

INVESTIGATIONS ON THE PRODUCTION OF PECTIC  
ENZYMES DURING THE ROTTING OF  
*SHOREA ROBUSTA* WOOD BY *IRPEX DESTRUENS*

By

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*Irpex destruens*, brings considerable damages to *Shorea robusta* timber. The secretion of pectic enzymes by this fungus during its pathogenesis was investigated. Of the three pectic enzymes viz., Pectin Methylesterase (PME), Pectin Polygalacturonase (PG) and Pectin Depolymerase (PD), the fungus showed little PME activity and very meagre PG and PD activities.

INTRODUCTION

The production of pectic enzymes by fungi during rotting of wood has been reported by a number of investigators. Zeller (1916) demonstrated the production of pectinase by the wood-destroying fungus *Lenzites saepiaria*. Pectinase production by *Fomes fraxineus* (Bull) Cooke, was studied by Montgomery (1936). In 1937, Bose and Sarkar estimated the activities of pectinase produced by the different polypores. Gupta (1960, 1962) also worked on the secretion of pectic enzymes by the parasitic fungus *Fusarium orthoceras* App. and W. Var ciceri Padwick. It may, therefore, be of interest to study whether *Irpex destruens* produces any pectolytic enzyme during decay of wood.

## MATERIAL AND METHODS

Fresh fruit-bodies of fungi along with the infected and non-infected wood of *Shorca robusta* were collected and wood pices were washed several times with distilled water. For the extraction of enzymes the tissues (2 gm. each, separately for the three samples,) were ground in a blender after the addition of 100 ml. of ice cold distilled water, for about 2 hours. The whole operation was performed at 0°C. The mixtures were kept overnight at 0°C, and then filtered through several layers of muslin. The filtrates then were centrifuged at 10,000 RPM at 0°C for 20 minutes. The supernatant was adjusted to the desired pH with IN-NaOH/HCl, constituted the enzyme extract. Enzyme preparations were assayed immediately.

For the process of enzyme assay three techniques were adopted (Baruah, 1941; Kertesz, 1951; Cole and wood, 1961; Gupta and Gupta, 1966; Singh and Hussain, 1968).

- (1) Measurement of the loss of coherence of blocks of plant tissues.
- (ii) Determination of the changes in viscosity of 1% pectin solution during hydrolysis by the enzyme.
- and (iii) Determination of the increase in free carboxyl groups in 1% solution of pectin by the activity of the enzyme.

To study the loss of coherence of plant tissues five different types of plant tissues, viz, tomato, lemon, orange, apple and *shores robusta*, were taken. Blocks (2 cms. x 1.0 cm x 1.0 cm) were made for each sample and each block was deeped in enzyme extract contained in petridish (separately for separate sample) and examined the degree of loss of coherence of the block of plant tissues at time intervals. Controls were taken with boiled enzyme extracts.

For the change in viscosity of the pectin solution 1 ml. each of the enzyme extracts was taken (separately for separate samples) in 5 ml. of 1% pectin M/V contained in a Oswalds viscosimeter. The temperature and pH were adjusted respectively at 25°C and 5.05, controls were maintained with distilled water

instead of enzyme extract and the relative viscosity was measured at intervals of 10 minutes.

To determine the increase in free carboxyl groups in 1% pectin, 4 ml each of the enzyme extracts (fungal fructifications, infected and non-infected host tissues) were taken separately, in 10 ml. of 1% pectin solution W/V with 2 ml. of N-NaCl. The temperature and H value were adjusted respectively at 250°C and 5.05. The higher pH value of the mixture was maintained by adding continually 0.1 N-NaOH, and the activity was measured as the amount of NaOH used in 1 hour after the addition of the enzyme; results are expressed as percentage of the total NaOH required for alkali saponification.

#### RESULTS

From the macerating technique it appears that enzyme extracts from the fungal fructification show slight pectinase activity while non-infected host tissue extract show minimum activity. The enzyme extract from the infected host tissues shows intermediate activity. But the pectinase activity shows negligible effect on the host wood (Table 1).

