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A STUDY ON SCREENING OF PSEDUMONAS FLUORESCENS S9 FOR THE PRODUCTION OF BIOACTIVE COMPOUNDS

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ABSTRACT

Background and Aim: This study was aimed to screen the bacteria, Pseudomonas fluorescens S9 isolated from Triticum aestivum (wheat) with plant growth promoting (PGP)activities. Plant growth promoting rhizobacteria (PGPR) are commonly used as inoculants for improving the growth and yield of agricultural crops, however screening for the selection of effective PGPR strains is very critical. **Methods:** The isolated strain was screened for the production of siderophores, indole acetic acid production, hydrogen cyanide production as well phosphate solubilization activities in vitro. **Results:** Pseudomonas fluorescens S9 was found to be an effective phosphate solubilizer showing a clear halo of 14.00 mm around its colony.The bacteria produced high levels of siderophore as a prominent orange halo appeared.The strain also showed positive results for IAA production by developing pink colour and showed positive results for HCN production by developing brown colour on the filter paper. **Conclusions:** Study indicated that the isolate , Pseudomonas fluorescens S9 showed all the multiple PGPR traits which may lead to growth of plants under indigenous conditions.

I.INTRODUCTION

Across world, there is a profound need to explore varied agro-ecological niches for the presence of native positive microorganisms. Many studies have been undertaken to understand the nature and properties of these unique microbes harboring potential plant growth promoting traits. With increasing awareness about the chemical-fertilizers-based agricultural practices, it is important to search for region-specific microbial strains which can be used as a potential plant growth promoter to achieve desired product. According to various reports PGPR strains are able to express multiple beneficial functions [Kloepper & Schrot, 1978]. The recognition of PGPR, a group of beneficial plant bacteria, as potentially useful for stimulating plant growth and increasing plant yields has evolved over the past several years to where today researchers are able to repeatedly use them successfully in field experiments. It is empirically proved that PGPR employ positive influence on the growth of different crops and plants [Wu et al., 2008; Bhattacharyya & Jha, 2012].

India, being blessed and enriched with a diverse agroecological condition, ensuring food and nutrition security to a majority of the Indian population through production and steady supply particularly in the recent past, is the second largest producer of wheat worldwide. Madhya Pradesh is one of the leading states in the production of this staple crop. Although the production is high still it gets infected from the pathogens. Environmental problems have raised great interest in environmentally friendly sustainable agricultural practices. The usage of Growth Promoting Rhizobacteria (PGPR) is a promising resolution for sustainable, environmentally friendly agriculture.The various mechanisms through which PGPR promote plant growth are modulation of the hormone balance in plants through the release of indole-3-acetic acid (IAA) and synthesis of 1-amino-cyclopropane-1-carboxylate (ACC) deaminase (Glick, 2014; Spaepen and Vanderleyden, 2011). Furthermore, PGPR make soil elements, such as iron, phosphorus and potassium, more accessible to plants by the release of siderophores, organic acids and enzymes (Ahmed and Holmström, 2014; Parmar and Sindhu, 2013; Rodriguez and Fraga, 1999).The present investigation was focused on screening of media for production of bioactive compounds by plant growth promoting rhizobacteria isolated from wheat. Thus, the main aim was to screen the strain obtained from wheat crop, thereby increasing the yield by maintaining soil fertility and ecological balance.

II. MATERIAL AND METHODS

Collection of samples:

The bacterial isolate (stored under 4°C) was procured from Department of Biotechnology, Career college, Bhopal.

Screening of bacteria for Phosphate solubilization

The bacterial isolate was then screened qualitatively for phosphate solubilization on the NBRIP medium (National Botanical Research Institute's phosphate growth medium) containing bromophenol blue and tricalcium phosphate (TCP) using point inoculation. The phosphate solubilization ability was analysed by the formation of a halo zone around colonies, indicating solubilization of tricalcium phosphate (Mehta and Nautiyal,2001). PSI (phosphate solubilization index) was calculated by using the following formula (Edi-Premono et al. 1996)

