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Role of fungal enzyme in wood rotting – I : activity of protease during decay of mango tree by the fungus – *Clytocybe multiceps*

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ABSTRACT

The activity of enzymes involved in the rotting action of Mango tree wood (log) by a fungus- *Clytocybe multiceps*, was studied .The degradation of cell wall was the main decaying change or the first step in the process of decaying which was brought about by the action of proteolytic enzymes in association with pectinolytic and cellulolytic enzymes.

Figure : 00 References : 30 Table : 01

KEY WORDS : *Clytocybe multiceps*, Decay, Mango tree, Protease enzyme, Wood rotting fungus.

Introduction

Decaying of wood (dead wood)is usually caused by a wide variety of fungi, bacteria, insects and physical factors of the environment [18]. Many forms of rots or decay are caused by a large number of wood rotting fungi. For undergoing the process of fungus-caused decay the fungi require a balance between atmospheric air and moisture. Decay does not occur in dry wood due to absence or lack of moisture. Again, decay cannot occur in completely saturated wood because air is essential to fungal growth and activity [4]. The plant cell wall is a complex structure consisting of cellulose , hemicellulose, pectic substances and proteinaceous compounds[1] . Plant pathogens have been shown to secrete enzymes capable of degrading the polysaccharides of plant cell walls[4]. A Botanist[16] suggested that plant pathogens would also secrete enzymes capable of degrading protein present in the plant cell wall. The degradation products as amino acids and amides serve as nitrogen and carbon sources to the pathogen[6,13,23,30]. Early investigators, using a Trichloroacetic acid precipitation assay , demonstrated the secretion of proteolytic enzymes by several plant pathogenic organisms[12,14,15,24]. Various workers[7,11,22,27,28] have reported the production of extracellular protease by pathogenic fungi. Another worker[19] reported that change of protein in healthy and infected tissues was largely attributed to the action of hydrolytic enzyme protease .

Proteinases are a complex group of enzymes varying greatly in their physiochemical and catalytic properties and playing important roles in the catabolic and regulatory processes of eukaryotes and prokaryotes alike [10,23]. These enzymes are divided into two major groups, peptidases and proteinases , on the basis of their nature of attack.

Coprinus cinereus has been shown to use protein as a source of carbon, nitrogen and /or sulphur[8]. This ability to use protein as a major growth substrates is correlated with production of extracellular proteinase activities [11]. In the early 1939, It was reported that wood destroying Basidiomycetes produce proteolytic enzymes and presumably could satisfy their requirements for nitrogen at least partly by utilisation of protein in wood[3]. It has also been suggested that a protease, lipase or the macerating enzyme of the pathogen acted upon an essential structural constituent of the protoplast membrane of host tissues to cause death. Macerating activity and death of cells were closely associated as in studies of other host-pathogen systems [21,28]. Though little is known about the mechanism controlling the production of fungal extracellular proteinases, the possibility of protease action in tissue maceration and in cell wall penetration is established. It was suggested that even if protease does not function in cell-wall penetration or separation , it may alter essential lipo-protein membrane permeability or disrupt other vital functions of the cell[25].

Materials and Methods

The assays of protease enzyme's activities were conducted with the modification of the method[17]. The

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TABLE-1 : Protease activity in the healthy and infected wood tissues , pileus and stipe of the fungal body at different pH levels, (showing the amount of Amino Acid released in mg /30 min.).

pH	Healthy wood (H)	Infected wood (If)	Pileus (P)	Stipe (S)
5	2.52	2.88	5.08	2.71
6	1.21	1.81	3.92	1.11
7	0.43	0.58	0.46	0.50

fungus *Clytocybe multiceps* is an edible mushroom found growing on decaying woods, timbers, tree-trunks, large branches or rarely on decayed root systems, in various parts of Manipur, mostly during rainy season from May to October. The fungus were collected intact carefully along with the host tissue , particularly those which grow on Mango tree/wood either in the decayed or fresh growing condition . Since the identification of the fungus and hyphae using microscope takes time, the collected fungi were cultivated in the Lab by using standard culture media , like Malt Extract Agar (Malt extract-25.0 g , Agar powder-15.0 g in1000ml.of distilled water) , Czapek's Dox Agar ($NaNO_3$ - 2.0 g, KH_2PO_4-1.0 g , $MgSO_4$.& H_2O-0.5 g , KCl – 0.5 g, $FeSO_4.7H_2O$ – 0.01g , Glucose – 30.00g , Agar powder- 15.00g, Distilled water- 1000ml.

For the study of action of cell wall degrading enzyme- protease the assays of protease enzyme activities were conducted. A known weight of the different materials to be tested were homogenised properly with distilled water and the mixture was centrifuged under cold condition. The collected supernatant was dialysed against several volumes of distilled water at 2-4^o C for 24 hrs., changing the water twice .

The dialysed solution was immediately used for the assay of proteolytic enzyme activities. The reaction mixture consisted of 5 ml of 1% casein (prepared in 0.1M PO_4 buffer), 2 .5 ml of PO_4 buffer , 205 ml of enzyme extract. Boiled enzyme was used as control for the expt. The assays were conducted at pH - 5, 6, & 7 at 30^{O}C . The reaction was continued for 30 min. and release of free amino acids was determined with the use of ninhydrin method [20].

Result and Discussion

From the present investigation , it has been observed that the activity of protease enzyme was more in the infected tissues than the healthy wood tissues . While for the fungus body the pileus of the fungal fructification produce the highest enzyme activity and the stipe was found to exhibit lesser enzyme activity. On the whole, the fungal body was found to produce the maximum enzyme activity as compared with the host tissues. The enzyme activity at different pH was calculated and found that , at lower pH, the host and pathogen produced maximum enzyme while the production was found to be lower with the increase in pH. This shows the occurrence of optimum pH during protease production.

The increase of enzymatic activities in the infected tissue was found to occur during the pathogenesis . Such increase in activity indicates the participation or involvement of proteolytic enzyme during pathogenesis as has been shown by the change in protein and amino acids in the infected tissues. Such change in protein content of tissue components has been brought as a result of fungal proteolytic activity. Increased enzyme activity in the infected tissues might be due to the secretion by the fungal mycelia which are in direct contact with the host tissues. The enzyme produced by the fungal hyphae help in the active degradation of nitrogenous compounds. It was reported that the production of extracellular enzyme- proteinase by fungi is found to be correlated with its ability to use protein as a major growth substrate[11]. The continued catabolism of protein in the presence of glucose and / or ammonium[9] shows regulation of production of these enzymes to be free of catabolite repression systems for proteinase production seen in lower fungi [5] .

Enzyme activity at different pH was recorded in the present study. The optimum pH was found to be pH-5. As the pH increased, the activity of enzyme was found to decrease. The different amounts of hydrogen ion (H^+) concentration might have affected the enzyme production by the pathogen. From the above study, it can be concluded that the degradation of cell wall of Mango wood (*Mangifera indica*) by the infection of the fungus , *Clytocybe multiceps* resulting to the decay is the result of pathogenesis brought about by the proteolytic enzymes associated with pectinolytic and cellulolytic enzymes produced by the fungus.

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