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FUNCTIONAL CHARACTERIZATION AND EVALUATION OF PLANT GROWTH POTENTIAL IN PGPR'S FROM SOIL

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ABSTRACT: Given increasing environmental contamination with the deterioration of soil health and due to the use of chemical fertilizers for increasing crop productivity, Plant growth-promoting bacteria or PGPR are being used as this present as a plant growth promoter and an alternative to chemical fertilizer. Plant rhizosphere is known to be preferred ecological niche for various types of soil microorganisms due to rich nutrient availability. Plant growth-promoting rhizobacteria (PGPR) are naturally occurring soil bacteria that aggressively colonize plant roots and benefit plants by several mechanisms like IAA production, siderophore production, phosphate solubilization, etc. Phosphate solubilizing microorganisms play an important role in supplementing phosphorus to the plants by several mechanisms like lowering of pH by acid production and ion chelation and thus benefit plant growth and development. The present study gave insight into the PGPR population from the different soil samples and obtained an overall 15 isolates. These isolates were then characterized by biochemical and functional criteria. The functional characterization revealed all the potential properties of the isolates that are necessary for an organism to be an effective PGPR. The experiment of seed bacterization on wheat seeds demonstrated the efficiency of these isolates in growth promotions, as all the isolates showed a good growth-promoting trait in the wheat seed germination. The study concluded that these isolates are very potential PGPRs and hence can be undertaken the consideration to be used at field scale for crop improvement. A future study could focus on the better functional characterization and molecular identification of these isolates as well as the application of these isolates on the stress tolerance experiments.

KEY WORDS: Rhizosphere, Crop improvement, PGPR, IAA.

I. INTRODUCTION

The first aim of agriculture was to ensure survival by producing the necessity for feeding also considered as subsistence farming. But nowadays, due to the continued and worrying growth of the world population, this primary objective of agriculture changed completely. Indeed, the world population is estimated at around 7 billion people and may reach 8 billion by 2020 (Glick, 2012). So, it is urgent to considerably increase agricultural production to reply to the strong food demand to reduce the risk of malnutrition and the increase of poverty. Agrochemical products such as chemical fertilizers, herbicides, fungicides, and insecticides are improperly and excessively used to increase crop yield. The use of these agrochemical projects a direct consequence on the environment pollution through groundwater and crop products contamination by heavy metals. These heavy metals areas known to be the major threat to major health problems as they are considered carcinogenic agents (Koo and Cho, 2009). Apart from this medical crisis, other consequences in the agricultural area such as natural ecological nutrient cycling interruption and soil biological community destruction are frequently reported (Karuppiah and Rajaram, 2011). To find ways to overcome such damage, several methods and applications are considered and among all the explored outcomes, the use of microorganisms currently called Plant Growth PromotingRhizobacteria (PGPR) is in pole position.

The naturally occurring soil bacteria that aggressively colonize plant roots and benefit plants by several mechanisms are termed as the Plant growth-promoting rhizobacteria (PGPR) (Cleyet- Marcel *et al*., 2001). The PGPRs mainly belong to diverse genera of *Azospirillum, Alcaligenes, Arthrobacter, Enterobacter, Erwinia, Acinetobacter, Bacillus, Burkholderia, Flavobacterium, Pseudomonas, Rhizobium,* and *Serratia.* All of these genera can exert beneficial effects on plant growth (Tilak *et al*., 2005). Unlike the case for nitrogen, there is no large atmospheric source for phosphorous that can be made biologically available although phosphorus is a major growth-limiting nutrient (Ezawa *et al.*, 2002). The soil microbes play a key role in soil Phosphorous dynamics or P dynamics and increase the subsequent availability of phosphate to plants (Richardson, 2001). Inorganic forms of Phosphorous are solubilized by a group of heterotrophic microorganisms excreting organic acids that dissolve phosphatic minerals, releasing P into solution (He *et al.*, 2002).

