Research Journal of Agricultural Sciences
An International Journal

Res. Jr. of Agril. Sci. **12**(1): 352–357

P- ISSN: 0976-1675 | **E**- ISSN: 2249-4538

www.rjas.org

Research Paper

Isolation and Characterization of Potential Plant Growth Promoting Rhizobacteria Isolated from Rhizospheric Soils of *Glycine max*

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Received: 06 Dec 2020 | Revised accepted: 08 Feb 2021 | Published online: 17 Feb 2021

A B S T R A C T

Plant Growth Promoting Rhizobacteria (PGPR) that acts as an alternative option for enhancing the fertility of agricultural fields to reduce the usage of chemical fertilizers. PGPR represents a wide variety of bacteria which colonize the root systems of plants and can stimulate plant growth by direct or indirect mechanisms. PGPRs are also well known for inducing resistance against various plant pathogens in different crops ranging from pulses, cereals, ornamentals, vegetables, plantation crops, spices etc. The present study was conducted to characterize the native PGPR from rhizosphere of *Glycine max* from the agricultural fields of Hadoti region, Rajasthan (Kota, Bundi, Baran, Jhalawar). Sixty samples were collected at the rate of 15 samples per district. Rhizobacteria strains were isolated from these samples. These isolates were analyzed for some of their PGPR traits like ammonia production, HCN, EPS production as well as enzymatic activity like cellulase and protease production following conventional methods. The microbiological investigations of these samples has shown the *Glycice max* rhizoshere in hadoti region contain high diversity of microorganism. Eight PGPR isolates were obtained from the soil from fields of Hadoti regions in Rajasthan. The isolated bacterial strains belonged to both gram negative and positive classes. Out of the eight isolates obtained, five isolates were gram negative and three are gram positive in nature. Out of the eight PGPR isolates, five (SBF38, TKF11, MBUF29, KBF41, CJF48) were found to efficient in terms of various plant growth promoting activities. The isolates CJF48 and TKF11 produced high ammonium whereas MBUF29 and KBF41 were found to be low. The HCN was produced by all the isolates (100%) except MBUF29. Siderophore production has been show by only two isolates SBF38 and KBF 41. None of the isolates show indole acetic acid production. The cellulase was produced by all the isolates except the MBUF29. The protease was produced by KBF41 and CJF48 except SBF38, MBUF29 and TKF11. These isolates were further identified and screened for their beneficial effect on other crops.

***Key words*:** Biofertilizers, PGPR, Phosphate solubilization, Production of phytohormones, Physiological characters

Soil is a major component of the Earth's ecosystem. Being a rich medium, it's a habitat for soil organisms, a recycling system for nutrients and organic wastes, a regulator of water quality, a modifier of atmospheric composition, and a medium for plant growth. It is the mixture of minerals, organic matter, gases, liquids and a myriad of micro- and macro-organisms that can support plant life. Since soil has a tremendous range of available niches and habitats, it contains most of the earth's genetic diversity.

According to a report of Indian Council of Agricultural Research, 2018, with an increase in population, limited availability of agricultural land, small land holdings and declining soil fertility, India is under serious threat of losing its food surplus status in the near future. The demand for food grains is expected to increase from 192 million tons in 2000 to 355 million tons in 2030. Hence, to mitigate this problem and obtain higher plant yields, farmers have become increasingly dependent on chemical sources of fertilizers. These chemical fertilizers are not just costly but deplete nonrenewable resources of soil minerals and poses human and environmental hazards [1]. In modern cultivation process indiscriminate use of nitrogenous and phosphorus fertilizers, has led to substantial pollution of soil, air and water. Over use of these chemicals exerts deleterious effects on soil microorganism, affects the fertility status of soil and also pollutes environment [2]. The long-term use of these fertilizers often leads to reduction in pH and exchangeable bases, making them unavailable to crops and thus declining the productivity of crop. Hence, the concept of PGPR induced soil fertility.

