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Periodic Research

Estimation of poly-methyl-galacturonase enzyme activity of Fusarium species in North Bihar

Abstract

Fusarium is very important phytopathogenic fungi. It causes various diseases in plants and result in heavy yield losses of crop. Pectinase are a big group of enzymes that breakdown pectic polysaccharides of plant tissue into simpler molecules like galacturonic acids. The enzyme makes plant cell wall more susceptible to infectious by various fungi and bacteria. To identify the role of pectolytic enzyme in pathogenesis, polymethylgalacturonase (PMG) activity of 6 (six) *Fusarium* species were evaluated using Viscometric assay. In the culture filterate of 14 days old culture enzyme activity was found to be high. Enzyme production was correlated with the growth of mycelium. Maximum enzyme activity was detected in *Fusarium oxysporum f.sp. lentis, followed by Fusarium udum, Fusarium solani, and Fusarium oxysporum f.sp. lini.*

Keyword: Fusarium, Pectinase, Polymethylgalacturonase, polygalacturonase, pectolytic enzyme

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Introduction

The genus *Fusarium* was first introduced by link (1809) as Fusisporium. *Fusarium* is a large genus of group ascomycetes fungi. It can be recovered from plants and soil worldwide as pathogens, endophytes and saprophytes (Booth, 1971; Burgess et al., 1994; Nelson et al., 1994; Summerell et al., 2003; Salleh, 2007). Phytopathogenic fungi can produce highly specific enzymes utilized for degrading the cell walls either in the plant infected tissue (in-vivo) or under culture conditions (in-vitro).

Pectinase produced by *Fusarium* render plant cell walls more susceptible to attack by other cell wall degrading enzyme (Bateman and Basham,(1976), Collmer and Keen (1986), Garibaldi and Bateman (1971), Liao and Chaterjee (1988)). Pectic enzymes have been grouped according to the following criteria: (i) The mechanism by which α-1,4 glycosidic bond is split (i.e. hydrolytic or lyitic) enzyme specific for a substrat (pectin or pectic acid) and (3) position at the pectic chains at which cleavage occurs (Bateman and Miller (1966), Rombouts and Pilink (1972). Additional specificity of pectin-degrading enzyme is determined by the degree of methylation of the chains, hence polymethylgalacturonase (PMG) for Pectins and less methylated pectinic acid and polygalacturonase for pectic acid.

The major components of the cell wall are Cellulose, Hemicelluloses, Pectic substances and Lignin. Whereas Pectic compounds form the middle lamella of plants cell wall. They have gelatinous consistency and act as cement like substances to bind the cells together. Polygalacturonic acid (homopolymer of 1,4-α-D-galactosyluronic acid) and rhamnogalacturonan (heteropolymer of repeating 1,2-α-L-rhamnosyl-1,4-α-D-galactosyluronic acid disaccharide units) are the two fundamental constituents of pectin. The Pectic substances are also characterized by the degree to which their free carboxyls are esterified with methyl group. The esterification varies with the age of the species of the plants concerned. The polymers of galacturonic acid residues are linked in 1-4 fashion. The galacturonic acid polymers have been subdivided into three general groups according to their chain length and degree of methyl esterification as suggested by Kertesz (1951). Pectins are complex polysaccharides consisting of partially methylesterified α - (1,4) linked homogalacturonic acid backbone and branched neutral sugar side chains. They are important components of cellwall and middle lamella, and can be found in fruits and vegetables. Enzymes cleaving pectic substances are called as pectinolytic enzymes or pectinases Such as Polymethygalacturonase (PMG) and Polygalacturonase (PG) (Saad et al 2007).

The role of these enzymes in pathogenesis is uncertain and still a matter of debate. However from the works of pathologists and biochemists,

the exact role of this enzyme in pathogenesis have become clear to some extent {(Bosham and Batman, 1975), Diamond (1967), Hall and wood (1973), Wood (1960) and Waggnor and Diamond (1955)}. In the present investigation it was decided to study the role of pectolytic enzymes in the wilt pathogen of *Fusarium* species.

