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CHARACTERIZATION STUDY OF FLUORESCENT PSEUDOMONADS ISOLATES

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ABSTRACT

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 and rhizoplane of castor plant grown under different types of soils of Patan and Banashkantha districts. Out of twenty 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 thirty samples from seven villages of Banashkantha district. All the bacterial isolates characterized on the basis of morphological, physiological and biochemical tests and placed under the group of "Fluorescent Pseudomonads" because all the isolates produced yellow green pigments on King's B medium which fluoresced under ultraviolet light and found to be gram negative, rod shaped, urease positive and phosphate solubilization positive. To know the relation of concentration of hydrogen ion, electrical conductivity and organic carbon content with population of fluorescent pseudomonads each soil sample was analyzed. There was no difference in pH values, but the EC value's range and average was on higher side in isolates recovered soil samples than not recovered soil samples. Organic carbon content was comparatively found more in isolates recovered soil samples than not recovered soil samples of both the districts.

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KEY WORDS: Isolation, Fluorescent Pseudomonads isolates

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.

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 Banashkantha 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.

Identification

All the rhizobacterial isolates were identified with the help of morphological, cultural and biochemical characteristics as per the "Bergey's Manual of Determinative Bacteriology." The cultures were tested for characters viz., colony and cell morphology, gram reaction and urease activity.

Soil analysis

Soil samples collected from different locations of Patan and Banaskantha districts were analyzed for physico-chemical properties of soil. Concentration of hydrogen ion (pH) was determined by Buckman's electrode pH meter, Electrical Conductivity (EC) by EC meter and organic carbon per cent by Walklly and Black's rapid titration methods (Jackson, 1973).

RESULTS AND DISCUSSION

Isolation of fluorescent pseudomonads ISOLATES from rhizosphere and rhizoplane

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Fifteen fluorescent bacterial isolates were obtained on selective medium viz., King's B medium from the rhizosphere and rhizoplane of castor by dilution plating method (10^6 cfu ml–1) after incubation period of 24-48 hours at $30^o \pm 1^oC$ 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 Banashkantha district (Table 1 and 2; Plate 1). 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).

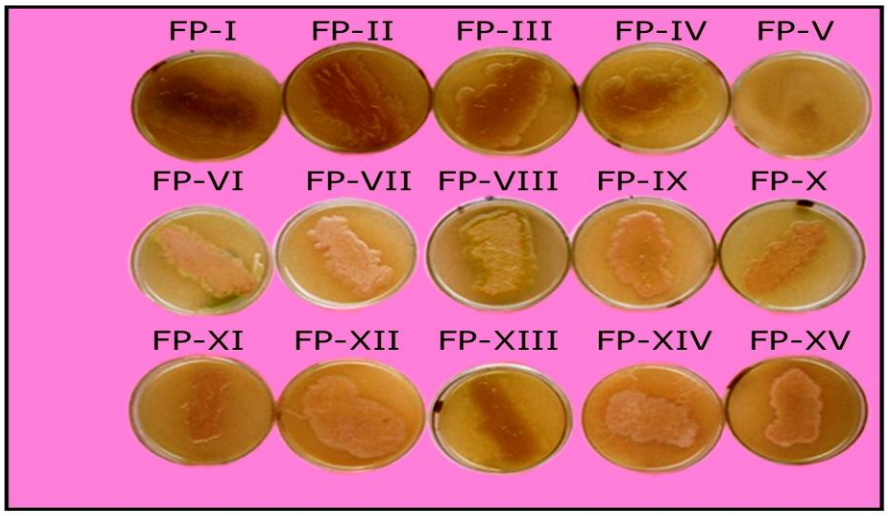

Plate 1 : Different isolates of Fluorescent *Pseudomonads*

Characterization of fluorescent pseudomonads isolates

Fluorescent pseudomonads are characterized by their production of yellow green pigments, termed pyoverdines, which fluoresce under ultraviolet irradiation and function as siderophores (Paulitz and Loper, 1991). Hence, characterization of all the isolates was done on the basis of

morphological, physiological and biochemical tests (Table 3) and grouped as "Fluorescent Pseudomonads" because all the isolates produced yellow green pigments on King's B medium, which fluoresced under ultraviolet light and were found to be gram negative, rod shaped, urease positive and phosphate solubilization positive. Pseudomonads are known to be largest group of rhizosphere bacteria and are not only specific for the inner most rhizosphere but are also present at some distance from the root (Kloepper *et al.,* 1980).

Relationship of fluorescent pseudomonads isolates with basic soil chemical properties

Each soil sample was analyzed for basic soil chemical properties *viz.,* pH, EC and organic carbon content, to know the relation of such characters on the population of fluorescent pseudomonads. The data presented in Table: 4 revealed that soil samples both from isolates recovered and not recovered showed neutral or slightly alkaline condition. There was no difference in pH values. Whereas, EC value's range and average was on higher side in isolates recovered soil samples than not recovered soil samples. Population of certain fluorescent pseudomonads increasing particularly in alkaline soils where available iron is very limited (Mishaghi *et al.,* 1982). Organic carbon content in the soil enhancing the population of microorganisms in the plant rhizosphere and rhizoplane. This is reflected from the data that per cent organic carbon content was found comparatively more in isolates recovered soil samples than not recovered soil samples of both the districts. According to Rovira and Sands (1971), fluorescent pseudomonads are poor competitors in soil and thus a reduction in the competition by other microorganisms is expected to favour these pseudomonads. So this may be the reason that higher number of isolates recovered from the samples of Patan district than Banashkantha district. Pseudomonads typically grow well at neutral or slightly alkaline condition pH (7.0 – 8.5) (Stolp and Gadkari, 1981) with activity being sharply curtailed at pH less than 5.5 (Brady, 1984). Production of siderophores might see unexpected in a soil at pH 5.3 and with 59.8 ppm DTPA extractable iron.

