Innovations

Studies on Bacterial Diversity and PGP Activity from Soils of North-Western Part of India

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Abstract

In the current research, attempts were made to isolate the potential plant growth-promoting bacterial (PGPRs) strains from the agricultural field soils from Udhampur (Jammu and Kashmir) and Gharuan (Punjab). A total of 9 bacterial strains were isolated, out of which 5 were isolated from the soil of the Jammu region and 4 from the Punjab region. In plant growth promotion tests out of the total, 55% of isolateswere found positive for HCN production while only 66% of isolates were positive in siderophore production. 55% and 88% of isolates show phosphate and zinc solubilization respectively and IAA was recorded only in the 66% of isolates. Isolates were identified at the molecular level using amplification and sequencing of 16S rRNA sequences. The DNA sequences were submitted to NCBI GenBank and accession numbers were obtained. A total of 5 isolates exhibited 4 or more than 4 plant growth-promoting traits and were inoculated with wheat varieties DBW 327, PBW 752, and Barley PL 426 grown in sterile pots containing soil and sand mixture. It was observed that the inoculated crop varieties show significant increases in 9.3%,15% and 9.9% height, 25%, 29%, and 40.2% increment in wet weight and 8.6%, 1.7% and 4.1% increment in chlorophyll content in the Wheat DBW-327, Wheat PBW-752 and Barley PL-426 crop varieties respectively than the uninoculated control plants. These isolates could become very crucial for the growth and yield of Wheat and Barley crops with sustainable agriculture. However, field trials are required to uncover the potential of isolates for plant growth and yield in an open uncontrolled environment.

Keywords: *Chemical fertilizers, PGPRs, Wheat, Barley, sustainable agriculture.*

Introduction

Over several decades the world population has grown very rapidly and currently, it is growing at the rate of around 1.05% per year. Fulfilling the demand for food for the increasing global population is a major challenge (World Population Prospects, 2019). In the 1960s, the first Green Revolutionwas introducedwith the use of intensive chemical fertilizer, which led to a significant increase in food production but consequently, the overuse of chemical fertilizers led to a major risk to soil, human, and plant health (Eliazer*et al.,* 2019). Nowadays chemical fertilizers and heavy metals are concerned with high cancer risk, so we require a safe alternative to chemical fertilizers to reduce the bad impact and maintain the sustainability of the soil focusing on the high yield of the crops (Sharma et al., 2023; Kumar *et al.,* 2019). Crops like Wheat and Barley are the major staple food crops and major sources of food in countries including India and they account for 70% of the food produced worldwide(Kianpour and Sobhanardakani, 2018).However, intensive human activities like the disposal of household and industrial waste and agricultural practices including the use of chemical fertilizers harmed soil fertility, plant health, and the microbiome of soil (Bhunia*et al.,* 2021). We need the best replacement for chemical fertilizers which have the less side effects on the soil as well as human health.

To alleviate the use of chemical fertilizers, soil bacteria have proven to be the best replacement. These bacteria when improving the growth and development of the plant as well as improving the health of the soil are known as plant growth-promoting bacteria (PGPBs) (Bai *et al.,* 2020). In the rhizosphere, only 1- 2% of bacteria can promote plant growth. They play an important role in regulating soil fertility, nutrient cycling and reducing the environmental impact of chemical fertilizers (Beneduzi*et al.,* 2012). The potential microbes stimulate the growth and development of the plant by direct and indirect mechanisms. In the direct mechanism of action, they can fix the atmospheric nitrogen, produce phytohormones like Auxin, Gibberellins, Cytokinins etc., and solubilize zinc and phosphate complexes by producing organic acids (Khatoon *et al.,* 2020). In the indirect mechanism of action, they reduce the impact of heavy metals by producing siderophores,competing with phytopathogens which protect plants from biotic stresses and producing many lytic enzymes to degrade the organic wastes to enhance the amount of organic matter in the soil (Vocciante*et al.,* 2022). These PGPBsform symbiotic relationships with the plants and reside either in the rhizosphere or enter into the plant roots (Santoyo and Gustavo, 2022).This seems to be a major utility of the second green revolution that prioritizes sustainable agricultural practices and minimizes reliance on harmful chemical inputs (Clay *et al.,* 2020; Pandey*et al.,* 2018a).

This study aims to isolate the potential plant growth-promoting bacteria from the agricultural fields where organic fertilizers are used and from the fields where chemical fertilizers are used to fulfil the nutritional requirements of the plants. wheat and barley plants were tested with isolates in the pots without any applied source of nutrientsin the soil.

