EFFECT OF TEMPERATURE ON FLUORESCENT PSEUDOMONADS ISOLATES

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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. The optimum growth temperature is between 25-30 degrees Celsius. Fifteen fluorescent pseudomonads isolates were obtained on King's Medium B from the rhizosphere of plant roots of castor. The effect of different temperature levels revealed that there was no suppressing or retardant effect on the growth of fluorescent pseudomonads isolates at 10°, 20°, 30° and 40°C temperature levels. Growth of isolates FP-I, FP-IV, FP-V, FP-VIII, FP-IX and FP-XV was faster in comparison to other isolates at all the four temperature levels.

KEY WORDS: Fluorescent Pseudomonads isolates, isolation**,** temperature, castor

INTRODUCTION

Fluorescent pseudomonads are 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 better bio-control agents because they are already closely associated with and/or adapted to the plant or plant parts as well as the particular environmental conditions in which they function. The screening of such locally adapted strains has yielded improved bio-control agents (Cook, 1993). Temperature is a key factor influencing colonization by *rhizobacteria* and expression of bio-control mechanisms. Soil temperature influences biological control by affecting the natural disease suppressiveness of soils.

Murakami *et al.* (1997) reported that when soil temperature was lower than 35°C the bacteria survived for at least 20 days. However, if soil temperature mounted higher than 43°C for 2 hours per day, the bacteria died completely within 76 days.

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 of Gujarat, where the castor is commonly grown. Healthy plants (60-75 days) of castor (*Ricinus communis* L.) uprooted carefully along with adhering soil and were 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 then 10 g of root bits were transferred to 90 ml sterilized distilled water. The flasks were placed on a rotary shaker for 1 hr to allow roots 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 $30^{o} \pm 1^{o}C$ 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 purified further 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.

Effect of Different Temperatures on Fluorescent Pseudomonads Isolates

Effect of different temperature on growth of different fluorescent pseudomonads isolates under 10^{o}, 20^{o}, 30^{o} and $40^{o}C$ incubation temperatures was studied. A loopful of 24 hours old culture of bacterial isolates was streaked centrally on King's Medium B plate and incubated for respective temperatures. Three replications were kept for each treatment. The growth of bacterial isolates was recorded at 24, 48 and 72 hours after incubation period.

RESULTS AND DISCUSSION

Isolation of Fluorescent Pseudomonads Isolates from Rhizosphere and Rhizoplane

Fifteen fluorescent bacterial isolates were isolated on selective medium *viz.,* King's Medium B from the rhizosphere and rhizoplane of castor by dilution plating method (106 cfu ml^{-}

[1]) after incubation period of 24-48 hours at 30° ± 1°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 fluorescent pseudomonads isolates (FP-X to FP-XV) were isolated 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).

Effect of Different Temperatures on Fluorescent Pseudomonads Isolates

All the fluorescent pseudomonads isolates were studied for their sensitivity against 10°, 20°, 30° and 40°C temperature levels. Centrally inoculated Kings Medium B plates incubated for 72 hours at respective temperature levels. The growth reaction was presented in Table 1 and Plate 1 divulged that there was no suppressing or retardant effect on the growth of fluorescent pseudomonads isolates. Growth of isolates FP-I, FP-IV, FP-V, FP-VIII, FP-IX and FP-XV was obtained after incubation period of 24 hours at all the four temperature levels, whereas growth of remaining isolates was noticed after 48 hours of incubation period. The results reflected that all the isolates can be sustaining their growth from 10° to 40°C.

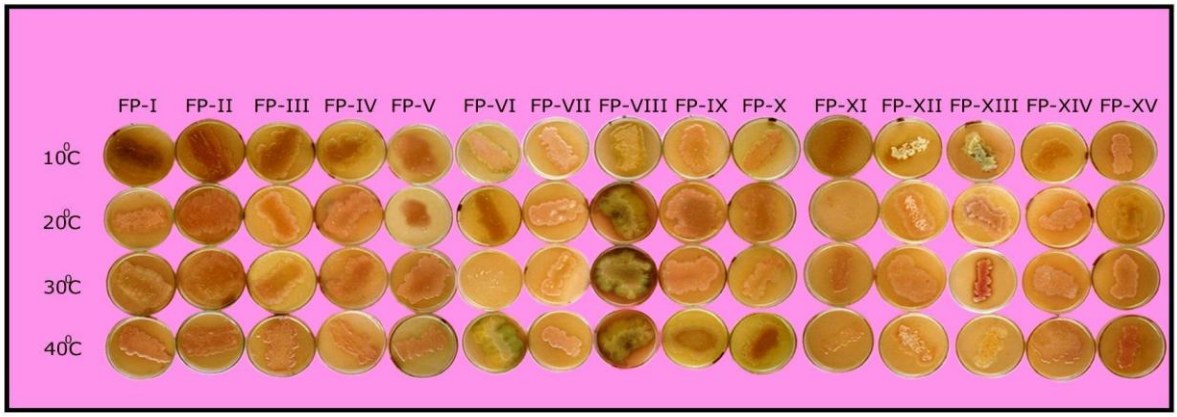

Plate 1 : Growth of Fluorescent *Pseudomonads* isolates at different temperatures

CONCLUSION

The present study reflected that there was no suppressing or retardant effect on the growth of fluorescent pseudomonads isolates. Growth of isolates FP-I, FP-IV, FP-V, FP-VIII,

FP-IX and FP-XV was obtained after incubation period of 24 hours at all the four temperature levels, whereas growth of remaining isolates was noticed after 48 hours of incubation period.

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Table 1: Growth of fluorescent pseudomonads isolates at different temperatures

Sr. No.	Isolates	Temperature (^{o}C)											
		10^{o}C			20^{o}C			30^{o}C			40^{o}C		
		Hours			Hours			Hours			Hours		
		24	48	72	24	48	72	24	48	72	24	48	72
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	+	+	+	+	+	+	+	+	+	+	+	+

Where, + = Growth, – = No Growth

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