Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 24, No. (3) : 2022 : 590-595

ISSN-0972-3005

DOI No.: http://doi.org/10.53550/AJMBES.2022.v24i03.0028

IN VITRO SCREENING OF PLANT GROWTH PROMOTING RHIZOBACTERIA FROM THE RHIZOSPHERIC SOILS OF WHEAT IN THE NORTHWEST REGION OF THE WESTERN HIMALAYAS

INDU BHARDWAJ[1], VIJAY KUMAR[2*], RICHA VERMA[3] AND SAHIL KUMAR[4]

[2*]Assistant Professor, Microbiology
[1,3,4]Research Scholars, Microbiology Research Laboratory
Division of Microbiology, School of Pharmaceutical and Health Sciences
Career Point University, Hamirpur (Himachal Pradesh) India-176041

(Received 7 May, 2022; Accepted 22 June, 2022)

Key words: *Biofertilizer, Bioformulation, Himachal Pradesh, PGP attributes, Rhizosphere*

Abstract– Plant growth promoting bacteria are bacteria that colonize the root surfaces and closely adhere to the soil interface, known as the rhizosphere. In the present study PGPR's were isolated from agricultural field of wheat (*Triticum aestivum*) Salouni and Ladraur sites of Hamirpur district of Himachal Pradesh. Total 20 bacterial isolates were isolated from two different sites. In vitro screening was used to identify the potential of isolates as plant growth promoting rhizobacteria inoculants based on their capacity to solubilize phosphate, protease production (lytic enzyme), and HCN synthesis. Out of 20 bacterial isolates, selected seven bacterial isolates were inoculate into carrier (cocopeat powder and charcoal powder). The purpose of this study was to isolate and characterize the most potent PGPR, as well as to evaluate their performance in terms of HCN production, protease production, and P-solubilization, and making consortia-based bioformulation by using these selected bacterial isolates. In this investigation, we evaluated the plant growth promoting activity of two different carriers such as charcoal and cocopeat based formulation of plant growth promoting rhizobacteria.

INTRODUCTION

The cultivation of wheat come before 5000 years back during the age of Indus valley civilization where the primary original species was *Triticum sphaerococcum* commonly referred to as Indian wheat has now disappeared and replaced by present day species- *Triticum aestivum* or common wheat or the common bread wheat, *Triticum durum* or the Macaroni wheat and *Triticum diococcum* or the Emmer wheat (farmer.gov.in). The Indian bread wheat or durum wheat verities possess low levels of grain iron and zinc. Therefore, there is a requirement of enhancing the iron, zinc, and other micronutrient content in wheat through biofortification (nfsm.gov.in). To circumvent the issue of soil fertility decreases by chemical fertilizer. Soil nutrient level gets decreased over time when crop plants get harvested, and these nutrients get restore either through natural decomposition process or by adding fertilizers. So PGPRs can be of more interest as they enhance the plant growth and yield in an ecofriendly manner Shrivastava *et al.,* (2015). The word PGPR was coined by Kloepper and Schroth in 1978 Verma *et al.,* (2019). They can stimulate the plant growth, increase the crop yield, reduce the fertilizer requirements, and protect plant from pathogens via secretion of various plants growth promoting substances as well as biofertilizer Haridom *et al.,* (2008). Several bacterial species have been utilized as PGPR such as *Agrobacterium, Arthrobacter, Azotobacter, Azospirillum, Bacillus, Burkholderia, Caulobacter, Chromobacterium, Erwinia, Flavobacterium, Micrococcus, Pseudomonas*, and *Serratia* [Bhattacharya and Jha (2012)]. In the present outline development of carrier-based formulation of bio-inoculant is an industrial skill to renovate a laboratory documented bacterium to a commercial profitable field product Pahari *et al.,* (2017). Studies have shown where cocopeat based formulations of

Pseudomonas, *Bacillus subtilis*, charcoal-based formulation of *Pseudomonas fluorescence* have enhanced the growth of maize, wheat, jowar and bajra plants [Gunjal and Kapadnis (2019)]. Formulation should contain ingredients (cocopeat powder, and charcoal) in a suitable carrier with additives that will help in the stabilization and perform as protective shield of the bacterial cell during storage. A major purpose of bacterial inoculant formulation is to provide suitable microhabitat for survival in the soil ecosystem Pahari *et al.,* (2017).