Material and Methods

Sample collection

Two samples of soil were collected from the agricultural field of village Moud of Udhampur district,Jammu and Kashmir having Lat-Long (33.00, 75.15) and from the agricultural field of Gharuan village near Chandigarh University, Punjab having Lat.-Long. (30.77, 76.55) soil samples were collected at a depth of 3 inches.

Isolation of microbes

Microbes were isolated from the soil samples by serial dilution method. 1 ml of 10^{-6th} dilution was spread on the nutrient agar media (NAM) and incubated at 37± 2°C for 24 hours. Colonies were sub-cultured on different NAM plates to get the pure cultures of the colonies. The subcultures were maintained for further research inoculation in the media containing equal volumes of nutrient broth and 30% glycerol and preserved at 4°C in the refrigerators after overnight incubation.

Biochemical Identification

Bacterial isolates were identified based on the morphology and biochemical tests like gram staining, catalase production, oxidase test, urease test, etc.

***In-vitro* PGP characterization**

The isolates were screened by plant growth promotion tests to get the potential bacterial isolates having plant growth promoting and biocontrol traits like HCN, Siderophore production, IAA production, phosphate and zinc solubilization. The most promising isolates were sent for molecular identification.

Molecular characterization

The isolates were identified at the molecular level by amplification and sequencing of 16s rRNA by using the universal primer pairs 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-GTTACCTTGTTACGACTT-3').Sequencing was carried out at the National Bureau of Agriculturally Important Microbes (ICAR-NBAIM, Mau, India). $BLAST_N$ was used to compare the sequences of the isolates to determine the similarity percentile.

Phylogenetic study of bacterial isolates

By using the ClustalW program (MEGA version 11) the alignment of gene sequence was done (Fitzgerald *et al.,* 2020). The test of phylogeny was carried out with the help of the bootstrap method having 1000 no. of bootstrap replications of the sequences.The evolutionary history was inferred using the Neighbor-Joining method. The obtained gene sequences of 16S rRNA were deposited in the NCBI database to attain their accession numbers given in Table5.

Analysis of the effect of Bioinoculants on plant growth

The fertile loamy soil was collected from the field near Chandigarh University, Punjab and mixed with the sand in a ratio of 3:1. The soil was sterilized at 121^{O}C for 15 minutes in an autoclave. Wheat DBW 327, Wheat PW 752 and Barley PL 426 varieties were obtained from the seed distributors of Kharar, Punjab and 5 Seeds of wheat and barley were sown on 3 March 2024 in each prepared pot and pots were placed inthe greenhouse of Chandigarh University during the Rabi season. The pots were inoculated with the 5 most potential bacterial strains 2 *Pseudomonas,* 1*Burkholderia,* 1*Enterobacter*and1 *Rhizobium*. 5 ml of broth was used to inoculate crop varieties in each pot. Shoot height was measured after 15 days of treatment and then the whole plant height, weight and chlorophyll content were observed after 35 days of treatment.

Results

The agricultural field soil from Udhampur, Jammu and Kashmir (S1) and Gharuan, Punjab (S2)was used to isolate the plant growth-promoting bacteria. Both the soilsamples were characterized by their physicochemical properties and a difference in their pH, electrical conductivity, organic carbon and organic matterare observed and noted in (table 1). Colonies were counted and differentiated based on their morphological features (table 2). A total of 16 colonies were sub-cultured to get their pure culture based ontheir differences in morphological characteristics. 9 isolateswere further processed out of 16forbiochemical characterization.

Table 1. Physiochemical Properties of Soil

Sample no.	Soil colour	pH	Electrical conductivity	Organic carbon (%)	Organic matter (%)	Total nitrogen (%)
S1	Dark brown	7.3	0.34	0.59	0.050	1.88
S2	Brown	7.8	0.28	0.51	0.037	0.97

Table 2. Number of bacterial isolates showing different colony morphological characteristics

Sample	Shape of Bacterial Colonies					
	Small regular	Small irregular	Mucoid regular	Mucoid irregular	Large Regular	Large irregular
S1	17	6	43	16	5	9
S2	23	4	7	21	13	27

Biochemical characterization

The biochemical characterization of the isolatesshowed that9 strains were found to be in a rod shape, in gram staining all were found gram-negative except S1B4 and the majority of them were positive for citrate test (except S2B3), positive in oxidase production (except S1B4, S2B7 and S2B8), all are positive in catalase test and all are motile. Only 3 strains were positive for urease production (table3).