The term rhizosphere was introduced for the first time by [3]. Since then, the studies on the major influence of the rhizosphere microorganisms in protecting the health of plants in ecofriendly manner have become important concern [4]. These microorganisms can also positively affect plant growth thus also referred to as a plant growth promoting rhizobacteria (PGPR) [5]. PGPR are involved in various biotic activities of the soil ecosystem to improve soil nutrient turn over as well sustainable for crop production [6]. In recent years attention has been paid towards the use of replace agrochemicals (fertilizers and pesticides) for the plant growth promotion by a variety of mechanisms PGPR affect plant growth by a variety of mechanisms: indirect or direct. The direct promotion of

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plant growth by PGPR includes either providing the plant with a compound that is synthesized by the bacterium, for example phytohormones, or facilitating the uptake of certain nutrients from the environment. Also, PGPR induces production of auxin, nitrogen fixing associated with roots and decrease of plant ethylene levels thus enhancing plant growth. The indirect promotion of plant growth occurs when PGPR lessen or prevent the deleterious effects of one or more phytopathogenic organisms by producing antagonistic substances or by inducing resistance to pathogens [7]. Thus, along with acting as an effective fertilizing agent, PGPR also act as bio-control agents. An understanding of plant growth promoting rhizobacteria and their interactions with biotic and abiotic factors is an important technique to reduce chemical fertilizers application and being economically, environmentally beneficial it acts as a step towards sustainable farming [8-9].

Based on the above-mentioned gaps, the present paper focuses on isolation and biochemical identification of rhizobacteria from *Glycine max* rhizophere from various soil samples of Kota, Bundi, Baran, Jhalawar districts of Rajasthan and its characterization for plant growth promoting activities. Thus, the study paves an eco-friendly alternative to enrich field fertility without the use of recalcitrant chemical fertilizer.

MATERIALS AND METHODS

Collection of soil samples

Soil samples were collected from rhizosphere of *Glycine max* from the agricultural fields of Hadoti region, Rajasthan (Kota, Bundi, Baran, Jhalawar). For this purpose, the plants were uprooted carefully, shoot portion cut off and roots along with the rhizospheric soil were aseptically kept in small plastic bags and were taken to the laboratory and stored at 4°C in sterile sealed polythene [10].

Isolation of rhizobacteria

Isolation of rhizobacteria from the soil sample was done by serial dilution method. 1µl from each serially diluted tube was spreaded over the nutrient agar plates and incubated at 37°C for 24-48 hours. After incubation colonies developed were further sub-cultured on the freshly prepared nutrient agar plates. After subcultures pure colonies were obtained. The isolates were successfully maintained under aseptic conditions in solid agar medium and were stored under controlled conditioned in glycerol stock at -20°C [11].

Morphological characterization

The isolated pure colonies were initially characterized by staining methods (simple and gram staining) and morphological characterization was carried out by identifying culture characteristics of the pure isolates (colony morphology, motility etc.).

Characterization of isolated rhizobacteria for plant growth promoting activities

To characterize isolated rhizobacteria for PGPR activity the following tests were conducted viz. Phosphate solubalisation, HCN production, Ammonium Production, Exopolysaccharide Production, Siderophore activity, Acetic acid production, Cellulase production, Protease Production.

Biochemical characterization of isolated cultures

After characterization for plant growth promoting activities, potential isolates were identified by biochemical characterization using various tests viz. nitrate test, carbohydrate fermentation test, urease test, catalase test, oxidase test, indole test, MRVP test, citrate utilization test, amylase production test.

RESULTS AND DISCUSSION

PGPR colonize roots of plant and exert beneficial effects on plant growth and development by diverse mechanisms. The precise mechanism by which PGPR enhance plant growth and productivity is not clearly established, though many hypothesis like phosphate solubilization, production of phytohormones, promotion of the mineral nutrient uptake and suppression of soil borne pathogens are typically believed to be involved [12].

In the study, a total of eight pure bacterial cultures were isolated using serial dilution method collected from Hadoti region. The isolated rhizobacteria were morphologically characterized by simple and gram staining techniques (Table 2). Then isolates were further analyzed various PGPR characteristics (Table 3) then biochemical characterization for further identification of the best potent bacterial isolate was done (Table 4).