Materials and Methods

Isolation of Pathogens

Diseased samples of roots, stems, leaves, cobs and seeds were used for isolation of pathogens. For the study of mycoflora associated with the different disease samples, agar plate method and standard blotter test method were followed (Musket 1948; de Tampe 1963). In agar plate method 100 samples were placed at equal distance in petriplates of 6 inches diameter containing Potato Dextrose Agar (PDA) medium. For blotter method, 25 samples were placed at equal distance in the same size petriplates on moistened blotting paper. The petriplates were incubated at room temperature (28±1 ºC) under the 12 hour light and dark cycle which has been recommended as general procedures for testing samples for fungal pathogens (Anonymous 1976). The mycoflora that appeared were isolated separately by single hyphal techniques and identified.

Preparation of Extracellular Pectic Enzyme

The Richard's medium was selected as the best medium for growth and sporulation of the *Fusarium*. It was used in exocellular pectolytic enzymatic studies. Sucrose was the carbon source in the medium was established by apple pectin. 100 ml of medium was placed in 250 ml Erlenmeyer flask. The pH of medium was adjusted to 6.0 before autoclaving at 15 psi (pound per square inch) for 15 to 20 minutes. The flasks were incubated with 2 ml spore mycelial disc of 7 days old culture and incubated at room temperature (27±1 ºC). The fungal mats were removed by filtration. The remaining culture filtrate was made by cell free centrifuging at 600 rpm for 30 minutes. The supernatant fraction was used immediately for estimation of pectinase enzyme. In all the experiments following series were maintained.

Series I : Reaction mixture.
Series II : Inactive enzyme.
Series III : Enzyme blank.
Series IV: Substrate blank.

Triplicates were maintained for each of the series. Estimation was also carried out in the triplicate series and the mean values were presented in the table. Inactivation of enzyme was carried out by keeping it in boiling water (Water Bath) for half an hour and cooling it rapidly in running tap water.

Estimation of polymethylgalacturonase (PMG) activity

Polymethylgalacturonase (PMG) hydrolyses esterified pectic substances more rapidly than pectic acid. This results in loss of viscosity of the pectic substances. The enzyme activity was assayed viscometrically of the apple pectin (Fluka, A.G.) at 1 percent level Hancock (1964). Viscometer (Corning India Limited) was used throughout the experiment. The reaction mixture was composed of 8 ml of 1 percent Pectin solution substrate + 2ml of Acetate buffer at pH 6.0 and 4 ml of culture filtrate (enzyme source). It was thoroughly mixed and placed in a water bath maintained at 27±1ºC. Reaction mixture with heat inactivated culture filtrate and active culture filtrate was served as control. The percentage loss in viscosity was calculated from the following formula:

$$V = \frac{T0-T}{T0-TH2O} \times 100$$

Where V = Percent loss in viscosity.
T0 = Flow time in seconds at zero
TH2O = Flow time of Distilled water.

The enzymatic activity is expressed as 100/time taken for the enzyme to produce 25 percent of total possible loss of viscosity.

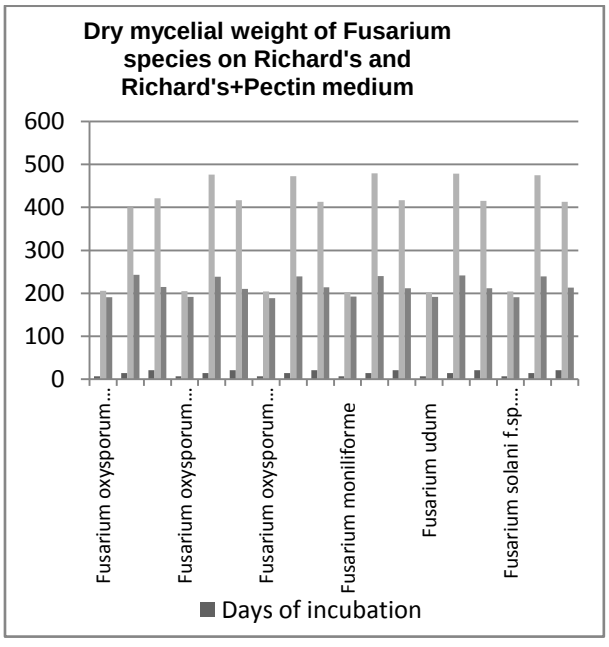
Enzyme activity= 100/t (time for 25% loss of viscosity).