Table 3. Morphological and biochemical features of isolated bacterial strains

Isolates	Gram staining	Citrate test	Oxidase test	Catalase test	Urease test	Motility	Shape
S1B2	-	+	+	+	-	+	Rod
S1B3	-	+	+	+	-	+	Rod
S1B4	+	+	-	+	-	+	Rod
S1B7	-	+	+	+	+	+	Rod
S1B8	-	+	+	+	+	+	Rod
S2B2	-	+	+	+	-	+	Rod
S2B3	-	-	+	+	+	+	Rod
S2B7	-	+	-	+	-	+	Rod
S2B8	-	+	-	+	-	+	Rod

In vitro PGP characterization

Various plant growth-promoting tests were performed to explore the ability of strains for their plant growth-promoting traits. Among all, 5 isolates (S1B3, S1B4,S1B7, S2B3 and S2B7) showed 4 or more than 4 PGP traits.Isolates S1B8 and S2B7were found positive for 2 PGP traits (IAA, zinc solubilization and zinc and phosphate solubilization respectively) while two isolates S1B2 and S2B2 were positive for 1 PGP trait zinc solubilization and siderophore production respectively(table 4). Isolates showing maximum PGP traits were further selected for inoculation with the seeds of wheat and barley.

Table 4. Evaluation of PGP traits of the selected isolate

Strains	HCN Production	IAA Production	Zinc solubilization	Phosphate solubilization	Siderophore
S1B2	-	-	+	-	-
S1B3	+	+	+	+	+
S1B4	+	+	+	+	+
S1B7	+	+	+	-	+
S1B8	-	-	+	+	-
S2B2	-	-	-	-	+
S2B3	+	+	+	+	+
S2B7	-	+	+	-	-
S2B8	+	+	+	+	+

Molecular Identification

The strains were identified at the molecular level by using 16s rRNA sequencing. The 16s rRNA gene is present in genomes of bacteria and its sequence is highly conserved with variable regions that can be used for species differentiation. By amplifying and sequencing this gene, all 10 bacterial strains were identified by performing BLAST of the sequences. The bacterial species which show the most similarities with the sequence of the isolated strains were selected which shows that S1B4(PP832579), S1B2 (PP832580), S1B3 (PP832586)and S2B7 (PP832583) strains belonging to the *Pseudomonas* genus, S1B7 (PP832581) and S1B8 (PP832582) belonging to the *Burkholderia*genus, S2B8 (PP832585) belongs to the *Enterobacter* genus,S2B3 (PP832584) shows similarities with *Rhizobium* genus while S2B2 (PP832587)showed similarities with *Comamonas* genus.table 5.

Table 5. Molecular identification of isolates by 16S rRNA sequencing

Sr. No.	Strain	Identified as	NCBI Accession no.	Query cover	E-Value	% identity
1.	S1B4	*Pseudomonas sp.*	PP832579	100%	0.0	99.43%
2.	S1B2	*Pseudomonas sp.*	PP832580	99%	0.0	99.24%
3.	S1B3	*Pseudomonas sp.*	PP832586	99%	0.0	99.24%
4.	S1B7	*Burkholderiacepacia*	PP832581	100%	0.0	99.17%
5.	S1B8	*Burkholderiathailandensis*	PP832582	99%	0.0	99.49%
6.	S2B2	*Comamonas sp..*	PP832587	99%	0.0	99.21%
7.	S2B3	*Rhizobium tropici*	PP832584	99%	0.0	99.94%
8.	S2B7	*Pseudomonas putida*	PP832583	100%	0.0	100%
9.	S2B8	*Enterobacterhormaechei*	PP832585	100%	0.0	99.87%

Phylogenetic Study

The phylogenetic characterization was undertaken by BLAST analysis of 16s rRNA gene sequences of 9 isolates which revealed that 4 isolates fell within the genus *Pseudomonas* with 98-100% sequence similarity, 2 isolates fell within the genus *Burkholderia*with 99-100% sequence similarity, 1 isolate fell within the genus *Enterobacter* with 98-100% sequence similarity, 1 isolate belongs to the genus *Rhizobium* with 98.94% sequence similarity and 1 belongs to the genus *Comamonas* with 99.21% similarity. The phylogenetic tree of isolates showed that all isolates are clustered in different subgroups along with their reference strains of genus *Pseudomonas, Burkholderia, Rhizobium, Enterobacter,* and*Comamonas.*(figure 1).

