EVALUATION OF FLUORESCENT PSEUDOMONADS ISOLATES FOR PHOSPHATE SOLUBILIZING ACTIVITY

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ABSTRACT

The pathogen is soil-borne and application of fungicides is very expensive and also polluting the ecosystem. Several strains of fluorescent pseudomonads isolates have been reported to suppress soil-borne diseases caused by fungal pathogens. Fluorescent pseudomonads are Gram-negative rod shaped bacteria that inhabit soil, plants, and water surfaces. Fifteen fluorescent pseudomonads isolates were obtained on King's B medium from the rhizosphere of plant roots. All the fluorescent pseudomonads isolates displayed positive phosphate solubilizing reaction by formation of clear inhibition zone around the colony on Pikovskaya's medium.

KEY WORDS: Isolation**,** Fluorescent Pseudomonads isolates and phosphate solubilizing activity

INTRODUCTION

Fluorescent pseudomonads are a free-living bacterium, commonly found in soil and water. However, it occurs regularly on the surfaces of plants and occasionally on the surfaces of animals. The microorganism isolated from the root or rhizosphere of a specific crop may be better adapted to that crop and may provide better control of diseases than organisms originally isolated from other plant species. Such plant associated microorganisms may make better Bio-control agents because they are already closely associated with and adapted to the plant or plant part as well as the particular environmental conditions in which they must function. The screening of such locally adapted strains has yielded improved Bio-control in some cases (Cook, 1993). Phosphate solubilizing activity of pseudomonads bacteria is prime important as it secrete an organic acid and lower down the pH in vicinity and bring about solubilization of insoluble phosphate in soil.

The phosphate solubilizing capacity of the Plant Growth Promoting Rhizobacteria (PGPR) was tested *in vitro* using Pikovskaya's agar as well as broth by Samanta and Dutta (2004). Bhatia *et al.* (2005) have performed the phosphate solubilization activity of *Pseudomonas fluorescens* by spot inoculation on Pikovskaya's medium incubation at 28 ± 1^{o}C

for 4-5 days and found the positive indication by formation of clear inhibition zone around the colony.

MATERIALS AND METHODS

Collection of Soil and Plant Samples

Fifty soil and roots samples were collected from established castor field plots of different locations of Patan and Banaskantha districts where the castor is commonly grown. Healthy plants of castor (Ricinus communis L.) of 60-75 days growth were carefully uprooted along with adhering soil and was carried to the laboratory in polythene bags. The soil particles loosely adhering to the roots were gently teased out and used for isolation of rhizosphere bacteria. Soil particles adhering tightly to the roots were allowed to go with the roots for isolation of rhizoplane bacteria.

Isolation of Fluorescent Pseudomonads Isolates

Excess of soil adhering with roots was removed by gentle shaking. From each sample 10 g of closely associated rhizosphere was added to 250 ml flask containing 90 ml sterilized distilled water. For isolation of rhizoplane bacteria, roots were cut into approximately 2-3 cm long pieces and 10 g of root bits were then transferred to 90 ml sterilized distilled water. The flasks were placed on a rotary shaker for 1 hr to allow root associated bacteria to diffuse. Three replications were kept for each location and serial dilution of rhizosphere and rhizoplane samples were made up to 106. An aliquot of 0.1 ml from 106 dilution of each sample was spread plated over solidified King's medium B on which preferentially fluorescent pseudomonads recovered under aseptic conditions. The plates were incubated at 30 ± 1^{o}C for 24-48 hrs. Colonies of different morphology were examined for their fluorescence under ultraviolet light (240-340 nm). The colonies showing fluorescence was picked-up and were further purified by streaking on same medium plates. The purified cultures were finally transferred onto solid King's B medium and preserved at low temperature (4^{o}C) in refrigerator in the Department of Plant Pathology, C.P.College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, for further activities.

Evaluation of Fluorescent Pseudomonads isolates for phosphate solubilizing activity

The phosphate solubilizing capacity of isolates was tested *in vitro* using Pikovskaya's agar medium (Yeast extract 0.50 g, Dextrose 10.00 g, Calcium phosphate 5.00 g, Ammonium sulphate 0.50 g, Potassium chloride 0.20 g, Magnesium sulphate 0.10 g, Manganese sulphate 0.0001 g, Ferrous sulphate 0.0001 g, Agar 15.00 g and Distilled water 1 lit.). This test was performed by spot inoculation of test organism on Pikovskaya's medium. Petri plate containing only Pikovskaya's medium served as control. Three replications were kept for each treatment. The plates were incubated at 28^{o} ± 1^{o}C for five days. Formation of a clear inhibition zone around the colony was considered as positive reaction for phosphate solubilization.

