicology, 101, 787-795.

Khoso, M. A., S. Wagan, I. Alam, A. Hussain, Q. Ali, S. Saha, T.R. Poudel, H. Manghwar and F. Liu. 2024. Impact of plant growth-promoting rhizobacteria (PGPR) on plant nutrition and root characteristics: Current perspective. Plant Stress, 100341. https://doi.org/10.1016/j.stress.2023.100341.

Kumar, P.N., Kaushal. and R. Dubey. 2015. Isolation and identification of plant growth promoting rhizobacteria (*Pseudomonas spp.*) and their effect on growth promotion of *Lycopersicon esculentum* L. Academy Arena, 7, 44-51.

Liu, X.X., K. Zhou, Y. Hu, R. Jin., L.L. Lu, C.W. Jin. and X.Y. Lin. 2015. Oxalate synthesis in leaves is associated with root uptake of nitrate and its assimilation in spinach (*Spinacia oleracea* L.) plants. Journal of the Science of Food and Agriculture, 95, 2105-2116.

Malleswari, D. and G. Bagyanarayana. 2013. Plant growth promoting activities and molecular characterization of rhizobacterial strains isolated from medicinal and aromatic plants. *IOSR I*nternational Journal of Pharmacy and Biological Sciences, 6, 30-37.

Miano, T.F. 2016. Nutritional value of *Spinacia oleraecea* spinach-an overview. International Journal of Life Sciences and Review (IJLSR), 2(12): 172-174.

Naseem, A., S. Akhtar, T. Ismail, M. Qamar, D.E.S. Sattar, W. Saeed, T. Esatbeyoglu, E. Bartkiene and J.M. Rocha. 2023. Effect of growth stages and lactic acid fermentation on anti-nutrients and nutritional attributes of spinach (*Spinacia oleracea*). Microorganisms, 11(9): 2343. https://doi.org/10.3390/microorganisms11092343.

Ngo, B.T., K.D. Hayes, D.J. DiMiao, S.K. Srinivasan, C.J. Huerter. and M.S. Rendell. 2005. Manifestations of cutaneous diabetic microangiopathy. American Journal of Clinical Dermatology, 6, 225-237.

Olanrewaju, O.S., B.R. Glick. and O.O. Babalola. 2017. Mechanisms of action of plant growth promoting bacteria. World Journal of Microbiology and Biotechnology, 33, 197. DOI 10.1007/s11274-017-2364-9.

Pan, Z., P. He, D. Fan, R. Jiang, D. Song, L. Song, W. Zhou and He.Wentian. 2024. Global impact of enhanced-efficiency fertilizers on vegetable productivity and reactive nitrogen losses. Science of the Total Environment, 926, 172016. https://doi.org/10.1016/j.scitotenv.2024.172016.

Patricia, O., L. Zoue, R.M. Megnanou, R. Doue and S. Niamke. 2014. Proximate composition and nutritive value of leafy vegetables consumed in Northern Cote d'Ivoire. European Scientific Journal, 10, 212-227.

Qi, G.Z., Y. Pan, F.J. Sugawa, T. Andriamanohiarisoamanana, M. Yamashiro, K. Iwasaki, I. Kawamoto, Ihara. and K. Umetsu. 2018. Comparative fertilizer properties of digestates from mesophilic and thermophilic anaerobic digestion of dairy manure: focusing on plant growth promoting bacteria (PGPB) and environmental risk. Journal of Material Cycles and Waste Management, 20, 1448-1457.

Qingwei, Z., T. Lushi, Z. Yu, S. Yu, W. Wanting, W. Jiangchuan, D. Xiaolei, H. Xuejiao and M. Bilal. 2023. Isolation and characterization of phosphate-solubilizing bacteria from rhizosphere of poplar on road verge and their antagonistic potential against various phytopathogens. BMC Microbiology, 23(1): 221. https://doi.org/10.1186/s12866-023-02953-3.

Rahati, S.M., A. Eshraghian, Ebrahimi. and H. Pishva. 2016. Effect of spinach aqueous extract on wound healing in experimental model diabetic rats with streptozotocin. Journal of the Science of Food and Agriculture, 96, 2337-2343.

Raman, D., N. Joshi and P.S. Rana. 2022. Effect of Organic and Chemical Fertilizers on the Nutritional Composition of Amaranthus spinosus. BioRxiv https://doi.org/10.1101/2022.06.02.494619.

Rejeki, F., S., E.R. Wedowati and D. Haryanta. 2023. Nutritional quality of spinach (*Amaranthus hybridus* L.) cultivated using black soldier fly (H*ermetia illucens*) waste compost. Cogent Food & Agriculture, 9(2): 2279742. https://doi.org/10.1080/23311932.2023.2279742.

Shah, B.K., N. Sohail, Nisar. and N. Ahmed. 2015. Screening of two varieties of spinach against grasshopper under field condition. Journal of Entomology and Zoology Studies, 3, 359-361.

Shaheen, S.M., M.J. Khan, S. Khan, Z. Jilani, M. Bibi, Munir. and M. Kiran. 2017. Effective microorganisms (EM) co-applied with organic wastes and NPK stimulate the growth, yield and quality of spinach (*Spinacia oleracea* L.). Sarhad Journal of Agriculture, 33, 30-41.