The main goal of this investigation is the isolation, screening, and characterization of wheat in Hamirpur district (Ladraur and Salouni), Himachal Pradesh, India, with a special focus on the isolation and selection of bacterial isolates to be used as potential bioformulations that are used as biofertilizers and play a role in enhancing plant growth and reducing the cost of chemical fertilizer.

MATERIALS AND METHODS

1. Soil Preparation

The soil samples used in this study were collected from the rhizosphere of Wheat fields (*Triticum aestivum*) from two locations of Hamirpur district (Ladraur and Salouni) of Himachal Pradesh, India. One site was selected for each location for the rhizospheric soil sampling. Soil samples were taken to the Microbiology Laboratory for bacterial isolation, purification, and *in vitro* screening of bacteria in the wheat rhizosphere. Sample were stored in plastic bags loosely tied to ensure sufficient aeration and to prevent moisture loss from the soil samples, Sukweenadhi *et al.,* (2019).

2. Bacterial Isolation and Purification

PGPR isolates were isolated from the rhizospheric soil sample by serial dilution and spread plate method using King's B agar medium at 37 °C. For rhizospheric PGPR, prepare soil suspension, serial dilutions were made up to 10^{-1} to 10^{-8}. On-prepared King's B agar plates, 0.1ml of each dilution was spreaded [Modi and Parmar (2017)]. After 24 hours of incubation, many colonies were randomly selected on the basis of colony morphology and further purified by streaking on King's B agar plates, Cassan *et al.,* (1977).

3. Morphological Characterization and Gram Staining

Morphological characteristics of isolates including colony morphology (pigment, shape, margin, and elevation). The production of pigments was checked on King's B agar at 37 °C after 24 to 48 hours. The cell wall composition of the isolates was determined by Gram staining. Crystal violet, Gram's iodine, Alcohol/ Acetone destaining reagents, and Safranin was used in the gram staining procedure [Cappuccino and Sherman (2008)].

4. Biocontrol activities of PGPR bacterial isolates

4.1 Production of Hydrogen cyanide (HCN)

The method suggested by Lorck (1948) and Castric (1977) was used to measure HCN production. Bacterial cultures were streaked on prepared plates of nutrient agar medium amended with glycine (1.4 g/l) to measure hydrogen cyanide production, Aarab *et al.,* (2016). A Whatman No. 1 filter paper strip was soaked in 0.5% picric acid, followed by 2% sodium carbonate, and placed on top of each petriplate. Plates were sealed with parafilm and incubated for four days at 28 °C. The change in color of the filter paper from yellow (-) to light brown (++), brown (+++), and dark brown (++++) indicated the presence of hydrogen cyanide [Modi and Parmar (2017)].

5. Growth promoting activities by the PGPR bacterial isolates

5.1 Protease Production

All isolates were screened out for protease production on skim milk agar (1% skim milk in nutrient agar medium) and autoclaved separately before pouring. Both flasks were mixed, and plates were poured. Each bacterial culture was spot inoculated on skim milk agar plates and incubated for 24-48 hours at 28 °C. A clear zone around the colony indicates that protease production is occurring [Modi and Parmar (2017)].

5.2 Phosphate solubilization

Each bacterial culture was inoculated in the center of Pikovskaya's agar plates containing tricalcium phosphate as an insoluble phosphate source. For 5-7 days, the plates were incubated at 28 ± 2 °C in an incubator. The plates were then examined for the formation of halozone or clear zone around the bacterial growth, Aarab *et al.,* (2016).

RESULTS AND DISCUSSION

1.1 Collection of rhizospheric soil samples

For PGPR isolation, rhizospheric soil samples were taken from the rhizosphere of wheat crops in the Hamirpur district of Himachal Pradesh, India, at different altitudes above mean sea level in Ladraur (807m) and Salouni (500m).

