The Bioscan 14(2): 159-162, 2019
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.com

ROLE OF PECTIC ENZYME IN THE ROTTING OF MANGO DEAD WOOD BY THE FUNGUS - *CLITOCYBE MULTICEPS*

BASUNDHARA TH.[1]* AND G.A. SHANTIBALA[2]
[1]PG-Department of Botany, D. M. College of Science, Dhanamanjuri University, Imphal - 795 001, INDIA
[2]Department of Life Sciences (Botany), Manipur University, Canchipur - 795 003, INDIA
e-mail: basunth5@gmail.com

KEYWORDS
Wood rotting fungus
Pectic enzyme
Polygalacturonase (PG)
Polymethyl esterase (PME)
Clitocybe multiceps

Received on :
11.01.2019

Accepted on :
25.04.2019

***Corresponding author**

ABSTRACT
Wood rotting is naturally caused by the action of insects, fungus, bacteria, nematodes, etc. Fungi produce many enzymes playing main role in the decaying of dead wood. To investigate the rotting action of enzymes produce by the fungus - *Clytocybe multiceps*, the fungus species was collected from various localities when they are grown vigorously during rainy season, brought to the lab for further study. The methodology adopted by Cole and Wood (1961) was followed. The study reveals the role of fungal enzymes in rotting of mango wood by the pectic enzyme produced by the fungus - *Clitocybe multiceps*. This fungus produce pectic enzyme which can degrade the layer of cell wall of host tissue leading to rotting. The stipe or stalk portion of the fungus is found to produce more amount of the wood - rotting pectic enzymes - polygalacturonase (PG), than the pileus portion. The cellulolytic enzymes - polygalacturonase and polymethylesterase (PME) were produced during pathogenesis of the wood rotting action of the dead tree wood by the fungus. The main aim of the present work is to investigate the role or action of pectic enzyme in the decay of dead mango wood tissue and is discussed.

INTRODUCTION

Fungal enzymes like Pectolytic and cellulolytic enzymes produce by phytophagous fungi are the best known enzymes associated with plant diseases mainly for the soft rot diseases. Both these enzymes are known to be produced by many phytopathogenic fungi, bacteria and nematodes in nature (Mehta, A., *et al.*, 2007). A characteristic feature of many phytopathogenic organisms is their ability to produce an array of enzymes capable of degrading the complex polysaccharides of plant cell wall and the membrane constituents (Simbo Aboaba, 2009). Many species of macro-fungi including species of *Clitocybe* (*C. fragrance*) are found to be associated and responsible for the post-harvest decay of bamboo. Decay and biodeterioration of culms are caused mainly by fungi that include - soft rot, white -rot and brown rot (Saharia and Sarma, 2012). The degradation of pectic substances is accomplished by a variety of enzymes and the activities of pectolytic and cellulolytic enzymes in the process of cell wall degradation during pathogenesis was well studied and established by many workers (Bateman and Basham, 1976, Mehta and Mehta,1985, Singh and Shukla, 1999, Sharma, 2000). Among the various diseases of arecanut, the basal-stem rot, a slow decline disease is the most dreaded disease caused by the fungus - *Ganoderma lucidum* (Chakrabarty, Acharya and Sarma, 2014). Naturally decaying of dead wood or log occurs mainly by the action of many enzymes produced by a large number organisms and the initial action of decay is the damaging or degrading of cell wall by the action of enzymes secreted by fungal pathogens. The presence of cellulolytic enzymes is the most important biochemical offensive weapon of most fungi, which help the pathogen in penetration, establishment and subsequent colonisation within the host (Batman and Miller,1966; Brown, 1965). The enzymes are produce both in vivo and in vitro. The enzymes dissolving the cell wall are mostly pectolytic and cellulolytic enzymes (Gupta, 1979; Mahadevan, 1966). Bateman and Basham (1976) studied that pectic enzymes apparently loosen the cell wall structures and thereby render other wall polymers susceptible to enzymatic attack. Attack on the plant tissue by a pathogen is often initiated by pectic enzymes because the pectic substances are most readily accessible. These enzymes also caused extensive breakdown of cell wall and maceration of infected host tissue (Albersheim et al. 1969; Bateman and Miller,1966). The enzymes responsible for maceration of plant tissues are also responsible for the death of the plant cells (Basham and Bateman, 1975; Mount *et al.* 1970). Rao and Singh (1978) confirmed the production of pectic enzyme by *Sclerotium rolfusii* only in the presence of pectin and found a close correlation between the growth of the pathogen and pectic enzyme production. The involvement of pectolytic enzyme in the damage of host plants by fungi has been well established by Bateman and Miller,1966; Weinhold and Motta 1973 and others. Many plant pathogenic fungi produce pectin-degrading enzymes in culture (Cooper, 1983; Keon et al., 1987). In plants these enzymes may cause tissue maceration and cell death (Dean and Timberlake, 1989). The present work studies the action of pectic enzyme produce by the edible white rot fungus - *Clitocybe multiceps* in the decaying action of mango tree wood.