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CONCLUSION

Fifteen fluorescent pseudomonads isolates were obtained on King's B medium from the rhizosphere and rhizoplane of 60 to 75 days old castor plant grown under different types of soils of Patan and Banashkantha districts. Out of twenty 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 thirty samples from seven villages of Banashkantha district. All the bacterial isolates characterized on the basis of morphological, physiological and biochemical tests and placed under the group of "Fluorescent Pseudomonads" because all the isolates produced yellow green pigments on King's B medium which fluoresced under ultraviolet light and found to be gram negative, rod shaped, urease positive and phosphate solubilization positive.

To know the relation of concentration of hydrogen ion, electrical conductivity and organic carbon content with population of fluorescent pseudomonads, each soil sample was analyzed. There was no difference in pH values, but the EC value's range and average was on higher side in isolates recovered soil samples than not recovered soil samples. Organic carbon content was comparatively found more in isolates recovered soil samples than not recovered soil samples of both the districts.

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Table 1 : Details of villagewise isolates obtained from the Patan District

Sr. No.	Village	No. of samples collected	No. of isolates obtained	Isolates designated as
1.	Patan	02	02	FP-I, FP-II
2.	Anavada	03	01	FP-III
3.	Khado	01	01	FP-IV
4.	Rajpur	02	01	FP-V
5.	Matarvadi	02	00	-
6.	Charup	02	01	FP-VI
7.	Paldi	02	00	-
8.	Vagdod	02	01	FP-VII
9.	Brahmanvada	02	01	FP-VIII
10.	Vadu	02	01	FP-IX
	Total	20	09	

Table 2 : Details of villagewise isolates obtained from the Banaskantha district

Sr. No.	Village	No. of samples collected	No. of isolates obtained	Isolates designated as
1.	Sardarkrushinagar	19	04	FP-X, FP-XI, FP-XII, FP-XIII
2.	Vaghrol	02	00	-
3.	Ganeshpura	01	01	FP-XIV
4.	Dantiwada Colony	03	00	-
5.	Chandisar	02	00	-
6.	Deesa	02	01	FP-XV
7.	Rasana	01	00	-
	Total	30	06	

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Table 3 : Characterization of fluorescent pseudomonads isolates

Sr. No.	Character	ISOLATES														
		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	FP-XV
[A] MORPHOLOGICAL																
1.	Colony colour	Yellow Brown	Light Brown	Light Brown	Yellow Brown	Cream Brown	Cream Yellow	Cream Yellow	Light Yellow	Yellow Brown	Light Brown	Cream Brown	Yellow Brown	Cream Brown	Yellow Brown	Light Brown
2.	Cell shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
3.	Pigment colour	Light Brown	Brown	Brown	Light Brown	Brown	Light Brown	Light Yellow	Brown	Brown	Light Yellow	Light Brown	Light Brown	Light Brown	Light Brown	Brown
4.	Media colour	Brown	Brown	Brown	Brown	No change	No change	No change	No change	Brown	Brown	Brown	Light Brown	Light Brown	Brown	Brown
5.	On UV light pigment colour	Yellow Green	Yellow Green	Yellow Green	Yellow Green	Yellow Green	Yellow Green	Yellow Green	Yellow Green	Yellow Green	Yellow Green	Yellow Green	Yellow Green	Yellow Green	Yellow Green	Yellow Green
[B] PHYSIOLOGICAL																
6.	Growth measurement	Fast	Slow	Slow	Fast	Fast	Slow	Slow	Fast	Fast	Slow	Slow	Slow	Slow	Slow	Fast
[C] BIOCHEMICAL																
7.	Gram reaction	–ve	–ve	–ve	–ve	–ve	–ve	–ve	–ve	–ve	–ve	–ve	–ve	–ve	–ve	–ve
8.	Phosphate solubilization	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9.	Urease	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+ = Growth ; – = No Grow																

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Table 4 : Basic chemical properties of soil*

Sr. No.	Particulars		pH	EC (dSm^{-1})	Organic carbon (%)
[A]	**PATAN DISTRICT :**				
	(1)	Isolates recovered samples	7.87** (7.81-7.92)***	0.17 (0.15-0.19)	0.30 (0.28-0.32)
	(2)	Not recovered samples	7.89 (7.80-7.92)	0.15 (0.10-0.19)	0.20 (0.13-0.27)
[B]	**BANASKANTHA DISTRICT :**				
	(1)	Isolates recovered samples	7.76 (7.70-7.82)	0.17 (0.16-0.19)	0.28 (0.26-0.30)
	(2)	Not recovered samples	7.30 (7.72-7.82)	0.15 (0.11-0.18)	0.23 (0.20-0.26)

* Soil from which the samples were taken
** Expressing average values
*** Expressing range values