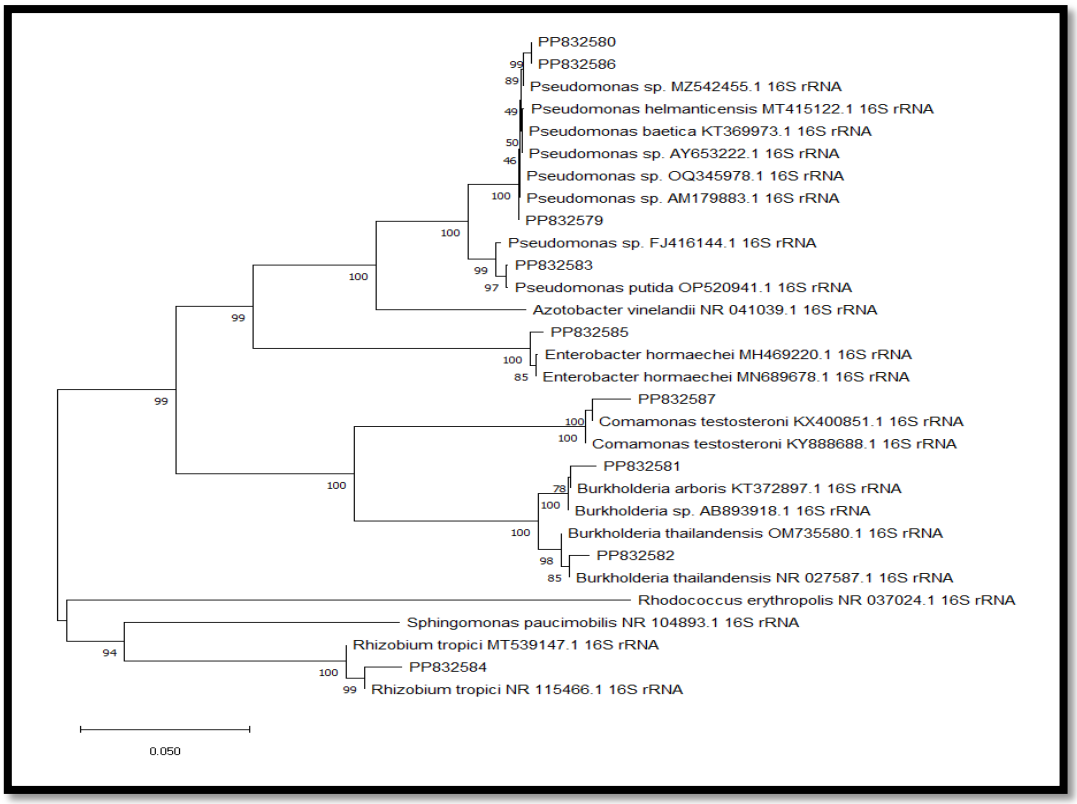

Figure 1. 16S rRNA phylogenetic tree; Phylogenetic tree of 16S rRNA sequences highlighting the position of bacterial strains relative to same and other species within the genus. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Numbers above branches are support values from 1000 bootstrap replicates. 0.050 on the scale bar represents 4 substitutions in 100 bp. Evolutionary analyses were conducted using Neighbor-Joining method in MEGA11.

Inoculation effect on plants

After 35 days of seed sowing, the plant height (root and shoot height), wet weight, dry weight and total chlorophyll content of leaves were observed.In wheat variety PBW-752 (table 6), plants inoculated with isolate S2B3 showed maximum plant height (23 inches) followed by plants treated with S1B7 (20 inches), S2B8 (17 inches),S1B3 (14inches) and S1B4 (11 inches). In wheat variety DBW-327 (table 7) the maximum height was observed in a pot inoculated with S1B4 (19.3 inches) followed by S2B8 (18 inches), S1B3 (17 inches), S2B3 (15.5 inches) and S1B7 (13.5 inches) whereas in barley variety PL-426 (table 8) the maximum height was found in the plants inoculated with S1B3 (18.4 inches), S2B3 (17 inches), S1B4 (16.2 inches), S2B8 (15 inches) and S1B7 (12.2 inches). The minimum plant height was recorded in uninoculated plants of wheat and barley varieties.

Maximum wet weight was noticed in the wheat variety PBW-752 (0.649 g) (table 6) inoculated with S2B3 isolate followed by S1B7 (0.596 g), S2B8 (0.456 g), S1B3 (0.382 g) and S1B4 (0.329 g). In wheat variety DBW-327 (table 7) the maximum wet weight was seen in the plants inoculated with S1B4 isolate (0.517 g) followed by S2B3 (0.467 g), S1B3 (0.441 g), S2B8 (0.406 g), and S1B7 (0.349 g) whereas isolate S2B3 showed maximum weight (0.625 g) in barley PL 426 (table 8) followed by S1B3 (0.495 g), S1B7 (0.401 g), S1B4 (0.382 g) and S2B8 (0.336 g). minimum plant wet weight in wheat and barley plant varieties were observed in control plants.