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The PGPR is known to affect plant growth through direct promotion by means of producing and secreting plant growth-promoting substances or by eliciting root metabolic activities by supplying biologically fixed nitrogen and through indirect promotion by acting against phytopathogenic microorganisms. Apart from this, they are known as the abundant producers of the exopolysaccharides (EPS) which reduce the toxic ion uptake including sodium and produce stress-specific proteins in plants under salt stress (Nadeem *et al*., 2006).PGPR can be used as the agricultural inputs with plant growth-promoting attributes and as a biological control to reduce plant diseases in various crops. PGPR has been commercialized as microbial bio inoculants or biofertilizers to increase crop production (Adesemoye and Kloepper, 2009). PGPR offers an attractive strategy for replacement and reduction of the heavy application of chemical pesticides and fertilizers (Banerjee *et al*., 2006). Hence, the present work was aimed to isolate, screened and characterize the PGPR from rhizospheric soils that can be utilized in the future for increasing growth and yield of plants.

II. MATERIALS AND METHOD

Collection of Soil samples

A total of 8 soil samples were collected from different fields of AREA. Among the collected samples 4 samples were rhizospheric soil collected from the root portion of the uprooted plants and the rest samples were non-rhizospheric. The samples were collected from the top layer of soil and stored in sterile bags.

Isolation of PGPR Bacteria

The isolation of bacteria from collected soil samples was carried out the serial dilution method. For this 1gm of soil was mixed with 10 ml of 0.85% of saline water and vortexed properly. Then the serial dilution was carried up to 8 dilutions. The isolation was carried out by plating 100µl of the suspension on sterile nutrient agar plates and incubated them on 35-37ºC for 24 hours. After the incubation period, NA plates were observed for morphological appearances and the number of bacterial colonies. From each soil sample, the most dominating isolates were transferred into a pure culture. Bacterial isolates having a different morphological appearance on agar plates were selected and maintained on nutrient agar slants and 50% glycerol at -80°C. All the isolates were morphologically characterized as per the method described in Bergey's manual of determinative bacteriology (Holt *et al*., 1994).

Biochemical characterization

The isolates were characterized for their biochemical pathways based on some selective biochemical tests selected considering the Bergey's manual. The biochemical test includes methyl red test, Voges-Proskauer test, catalase test, citrate utilization test, indole test, oxidase test, and sugar fermentation test. For all the biochemical test their specified media was prepared and inoculated with the respective pure cultures of all the isolates. For all the test the result interpretation was done based on the detection reagent used. The reagent includes MR reagent and VP I and II reagent, Hydrogen peroxide, Bromothymol blue, phenol red Kovac's reagent, etc. The biochemical characterization helps in a partial identification of the species of the microbes under study.

Functional characterization

The functional diversity amongst recovered isolates was studied by a qualitative screening of their ability to produce certain enzymes and compounds that confer their plant growth-promoting property and potential. For this purpose, several tests and analyses were conducted for the better characterization of the isolates.

Phosphate Solubilization

Phosphate or phosphorus is the second most important after nitrogen in mineral nutrients that is most commonly required for the optimum growth of crop plants. Ironically soils may have large reserves of total phosphorus but the amount available to plants is usually a tiny proportion of this total (Stevenson and Cole, 1999). Many soil microorganisms can solubilize the unavailable form of bound phosphorus (Kokalis *et al*., 2006). The isolates were checked for this ability and for that the plates were prepared with Pikovaskya's medium. The isolates were spot inoculated on the plates and incubated in an incubator at 28ºC for 3 to 5 days. The formation of a clear zone around the microbial colonies indicated phosphate Solubilization ability of the isolates.

Indole Acetic Acid Production

IAA production was detected by the modified method as described (Brick *et al*., 1994). Diverse soil microorganisms including bacteria, fungi, and algae are also capable of producing physiologically active quantities of auxin. This ability makes any microbes an efficient PGPR and hence the isolates were screened for their ability to synthesize the auxins. The entire cultures were incubated in the peptone broth enriched with tryptophan broth to check for the production of indole acetic acid a precursor of auxin which is an important plant hormone. The qualitative estimation of IAA was performed by using the Salkwouski method by using the reagent (50 ml, 35% of per Chloric acid, 1 ml of 0.5 M FeCl3solution). The supernatant from the broth and

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reagent were mixed in a 2:1 ratio and the mixtures were incubated at room temperature for 30 min and observed for pink color indicating the positive result for the test.