Isolation of rhizobacteria: Isolation of bacteria from the rhizosphere of Wheat (*Triticum aestivum*) were done by serial dilution and spread plate method using King's B agar medium. Total, 20 PGPR's isolates were isolated on King's B agar medium. The morphological characters of bacterial isolates, i.e., pigmentation, form, elevation, margin, shape, and gram-reaction were noted down (Table 1 and Table 2).

2. Morphological identification of PGPR's isolated from rhizospheric soil of wheat

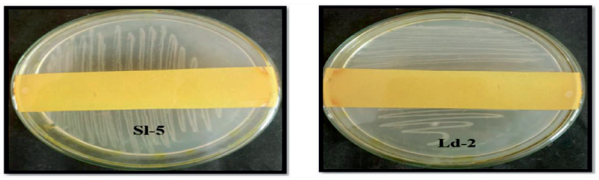
The morphological characters of bacterial isolates i.e., color, form, elevation, margin, shape, and gram reaction of bacterial isolates were noted down.

3. Biocontrol and growth promoting activities of bacterial isolates

3.1 HCN Production

3. Biocontrol activities of bacterial isolates

Out of nine isolates, isolated from Ladraur, maximum production of HCN (++++) was shown by Ld-2 to change the color of filter paper strip yellow (-) to dark brown (++++) (Table 3 and figure 1 (B)). On the other hand, out of eleven isolates, isolated from Salouni site, maximum production of HCN (+++) was shown by Sl-5 to change the color of filter paper strip yellow (-) to brown (+++) (Table 3 and figure 1 (A)). In the research conducted by Amaresan *et al*., (2011), 8 isolates identified by using a microbial identification system (Biolog). Out of 8 isolates, six belong to genus *Bacillus* and *two* are



(A). Salouni (B). Ladraur

Fig. 1. (A) Shows the HCN production by Sl-5 bacterial isolates from Salouni site and (B) Shows the HCN production by Ld-2 bacterial isolates from Ladraur site

Table 1. Colony Morphology of bacterial isolates from the site Salouni

Sr. No.	Isolates	Pigment	Elevation	Margin	Form	Gram reaction	Shape
1	Sl-1	White	Raised	Entire	Round	+	Bacilli
2	Sl-2	Cream	Convex	Entire	Round	-	Bacilli
3	Sl-3	Cream	Flat	Entire	Round	-	Bacilli
4	Sl-4	Cream	Flat	Filiform	Filamentous	-	Bacilli
5	Sl-5	Cream	Flat	Entire	Punctiform	-	Bacilli
6	Sl-6	White	Raised	Entire	Circular	-	Bacilli
7	Sl-7	White	Raised	Entire	Round	-	Cocci
8	Sl-8	White	Raised	Entire	Circular	-	Bacilli
9	Sl-9	Off-white	Raised	Entire	Circular	-	Cocci
10	Sl-10	Cream	Raised	Undulate	Circular	-	Bacilli
11	Sl-11	Cream	Flat	Undulate	Circular	-	Cocci

Table 2. Morphological characteristics of bacteria isolated from site Ladraur

Sr. No.	Isolate	Pigment	Elevation	Margin	Form	Gram reaction	Shape
1	Ld-1	Cream	Raised	Entire	Round	-	Bacilli
2	Ld-2	Cream	Raised	Entire	Round	-	Bacilli
3	Ld-3	White	Flat	Entire	Circular	-	Bacilli
4	Ld-4	White	Flat	Undulate	Punctiform	-	Cocci
5	Ld-5	Whitish	Flat	Entire	Round	-	Bacilli
6	Ld-6	White	Flat	Entire	Circular	+	Bacilli
7	Ld-7	Off-white	Flat	Entire	Punctiform	-	Bacilli
8	Ld-8	Off-white	Raised	Entire	Punctiform	-	Bacilli
9	Ld-9	White	Flat	Undulate	Round	-	Bacilli