Similarly, the dry weight of the plants was observed and we found that the wheat variety PBW-752 (table 6) showed maximum dry weight (0.23 g) inoculated with S2B3 isolate followed by S2B8 (0.21 g), S1B7 and S1B3(0.20 g), and S1B4 (0.19 g) whereas in wheat variety DBW-327 (table 7) isolate S2B3 (0.20 g) maximum dry weight and followed by S2B8 (0.28 g), S1B4 (0.17 g), S1B3 (0.16 g) and S1B7 (0.12 g). In barley variety PL-426(table 8) inoculate S2B3 showed maximum dryweight (0.21 g) followed by S1B3 (0.19 g), S2B8 (0.15 g), S1B4 (0.14 g) and S1B7 (0.10 g). Uninoculated plants showed the minimum dry weight in all the plants.

Subsequently, total chlorophyll content was observed in the wheat and barley varieties and it is observed that wheat variety DBW-327 (table 7) showed maximum chlorophyll content (26.2 mg/100 g) with inoculation of isolate S1B4 followed byS2B8 (25 mg/100 g), S1B3 (21.7 mg/100 g), S2B3 (21.5 mg/100 g) and S1B7 (20.8 mg/100 g) whereas in wheat variety PBW-752 (table 6) isolate S2B3 showed (26 mg/100 g) followed by S1B7 (25.6 mg/100 g), S1B3 (23.2 mg/100 g), S2B8 (21.3 mg/100 g) and S1B4 (19.4 mg/100 g). In barley PL 426(table 8) isolate S1B4 showed maximum chlorophyll content (21.8 mg/100 g), S1B3 (20.9 mg/100 g), S1B7 (19.2 mg/100 g), S2B3 (21 mg/100 g) and S2B8 (18.3 mg/100 g). Minimum chlorophyll content was observed in the uninoculated control plants of all three varieties.

Table 6. Effect of microbial inoculation on Wheat variety PBW 752

Treatment	Plant height (inches)	Plant wet weight (gm)	Plant dry weight (gm)	Total Chlorophyll content (mg/100 g)
CONTROL	8	0.351	0.07	19.6
S1B3	14	0.382	0.19	23.2
S1B4	11	0.329	0.20	19.4
S1B7	20*	0.596*	0.20	25.6*
S2B3	23*	0.649*	0.23*	26*
S2B8	17	0.456	0.21*	21.3

Table 7. Effect of microbial inoculation on Wheat variety DBW 327

Treatment	Plant height (inches)	Plant wet weight (gm)	Plant dry weight (gm)	Total Chlorophyll content (mg/100 g)
CONTROL	10	0.267	0.11	16.4
S1B3	17	0.441	0.16	21.7
S1B4	19.3*	0.517*	0.17	26.2*
S1B7	13.5	0.349	0.12	20.8
S2B3	15.5	0.467	0.20*	21.5
S2B8	18*	0.406*	0.18*	25*

Table 8. Effect of microbial inoculation on Barley varietyPL 426

Treatment	Plant height (inches)	Plant wet weight (gm)	Plant dry weight (gm)	Total Chlorophyll content (mg/100 g)
CONTROL	8.5	0.223	0.10	16.9

S1B3	18.4	0.495*	0.19*	20.9
S1B4	16.2*	0.382	0.14	21.8*
S1B7	12.2	0.401	0.10	19.2
S2B3	17*	0.625*	0.21*	21*
S2B8	15	0.336	0.15	18.3

Discussion

In this researchhigherbacterial diversity was reported from the soil samples from Jammu.However, increasedplant growth promotional activities were reported from thebacterial strains obtained from the soil samples from Punjab. To the best of our knowledge, no such study with respect to the comparison of bacterial diversity and their PGP activityis done earlier from the soil sample of these two states. Plant growth-promoting bacteria can promote the growth of plants by multiple PGP traits like auxin production, phosphate and zinc solubilization, siderophore production,HCN production etc. Many studies reported positive results of inoculation of the PGPRs with the Wheat and Barley crops (Ahad and Pandey, 2024; Devi *et al.,* 2023).

