Research Journal of Recent Sciences _____ ISSN 2277-2502
Vol. **1(6),** 58-61, June **(2012)** *Res.J.Recent Sci.*

Short Communication

Rhizobacteria from Rhizosphere of Sunflower (*Helianthus annuus* L.) and their effect on Plant Growth

Raval A.A.[1] and Desai P.B.[2]*
[1]Department of Microbiology, Arts, Science and Commerce College, Kamrej Cross Roads, Surat, INDIA
[2]Dept. of Microbiology, Shree Ramkrishna Inst. of Applied Sciences, MTB College Campus, Athwalines, Surat, INDIA

Available online at: www.isca.in
(Received 26th February 2012, revised 10th March 2012, accepted 30th March 2012)

Abstract

*Rhizosphere harbors a vast population of bacteria; among them a beneficial group is the Plant Growth Promoting Rhizobacteria (PGPR) that help plant growth promotion. Sunflower is an important oilfield crop and has also been used in bioremediation and phytoremediation studies. Several of these bacteria were isolated from different sites, the bulk soil, rhizosphere and endorhizosphere regions of this experimental plant and their effects on plant growth were studied. The stimulation of plant growth is attributed to various plant growth promoting mechanisms. Study of the direct mechanisms i.e. production of phytohormones, solubilization of Phosphate and the indirect mechanisms- antifungal activity against plant pathogenic fungi, production of lytic and detoxification enzymes, Siderophore production, production of Ammonia and HCN were also carried out. Among the isolates, about 30 rhizobacteria that were positive for several of these plant growth promoting mechanisms were selected for plate germination and pot assay experiments. The bacteria mainly belonged to the **Azotobacter**, Pseudomonads, and the Bacillus group. From the plate experiments seven of the bacteria were selected i.e. four rhizosphere isolates, one endorhizosphere isolate and two soil isolates gave promising results when compared to control. All of the isolates also showed elongation of roots. Three had elongated shoots as compared to control in pot trial experiments. These isolates will be further tested for field experiments.*

Keywords: Rhizobacteria, phytohormone, antifungal, bioremediation.

Introduction

Rhizosphere is a zone of diverse bacterial population. This soil-plant root eco zone has been an area of research for many years. The rhizosphere bacterial population is influenced by several biotic and abiotic factors. The root exudates[1] of the plant also have a profound effect on the rhizoflora. One subpopulation among these rhizobacteria is the PGPR[2], the plant growth-promoting rhizobacteria, which are the beneficial ones that stimulate plant growth by an array of mechanisms. These bacteria are of agricultural importance because they encourage improving plant health and growth, suppress disease-causing microbes and accelerate nutrient availability and assimilation[3].

The various traits exhibited by these PGPR make them valuable. These PGPR may help to provide nitrogen and make available phosphorus to the plant, produce phytohormones like indole acetic acid (IAA), gibberellic acid (GA), cytokinins, produce other antagonistic substances like ammonia, hydrocyanic acid (HCN), release certain lytic and detoxification enzymes[4]. So the potential PGPR were screened *in vitro* for such multiple plant growth-promoting traits and their effect on growth was observed by conducting plate and pot experiments. These root colonizing bacteria were isolated from sunflower (h*elianthus annuus*) rhizosphere. The plant has several uses, mainly the oil is important. The oil contains lecithin, tocopherols, carotenoids, waxes and a high vitamin E content. It is a combination of monounsaturated and polyunsaturated fats with low saturated fat levels. Oil can be used in cosmetic formulations and appears to have skin health benefits as it forms a barrier that resists infection over the skin[5,6]. Along with these properties *Helianthus annuus* has also been used in several bioremediation and phytoremediation studies as it is a high biomass and hyper accumulator plant[7,8].