Table 3. Evaluation of different PGP attributes of rhizobacteria isolated from site Salouni and Ladraur

Sr. No.	Isolates	HCN Change of color (Yellow to brown)	Protease production plate (mm dia)	Phosphate-solubilization plate (mm dia)
1	Ld-1	-	0	11
2	Ld-2	++++	9	18
3	Ld-3	-	7	8
4	Ld-4	-	0	9
5	Ld-5	-	11	6
6	Ld-6	-	16	17
7	Ld-7	-	6	13
8	Ld-8	-	0	12
9	Ld-9	-	8	-
10	Sl-1	-	7	8
11	Sl-2	-	0	6
12	Sl-3	-	7	9
13	Sl-4	-	11	13
14	Sl-5	+++	9	16
15	Sl-6	-	0	11
16	Sl-7	-	0	12
17	Sl-8	-	13	-
18	Sl-9	-	11	-
19	Sl-10	-	0	-
20	Sl-11	-	6	-

Serratia which obtained from the chilli plants in the tropical regions of Andaman and Nicobar Islands, India has the potential as a plant growth promoting activity with the ability of isolates to produce IAA, and siderophore, by *in vitro* screening. These 8 bacterial showed antagonistic activity against *Sclerotium rolfsii, Fusarium oxysporum*, and *Pythium* species.

***HCN production colour change from Yellow to brown to dark brown**

Yellow : - Light brown : ++ Brown: +++ Dark brown : ++++

3.2 Determination of Protease production

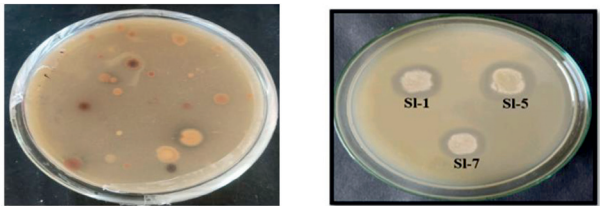
Protease activity

Protease activity shown by bacterial isolates from Ladraur site was expressed in the range of 6 to 16 mm diameter of clear zone around the bacterial growth on skim milk agar plate. Out of nine isolates, 6 isolates shown protease activity. Maximum activity was shown by Ld-6 (16 mm) followed by Ld-5 (11mm) and Ld-2 (9mm) [Table 3]. Whereas protease activity shown by bacterial isolates from Salouni site was expressed in the range of 6 to 13 mm diameter of clear zone around the bacterial growth on skim milk agar plate. Out of eleven isolates, 7 isolates shown protease activity. Maximum activity was shown by Sl-8 (13 mm) followed by Sl-4 (11 mm) and Sl-9 (11 mm) [Table 3]. Aarab *et al*., (2015), *Aeromonas*, *Pseudomonas*, *and Enterobacter* which isolated 305 bacteria from the rice fields in Northwestern Morocco, have the potential as a plant growth promoting activity with the ability of six isolates to produce IAA, 3 bacteria were positive for HCN activity and while, all bacteria show siderophore production except isolate P66, and P-solubilizing activity.

4. Growth promoting activities by bacterial isolates

4.1 Phosphate solubilization

Phosphate solubilization activity shown by bacterial isolates from Ladraur site was expressed in the range of 6 to 18 mm diameter of halozone on PVK agar plate. Out of nine isolates, only 8 isolates showed phosphate solubilization activity. Maximum P-solubilization was shown by Ld-2 (18 mm) followed by Ld-6 (17 mm) and Ld- 2 (13mm) [Table 3 and figure 2 (A)]. Whereas phosphate solubilization activity shown by bacterial isolates from Salouni site was expressed in the range of 6 to 16 mm diameter of halozone on PVK agar plate. Out of eleven isolates, only 7 isolates showed phosphate solubilization. Maximum P-solubilization was shown by Sl-5 (16 mm) followed by Sl-4 (13mm) and



(A). Ladraur (B). Salouni site

Fig. 2. (A) Phosphate solubilization activity shown by bacterial isolates from Ladraur site and Salouni site