Phosphorous is the most important component of nucleic acids and plays an important role in the regulation of protein synthesis, cell division, energy transfer and the development of newly formed tissues, respiration, root development and speeding up the growth and development of plants. phosphorous is required to produce high yields of crops but in the soil normally it is available in a complex form that plants cannot uptake normally. In our study, out of selected isolates for inoculation*Pseudomonas, Rhizobium tropici,* and*Enterobacterharmocheii*solubilize phosphate and promote growth of the Wheat and Barley crops.Our study with results of *Pseudomonas* shows similarities with the research ofIbanez*et al.*(2021) for Barley crops and Elhaissoufi*et al.*(2020) for Wheat.Tyagi and co-workers(2022) and Gupta *et al.*(2021) also found *Rhizobiumtropici* positive for phosphate solubilization.

The availability of zinc in the form of complexes can affect the low yields of the crops. the zinc-solubilizing bacteria can enhance growth and development by solubilizing and making them available for the plant's uptake. In our study, 90% of isolates show positive results for zinc solubilizing. The most promising 5 isolates show similarities with the findingsof Zahra *et al.,*(2023); Devi *et al.,*(2023); Upadhyay *et al.,*(2021) andSammauria*et al.,*(2020) with respect to the zinc solubilization.

Auxin is the most important plant hormone which plays an important role in the signalling of plant development, regulation, cell elongation, cell division and cell differentiation. In our study, we found *Pseudomonas* sp., *Burkholderiacepacia*, *Rhizobiumtropici* and *Enterobacterharmocheii* are positive for IAA production in vitro and promote the growth of the Wheat and Barley crops but *Rhizobium tropici* is found to be as the most promising isolate as it promotes more growth than the others. Parewa*et al.,*(2014) also found *Rhizobium tropici* positive for auxin production. Pandey*et al.,*(2023) also reveals that *Rhizobium* strains could become wonderfulauxin producers.

PGPB also raise competition for the pathogens for essential nutrients and elements. One of the vital elements for plants as well as for microbes is iron. PGPBs secrete siderophores in soil and chelate the available iron and make it unavailable for pathogens. Siderophore is alow molecular weight (500-1000 da) compound. In addition,it also promotes induced systemic resistance (ISR)

and promotes plant growth and development. According to (Datta and Chakraborty, 2014) siderophore can increase the availability of soluble metals in addition to forming stable complexes with heavy metals. In this study found that our 6 isolates can be able to produce siderophores and the same results are described by many researchers (Jha*et al.,* 2021; Roslan*et al.,* 2020; Pandey*et al.,* 2018b; Datta*et al.,* 2014).

PGPRs also protect plants from biotic stressors (pathogens) by producing HCN. So PGPRs are the best replacement for pesticides and are used as an eco-friendly approach for sustainable agriculture. In this work, we get 5 isolates having HCN-producing ability. Similar work for the same isolates is done by different researchers for *Enterobacterhormoachei*(Sandilya*et al.,* 2022; Katiyar*et al.,* 2017), *Pseudomonas* sp. (Reetha*et al.,* 2014),*Rhizobiumtropici* (Bhat*et al.,* 2020), *Burkholderiacepacia* (Heo*et al.,* 2022).

Conclusion

In the current study, PGPRs were isolated from the soil samples of two different states *i.e.* Jammu-Kashmir and Punjab, India. A total of 9 bacterial strains were isolated from these soil samples.Out of the total, 5 isolates (two isolates of *Pseudomonas genus,*one*Burkholderiacepacia,*one *Rhizobium sp., and*one*Enterobacterharmocheii)* showed multiple PGP traits *invitro.* Inoculation of these potential bacterial isolates in the wheat varieties (Wheat DBW 327 and Wheat PBW 752) and Barley PL 426 enhanced plant height, plant dry weight, and chlorophyll content in the pot experiment under natural sunlight conditions in the net house without application of chemical fertilizers. The results suggest that these potential strains may be used as an alternative to chemical fertilizers, in sustainable agriculture and may reduce the risks associated with chemical fertilizers and human health. However, field trials are required to evaluate the efficacy and contribution of the isolated microbes towards crop yield and yield-related traits.

Additional research could concentrate on evaluating the strain's potential to boost productivity and exploring the correlations between gene function. These strains could undergo testing in diverse field environments to assess their impact on plant growth promotion and their biocontrol efficacy against various phytopathogens under different field conditions. Stress response and gene expression analyses could be conducted to investigate any protective effects of the formulations on crops. Bio-formulations could be developed and field-tested to explore the potential for commercialization.

