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Characterization of cotton phylloplane bacteria antagonistic to bacterial blight disease of cotton

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A resident phylloplane bacterium, Plb-29 was characterized upto the molecular level and was found to be a potent bio-control agent against *Xanthomonas axonopodis* pv. *malvacearam (Xam),* the causal organism of bacterial blight of cotton. The bacterium exhibited a pseudomonad morphology with a GC centent of 64.9%.

Key Words : *Xanthomonas,* phylloplane ; GC content

INTRODUCTION

Cotton (*Gossypium* sp.) one of the important non-food agricultural commodities covers on acreage of 9.1 million hectares in India, with an annual production of about 14.5 million bales. The cotton leaves serve as a residence of many bacteria and they are termed as phylloplane bacteria (Plb). Out of the 46 isolates of Plbs obtained, the one which exhibited maximum antagonistic activity against *Xanthomonas axonopodis* pv *malvacearam (Xam)*, was characterized at a molecular level, to identify its genus, so that it can be subsequently used as a potential bio-control tool.

MATERIALS AND METHODS

Isolation of Plbs

The Plbs were isolated by using wash method (Voznyakoyskaya and Khydyakov, 1960) and christened as Plb-1, Plb-2 and so on.

Antagonism studies

In vitro studies were made by the dual culture method using each of the Plbs and *Xanthomonas axonopodis* pv. *malvacearam (Xam).* The diameter of inhibition zone gave the degree on antagonism.

Morphological characters, Gram reaction and flagellation

Morphological characters were studied following the standard procedures and staining reagents as described in Manual of Microbiological Methods (S.A.B., 1957)

Colony characters and fluorescence

Colony characters were studied on nutrient-sucrose-agar [Sucrose-5 g ; Yeast extract-4 g ; Peptone-4 g ; Beef extract-2 g ; Agar-20 g ; Distilled Water-1000 ml] plates. For fluorescent studies King's A and King's B medium were prepared according to King *et al.* (1954)

Biochemical characters

Gelatin liquefaction : Stab inoculations were made into nutrient medium containing gelatin and incubated at 20^{0}C. The liquefaction of gelatin column was observed at intervals upto 1 month.

Catalase production : A loopful of bacterial growth from a 24 h slant was taken and placed into a drop of 20 volume hydrogen peroxide on a clean glass plate. Production of bubbles indicate a positive result.

Tyrosinase activity: Dye's (1962) method was used. 24 h old culture was stab inoculated and observations were recorded for the browing of medium after 7 days of incubation.

Lipolytic activity : Standard method as described by Sierra (1957) was followed.

Oxidase test : A filter paper saturated with 1% freshly prepared tetramethyl-para-phenylene-diamino-dihydrochloride was streaked with 24 h old culture. For positive reaction a red or purple colouration is expected within 10 seconds.

Arginine dihydrolase test : 5 ml of Thornley's 2-A medium (Thornley, 1960) was dispensed in test tubes and 24 h old culture was stab inoculated. The medium was then covered with sterile liquid paraffin. A change of the medium colour from pale pink to red indicates positive activity.

For all biochemical tests, uninoculated controls were kept.

Isolation of genomic Deoxyribonucleic Acid (DNA) to estimate the GC content

Genomic DNA was isolated by using the protocol of Ausubel *et al.* (1995). DNA of *Escherichia coli* was taken as a standard. GC is calculated by using the formula % GC = 2.44 (T_m-69.3)

RESULTS AND DISCUSSION

A total number of 46 Plbs were isolated and out of these 3 Plbs, namely Plb-7, Plb-29 and Plb-33 were found to be antagonistic towards *Xam* when tested in dual culture method. The inhibition zone exhibited by Plb-29 (i.e. 12 mm in diameter) was more than both Plb-7 (3 mm) and Plb-33 (4 mm) (Table 1)

Table 1 : Selection of Plbs based on antagonistic properties

Isolate No.	Zone of Inhibition (mm)			
	R_1	R_2	R_3	Mean
Plb-7	2.8	3.2	3	3
Plb-29	12.5	14	9.5	12
Plb-33	3.5	3.5	5	4

* 3 replication of each Plb is taken.