Sl-7 (12mm) [Table 3 and figure 2 (B)]. From Salouni site most of the isolates were found positive for P-solubilization. Moreover, Modi *et al.*, (2017) isolated 6 strains of *Pseudomonas*, 4 strains of *Azotobacter* and 2 strains of *Bacillus* from rice rhizospheric soil from different villages of Bardoli, Gujarat, India. Gram positive and Gram-negative bacteria like *Pseudomonas*, *Bacillus* and *Burkholderia* etc. are among the most potent phosphate solubilizers (Saiyad *et al*., 2015). Several studies in past suggested that application of PSB improves plant P nutrition and increases the yield of cereals including wheat (Afzal and Bano, 2008). The purpose of this study was to isolate and characterize the most potent PGPR, as well as to evaluate their performance in terms of HCN production, protease production, and P-solubilization, and making consortia-based bioformulation by using these selected bacterial isolates.

5. Bioformulation

On the basis of PGP attributes, the selected bacterial isolates are subsequently transferred into these carriers (cocopeat and charcoal). This type of formulation is referred to as consortium-based formulation, and it functions as a biofertilizer (Gunjal and Kapadnis, 2020). After bacterial inoculation to the carrier, the maximum viable count was observed in the cocopeat powder. Cocopeat-based formulations depict higher plant growth promoting activity as compared with another carrier. Thus, cocopeat-based formulation is often used for plant growth promoting activity of varied crops. Then these consortia are then introduced to plants to monitor their actions, such as enhancing crop yields and encouraging plant development and reduced the cost of chemical fertilizer and plays a role in sustainable agriculture. The maximum microbial diversity shown on cocopeat powder as compared to charcoal powder different site of Hamirpur District of Himachal Pradesh.

CONCLUSION

A total of twenty isolates were recovered from the Salouni and Ladraur site in the Hamirpur district of Himachal Pradesh. These isolates were isolated on King's B agar media from rhizospheric soil samples of wheat, bacteria were showed different plant growth promoting activities such as HCN, Protease activity, and Phosphate solubilization activity. There were 20 bacterial isolates that were successfully purified from the rhizospheric region of wheat (*Triticum aestivum*) Salouni and Ladraur sites. Out of 20 isolates, 15 isolates were positive for P-solubilization in the range of 10-30 mm zone on PVK agar. In case of lytic enzyme activity 13 bacterial isolates were positive for protease production. In case of HCN production 2 bacterial isolates were found positive for hydrogen cyanide activity. Maximum P-solubilization was shown by the isolates from two different site were Ld-2 (18 mm) and Sl-5 (16 mm). In case of protease, maximum protease activity was shown by the isolates Ld- 6 (16 mm) and Sl-8 (13 mm) on skim milk agar plates. In case of HCN production, maximum HCN activity was shown by Ld-2 (++++) and Sl-3 (+++) to change the color of filter paper strip yellow (-) to brown (+++), dark brown (++++) to light brown (++).

In vitro, this investigation shows that a potential PGPR plays a critical role in protease activity, HCN and phosphate solubilizing activity. It is stated that this is a fundamental study that has offered insight into the bacterial community found in rhizosphere of wheat in Salouni and Ladraur sites of Himachal Pradesh, India.

ACKNOWLEDGMENT

Authors are thankful to Career Point University, Hamirpur (H.P.), India for funding the research work. Thanks, are also due to the Head, Division of Microbiology, Career Point University, Hamirpur (H.P.) for providing the necessary facilities required for conducting the research work.

Conflict of Interest Statement

The authors declare that they have no conflicts of Interest.

REFERENCES

Aarab, S., Ollero, F.J., Megías, M., Laglaoui, A., Bakkali,

M. and Arakrak, A. 2015. Isolation and screening of bacteria from rhizospheric soils of rice fields in Northwestern Morocco for different plant growth promotion (PGP) activities: An in vitro study. *Int J Curr Microbiol App Sci*. 4(1) : 260-269.