RESULTS AND DISCUSSION

Isolation of Fluorescent Pseudomonads Isolates from Rhizosphere and Rhizoplane

Fifteen fluorescent bacterial isolates were isolated on selective medium viz., King's B medium from the rhizosphere and rhizoplane of castor by dilution plating method (106 cfu ml–1) after incubation period of 24-48 hours at $30^{o} \pm 1^{o}C$ and examined the fluorescence under ultraviolet light (200-340 nm). These isolates were designated as FP-I, FP-II, FP-III, FP-IV, FP-V, FP-VI, FP-VII, FP-VIII, FP-IX, FP-X, FP-XI, FP-XII, FP-XIII, FP-XIV and FP-XV. Out of 20 samples collected from ten villages of Patan district, nine fluorescent pseudomonads isolates (FP-I to FP-IX) were obtained, whereas six isolates (FP-X to FP-XV) were gained from 30 samples from seven villages of Banaskantha district. These results are in accordance with the methodology adopted by Vidhyasekaran and Muthamilan (1995), Gupta *et al.* (2000), Yeole and Dube (2001), Gholve and Kurundkar (2004), Samanta and Dutta (2004) and Sen *et al.* (2006).

Evaluation of Fluorescent Pseudomonads isolates for phosphate solubilizing activity

The phosphate solubilizing activity of the fluorescent pseudomonads isolates were tested by spot inoculation of isolate on Pikovskaya's medium. After incubation period of five days at room temperature ($28^{o} \pm 1^{o}C$), all the isolates displayed positive reaction by formation of clear inhibition zone around the colony (Table 1; Plate 1). Similar results were found by Kundu *et al.* (2002), Samanta and Dutta (2004), and Bhatia *et al.* (2005). Pseudomonads bacteria secrete an organic acid and lower down the pH in vicinity and bring about solubilization of insoluble phosphate in soil was described by Gaur (1990).

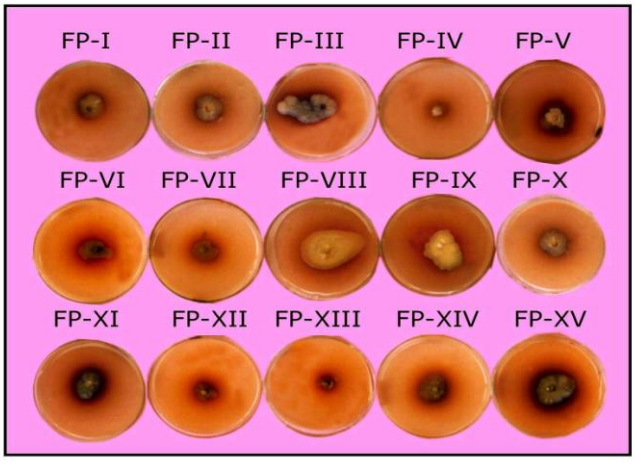

Plate 1 : Phosphate solubilizing activity of Fluorescent *Pseudomonads*

CONCLUSION

The phosphate solubilizing activity of the fluorescent pseudomonads isolates were tested by spot inoculation of isolate on Pikovskaya's medium. After incubation period of five days at room temperature (28^o + 1^oC), all the isolates displayed positive reaction by formation of clear inhibition zone around the colony.

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Table 1: Phosphate solubilizing activity of fluorescent pseudomonads isolates

Sr. No.	Isolate	Reaction*
1.	FP-I	+
2.	FP-II	+
3.	FP-III	+
4.	FP-IV	+
5.	FP-V	+
6.	FP-VI	+
7.	FP-VII	+
8.	FP-VIII	+
9.	FP-IX	+
10.	FP-X	+
11.	FP-XI	+
12.	FP-XII	+
13.	FP-XIII	+
14.	FP-XIV	+
15.	FP-XV	+
***** + = *Growth*		

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