Siderophores production

Siderophores are low molecular weight chelating agents with a high affinity for the ferric iron. Bacterial isolates were assayed for Siderophore production on the Chrome azurole S agar medium (Sigma, Ltd.) Described (Schwyn and Neilands, 1987). The culture isolates were streaked on the surface of the CAS agar medium and incubated at 28± 10C for 48 to 78 hours. Siderophore production was indicated by orange halos around the colonies was considered as positive for Siderophore production.

Hydrogen Cyanide Production

All isolates were screened for hydrogen cyanide production following the method described by (Baker and Schippers, 1987). Each isolate was streaked on nutrient agar medium added with glycine (4.4 g/L). The agar was covered with a Whatman number 1 filter paper previously soaked in a specific solution (0.5% picric acid and 2% sodium carbonate w/v). Plates were sealed with parafilm paper and incubated at 36 ± 2ºC for 4 days. The appearance of orange or red color indicates the production of hydrogen cyanide.

Ammonia Production

To assess the production of ammonia, each isolated strain was grown in peptone broth (10mL) and incubated at 36 ± 2˚C for 48 to 72 h. After incubation, 0.5mL of Nessler's reagent was added to the bacterial suspension. The development of brown to the yellow color indicated ammonia production (Cappuccino and Sherman, 1992).

Evaluation of the growth-promoting ability of isolates on wheat seeds

The evaluation experiment was carried out using the wheat seeds and all the isolates were undertaken in the studies. The seeds were surface sterilized by washing with 70% ethanol for 1 min followed by three times washings with sterile distilled water. Thereafter, the seeds were treated with1.5% sodium hypochlorite (NaOCl) solution for 5 min followed by six times of successive washings in sterile water to remove all traces of the disinfectant (Rudolph *et al*., 2015). To check the efficacy of the sterilization process, few seeds were placed on plates containing NA medium for 4 days and the plates were monitored for any microbial growth. To prepare the bacteria inoculum, log-phase cultures (OD600 of 0.6) of the selected bacterial isolates were used. The cultures were centrifuged at5000 rpm for 10 min and the pellets were washed three times with sterile distilled water and then resuspended in a final volume of 25 ml sterile distilled water. Sterilized seeds were imbibed in the bacterial suspension for 1 h (for control treatment, seeds were imbibed in sterile water). Imbibed seeds were allowed to dry under a laminar airflow cabinet for 2 h before transferring them into sterile plastic boxes lined with sterile filter papers soaked with 30 ml of distilled water. Three replicates for each treatment were performed with 5 seeds per isolate. All boxes were incubated at 22ºC under complete darkness for the first 3 days, then placed under a photoperiod cycle of14 h light–10 h dark for the other 7 days. Germination percentage was measured after 10 days and root length and diameter were recorded. The seed germination was monitored on filter paper wetted with distilled water as described above.

III. RESULT AND DISCUSSION

For the isolation of plant growth-promoting bacteria, different samples of non-rhizospheric and rhizospheric soils were collected. From all the collected samples the dominating colonies were selected and overall 15 different isolates from total samples were obtained. The colony to be selected were characterized and after isolation, their response to gram's staining and morphology was concluded, the results for this are summarized in table no. 1. Overall the isolates were diverse with gram-positive and negative rods and gram-positive coccus.

After this the biochemical characterization was conducted for all the isolates for various metabolic and enzymatic characteristics of the microbes, the results for this are summarized on the table no. 2. This was followed by the functional characterization of all isolates to determine their plant growth-promoting potential via various tests. The result of all the tests conducted depicted a positive potential ability of the isolates for plant growth promotion as the isolates gave a positive result for most of the tests conducted. All these tests undertaken reveals the enzymatic and functional potential of the isolates for promoting plant growth, the result for the same are summarized in table no. 3

After the functional characterization of isolates since all the isolates showed a promising potential hence all the isolates were carried forward for their assessment of the ability to promote growth in wheat seeds sample. The experiment results concluded after ten days of the procedure revealed a very much promising potential of the isolates in increasing the growth rate of wheat seeds germination as compared to the control seeds, results of this

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analysis are summarized in table no.4 and also followed by a graphical representation of the data to show the comparative improvement in root length and diameter between the isolates treated seeds and the control seeds, shown in graph no. 1 and 2. The overall conclusion driven form this analysis is that although all the isolates have good potential as plant growth-promoting microorganisms among them much better potential was shown by isolates 3, 4, 9, and 10.