The bacteria belonging to the genera *Acetobacter*, *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Azoarcus*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Derxia*, *Enterobacter*, *Gluconoacetobacter*, *Herbaspirillum*, *Klebsiella*, *Ochrobactrum*, *Pantoae*, *Pseudomonas*, *Rhodococcus*, *Serratia*, *Stenotrophomonas* and *Zooglea*[9] may act as selective candidates for plant growth promotion. These PGPR with multiple plant growth-promoting activities can further be used as biofertilizers, bioinoculants or bioenhancers. Again, *Pseudomonas* and *Bacillus* have been reported by several authors for plant growth-promotion. *Bacillus cereus*, *Pantoae* and *Pseudomonas aeruginosa* have been reported by several researchers for improvement of plant growth[10,11] and nutrition under salt stress in wheat plant. According to a study *Pseudomonas flourescens* biotype G, *Pseudomonas flourescens* and *P. putida* biotype A have been observed to improve the fresh and dry weight, root length and shoot length in the pea plant[12].

Experiments carried out on the *Cicer arietinum* plant also reported the effectiveness of PGPR isolates for significant increase in shoot length, root length and dry weight of shoot and root[13].

This paper reports on some of the plant growth-promoting mechanisms of the bacteria isolated from the vicinity of the roots of the *Helianthus annuus* plant and its effect on plant growth.

Material and Methods

Microbial inoculation and properties: Bacterial strains were isolated from various regions of the Sunflower plant i.e. bulk soil, rhizosphere and endorhizosphere regions. They were isolated on specialized media for *Azotobacter, Pseudomonas* and *Bacillus* species. These bacteria were then screened for the production of a phytohormone indole acetic acid (IAA) and determination of phosphate solubilization. Other antagonistic mechanisms studied were antifungal activity against four phytopathogenic fungi, production of lytic and detoxification enzymes, HCN, ammonia and siderophore production were also evaluated.

Auxin production by strains was determined colorimatrically by using the Salwoskii's reagent. The intensity of color was measured spectrophotometrically at 535 nm. From the color development in standard solutions of IAA a standard curve was established[14,15].

Solubilization of phosphate was measured by growing the isolates in a medium incorporated with inorganic phosphate (TCP). The ability of isolates to solubilize the phosphate was observed by formation of halo around the colonies[16,17].

Antifungal activity was determined by the circle method[3].

Hydrolytic enzymes are significant as they act as agents for prevention of plant diseases[18] by causing lysis of deleterious microbes in the close vicinity of the plant. The enzymatic activities such as protease and amylase activities were determined as described by Smibert and Kreig[19].

Hydrolysis of tween 20 was determined on modified Sierra agar. Hydrolysis of this compound was recorded as white precipitation around the colonies.

The production of HCN [20] and ammonia production[21] was also observed. Production of Siderophores was evaluated by Chrome Azurol-S Assay on CAS agar plates by observation of orange halo around the colonies[22,23].

Plate experiments were carried out by preparing bacterial inoculants after growing the selected isolates in the LB broth medium and incubating on a rotary shaker at 28°C for 24-48 hrs. Seeds were then bacterized [24]. Non-bacterized seeds were used as control. The results of seed germination assay in plates are shown in figure-2.

Results and Discussion

We have observed that seven among the 30 isolates possessed several of the plant growth-promoting mechanisms and have also promoted plant growth in plate and pot experiments. Indole acetic acid production was the highest in M6S3 (58 µg/ml) and isolate M7S1 gave 52 µgm/ml (table-1). All of the isolates also showed solubilization of inorganic phosphate by producing a halo around the colonies (table-1). Table-2 shows the production of hydrolytic enzymes by the isolates. Isolate M6S3 also showed Siderophore production (table-3) and possessed antifungal activity against the tested phytopathogenic fungi (figure-1). HCN production was shown by three of the isolates, one bluish green pigmented *Pseudomonas* and the other two belonged to *Bacillus* spp.

