ISOLATION AND SCREENING OF POTENTIAL PGPR ISOLATES FROM SUNFLOWER

S.MAHALAKSHMI, M.VIJAYAPRIYA & N.PANDEESHWARI
Assistant Professors
Department of Microbiology
Annamalai University
Annamalainagar - 608 002, Tamil Nadu

Abstract

Sunflower is one of the most important oil crops in globally. It produces the high quality oil for human consumption as well as technical purposes. The research was planed at assessment of the diversity of rhizobacteria are associated with sunflower and assessment of their ability of plant growth promoting substance for enhancement of crop yield. Among the twenty PGPR isolates were isolated from rhizosphere soil of sunflower in different location using selective media in each species. The production of siderophores, indole acetic acid and phosphate solubilisation ability for each isolate were studied. Out of twenty isolates, eight isolates were selected for their efficiency of nitrogen fixation; siderophore production and phosphate solubilzation ability were assessed. The *Burkholderia* is predominant genera was isolated from soil. The siderophores production is widely found all isolates, phosphate bacteria is found only 19.8 per cent. All the three PGP traits were exhibited only 8 per cent of the isolates where as belonged to the genus of *Burkholderia.*

Key words: Sunflower, PGPR, diversity, burkholderia, enterobacter, etc.

1. Introduction

Sunflower (*Helianthus annuus* L.) is a major oilseed crop grown under a wide range of agro-environmental conditions. It is one of the most important oilseed crops in globally. Sunflower oil has a light colour, a bland flavor, a high smoke point, and consists of a comparatively high concentration of polyunsaturated fatty acid and linoleic acid. Plant growth promoting rhizobacteria (PGPR) are heterogenous bacteria that can be found in rhizosphere region *viz. Klebsiella, Azotobacter, Pseudomonas, Azospirillum, Bacillus, Serratia* and *Burkholderia. Enterobacter* are considered as PGPR traits that enhance the plant growth promoting substance by producing plant hormones like gibberellic acid, indole acetic acid and providing nutrients to host plant by PGPR traits as well as nitrogen fixation to atmospheric nitrogen. The antibiotic production, lytic enzymes, hydrogen cyanide and catalase involved by the direct mechanism which acts as a biological control of plant pathogens and microbes. Gray and Smith (2005) reported that free living bacterium have been found to display beneficial effects on numerous crops as well as the species of general such as *Burkholderia , Azobacter, Azospirillum, Arthrobacter, enterobacter, Herbaspirillum, Pseudomonas, Rhizobium*, *Bacillus,* and *Serratia.* The soil contains much amount of nutrients are transferred through rhizosplane where as the functional and structural diversity of bacteria has a significant implication for growth promotion of plant. Sunflower is high adaptability to diverse environmental conditions. The variability in the association of PGPR may be due to various environmental factors that may affect their growth and exert effect on plant. The maximum

growth promoting interaction between PGPR and nursery seedlings it is important to discover the rhizobacteria exerting their effects are altered by various environmental factors, including the presence of other microorganisms. Therefore, it is necessary to develop efficient PGPR strains in field conditions and attempt to explore soil microbial diversity for PGPR having combination of PGP activities and well as to adapt in particular soil environment. The present scenario of this study, design certain rhizosphere PGPR strains occurs in sunflower and to screening the potential stains can be used as bioinoculants for sunflower.

2. Materials and methods

The rhizosphere soil samples were collected from 20 various locations in sunflower and were analyzed for clay, organic matter Content PH, P , K , Fe, Ca, Mg , and Al by standard method. Soil samples were placed in individual sterile 500 ml erlenmayer flasks containing 90 ml of sterile saline solution (0.85% Nacl). Samples were incubated at 28◦C under agitation for 16 h. Aliquots of 0.1 ml of three fold serial dilutions are inoculated, in triplicate, into vials containing 4 ml of semi-solid N-free medium (0.18% agar-agar), either NFb, LGI P (Dobereiner 1988). Five days after incubation at 28°c, those vials showing a pellicle formation on surface of the medium are considered to be positive for growth of bacteria and were used for inoculated to other vials containing the same semi-solid N-free medium previously utilized. The cultures from the positive vials were subjected to further purification steps by streaking them on to selective medium as was used in the semi -solid vials, but containing 20mgl^{-1} yeast extract and incubated at 28°C for 2 days and over the period of incubation the distinct colonies are grown in liquid LB medium at 28°C (Sambrook and Russel 2001). The PGPR strains were characterized by their morphological and biochemical characteristics such as hydrolysis of starch, liquid and chitin, utilization of glucose, sucrose, mannitol, citrate and catalase reactions) using standard methods. In vitro screening of plant growth promoting rhizobacterial isolates for their pgp activities.