PSI = Colony diameter + Halo diameter
Colony diameter

Determination of Indole Acetic Acid (IAA)production

IAA production by bacterial strains was estimated based on the method of Gordon and Weber (1951). A loopful of culture was inoculated in 10 ml of Luria broth supplemented with L-tryptophan and was incubated for 72 hrs at 30°C. Then the culture was centrifuged at 10,000 xg for 10 min., and the supernatant was collected. 1 ml of supernatant was allowed to react with 2 ml of Salkowsky reagent (1 ml of 0.5 M FeCl3 in 50 ml of 35% HClO4) at 30°C for 30 min. Development of pink colour was the indication for the presence of IAA (Patten and Glick, 1996)

Determination of Hydrogen Cyanide (HCN) production

Screening of bacterial isolate for hydrogen cyanide (HCN) production was done as per the methodology described by Castric (1975). Bacterial isolate was streaked on nutrient agar plate containing 4.4 g per litre of glycine. A Whatman filter paper No.1 soaked in 0.5% picric acid solution (in 2% Sodium Carbonate) was placed on top of the plate. The plates were sealed with parafilm and was incubated at 30 ± 0.1°C for 4 days. Development of light brown to dark brown colour on the filter paper was the indication for the presence of HCN production.

Determination of Siderophore production

The bacterial isolate was qualitatively screened for siderophore production by spot inoculation on the surface of Chrome azurole' S agar medium according to the method of Alexander and Zuberer (1991) and was incubated at 48°C for 3 days.The development of orange halos around the bacterial colonies after incubation was considered as a positive sign for siderophore production.

III. RESULT AND DISCUSSION

Associative and free-living microorganisms may contribute to the nutrition of plants through a variety of mechanisms, including direct effects on nutrient availability, providing the plant with plant growth promoting substances that are synthesized by the bacterium or by facilitating the uptake of certain plant nutrients from the environment (Andrews et al. 2003; Ahmad et al. 2006; Raaijmakers et al. 2009). A particular bacterium may affect plant growth using any one, or more, of these mechanisms. Moreover, a bacterium may provide different benefits at various times during the life cycle of the plant (Glick 2005). This study reports the characterization of plant growth promoting rhizobacteria having multiple plant growth promoting activity. Four plant growth promoting characteristics were evaluated for the isolate in this study.

In current study Pseudomonas fluorescens S9 was found to be an effective phosphate solubilizer showing a clear halo of 14.00 mm around its colony after 24 hrs of incubation. Phosphorus solubilizing bacteria (PSB) play important role by enhancing its availability to plants through release from inorganic and organic soil P by solubilization and mineralization. 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). Pseudomonas fluorescens S9 showed positive results for IAA production by developing pink colour. Under in vitro conditions, IAA was synthesized as secondary metabolite by bacteria upon induction with tryptophan. Earlier studies stated the production of IAA by various rhizosphere isolates such as Enterobacter sp, Klebsiella sp, Azotobacter sp and Pseudomonas sp. There are evidences that the IAA concentration increased as the tryptophan concentration in the medium was increased.Pseudomonas fluorescens S9 showed positive results for HCN production by developing brown colour on the filter paper .HCN production by Pseudomonas is involved in the suppression of pathogens. Sometimes PGPR exert their biocontrol ability on phytopathogens also by some other means like secreting the fungal cell wall degrading enzymes like chitinase and beta 1,3 glucanase together with the potentiality of HCN secretion as their secondary metabolite (Chandra et.al.2007).The orange halos developed around the bacterial colonies after incubation showed that Pseudomonas fluorescens S9 produced high levels of siderophore. Siderophores are low molecular weight bio-molecules secreted by micro-organisms in response to iron starvation for acquisition of iron from insoluble forms by mineralization and sequestration [Lankford, 1973].

Current study shows that Pseudomonas fluorescens S9 strain is an efficient PGPR isolate. This PGPR strain holds good prospects in future for sustainable agricultural practice with minimal chemical inputs and enhancing organic farming. Production and utilization of biofertilizer formulation using these rhizobacterial strains in agricultural fields can increase soil fertility and can increase the yield by the multifaceted PGP action of these PGPR isolates. These beneficial effects can considerably reduce the use of chemical fertilizers. Therefore, screening of the rhizobacteria for its in vitro potential of growth regulator production offers a reliable base for choice of effective PGPR.

DECLARATIONS

Ethical approval and consent to participate:Not applicable.

Consent for publication:Not applicable.

Competing interests: The authors declare no competing interests.

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