Amaresan, N., Kumar, K., Madhuri, K. and Usharani, G.K. 2016. Isolation and characterization of salt tolerant plant growth promoting rhizobacteria from plants grown in tsunami affected regions of Andaman and Nicobar Islands. *Geomicrobiology Journal*. 33(10): 942-947. https://doi.org/10.1080/01490451.2015.1128994

Bhattacharyya, P.N. and Jha, D.K. 2012. Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World Journal of Microbiology and Biotechnology*. 28(4): 1327-1350. https://doi.org/10.1007/s11274-011-0979-9.

Cappuccino, J.G. and Sherman, N. 2008. Microbiology: a laboratory manual (Vol. 9).

Cassán, F.D., Okon, Y. and Creus, C.M. 2015. Handbook for Azospirillum. *Springer, Basel.* 10: 978-3.

Gunjal, A.B. and Kapadnis, B.P. 2019. Agroindustry By-Product Based Formulations of Burkholderia gladioli for Plant Growth. In *Proc Indian Natn Sci Acad*. 85 (3): 689-695). doi: 10.16943/ptinsa/2019/49646

Gunjal, A.B. and Kapadnis, B.P. 2020. Pressmud based formulations of plant growth promoting rhizobacteria as eco-friendly approach for the plant growth. *International Journal of Agricultural Sciences and Veterinary Medicine.* 8(3): 10-13

Hardoim, P.R., van Overbeek, L.S. and van Elsas, J.D. 2008. Properties of bacterial endophytes and their proposed role in plant growth. *Trends in microbiology*. 16(10): 463-471. Doi: https://doi.org/10.1016/j.tim.2008.07.008

Wheat: The Crop for Ensuring Indian Food Security https://farmer.gov.in/imagedefault/pestanddiseasescrops/wheat.pdf

https://www.nfsm.gov.in/StatusPaper/Wheat2016.pdf

Lorck, H., 1948. Production of hydrocyanic acid by bacteria. *Physiologia Plantarum*, 1(2): 142-146. https://doi.org/10.1111/j.1399-3054.1948.tb07118.x

Modi, K., Patel, P. and Parmar, K. 2017. Isolation, screening, and characterization of PGPR from rhizosphere of rice. *Int J Pure Appl Biosci*, *5*, pp.264-270. Doi: http://dx.doi.org/10.18782/2320-7051.2887

Pahari, A., Pradhan, A., Maity, S. and Mishra, B.B. 2017. Carrier based formulation of plant growth promoting Bacillus species and their effect on different crop plants. *Int J Curr Microbiol App Sci*, *6*, pp.379-385. https://doi.org/10.20546/ijcmas.2017.605.043

Shrivastava, S., Egamberdieva, D., Varma, A. 2015. Plant Growth-Promoting Rhizobacteria (PGPR) and Medicinal Plants: The State of the Art. In: Egamberdieva, D., Shrivastava, S., Varma, A. (eds) Plant-Growth-Promoting Rhizobacteria (PGPR) and Medicinal Plants. Soil Biology, vol 42. Springer, Cham. https://doi.org/10.1007/978-3-319-13401-7_1.

Sukweenadhi, J., Purwanto, M. G. M., Hardjo, P.H., Kurniawan, G. & Artadana, I. B. M. 2019. Isolation and in vitro screening of plant growth promoting rhizobacteria from Barak Cenana red rice. In: Mahat, A. N., Wahab, Ab. R., Huyop, Z. F., Keyon, S. A. A., Attan, B. N., Chandren, S. & Gunam, B. W. I. (eds.) AIP 2019: Biosciences and Medical Engineering (ICBME2019): AIP Conference Proceedings. AIP 2019, 11–12 April 2019, Bali, Indonesia. 2155 (1), 020037.

Verma, M., Mishra, J., Arora, N.K. 2019. Plant Growth-Promoting Rhizobacteria: Diversity and Applications. In: Sobti, R., Arora, N., Kothari, R. (eds) Environmental Biotechnology: For Sustainable Future. Springer, Singapore. https://doi.org/10.1007/978-981-10-7284-0_6.