Figure 2 shows isolate M1R2, M7S1, M7ER1, M11R2, M12R1 had elongated roots. Two of the isolates M6S3 and M11R2 showed shoot elongation as compared to control. Results revealed that shoot length increased in PGPR treated seeds over uninoculated control the highest root length recorded was 10.6 cm in isolate M12R1 followed by M7ER1 which was 9.9 cm. Isolate M6S3 showed the highest elongation of shoot (6.6 cm) among the isolates (figure-2).

Table-1
Production of IAA and solubilization of Phosphate by the isolates

Isolate No.	IAA µg/ml	Sol. of P
M1 R2	8.8	++
M6 S3	58.4	++
M7 S1	51.7	+
M7 R1	12	+
M7 ER1	6.0	+
M11 R2	3.4	++
M12 R1	1.5	++

Table-2
Production of lytic enzymes by the isolates

Isolate No.	Protease	Amylase	Lipase	Cellulase
M1 R2	Negative	Negative	Positive	Positive
M6 S3	Negative	Trace	Negative	Negative
M7 S1	Negative	Negative	Negative	Negative
M7 R1	Positive +	Negative	Positive ++	Negative
M7 ER1	Negative	Negative	Negative	Positive ++
M11 R2	Positive ++	Positive +	Positive ++	Positive ++
M12 R1	Negative	Positive	Positive +++	Positive +

Table-3
Production of other biocontroling factors by the isolates

Isolate No.	Siderophore	HCN	Ammonia
M1 R2	-	-	+
M6 S3	+	-	-
M7 S1	+	-	-
M7 R1	+	++	+
M7 ER1	-	-	++
M11 R2	-	+	++
M12 R1	+	++	+

Conclusion

Results suggest that PGPR are able to enhance the production of IAA, solubilize the phosphate and show antagonism towards pathogens thereby may improve the growth of *Helianthus annuus*. The use of such PGPR singly or in consortium can act as efficient bioinoculants which may be an approach to replace chemical fertilizers and pesticides for sustainable cultivation of *Helianthus annuus* in India and in other developing countries. Further investigations include efficiency test under green house and field conditions which are needed to evaluate the role of PGPR.

Acknowledgement

I am grateful to the management of Shri Bhartiya Vidya Mandal, the Principal, Arts, Science and Commerce College for their co-operation and support. I also acknowledge my sincere thanks to the Director, SRICEAS, for providing me excellent research facilities.

References

1. Kennedy I.R., Choudhary A.I. M.A. and KecSkes M.L., Non-symbiotic bacterial diazotrophs in crop farming systems: can their potential for plant growth promotion be better exploited, *Soil Biol Biochem* 3, **6(8)**, 1229-1244 **(2004)**

2. Kloepper J.W., Hume D.I., Scher F.M., Singleton C., Tipping B., Lallberte M., Frauley K., Kutchaw T., Simonson C., Liftshitz R., Zaleska I. and Lee L., Plant growth-promoting rhizobacteria on Canola (rapeseed), *Plant disease,* **72,** 42-46 **(1988)**

3. Cattelan A.J., Hartel P.G. and Fuhrman J. J., Screening for pgpr to promote early Soybean growth, *Soil Science Society of America Journal,* **63,** 1670-1680 **(1999)**

4. Chaihan M., Chunhaleuchanon S., Kozo A., Lumyong S., Screening of rhizobacteria for their plant growth promoting activities, *KMITL Sci. Tech. J.,* **8**(1) **(2008)**

5. Akbari P., Ghalavand A., Modarres Sanavy A.M. and Agha Alikhani M., The effect of biofertilizers, nitrogen fertilizer and farmyard manure on grain yield and seed quality of sunflower (*Helianthus annuus* L.), *Journal of Agricultural Technology,* **7(1),** 173-184 **(2011)**