Table no. 1: table showing the results for the colony characterization and cell morphology of the isolates

Isolate code	Colony Morphology					Gram's reaction	Cell shape
	Shape	Margin	Opacity	Color	Elevation		
PGP 1	Circular	Smooth	Opaque	Milky	Raised	+	Rods
PGP 2	Irregular	Undulate	Translucent	Whitish	Flat	-	Rods
PGP 3	Punctiform	Smooth	Opaque	Milky	Raised	+	Coccus
PGP 4	Irregular	Rough	Translucent	Off-white	Raised	+	Rods
PGP 5	Circular	Smooth	Opaque	Whitish	Raised	-	Rods
PGP 6	Irregular	Rough	Opaque	Milky	Convex	-	Rods
PGP 7	Circular	Smooth	Translucent	Yellowish	Flat	-	Rods
PGP 8	Punctiform	Smooth	Opaque	Off-white	Convex	+	Coccus
PGP 9	Irregular	Undulate	Opaque	Yellowish	Flat	+	Rods
PGP 10	Irregular	Rough	Translucent	Whitish	Convex	+	Coccus
PGP 11	Punctiform	Smooth	Opaque	Milky	Flat	+	Rods
PGP 12	Irregular	Undulate	Translucent	Yellowish	Convex	+	Coccus
PGP 13	Circular	Smooth	Translucent	Whitish	Convex	-	Rods
PGP 14	Punctiform	Smooth	Opaque	Off-white	Raised	-	Rods
PGP 15	Irregular	Rough	Translucent	Whitish	Flat	+	Rods

Table no. 2: table showing the results for the biochemical characterization of the bacterial isolates; (+) positive and (-) negative

Isolate code	Biochemical test								
	MR test	VP test	Catalase test	Citrate utilization test	Oxidase test	Indole test	Sugar fermentation test		
							G	M	S
PGP 1	-	+	+	+	+	+	+	-	+
PGP 2	-	+	+	+	+	+	+	+	+
PGP 3	+	-	-	+	_	+	+	+	-
PGP 4	+	-	+	+	+	+	+	-	+
PGP 5	-	+	+	+	+	+	+	+	+
PGP 6	+	-	+	+	+	+	+	+	+
PGP 7	+	-	+	+	+	+	+	-	+
PGP 8	+	-	-	+	-	-	+	-	+
PGP 9	+	-	+	+	+	+	+	+	+
PGP 10	-	+	+	+	+	+	+	+	+
PGP 11	-	+	+	-	+	+	+	+	-
PGP 12	-	+	+	+	+	+	+	+	-
PGP 13	-	+	+	+	+	+	+	+	+
PGP 14	+	-	+	-	+	+	+	-	+
PGP 15	+	-	+	+	+	-	+	-	-

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Table no. 3: table showing the results for the functional characterization of the bacterial isolates depicting the results for the PGPR activity of the isolates; (+) positive and (-) negative

Isolate code	Biochemical test				
	IAA production	Phosphate solubilization test	HCN production	Ammonia production	Siderophore production
PGP 1	+	+	-	+	+
PGP 2	+	+	+	+	+
PGP 3	+	+	+	+	+
PGP 4	+	+	+	+	+
PGP 5	+	-	+	-	-
PGP 6	+	-	+	+	+
PGP 7	+	+	-	+	+
PGP 8	-	+	+	+	+
PGP 9	+	+	+	+	+
PGP 10	+	+	+	-	+
PGP 11	+	+	+	-	+
PGP 12	+	+	+	+	+
PGP 13	+	+	+	+	+
PGP 14	+	-	+	+	+
PGP 15	-	+	+	+	+

Table no. 4: table showing the results for the growth evaluation experiment on wheat seeds conducted for all the isoaltes where the root length and diameter are given in mm as average

