Evaluation of Plant Growth Promoting Properties of Pseudomonas Aeruginosa

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Abstract- *Plant Growth Promoting Rhizobacteria (PGPR) can aid in stimulating plant growth by several mechanisms like phosphate solubilization, plant growth hormones, siderophore and antimicrobial compounds production etc. The present study aimed at evaluating the potentials of Pseudomonas aeruginosa for plant growth promotory attributes. Pseudomonas aeruginosa was found to solubilize phosphate with a solubilization index of 2.45. The bacteria produce high levels of siderophore as a prominent orange halo appears around its colony. The antifungal activity of P. aeruginosa was tested against Fusarium oxysporum species and the percent inhibition was observed as 64.41%. Thus Pseudomonas aeruginosa was found to be a potential rhizobacteria for improving plant growth by providing nutrients to plants as well as by its defense mechanism against phytopathogen.*

Keywords- Rhizobacteria, Pseudomonas aeruginosa, siderophore, phytopathogen.

I. INTRODUCTION

Plant growth promoting rhizobacteria (PGPR) resides in the rhizosphere region which is a narrow zone of soil that surrounds plant roots (Manthey et al., 1994). It is a group of favorable plant bacteria which are helpful to promote plant growth and also control phytopathogens thereby increasing yield of crops. Most common are the members of genera *Pseudomonas*, *Azotobacter*, *Arthrobacter*, *Klebsiella, Bacillus*, *Burkholderia*, *Serratia* and *Xanthomonas*. Among these, Pseudomonads are the major players and have been studied globally for both plant growth promotion and biological control of phytopathogens (Ramadan et al., 2016). These organisms rapidly colonize the rhizosphere and thus mediate improved plant growth by stimulation of the plant host defense mechanisms (Ahmad et al., 2008; Bhattacharyya and Jha, 2012).

Pseudomonas aeruginosa is a gram-negative, aerobic, ubiquitous bacteria present in agricultural soils. They have multiple characteristics that make them ideal for plant growth-promotion and biocontrol (Deshwal, 2012). This includes their ability to rapidly colonize and proliferate in the region of rhizosphere of the plants. They quickly utilize root and seed exudates and grow fast in laboratory conditions thus can be mass produced. They produce a broad spectrum of bioactive metabolites like siderophores, antibiotics, volatile compounds, and growth- promotory substances.

II. MATERIALS AND METHODS

Bacterial culture and growth condition

Pseudomonas aeruginosa culture was procured from the Department of Microbiology, BU, Bhopal and was maintained as 40% glycerol stocks at -20^{o}C in King's B medium.

Determination of Plant Growth Promontory Properties
Estimation of Phosphate solubilisation

Sterilized Pikovskaya's agar (Pikovskaya, 1948) was poured on to the sterilized petriplates and spot inoculated with *Pseudomonas aeruginosa* culture then incubated at 28 ± 2°C for 48 h. Formation of a clear zone around the colonies were considered as positive result for phosphate solubilization The Phosphate solubilization index was calculated0 by the formula as below (Edi- Premono et al, 1996).

$$\text{Phosphate Solubilization Index (SI)} = \frac{\text{(Colony diameter + Halo zone diameter)}}{\text{Colony diameter}}$$

Estimation of Siderophore production

Siderophore production was qualitatively assayed as described by Schwyn and Neilands (1987). *P. aeruginosa* culture was spot inoculated onto a chrome azurol S (CAS) agar plate and incubated at 28 °C for 48 h. The orange halo development around the bacterial culture is an indicative of positive test for siderophore production.

Estimation of Antifungal activity

100 ml LB medium was prepared, autoclaved and cooled. It was then inoculated with 1ml *Pseudomonas*

aeruginosa culture (24 hours old). The flask was incubated in shaker at 28ºC for 48 hours. The incubated culture was centrifuged at 10,000 rpm for 10 minutes to obtain cell free supernatant. *Pseudomonas aeruginosa* was tested for their inhibitory activity against fungus *Fusarium oxysporum* using disc diffusion method (Lahlali and Hijri, 2010). The fungal pathogen was grown on PDA plate till it covered the whole surface of the agar. With the help of a sterile borer, a disc of fungal pathogen was placed at the centre of dual media (PDA and NAM in equal ratio) plate. Then, filter paper discs (about 6 mm in diameter), containing 10µl bacterial supernatant was placed on the dual media surface. The Petri dishes were kept in refrigerator for 1 hour and then incubated at 28ºC for 96 hours. Dual media plate simultaneously inoculated with only the fungal pathogen (Parani et al., 2009) served as a control. The diameters of inhibition growth zones were measured. Zone between the bacteria and fungus was used as an indication for the extent of antagonism. The percentage inhibition of mycelia growth of the fungus was calculated using the following formula (Kra et al., 2009).