Table 1 Description of bacterial isolates

Isolate code	Location	Soil sample
MKF3	Mandana, Kota	Rhizospheric soil
TKF11	Thathedi, Kota	Rhizospheric soil
KKF8	Kewal nagar, Kota	Rhizospheric soil
SBF38	Salpura, Bara	Rhizospheric soil
MBUF29	Matunda, Bundi	Rhizospheric soil
KBF41	Kawai, Bara	Rhizospheric soil
JJF48	Jhalapatan, Jhalawar	Rhizospheric soil

Table 2 Morphological characterization of PGPR Isolates

Isolate	Simple Staining	Motility	Gram Staining
MKF3	Large Rod Shape	Motile	Gram Negative
TKF11	Large Rod Shape	Motile	Gram Negative
KKF8	Rod Shape, Chained	Motile	Gram Negative
SBF38	Rod Shape	Motile	Gram negative
MBUF29	Small Rod Shape	Non motile	Gram Positive
KBF41	Small Rod Shape	Non motile	Gram Positive
CJF48	Small Rod Shape	Non motile	Gram Negative
CJF53	Small Rod Shape	Non motile	Gram Positive

Fig 1 Pure culture of Rhizobacteria isolated from soil of Hadoti region

The potential PGPR isolated from the soil of Hadoti regions were found to have been distributed both as Gram Negative and Gram-Positive species on the basis of gram

staining results. The results are in accordance with the previous study reporting that to evaluate the biodiversity of plant growth-promoting rhizobacteria (PGPR) in Korea, 7,638 bacteria isolated from the rhizosphere of plant species growing in many different regions were screened. A large number of PGPR were identified by testing the ability of each isolate to promote the growth of cucumber seedlings. Out of which 68 Gram-positive (76%) and 22 Gram-negative (24%) isolates were assigned to 21 genera and 47 species [13]. In another study focused on the isolation and characterization of bacteria from the rhizosphere of *Spartina maritima* in the metal contaminated Odiel estuary out of 25 strains, 84% were identified as gram-positive, particularly *Staphylococcus* and *Bacillus*. Gram-negative bacteria were represented by *Pantoea* and *Salmonella* [14].

Furthermore, the isolates were tested for various parameters to ensure the best PGPR characteristics. Out of the eight isolated PGPR isolates, five PGPR isolates showed best activity in terms of various parameters which are as discussed below (Table 3).

Table 3 Characterization of isolated rhizobacteria for plant growth promoting activities

Isolates	Phosphate solubilization	Hydrogen Cyanide Production (HCN)	Ammonium Production	Exopolysaccharide Production	Siderophore activity	Acetic Acid Production	Cellulase Production	Protease Production
SBF 38	-	+	Nil	2.08511gm	+	-	+	-
TKF 11	_	+	High	1.99978gm	-	-	+	-
MBUF29	-	-	Low	0.00885gm	-	-	-	-
KBF 41	-	+	Low	2.11347gm	+	-	+	+
CJF 48	_	+	High	1.99978gm	-	-	+	+
Control	-	-	Nil	-	-	-	-	-

Phosphate solubilization

It is a key factor of soil enrichment employed by PGPR strains by adopting to different strategies to make use of unavailable forms of phosphorus and in turn also help in making phosphorus available for plants to absorb [15]. In the present study no PGPR showed this activity.

Hydrogen Cyanide Production (HCN)

In HCN Producing bacteria, amino acid glycine is main precursor of cyanide synthesis. Another PGPR characteristics analyzed in this study was hydrogen Cyanide Production (HCN). In the present study, 4 PGPR (SBF-38, TKF-11, KBF-41 and CJF-48) isolates showed HCN production activity marking the utility of the PGPR isolate as potential biocontrol agent, antibiotics, lytic enzyme secretion and elicitation of induced systemic resistance (ISR) in plant as compare to others. As in accordance with previous works [16] also reported the production of DAPG and HCN by *Pseudomonas* contributing to the biological control of bacterial canker of tomato. Recent studies have also reported the benefit of HCN production in promoting the mobilization of elements from soil thus enhancing uptake of minerals in plants [17-19].