In the viscosity measurement it is observed that the extracts from the non-inflected host tissues show a gradual decrease in viscosity upto the 50th minutes, the same is applicable in the case of infected host tissue extracts; but in the case of fungal extract the gradual fall in viscosity is found upto the 40th minute and then it goes up (Table 2).

The result of the free carboxyl determination shows negligible response. There is slight increase in the utilization of NaOH in the infected host wood extract (0.15 ml) over the non-inflected host wood extract (0.05 ml) and then declines to 0.10 ml in the fungal fructification as seen in (Table 3).

**Table 1. Maceration of tissues by Pectinase from Ipex destruens**  
 Dead healthy host tissues extract      Infected host tissue extract      Fungal fructification extract

Time interval in minutes	Lemon	Oran-Potato	Apple	Tomato	Healthy host wood	Lemon	Oran	Potato	Apple	Tomato	Healthy host wood	Lemon	Oran	Potato	Apple	Tomato	Healthy host wood	
0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
120	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
180	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
300	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
After 12hrs.	-	-	+	+	-	-	-	-	±	+	+	-	-	+	+++	+++	+	+
After 24hrs.	-	-	++	++	+	-	-	+	+++	+++	±	-	-	+	+++	+++	+++	+

Temperature—20-30°C  
 - indicates no reaction ;  
 +, indicates partial reaction.  
 ++, indicates actual reaction.

Bolled enzyme extracts from the three sources did not show any reaction on any of the tissue blocks.

**Table 2.** Change in viscosity of one percent pectin solution caused by the pectinolytic enzyme of *Irpex destruens* extracts; infected and non-infected host tissues of *Shorea robusta*

Time in minutes	Relative viscosity percent		
	Non-infected host tissues	Infected host tissues	Fungal fructification
0	100	100	100
10	82.25	81.05	81.63
20	82.05	29.82	81.33
30	80.95	79.76	81.11
40	80.37	78.98	80.57
50	79.40	78.86	80.79
60	78.94	78.56	81.49
70	80.85	78.60	81.51

**Table 3.** Determination of increase in free carboxyl groups in 1% pectin by the activity of PME 1 hour

Samples	Volume of NaOH utilized (ml)
Dead healthy wood extract	0.05
Infected host wood extract	0.15
Fungal fructification extract	0.10

#### DISCUSSION

It appears from the findings that the fungus shows very negligible pectic enzymes activities. In the case of viscosity measurement both infected and non-infected the host wood extracts show gradual rise in viscosity after 60 minutes. the gradual rise in viscosity may be from the side reaction, interection between the phenolic substances present in high quantities (Singh and Baruah, 1970), and pectic enzymes (Cole and Wood 1961). As the enzyme extracts were crude ones there might be other substances, presumably phenolic com-

pounds in association with the enzymic protein. Cole and Wood (1961) reported that the products formed on the oxidation of some of the phenolic substances present in the host, inactivated the pectic enzymes. In the present case also presence of phenolic substances in the crude enzyme extracts might cause the inactivation of the pectic enzymes. Moreover, the products formed due to the oxidation of these phenolic substances might cause the increase in viscosity.

From the findings of viscosity measurement it is clearly seen that the activity of the pectic enzyme particularly PD is very feeble. Of the three enzyme extracts, maximum activity is seen in the infected host tissue extracts. This may be due to the secretion of enzyme (Weimer and Haster, 1923) by the fungus to the infected areas during infection. The infected host tissues therefore, contain much higher concentrations of enzymes than that found in the fungal fructifications. The enzyme extracts from the fungal fructifications show the fall in the viscosity perceptibly during the first 20 minutes.

The results also reveal that there is very negligible pectin methyl-esterase (PME) activity in all cases. Measurement of the loss of coherence of blocks of plant tissues, show that: (i) there is partial action of the enzyme extracts on apple and tomato tissues after 12 hours. (ii) there is no marked action on the other tissues including non-infected wood stem block.

It appears that *Irpex destruens* shows little PME and very little macerating enzyme and PD activity also indicates that in the breakdown of timber pectolytic enzymes are not implicated to any limit.

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