PRODUCTION OF PROTEASE ENZYME FROM RHIZOSPHERIC FUNGI OF BT AND NON BT COTTON VARIETIES

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ABSTRACT

The proteolytic enzyme producing capability of fungi isolated from Bt and non-Bt cotton rhizosphere were screened and results were observed. Fungi isolated from the rhizosphere of Bt cotton varieties viz. *Trichoderma viride, Alternaria alternata* and *Fusarium oxysporum* shown maximum production of protease. *Alternaria alternata* isolated from rhizosphere of Bt cotton varieties shown maximum production of protease enzyme. It seems that the fungi inhabiting rhizosphere of Bt cotton plants are well makeup with the gene capable of secreting protease enzymes and permitting the presence of fungi in rhizosphere of Bt cotton plants.

Key words: Protease, Rhizosphere of Bt and non- Bt cotton.

INTRODUCTION

It is established that most of the fungi present in the soil relay on the exudates of the plants. The exudates of plants are chemically complex in nature containing carbohydrates, lipids, proteins and some other secondary metabolites. The exudates of Bt plants are found to be rich in endotoxin i.e. protein Shen *et al.* (2006) and fungi residing in the vicinity of rhizosphere of Bt plants should be well equipped with proteolytic enzymatic makeup in order to relay on the exudates of Bt plants. By keeping this object the experiment was conducted in which the proteolytic enzyme producing capability of fungi isolated from Bt and non-Bt cotton rhizosphere were screened and results were observed.

MATERIALS AND METHODS

Production of protease

Production of protease (s) was made by growing the fungi on liquid medium containing glucose 10 g, gelatin 10 g, dipotassium hydrogen phosphate 1.0 g, $MgSO_4 7H_2O$, 500 mg and distilled water 100 ml pH of medium was adjusted at 5.5 Twenty five ml of medium was poured in 100 ml Erlenmeyer conical flask and autoclaved at 15 lbs pressure for 20 minutes. The flasks on cooling were inoculated separately with 01 ml standard spore / mycelial suspension of test fungi prepared from 7 days old cultures grown on PDA slants. The flasks were incubated for 6 day at 25 + 1°C with diurnal periodicity of light. On 7th day, the flasks were harvested by filtering the contents through Whatman's filter No.1. The filterate were collected in the pre sterilized bottles and termed as crude enzyme preparation.

Assay method (cup-plate method).

Determination of protease (s) activity was done with the help of cup plate- method, adopted by. A basal medium was prepared by adding 2% (w/v) agar and one percent (w/v) gelatin. pH of the medium was adjusted at 5.6 with Mellavainels buffer. Then it was sterilized at 15 lbs pressure for 15 minutes. About 15 ml of the medium was poured in pre sterilized petriplates under aseptic condition. On solidification 6 mm diameter cups / cavities were made in the centre of each of the agar plate with a sterilized cork borer (9mm). The cups/ cavities were filled carefully with about 0.5 ml of culture filtrate (crude enzyme preparation). The plates were incubated at 25°C for 24 hours. Then the plates were flooded with 15 percent mercuric chloride in 7 NH_4cl. After 10 minutes of standing, a clear transparent zone indicated the hydrolysis of gelatin by extra cellular proteolytic enzymes, where as the rest of the region of the petriplates became opaque due to the coagulation of gelatin (protein) by mercuric chloride Diameter of the clear zone was used as measure (mm) of protease activity, while non appearance of clear zone considered absence of protease (s) in the culture filtrates.

RESULTS AND DISCUSSION

It is clear from table that all three rhizospheric fungi viz. *Alternaria alternata, Fusarium oxysporum and Trichoderma viride* of Bt and non Bt cotton varieties showed the maximum production of protease on gelatin containing medium. Among the isolates of Bt and non Bt varieties, the isolates of Bt varieties shown maximum production of protease enzyme. Among three isolated

fungi *Alternaria alternata* showed maximum production of protease enzyme.

The fungi inhabiting the rhizosphere of plants, these fungi relay on the exudates released by the plants (Bhauvaneshwari and Subbarao 1957). Generally exudates are rich in protein, carbohydrate and some stimulators and act as a food for the relaying organism (Parkinson 1955). The organisms secrete enzymes in order to digest the chemicals present in exudates. It is well established that the exudates of Bt cotton plants are rich in endotoxin as studied by Rui *et al* (2005) which is a protein. The fungi inhabiting their rhizosphere of Bt cotton plants should have the genetic makeup capable of digesting such protein rich exudates. The enzyme protease which is responsible for digesting protein containing material was studied in the fungi inhabiting rhizosphere of Bt cotton plant and protease production capacity. It was compared with fungi inhabiting rhizosphere of non-Bt cotton plants.

The experiment clearly revealed that the fungi present in rhizosphere of Bt cotton plants viz. *Trichoderma viride*, *Fusarium oxysporum* and *Alternaria alternata* shown maximum production of protease enzyme as compared to that of fungi isolated from the rhizosphere of non-Bt cotton plants. It seems that the fungi inhabiting rhizosphere of Bt cotton plants are well makeup with the gene capable of secreting protease enzymes and permitting the presence of fungi in rhizosphere of Bt cotton plants.

These results inclined to conclude that the exudates of Bt cotton might be rich in other substance capable of modifying genetic makeup of organisms living in the vicinity of such exudates.

Table 1: Production of protease enzyme in different rhizospheric fungi of Bt and non Bt cotton varieties

Rhizospheric fungi	Zone in mm	
	Gelatin	Glucose nitrate medium
Isolated from Bt varieties		
Alternaria alternata	20	12
Fusarium oxysporum	17	10
Trichoderma viride	12	08
Isolated from non-Bt varieties		
Alternaria alternata	17	14
Fusarium oxysporum	15	13
Trichoderma viride	10	10

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