References

1. *Ahad, M. and andPandey, R. P (2024). Isolation and Inoculation Effect of Trichoderma reesei on Growth and Yield of Barley. Asian Journal of Agricultural and Horticultural Research,11 (1), 15-19.*

2. *Bai, Y. C., Chang, Y. Y., Hussain, M., Lu, B., Zhang, J. P., Song, X. B., Lei, X. S, and Pei, D(2020). Soil chemical and microbiological properties are changed by long-term chemical fertilizers that limit ecosystem functioning. Microorganisms,8(5), 694.*

3. *Beneduzi, A., Ambrosini, A and Passaglia, L. M (2012). Plant growth-promoting rhizobacteria (PGPR): their potential as antagonists and biocontrol agents. Genetics and Molecular Biology,35,1044-1051.*

4. *Bhat, M. A., Kumar, V., Bhat, M. A., Wani, I. A., Dar, F. L., Farooq, I., Bhatti, F., Koser, R., Rahman, S and Jan, A. T (2020). Mechanistic insights of the interaction of plant growth-promoting rhizobacteria (PGPR) with plant roots toward enhancing plant productivity by alleviating salinity stress. Frontiers in Microbiology,11,1952.*

5. *Bhunia, S., Bhowmik, A., Mallick, R and Mukherjee, J (2021). Agronomic efficiency of animal-derived organic fertilizers and their effects on biology and fertility of soil: A review. Agronomy,11(5), 823.*

6. *Clay, N and Zimmerer, K. S (2020). Who is resilient in Africa's green revolution? Sustainable intensification and climate smart agriculture in Rwanda. Land Use Policy,97,104558.*

7. *Datta, B and Chakrabartty, P. K (2014). Siderophore biosynthesis genes of Rhizobium sp. isolated from Cicerarietinum L. 3 Biotech,4(4),391-401.*

8. *Devi, K., Sharma, D., Singh, R. and Pandey, R.P.(2023). Screening and characterization of plant growth promoting rhizobacteria and their effect on growth and yield of barley (Hordeumvulgare L.). Crop Research,58(5),207-215.*

9. *Devi, S. P., Ranjithkumar, R., Djearamane, S., Tey, L. H., Wong, L. S., Kayarohanam, S., Arumugam, N., Almansour, A. I and Perumal, K(2023). Organic Remobilization of zinc and phosphorus availability to plants by application of mineral solubilizing bacteria Pseudomonas aeruginosa. Heliyon,9(11).*

10. *Elhaissoufi, W., Khourchi, S., Ibnyasser, A., Ghoulam, C., Rchiad, Z., Zeroual, Y., Lyamlouli, K and Bargaz, A (2020). Phosphate solubilizing rhizobacteria could have a stronger influence on wheat root traits and aboveground physiology than rhizosphere P solubilization. Frontiers in Plant Science,11,979.*

11. *Eliazer Nelson, A. R. L., Ravichandran, K and Antony, U (2019). The impact of the Green Revolution on indigenous crops of India. Journal of Ethnic Foods,6(1), 1-10.*

12. *Gupta, R., Noureldeen, A and Darwish, H (2021). Rhizosphere mediated growth enhancement using phosphate solubilizing rhizobacteria and their tri-calcium phosphate solubilization activity under pot culture assays in Rice (Oryza sativa). Saudi Journal of Biological Sciences,28(7),3692-3700.*

13. *Heo, A. Y., Koo, Y. M and Choi, H. W (2022). Biological control activity of plant growth promoting rhizobacteriaBurkholderiacontaminans AY001 against tomato fusarium wilt and bacterial speck diseases. Biology,11(4),619.*

14. *Ibáñez, A., Diez-Galán, A., Cobos, R., Calvo-Peña, C., Barreiro, C., Medina-Turienzo, J., Sánchez-García, M and Coque, J. J. R(2021). Using rhizosphere phosphate solubilizing bacteria to improve barley (Hordeumvulgare) plant productivity. Microorganisms,9(8),1619.*

15. *Jha, A and Popat, T (2021).Burkholderiacepacia BAM-12 isolated from Mungbean field in Rajasthan augments plant growth in agricultural field soil of Gujarat, India. Journal ofMicrobiology, Biotechnology and Food Sciences,11(1),e3222-e3222.*

16. *Katiyar, D., Hemantaranjan, A., Singh, B and Malakar, A (2017). Isolation and characterization of plant growth promoting rhizobacteriaEnterobacterhormaechei and their suppression efficacy against Colletotrichumfalcatum in combination with Chitosan. International Journal of Plant and Soil Science,14(3),1-12.*

17. *Khatoon, Z., Huang, S., Rafique, M., Fakhar, A., Kamran, M. A and Santoyo, G(2020). Unlocking the potential of plant growth-promoting rhizobacteria on soil health and the sustainability of agricultural systems. Journal of Environmental Management,273,111118.*

18. *Kianpour, S and Sobhanardakani, S (2018). Evaluation of Zn, Pb, Cd and Cu concentration in wheat and bread consumed in human city. Journal of Food Hygiene,7, 81-98*