Singh, M., V.P. Singh. and K.S. Reddy. 2001. Effect of integrated use of fertilizer nitrogen and Farmyard manure or green manure on transformation of NP and S productivity of ricewheat system on vertisols. Journal of Indian Society of Soil Sciences, 49, 430-435.

Singh, M.A., S.K. Awasthi, R. Soni, R.K. Singh, Verma. and A. Kalra. 2015a. Complementarity among plant growth promoting traits in rhizospheric bacterial communities promotes plant growth. Scientific Reports, 5, 15500.

Singh, R., B. Pathak and M. Fulekar. 2015b. Characterization of PGP traits by heavy metals tolerant *Pseudomonas putida* and *Bacillus safensis* strain isolated from rhizospheric zone of weed (*Phyllanthus urinaria*) and its efficiency in Cd and Pb removal. International Journal of Current Microbiology and Applied Sciences, 4, 954-975. DOI: 10.1038/srep15500.

Singh, S.K., X. Wu, C. Shao. and H. Zhang. 2022. Microbial enhancement of plant nutrient acquisition. Stress Biology, 2(1): 1-14. https://doi.org/10.1007/s44154-021-00027-w.

Sritongon, N., S. Boonlue, W. Mongkolthanaruk, S. Jogloy and N. Riddech, N. 2023. The combination of multiple plant growth promotion and hydrolytic enzyme producing rhizobacteria and their effect on Jerusalem artichoke growth improvement. Scientific Reports, 13(1): 5917. https://doi.org/10.1038/s41598-023-33099-x.

Stewart, M.W., W.D. Dibb. E.A. Johnston. and J.T. Smyth. 2005. The Contribution of Commercial Fertilizer Nutrients to Food Production. Agronomy Journal, 97, 1-6. https://doi.org/10.2134/agronj2005.0001.

Tahir, H.A., Q. Gu, H. Wu, W. Raza, A. Hanif, L. Wu, M.V. Colman. and X. Gao. 2017. Plant growth promotion by volatile organic compounds produced by *Bacillus subtilis* SYST2. Frontiers in Microbiology, 8, 171. https://doi.org/10.3389/fmicb.2017.00171.

Tirry, N., N.T. Joutey, H. Sayel, A. Kouchou, W. Bahafid, M. Asri. and N. El Ghachtouli. 2018. Screening of plant growth promoting traits in heavy metals resistant bacteria: prospects in phytoremediation. Journal of Genetic Engineering and Biotechnology, 16, 613-619.

Tsegaye, B., C. Balomajumder and P. Roy. 2019. Isolation and characterization of novel lignolytic, cellulolytic, and hemicellulolytic bacteria from wood-feeding Termite *Cryptotermes brevis*. International Microbiology, 22, 29-39.

Tsegaye, Z.B., G. Gizaw, A. Tefera, S. Feleke, T. Chaniyalew, Alemu. and F. Assefa. 2019b. Isolation and biochemical characterization of plant growth promoting (PGP) bacteria colonizing the rhizosphere of Tef crop during the seedling stage. Journal of Plant Science and Phytopathology, 3, 013-027.

Ugulu, I. 2012. Fidelity level and knowledge of medicinal plants used to make therapeutic Turkish baths. Studies on Ethno-Medicine, 6, 1-9. https://doi.org/10.1080/09735070.2012.11886413

.

Wang, Y., G. Zhang, Y. Huang, M. Guo, J. Song, T. Zhang, Y. Long, B. Wang and H. Liu. 2022. A potential biofertilizer—siderophilic bacteria isolated from the rhizosphere of Paris polyphylla var. yunnanensis. Frontiers in Microbiology, 13, 870413. doi:10.3389/fmicb.2022.870413.

Wei, X., B. Xie, C.Wan, R. Song, W. Zhong, S. Xin and K. Song. 2024. Enhancing soil health and plant growth through microbial fertilizers: Mechanisms, benefits, and sustainable agricultural practices. Agronomy, 14(3): 609.https://doi.org/10.3390/agronomy14030609.

Weinand, T., A. El-Hasan and F. Asch. 2023. Role of *Bacillus* spp. Plant Growth Promoting Properties in Mitigating Biotic and Abiotic Stresses in Lowland Rice (*Oryza sativa* L.). Microorganisms, 11(9): 2327. https://doi.org/10.3390/microorganisms11092327

.

Yulikasari, A., B.V. Tangahu, E. Nurhayati, I. Arliyani, M. Mashudi, H.S. Titah, Y.M. Lam, Y. Yang, H. In and M.M. Soesilo. 2024. Identification of the morphological features of indigenous microbial from bauxite residue disposal areas in Indonesia. In *E3S Web of Conferences* (Vol. 557, p. 03003). EDP Sciences,24 .https://doi.org/10.1051/e3sconf/202455703003.

Zhou, D., X.F. Huang, J.M. Chaparro, D.Y. Badri, D.K. Manter, J.M. Vivanco. and J. Guo. 2016. Root and bacterial secretions regulate the interaction between plants and PGPR leading to distinct plant growth promotion effects. Plant and Soil, 401, 259-272.