Inhibition Percentage= (A - B) x 100 / A

Where, A and B are the average diameter of fungal growth on control medium and diameter of fungal growth with bacterial supernatant disc on medium respectively.

III. RESULTS AND DISCUSSION

Pseudomonas aeruginosa is found to be an efficient phosphate solubilizer as a clear zone of 12.00 mm around the *Pseudomonas aeruginosa* colony was found after incubation period and 2.45 was calculated as the solubilization index (SI) of the bacteria. The clear zone formation which appears around the colony might be the result of production of organic acids by phosphate solubilizing bacteria (Paul and Sinha, 2013). Siderophore production is one of the biocontrol mechanism possessed by PGPRs under iron limiting condition (Sasirekha and Srividyab, 2016; Dimkpa, 2016). The CAS assay is a functional assay based on the high affinity of siderophores for iron. When a siderophore removes the iron from the Fe-CAS-hexadecyltrimethylammonium bromide (HDTMA) complex, its color turns from blue to orange. Orange halos around the colonies on CAS agar plates are indicative of siderophore production. In this study *Pseudomonas aeruginosa* produced high levels of siderophore. The plates were observed for the antifungal activity of *P. aeruginosa* against growth of pathogen in the treated and control plates and percent inhibition was measured. *Pseudomonas aeruginosa* showed good activity against phytopathogen *Fusarium oxysporum.* The maximum percent inhibition against *Fusarium oxysporum* was observed as 64.41% by *Pseudomonas aeruginosa.*

REFERENCES

[1] Ahmad F., Ahmad I., Khan M.S. (2008), Screening of free living rhizosphereic bacteria for their multiple plant growth promoting activities, Microbiololgy Research, 163:173-181.

[2] Bhattacharyya P.N., Jha D.K. (2012), Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J Microbiol Biotechnol 28: 1327-1350.

[3] Deshwal K. (2012), *Pseudomonas aeruginosa* as biological control agent against plant pathogenic fungus *Sclerotina sclerotiorum,* International Journal of Plant, Animal and Environmental Sciences, 2(1):14-17.

[4] Dimkpa C., (2016), Microbial siderophores: Production, detection and application in agriculture and environment. Endocytobiosis and Cell Research, 27(2)7-16.

[5] Kra K.D., Diallo H.A., Kouadio Y. J., Journal of Applied Biosciences. 24(2009)1488.

[6] Lahlali R. and Hijri M. (2010), Screening, identification and evaluation of potential biocontrol fungal endophytes against *Rhizoctonia solani* AG3 on potato plants, FEMS Microbiol Lett, 311:152–159.

[7] Manthey J. A , Crowley DE , Luster DG (eds) Biochemistry of metal micronutrients in the rhizosphere. Lewis Publishers, Boca Raton, FL, 81-92.

[8] Parani K., and Saha B.K.; Studies on interaction of Serratia marcescens strain (SR1) with fungal pathogens.J.Agri.Environ.Sci., 2009, 5(2): 215- 218.

[9] Paul D. And Sinha S.N. (2013), Phosphate solubilization potential and phosphatase activity of some bacterial strains isolated from thermal power plant effluent exposed water of river Ganga, CIBTech J. Microbiol., 2:1-7.

[10] Pikovskaya R.I. (1948), Mobilization of phosphorous in soil in connection with vital activity of some microbial species, Mikrobiologiya, 17:362-370.

[11] Premono M.E., Moawad A.M., Vleck P.L.G. (1996). Effect of phosphate solubilizing *Pseudomonas putida* on the growth of maize and its survival in the rhizosphere, Indonesian Journal of Crop Science, 11(1):13-23.

[12] Ramadan E.M., Abdelhafez, A.A., Hassan E.A., Fekria S.M. (2016). Plant growth promoting rhizobacteria and their potential for biocontrol of phytopathogens, African Journal of Microbiology Research 10(15):486-504.

[13] Sasirekha B. And Srividyab S. (2016), Siderophore production by *Pseudomonas aeruginosa* FP6, a biocontrol strain for *Rhizoctonia solani* and *Colletotrichum gloeosporioides* causing diseases in chilli, Agriculture and Natural Resources 50:250-256.

[14] Schwyn B. and Neilands J.B. (1987), Universal chemical assay for the detection and determination of siderophores, Analytical Biochemistry, 160(1): 47-56.