6. Nair A.T., Pharmaceuticals in Environment: A review on its effect, *Res. J. Chem. Sci*., **2(1)**, 103-105 **(2012)**

7. Zadeh B.M., Firozabadi G.R.S., Alikhani H.A., Hosseinin H.M., Effect of Sunflower and *Amaranthus* Culture and Application of Inoculants on Phytoremediation of the Soils Contaminated with Cadmium, *American-Eurasian J. Agric. and Environ. Sci.*, **4 (1),** 93-103 **(2008)**

8. Faisal M., Hasnain S., Growth improvements of Sunflower seedlings by Cr (VI)-resistant bacteria, *Iranian Journal of Biotechnology*, **3(2)**, **(2005)**

9. Babalola O.O., Beneficial bacteria of agricultural importance, *Biotechnol Lett*. **32,** 1559-1570 **(2010)**

10. Egamberdiyeva D., Plant growth promoting properties of rhizobacteria isolated from wheat and pea grown in loamy sand soil, *Turk J Biol,* **32(1),** 9-15 **(2008)**

11. Chauhan R.R., Chaudhary R., Singh A. and Singh P.K., Salt tolerance of sorghum bicolor Cultivars Germination and Seedling Growth. *Res. J. Recent Sci.,* **1(3)**, 1-10 **(2012)**

12. Zahir Z.A., Munir A., Asghar H.N., Shaharoona B., Arshad M., Effectiveness of rhizobacteria containing ACC deaminase for growth promotion of peas (*Pisum sativum*) under drought conditions, *J. Microbiol Biotechnol* **18(5),** 958-963 **(2008)**

13. Mishra M., Kumar U., Mishra P.K., Prakash V., Efficiency of plant growth-promoting rhizobacteria for the enhancement of *Cicer arietinum* L. growth and germination under salinity, *Advances in biological research* **4(2),** 92-96 **(2010)**

14. Gordon S.A. and Paleg L.G., Quantitative measurement of indole acetic acid. *Plant Physiology,* **10**, 37-48 **(1957)**

15. Brick J.M., Bostock R.M., Silverstone S.E., Rapid *In situ* assay for indole acetic acid production by bacteria immobilized on a nitrocellulose membrane. *App. Environ. Microbiol*. **57,** 535-538 **(1991)**

16. Pikovskaya R.I., Mobilization of phosphorus in soil in connection with the vital activity of some microbial species, *Mikrobiologiya* **17,** 362-370 **(1948)**

17. Mehta S., Nautiyal C.S., An efficient Method for Qualitative Screening of phosphate-Solubilizing Bacteria, *Current Microbiology,* **43,** 51-56 **(2001)**

18. Van Loon L.C., Plant responses to plant growth-promoting rhizobacteria, *Eur J Plant pathol,* **119,** 243-254 **(2007)**

19. Smibert R.M. and Kreig N. R., Phenotypic characterization in: Gerhardt P. et al. (Eds.) Methods for general and molecular bacteriology, *American Society for Microbiology*, Washington D.C., 607-654 **(1994)**

20. Baker A.W., Schippers B., Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Psudomonas* spp, Mediated plant growth stimulation, *Soil Biol Biochem* **19,** 451-457 **(1987)**

21. Dye D.W., The inadequacy of the usual determinative tests for identification of *Xanthomonas* spp. *NZT Sci*. **5,** 393-416 **(1962)**

22. Schwyn B. and Neilands J.B., Universal chemical assay for the detection and determination of Siderophores, *Anal. Biochem*., **160,** 47-56 **(1987)**

23. Raaska L. and Viikari L., Mattila-Sandholm T., Detection of Siderophores in growing cultures of Pseudomonas spp. *Journal of Industrial Microbiology*, **11,** 181-186 **(1993)**

24. Wokoma E.C., Studies on plant growth promoting Rhizobacteria isolated from Choba rivers, state Nigeria. *Asian Jr. of Microbiol. Biotech. Env.Sc*., **10(4),** 739-747 **(2008)**