Ammonium production

In the present study, two PGPR (TKF-11 and CJF-48) isolates produced high ammonium compare to control bacterial culture. MBUF-29 and KBF-41 produced low ammonium as compare to SBF-38 nil. As reported in previous studies ammonia production by the plant growth promoting bacteria helps influence plant growth indirectly as well as directly thus these isolates are promising as biofertilising agent [20].

Many previous studies have reported ammonium production capability of various strains like *Bradyrhizobium*, *Sinorhizobium*, and *Mesorhizobium* (leguminous plant), Frankia with non-leguminous trees and shrubs where nitrogen is fixed to ammonia and make it available for the plant [21]. On the other hand, non-symbiotic nitrogen fixation in the form of ammonium is carried out by free living diazotrophs and this can stimulate non-legume plants growth such as in radish and rice by rhizospheric bacteria belonging to genera including *Azoarcus*, *Azotobacter*, *Acetobacter*, *Azospirillum*, *Burkholderia*, *Diazotrophicus*, *Enterobacter*, *Gluconacetobacter*, *Pseudomonas* and *cyanobacteria* (*Anabaena, Nostoc*) is reported to enhance plant growth due to ammonium production [22].

Exopolysaccharide production

PGPRs are also reported to show effective colonization of plant roots by EPS-production that helps the bacteria to hold the free phosphorous from the insoluble one in soils and circulating essential nutrients to the plant for proper growth and development and protecting it from the attack of foreign pathogens. In the present study PGPR isolates produced a maximum of 2.113gm (KBF-41) of EPS as compare to other isolates SBF-38- 2.085, TKF-11-1.999, MBUF-29-0.008, CJF-48-1.999 which is quite a significant amount in terms of beneficial effects of EPS.

Other innumerable functions performed by EPS producing microbes constitute shielding from desiccation, protection against stress [23], attachment to surfaces plant invasion, and plant defense response in plant–microbe interactions [24].

Siderophore activity

In the present study, siderophore activity was significantly shown by two (SBF-38, KBF-41) isolates. Furthermore, Siderophore activity was analyzed for the isolated PGPR strains as siderophores have been implicated for both direct and indirect enhancement of plant growth by plant growth promoting rhizobacteria. In accordance with the previous studies, the direct benefits of bacterial siderophores on the growth of plants have been demonstrated by using radiolabeled ferric-siderophores as a sole source of iron showed that plants are able to take up the labeled iron by a large number of plant growth promoting rhizobacteria including *Aeromonas, Azadirachta, Azotobacter, Bacillus, Burkholderia, Pseudomonas, Rhizobium, Serratia* and *Streptomyces* sp. and also showed enhanced chlorophyll level compared to un inoculated plants [25-26].

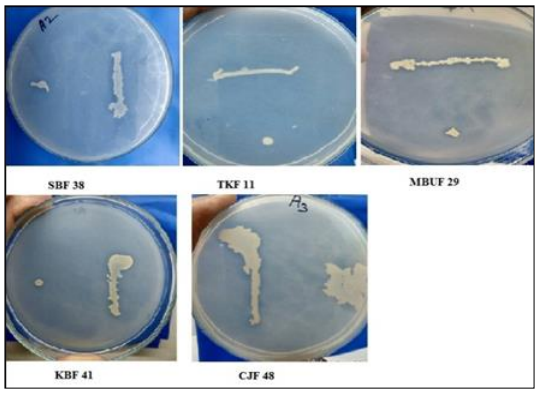
Acetic acid production

Even in the current study, no acetic acid production was observed by any of the bacterial isolates. In context of PGPR characteristics, it has also been reported in previous studies that the root growth promotion is induced by the free living PGPR by acetic acid production. In contrast, sometimes