Isolate code	After 10 dyas	
	Root length (mm)	Root diameter (mm)
Control	41.73	0.51
PGP 1	58.32	0.64
PGP 2	50.23	0.53
PGP 3	63.45	0.71
PGP 4	60.10	0.65
PGP 5	49.23	0.57
PGP 6	53.64	0.54
PGP 7	54.40	0.55
PGP 8	53.02	0.50
PGP 9	63.40	0.68
PGP 10	60.21	0.56
PGP 11	48.90	0.55
PGP 12	57.83	0.57
PGP 13	54.30	0.52
PGP 14	56.32	0.51
PGP 15	50.13	0.48

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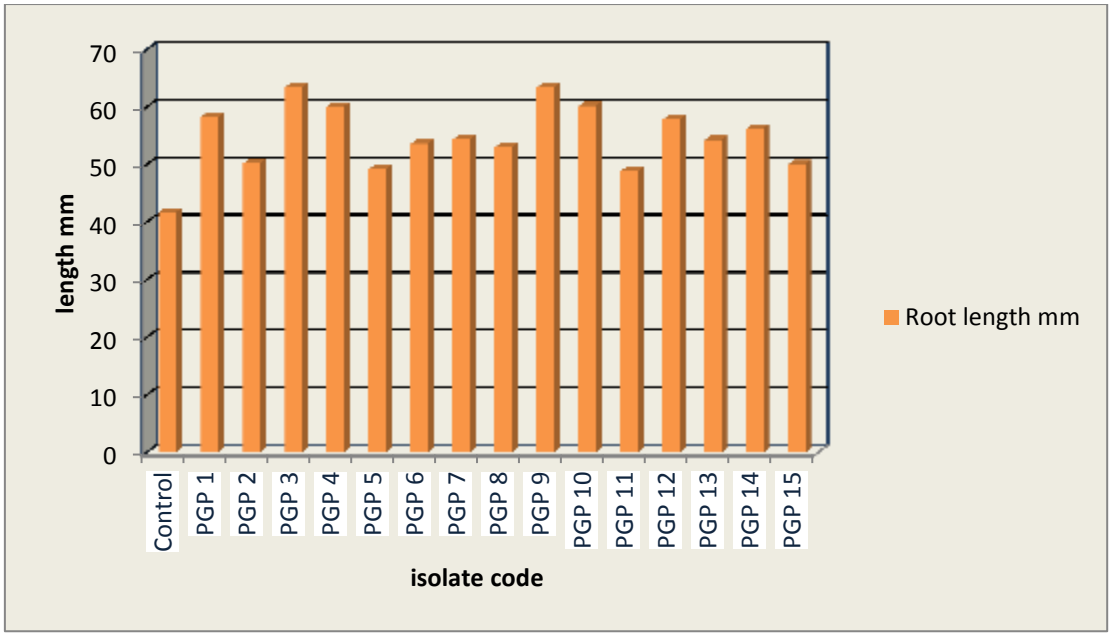
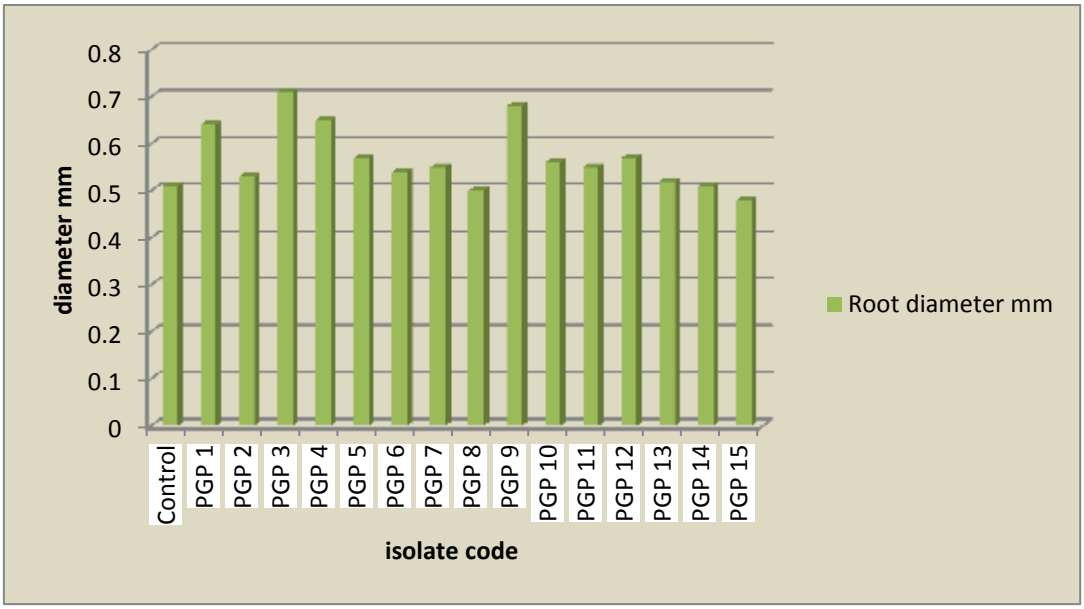