19. *Kumar, R., Kumar, R and Prakash, O (2019). Chapter 5 the impact of chemical fertilizers on our environment and ecosystem. Chief Ed,35(69),1173-1189.*

20. *Parewa, H. P., Yadav, J., Rakshit, A., Meena, V. S and Karthikeyan, N (2014). Plant growth promoting rhizobacteria enhance growth and nutrient uptake of crops. Agric Sustain Dev,2(2),101-116.*

21. *Pandey, R. P., Singh, P. K., Pundir, R. K., Srivastava, A. K., Gupta, V. K., Ramteke, P. W., and O'Donovan, A. (2023). Stress-tolerant Plant Growth-Promoting Mesorhizobiumciceri Isolates from MID-gangetic Plains. Applied Biochem and Microbiol,59(3), 349-360.*

22. *Pandey, R. P., Srivastava, A. K., Srivastava, A. K., &Ramteke, P. W. (2018a). Antibiotic Resistance in Mesorhizobiumciceri from Eastern Uttar Pradesh, India. Climate Change and Environmental Sustainability, 6(2), 114-118.*

23. *Pandey, R. P., Srivastava, A. K., Gupta, V. K., O'Donovan, A., &Ramteke, P. W. (2018b). Enhanced yield of diverse varieties of chickpea (Cicerarietinum L.) by different isolates of Mesorhizobiumciceri. Environmental Sustainability, 1(4), 425-435.*

24. *Reetha, A. K., Pavani, S. L and Mohan, S(2014). Hydrogen cyanide production ability by bacterial antagonist and their antibiotics inhibition potential on Macrophominaphaseolina (Tassi.) Goid. International Journal of Current Microbiology and Applied Sciences,3(5),172-178.*

25. *Roque, W. F., Guimarães, S. L and Bonfim-Silva, E. M (2021). Development of wheat plants coinoculated with rhizobium strains. RevistaBrasileira de EngenhariaAgrícola e Ambiental,25,758-763.*

26. *Roslan, M. A. M., Zulkifli, N. N., Sobri, Z. M., Zuan, A. T. K., Cheak, S. C and Abdul Rahman, N. A (2020). Seed biopriming with P-and K-solubilizing Enterobacterhormaechei sp. improves the early vegetative growth and the P and K uptake of okra (Abelmoschusesculentus) seedling. PloS One,15(7),e0232860.*

27. *Sammauria, R., Kumawat, S., Kumawat, P., Singh, J and Jatwa, T. K (2020). Microbial inoculants: potential tool for sustainability of agricultural production systems. Archives of Microbiology,202(4),677-693.*

28. *Sandilya, S. P., Jeevan, B., Subrahmanyam, G., Dutta, K., Vijay, N., Bhattacharyya, N and Chutia, M (2022). Co-inoculation of native multi-trait plant growth promoting rhizobacteria promotes plant growth and suppresses Alternaria blight disease in castor (Ricinuscommunis L.). Heliyon,8(12).*

29. *Santoyo, G(2022). How plants recruit their microbiome? New insights into beneficial interactions. Journal of Advanced Research,40,45-58.*

30. *Sharma, D., Devi, K. and Pandey, R. P. (2023). Isolation of Heavy Metal Tolerating Bacteria and Its Effect on Two different Wheat Varieties. Innovations,73(4),1117-1130.*

31. *Tyagi, S., Reddy, K., Haniya, K., Swarnalakshmi, K., Senthilkumar, M., Kumar, U and Annapurna, K (2022). Harnessing cereal–rhizobial interactions for plant growth promotion and sustainable crop production. In Nitrogen Fixing Bacteria: Sustainable Growth of Non-legumes,277-298.*

32. *Upadhyay, H., Gangola, S., Sharma, A., Singh, A., Maithani, D and Joshi, S (2021). Contribution of zinc solubilizing bacterial isolates on enhanced zinc uptake and growth promotion of maize (Zea mays L.). Folia Microbiologica,66,543-553.*

33. *Vocciante, M., Grifoni, M., Fusini, D., Petruzzelli, G and Franchi, E (2022). The role of plant growth-promoting rhizobacteria (PGPR) in mitigating plant's environmental stresses. Applied Sciences,12(3),1231.*

34. *Wang, Z., Zhang, H., Liu, L., Li, S., Xie, J., Xue, X and Jiang, Y (2022). Screening of phosphate-solubilizing bacteria and their abilities of phosphorus solubilization and wheat growth promotion. BMC Microbiology,22(1),296.*