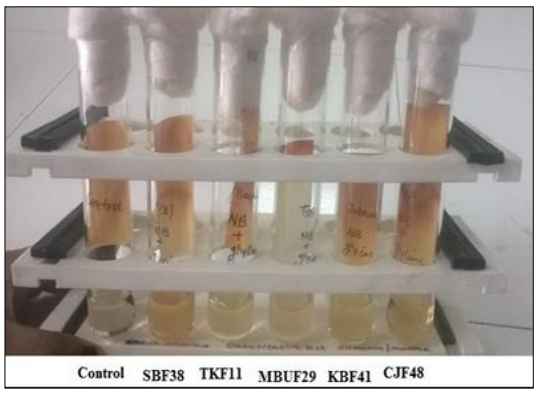
PGPRs show extremely low to zero level of acetic acid secretion like *Alkaligenes faecalis*, *Enterobacter cloacae*, *Acetobacter dizotrophicous*, species of *Azospirillum*, *Pseudomonas and Xanthomonas* sp. [27].

Cellulase and protease production

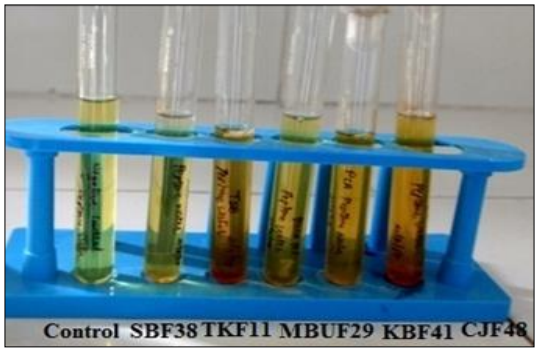
Another significant feature of PGPR is growth enhancement through enzymatic activity. In the present study the PGPR isolates showed significant activity in production of cellulose and protease enzymes. Four isolates (SBF-38, TKF-11, KBF-41 and CJF-48) were capable of showing cellulase activity, whereas two were (KBF-41 and CJF-48) showing protease activity thus proving as potential PGPR isolates. In this context previous works has reported that through these activities, PGPR play a very important role in plant growth promotion particularly to protect them from biotic and abiotic stresses by suppression of pathogenic fungi including *Botrytis cinerea*, *Sclerotium rolfsii*, *Fusarium oxysporum*, *Phytophthora sp.*, *Rhizoctonia solani*, and *Pythium ultimum* [28].