Graph no.1: graph showing the comparsion between the isolates treated and control wheat seeds increment in root length after 10 days of the procedure



Graph no.1: graph showing the comparsion between the isolates treated and control wheat seeds increment in root diameter after 10 days of the procedure

Several studies have reported the benefit of seeds inoculation by Plant Growth Promoting Rhizobacteria. This growth-promoting effect is influenced by biotic and abiotic factors including bacterial species and the soil types. It is in this context that this prospective study was realized in the prelude of the promotion of microbial biofertilizers based on native rhizobacteria. The study isolated an overall of 15 isolates of the PGPRs and undertook their biochemical and functional characterization. Ammonia production is an important characteristic of PGPR, which indirectly influences plant growth (Yadav et al., 2010). The hydrogen cyanide is part of powerful antifungal compounds produced by PGPR and involved in pathogen biological control (Hass and D' efago, 2005). Iron is an essential requirement of plants and microorganisms. Siderophore producing bacteria make it available to the plant and also these bacteria compete for iron with the soil-borne pathogen and play a role as a biocontrol agent (Looper and Buyer, 1991).IAA is involved in root initiation, cell division, cell enlargement, increase root surface area, and consequent access to soil nutrients by enhanced formation of roots (Gray and Smith, 2005). Auxin production has been proposed as a major means of attaining early growth

promotion in wheat (Khalid et al., 2004) along-with P-solubilization (Rajput et al., 2013). Phosphorus is the second most important nutrient, next to nitrogen (N) required for the growth of plants. A greater portion of phosphorus in soil is in the form of insoluble phosphates and cannot be used directly by the plants (Pradhan and Shukla, 2006). The beneficial effect of PGPR in maintaining adequate levels of mineral nutrients especially the P in crop production had been previously reported (Saravanan et al., 2007). The isolates obtained in the study have shown a very positive response to the functional characterization and hence depicted their importance and ability to be used as an effective PGPRs, the result for the same are summarized in table no. 3 given above. The present study has demonstrated an increase in plant growth by seed bacterization. It is well established that plant growth-promoting rhizobacteria increases the synthesis of gibberellins, which would have triggered the activity of specific enzymes including amylase to promote early germination, which brought an increase in the availability of starch assimilation (Bharathi et al.,2004). In the present study, the results depicted that all the isolates were acting as a good promoter for the seed growth as the seeds showed the root length and diameter differences compared to the control seeds. Among all these isolates few showed the commendable growth-promoting activity as per the observation. Use of PGPRs as stimulants of seed germination in medicinal and aromatic species can provide more uniformity in germination, seedling emergence and other growth stages in particular flowering, which is a critical time to achieve more bioactive secondary metabolites (Ghorbanpour et al., 2015).

IV. CONCLUSION

The present work is a basic study that provides insight into the bacterial community of the various soil samples and ultimately portraying their fertility. This study demonstratedefficientN2- fixing, P-solubilizing, and IAA-producing bacteria present among the natural population of these soil samples. These characteristics are considered as important PGP traits and have been found effective in positively improving the growth and N contents of tested wheat plants. It is very clear from the study that rhizospheric soil can provide a rich source of IAA producing bacteria and can produce a significant amount of IAA in a tryptophan-supplemented medium. It can be concluded from the above discussion that Plant growth-promoting rhizobacteria are very potential and active in increasing the yield and fertility of the soil by their specific characteristics and are increasingly used for agricultural productivity and production improvement. These isolates offer potential in field applications as PGP agents in wheat. Further studies should be focused on the detailed molecular and functional characterization of this PGPR for practical applications in the field.

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