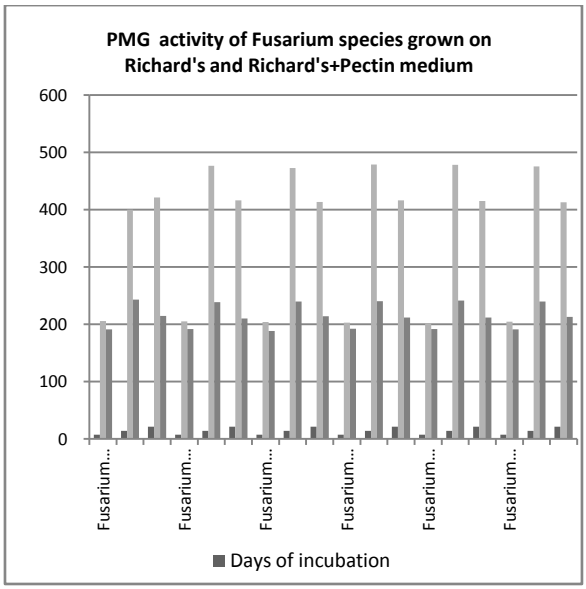
Results were obtained and presented in the table.

Table (7):- Polymethylgalacturonase (PMG) activity of Fusarium species.

- Average of three replicates

SNo	Fusarium spp.	Days of incubation	Mycelial dry weight in mg		*Enzyme activity in µg	
			Richard's Medium	Richard's + Pectin Medium	Richard's Medium	Richard's+ Pectin Medium
1	*Fusarium oxysporum f.sp. lentis*	7	205.65	190.82	-	4.08
		14	400	243	-	9.62
		21	421.3	214.33	-	6.05
2	*Fusarium oxysporum f.sp. lycopersici*	7	204.86	191.36	-	4.37
		14	476.5	238.5	-	8.86
		21	416.3	210	-	5.95
3	*Fusarium oxysporum f.sp. lini*	7	203.95	188.5	-	4.43
		14	472.66	239.4	-	9.21
		21	413.2	213.8	-	5.92
4	*Fusarium moniliforme*	7	202.87	192.1		4.38
		14	479	240.35	-	9.16
		21	416.3	211.8	-	5.86
5	*Fusarium udum*	7	201.65	191.76	-	4.3
		14	478.33	241.2	-	9.35
		21	414.85	211.65	-	5.92
6	*Fusarium solani f.sp. lentis*	7	204.3	190.81	-	4.23
		14	475.14	239.6	-	9.45
		21	412.86	213	-	5.98





Result and Discussion

After perusal of the table it was concluded that the enzyme production was adaptive in nature, that is, production of enzyme increased remarkably in the presence of substrate pectin. This adaptive nature of enzyme was also confirmed by Mukherji and Mazumdar (1974) and Vidyashekran et al. (1973).

Maximum enzyme activity was observed in culture filtrate of 14 days incubation, similarly maximum mycelial weight was also observed on 14 days incubation. Enzyme production was correlated with the growth of mycelium. Maximum enzyme activity was detected in *Fusarium oxysporum f.*sp*. lentis* followed by Fusarium udum, Fusarium solani and *Fusarium oxysporum f.sp. lini.*

Minimum enzyme activity was observed in *Fusarium oxysporum f.sp. lini.* More or less similar activity was observed in *Fusarium oxysporum f.sp. lycopersici* and Fusarium moniliforme. Similar enzyme activity was also detected by Dees and Sthanan (1962).

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