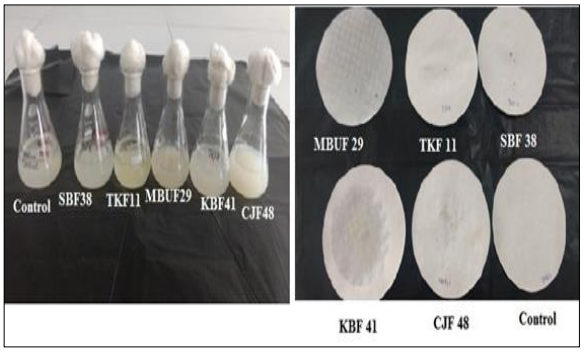
a) Phosphate solubilization



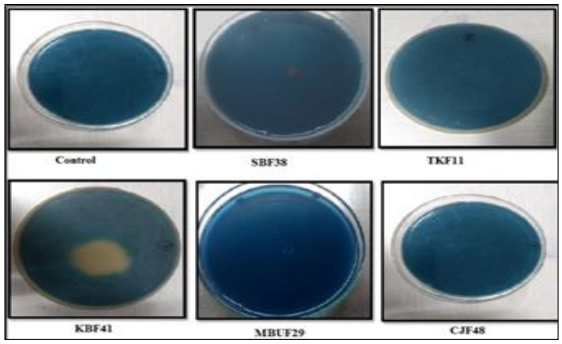
b) HCN production



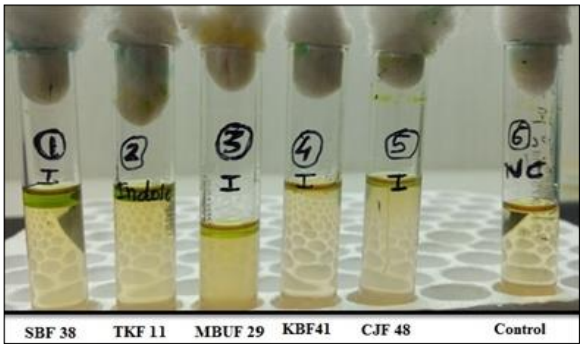
c) Ammonium Production



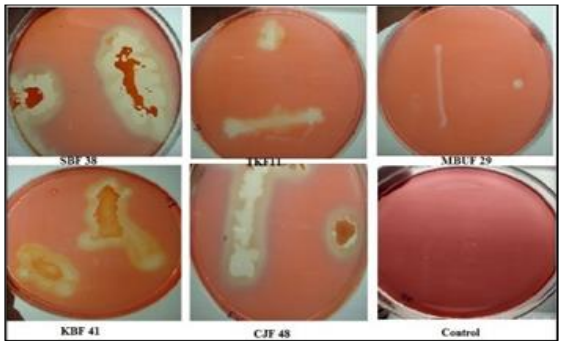
d) Exopolysaccharide Production



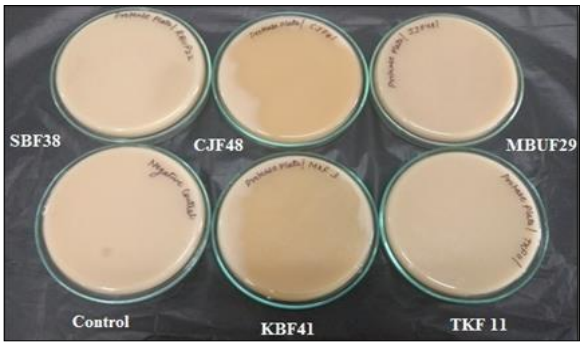
e) Siderophore activity



f) Acetic acid production



g) Cellulase production



h) Protease Production

Fig 2 The figure depicts various tests undertaken to characterize the plant growth promotion activity by isolated PGPR

After the characterization Plant Growth Promoting Rhizobacteria (PGPR) for plant growth promotion activities and correlating the results with previous reviews, five best PGPR isolates (Table 4) were characterized further using biochemical characterization, the results of which are listed below in (Table 3).

Table 4 Biochemical characterization of best potential PGPR

Isolates	Nitrate test	Amylase test	Urease test	Catalase test	Oxidase test	Indole test	MR test	VP test	Citrate utilization test	Carbohydrate fermentation test		
										Dextrose	Sucrose	Lactose
SBF 38	-	-	-	+	-	-	+	-	-	+	-	-
TKF 11	-	-	-	+	+	-	+	-	-	-	-	-
MBU29	-	-	-	+	-	-	+	-	+	-	-	-
KBF 41	+	+	-	+	+	-	-	+	-	+	+	-
CJF 48	+	+	-	+	-	-	-	+	-	+	-	-
Control	-	-	-	-	-	-	-	-	-	-	-	-

CONCLUSIONS

It can be concluded that the ever-increasing population and its demand has pushed farmers to use chemical fertilizers and pesticides to enhance the overall production which in turn is hampering soil quality in long run. Hence, PGPRs are beneficial alternative in this regards due to their plant growth promoting activity and their prominent biocontrol ability. In our study, eight PGPRs were isolated from the *Glycine max.* fields of Hadoti region, Rajasthan, out of which five were proved to be potential PGPR with respect to various plant growth promoting activities like Ammonium, HCN, EPS production or enzymatic activity viz. cellulose and protease production. Also, the morphological and biochemical characterization of potent isolates showed a combination of gram positive and negative strains. Thus, screening of PGPR from the soil of agricultural fields is a good tool to select effective PGPRs for biofertilizer development technology.

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