Assay for Indole Acetic Acid (IAA) production: The IAA production was detected by modified method as described by Brick, *et al.* (1991). The quantitative analysis of IAA was carryout by the method (Loper and Scroth, 1986) at various concentrations of tryptophan (0, 50, 150, 300, 400 and 500 mg/ml). Bacterial cultures were grown for 72 h (*Azotobacter*) and 48 h (*Bacillus* and *Pseudomonas*) on the respective media at 28ºC. Fully grown cultures were centrifuged at 3000 rpm for 30 min. The supernatant (2 ml) was mixed with two drops of orthophosphoric acid and 4 ml of the Salkowski reagent (50 ml, 35% of perchloric acid, 1 ml 0.5 M FeCl3 solution). Development of pink colour indicates the production IAA. Optimum density was taken at 530 nm by using spectrophotometer Spectronic 20 D+. The concentration of IAA production by cultures was measured by standard graph of IAA (Hi-media) obtained in the range of 10–100 mg/ml.

Production of NH_3: The PGPR strains were determined for ammonia production in peptone water. Freshly grown cultures are inoculated of 10 ml peptone water in each tube and incubated for 48–72 h at 2872 1C. Nessler's reagent (0.5 ml) was added in each tube. Development of brown to yellow colour was positive test for ammonia production (Cappuccino and Sherman, 1992).

Siderophore production: The PGPR strains were assayed for siderophore production on Chrome azurol S agar medium (Sigma, Ltd.) and the method described (Schwyn and Neilands, 1987). Chrome azurol S agar plates are divided into equal sectors and inoculated with PGPR isolates (10 ml of 10^6 CFU/ml) and it was incubated at 28ºC for 48–72 h and yellow–orange halo around development of the growth was considered as a positive for siderophore production.

Phosphate solubilization by PGPR isolates: The PGPR isolates were screened on Pikovskaya's agar plates for phosphate solubilisation, the method described (Gaur, 1990). Quantitative analysis of solubilization of tricalcium phosphate in liquid medium was method described (King, 1932). Further PGPR strains were inoculated in 25 ml Pikovskaya's broth and it was incubated of four days at 28ºC. The PGPR cultures were centrifuged at 15,000 rpm for 30 min. The 1 ml of supernatant was mixed with 10 ml of chloromolibidic acid and volume is made up to 45 ml with distilled water. Cholorostannous acid (0.25 ml) was added and the volume was made up to 50 ml with distilled water. The absorbance of the developing blue colour was read at 600 nm. The amount of soluble phosphorus was detected by using standard curve of KH_2PO_4.

3. Results

The morphological and biochemical characteristics of PGPR isolates from the rhizosphere soil of sunflower. The most abundant genera were found in association with rhizosphere of sunflower such as *Klebsiella*, *Enterobacter* and *Burkhloderia*. In addition to the above genera, some of the few bacterial isolates were isolated from the rhizosphere soil like *Agrobacterium*, *Azospirillum*, *Pseudomonas* and *Rhizobium*. Based on their dinitrogen fixing efficiency, the efficient six isolates belongs such as *Burkholdira*, *Klebsiella*, *Enterobacter*, *Pseudomonas*, *Azospirillum* and *Bacillus* were screened for their plant growth promoting activities.

TABLE 1

Production of Siderophore, IAA and Phosphate Solubilisation by PGPR Isolates

Isolate designation	IAA (150µ/ml)	Siderophore production	Phosphate solubilisation	ARA activity
SBR-8	1.27±0.31	+	+	1.26
SBR-4	2.13±0.15	+	+	0.61
SKA-6	1.77±0.21	+	-	0.56
SER-10	1.60±0.30	+	-	0.26
SER-2	1.47±0.25	+	-	0.66
SPS-4	2.60±0.20	+	+	0.39
SAZ-9	3.60±0.20	+	+	81.35
BS-3	ND	+	+	0.44

IAA-Indole Acetic Acid ARA – Acetylene reduction assay

Screening results of PGP traits are depicted in Table 1. The IAA production was show in PGPR isolates of *Azospirillum*, followed by *Pseudomonas, Burkholdira* and *Enterobacter*. The siderophore production simultaneously was exhibited by all the PGPR isolates were chitin hydrolysis for negative and where as the production of ammonia for positive.