The bacterium exhibited typical pseudomonad morphology of being rod-shaped and measuring about 0.6 x1-1.2 mm. It has a polar flagellum. The colonies were yellowish-white in colour, smooth, circular and raised, measuring about 2-3 mm in diameter. It produced neither yellowish-green fluores-

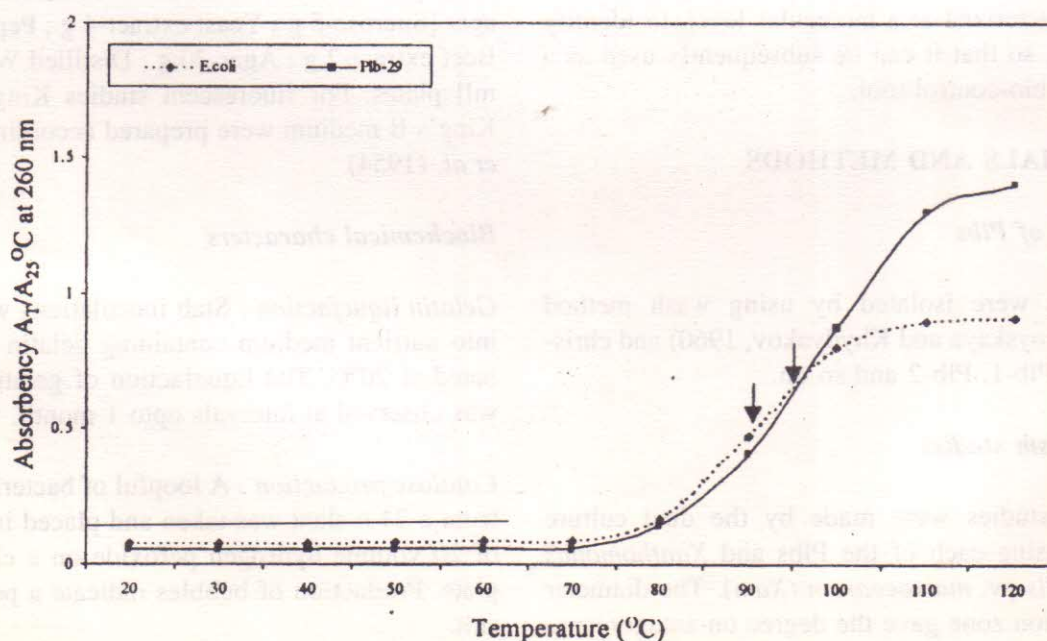

Fig. 1 : Determination of T_m value of Plb-29 against standard *E. coli.*

cent pigment of King's B or blue-green pigment on King's A medium. So, it does not belong to either *Pseudomonas aeruginosa* or *P. fluorescens* group. As Plb-29 was capable of liquefying gelatin, it is not a member of *P. putida* as the latter not only is unable to liquefy gelatin, but also produced yellowish-green fluorescent pigment on King's B medium (Stolp and Gadkari, 1981). Majority of the members of genus *Pseudomonas* show positive reaction for catalase and oxidase and negative response for tyrosinase and lipolytic activity. Plb-29 conformed to the above properties as mentioned by Holt *et al.* (1994) and manifested a positive arginine dihydrolase test. Thus, based on the morphological and biochemical properties, it can be tentatively placed in the *Pseudomonas* group.

The Guanine + Cytosine (GC) content of the bacteria's DNA was found to be 64.9% against the standard *Escherichia coli* (51.3%) (Fig. 1) Mandel (1966) analysed the GC content of 125 strains of *Pseudomonas* and stated that it ranges from 57-70%. As the GC content of Plb-29 is in accordance with the previous reports, it can unequivocally be placed under the genus *Pseudomonas*.

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