

**ISOLATION OF NATIVE ISOLATES OF FLUORESCENT
PSEUDOMONADS FROM CASTOR WILT FIELD IN NORTH GUJARAT
AREA**

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ABSTRACT

Fusarium wilt is a very serious disease in castor (*Ricinus communis* L.) growing areas of the state but particularly in north Gujarat. The pathogen is soil-borne and application of fungicides is very expensive and also polluting the ecosystem. Fifteen fluorescent pseudomonads isolates were obtained on King's B medium from the rhizosphere and rhizoplane of castor plant. 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 Banaskantha 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 Banaskantha 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

KEY WORDS: Isolation, King's B medium, Isolates of Fluorescent Pseudomonads

INTRODUCTION

The present days need is to develop and utilize the effective low cost eco-friendly technologies in the crop production programs. The increasing use of potentially hazardous fungicides for the management of

soil borne plant diseases have created the problem of resistance in plant pathogens, besides posed a serious problem of pollution in the ecosystem. Castor wilt is serious in Gujarat state and caused heavy losses in past and also causing in present. Up to 80 per cent wilt incidence was recorded in north Gujarat. (Patel *et al.*, 2003). Castor wilt is difficult to manage due to soil borne nature. The indigenous potential isolates of fluorescent pseudomonads from the rhizosphere and roots of field grown castor plants and their biocontrol capabilities against *Fusarium oxysporum* f. sp. *ricini* is effective and eco-friendly biological control as an alternative strategy for the castor wilt. Several strains of fluorescent pseudomonads isolates have been reported to suppress soil-borne diseases caused by fungal pathogens.

MATERIALS AND METHODS

1. 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 of 60-75 days growth were carefully uprooted alongwith 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.

2. 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 10^6 . An aliquot of 0.1 ml from 10^6 dilution of each sample was spread plated over solidified King's medium B (Protease peptone No. 3 20.00 g, Dipotassium hydrogen phosphate 1.50 g, Magnesium sulphate $7H_2O$ 1.50 g, Agar 20.00 g, Glycerol 15.00 ml and Distilled water 1 lit.), selective medium on which preferentially fluorescent pseudomonads

recovered under aseptic conditions. The plates were incubated at $30^{\circ} \pm 1^{\circ}\text{C}$ for 24-48 hrs. Colonies of different morphology were examined for their fluorescence under ultraviolet light (240-340 nm). The colonies showing fluorescence were 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°C) in refrigerator.

3. 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.

RESULTS AND DISCUSSION

Isolation of Fluorescent Pseudomonads Isolates from Rhizosphere and Rhizoplane

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 $30^{\circ} \pm 1^{\circ}\text{C}$ 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.



Plate 1a : Different isolates of Fluorescent *Pseudomonads*

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