4. Discussion

Bacteria associated with rhizosphere of several plants have been isolated and screened by many researchers, and also beneficial effects promoted by different strains are already well-established (Andrews *et al.*, 2003; Vessey 2003). Although strains of various bacteria, such as *Rhizobium radiobacter, Bacillus*, B. cereus, *B. licheniformis, B. pumilus, Pseudomonas, P. fluorescens, P. putida*, *P. vesicularis, Burkholderia cepacia, Flavobacterium odoratum, Stenotrophomonas maltophilia, Rhizobium, Achromobacter xilosoxidans, Azospirillum, Methylobacterium*, and *Dyella thiooxydans* were found to be associated with sunflower. Screening results of PGP traits are represented in Table 11. Production of IAA was showed in PGPR isolates of *Azospirillum* followed by *Pseudomonas, Burkholdira* and *Enterobacter*. The production of siderophore was simultaneously exhibit by all the PGPR isolates and also the phosphate solublization was most frequently encountered by *Bacillus* isolates followed by *Pseudomonas, Azospirillum* and *Enterobacter* where as negative result in chitin hydrolysis and positive result in production of ammonia. In the present study, rhizosphere soil samples were collected from sunflower filed in Cuddalore, screened for their diversity, presence of nitrogen-fixation by plant growth promoting rhizobacteria. The twenty PGPR isolates were identified, out of these, *Mycobacterium* was Gram-positive was found in rhizoshphere soil. Although the isolates from the genera *Klebsiella , Agrobacterium, Pseudomonas and Azospirillum* encompassed about 30 per cent of the bacteria encountered in sunflower roots and rhizosphere soil

5. Conclusion

In the present study, the general identification of microorganisms are already known for their ability of nitrogen fixation and majority of isolates displayed more than one of the PGP characteristics were analyzed. Sunflower is an important crop which produces good quality oil for human consumption, and has high potential as a new source of energy for renewable fuels. Although non-culturing DNA/RNA-based methods are now commonly used to examine bacterial diversity associated with plants. The isolation and culturing methods used in this study are particularly suited for obtaining microbial communities are capable of multiplication in *vitro* for the purposes of potential production of biofertilizers. Indeed, further studies are now being undertaken to assess these PGPR isolates under field conditions with the aim of developing viable biofertilizers for sunflower crops.

6. Reference

Bent, et al. (2001). Alterations in Plant Growth and in Root Hormone Levels of Lodgepole Pines Inoculated with Rhizobacteria. *Canadian Journal of Microbiology*, 47, 793-800

Brick, J.M., Bostock, R.M., & Silverstone, S.E. (1991). Rapid in Situ Assay for Indoleacetic Acid Production by Bacteria Immobilized on Nitrocellulose Membrane. *Applied and Environmental Microbiology*, 57, 535-538.

Cappuccino, J.C., & Sherman, N. (1992). *Microbiology: A Laboratory Manual*. New York: Benjamin/cummings Pub. Co., 125-179.

Döbereiner, J. (1988). Isolation and Identification of Root Associated Diazotrophs. *Plant Soil*, 110 (2), 207-212.

Gaur, A.C. (1990). *Physiological Functions of Phosphate Solubilizing Micro-Organisms.* In: Gaur, A.C. (Ed.), *Phosphate Solubilizing Micro-organisms as Biofertilizers*. New Delhi: Omega Scientific Publishers, 16-72.

Gray, E.J., & Smith, D.L. (2005). Intracellular and Extracellular PGPR: Commonalities and Distinctions in the Plant-bacterium Signaling Processes. *Soil BiolBiochem*, 37, 395-412.

King, J.E. (1932). The Colorimetric Determination of Phosphorus. *Biochem, Journal*, 26, 292

Loper, J.E., & Scroth, M.N. (1986). Influence of Bacterial Sources on Indole-3 Acetic Acid on Root Elongation of Sugarbeet. *Phytopathology*, 76, 386-389.

Sambrook J, Russel DW (2001). *Molecular Cloning: A Laboratory Manual*. NewYork: Ed.Cold Spring Harbor Laboratory Press.