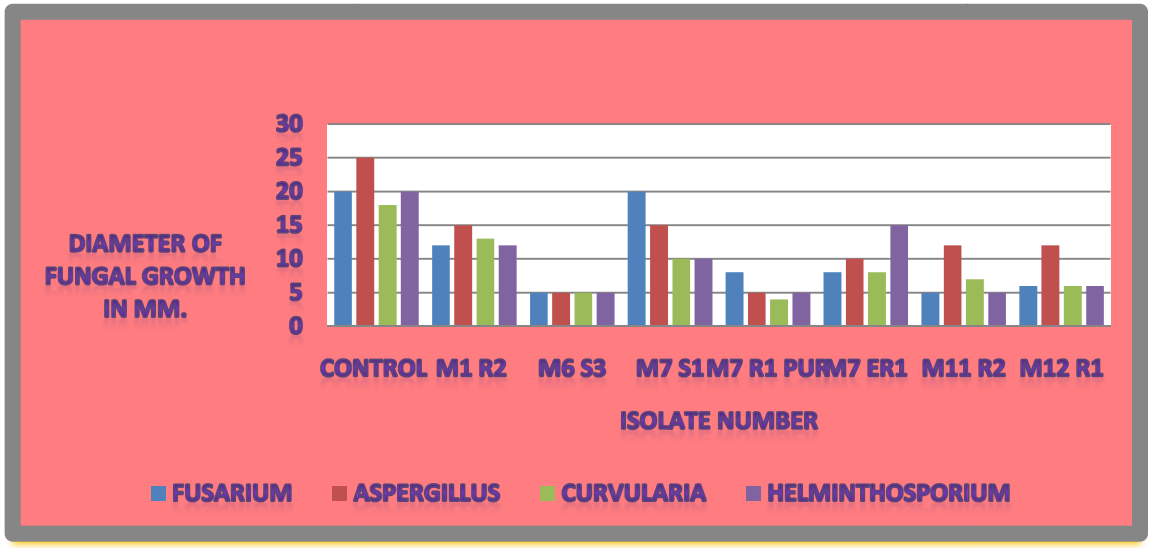

Figure-1
Antifungal activity of the isolates against phytopathogenic fungi

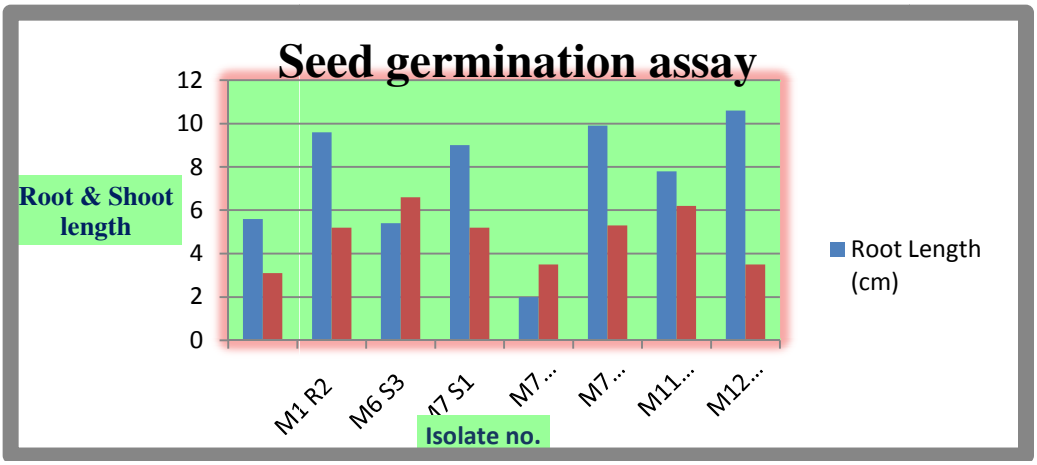

Figure-2
Results showing the comparison of root and shoot lengths of the isolates