MATERIALS AND METHODS

The wood rotting fungus - *Clitocybe multiceps* was found to grow during the rainy season, from May to October and they grow more vigorously during the three months of June, July and August. The fungus specimens were collected from different localities and taking intact along with their infected host wood tissue, inside plastic bags and brought to the lab for processing and further procedures. The method adopted by Cole and Wood (1961) was followed for this work. Enzyme extract was prepared by taking 2 gm. each of different tissues (infected by the fungus) and 100ml of ice-cold distilled water, then grinded in a mixer / blender for about 2 hrs. The mixtures were then filtered through several layers of muslin cloth with the application of pressure and the filtrates was centrifuged at 10,000 rpm for 30 minutes. The supernatant was then kept overnight at 0°C. Next to begin the enzyme analysis, the supernatant was adjusted to the desired pH with 1N NaOH or HCl. The reaction mixture made of 1 ml of enzyme extracts, that is of pileus and stipes of fungal fructification, infected and healthy host tissues was taken separately at pH 5.5 (distilled water in the control), 5ml of 1% pectin (pH 5.5) and 2ml of distilled water at 25°C. The percentage of loss of viscosity was measured at intervals of 10 minutes by means of Oswald's viscometer. The procedure adopted by Cole and Wood 1961 was followed to find out the loss of viscosity in the pectin solution. The viscosity of the reaction mixture is usually expressed as a percentage of the initial viscosity of 1% pectin solution. The time required to flow of the 1% pectin solution in the viscometer, is taken as the 100% viscosity. The reaction is completed only when the viscosity of the pectin solution is reduced to that of water. The results are shown in Table 1. To measure free carboxyl groups also the procedure adopted by Cole and Wood (1961) was followed. The reaction mixture was made of 4ml each of enzyme extracts of different tissues (infected), 10 ml of pectin (1% W/V) and 2 ml of N NaCl. Water was used in control in place of enzyme extracts. The pH of the mixture was kept at 7 by continually adding 0.02N NaOH and enzyme activity was measured as the amount of NaOH used in 30 minutes after adding the enzyme. The results are expressed as percentage of the total NaOH required for alkali saponification and results are shown in the Table 2.

RESULTS AND DISCUSSIONS

The present finding suggest that the said fungus - *Clitocybe multiceps* is able to produce the pectic enzymes which can degrade the different layers of cell wall and the presence of more of these enzymes in infected tissues is an additional evidence for the involvement of these enzymes in infected tissues performing their degrading action leading to rotting. Such similar findings were also made and reported by Ramaraj and Vidhyasekaran (1982) and Sharma and Kaul (1989) in infected apple.

Further the determination of free carboxyl groups shows the maximum activity of pectin methyl esterase in the infected host tissues as the tissues consumed more sodium hydroxide than the dead healthy wood. The consumption of sodium hydroxide by the pileus was found more than that of stipes. This shows the more increase enzyme activity of the pileus.

Analysis of the host tissue extracts showed that the enzymes polygalacturonase (PG) and polymethylesterase (PME) were produced during pathogenesis of the wood rotting action of the mango tree by the fungus - *Clitocybe multiceps*. The presence of higher polygalacturonase enzymes in the infected host wood tissues has been reported by many workers (Hancock and Miller, 1965; Khanna and Chandra, 1979; Sharma and Kaul, 1989; Umabala and Ramarao, 1979). The production of high enzyme activity by the infected tissues of Mango tree (*Mangifera indica*) is due to the exudation of the enzyme by the fungus to the infected tissues. Prasad and Sinha (1979) however, suggested that the increased enzyme activity of polygalacturonase and polymethyl esterase in the infected tissue might be due to the continued availability of the substrates in the host and or might also be due to the adaptability of the pathogens. Reports on higher PME activities in the infected host tissue has been reported by Umabala and Ramarao (1979); Sharma and Kaul(1989) and others. Increased PME in infected tissues might be of host origin which could function to demethylate the host proteins (Bateman,1963).The different pectolytic enzymes have a role in the pathological degradation process (Hancock and Miller, 1965). Endopolygalacturonase catalysed the hydrolysis of pectic acid which is formed by de-esterified activity of PME whereas exopolygalacturonase seems to be insignificant in pathogenesis.

Hence degradation of the pectic substances of the middle lamella and primary cell wall could simultaneously facilitate fungal movement in the middle lamellar region, expose other structural components of the primary cell wall to more rapid enzymatic degradation and provide a readily available carbohydrate source to the pathogen. Thus the presence of higher pectic enzymes in the infected tissues is due to the secretion of the fungus pathogens.

The analysis of pileus and stipes of the fungal body has shown different enzyme activity but the activity produce by them is not very high. The stipes produce higher polygalacturonase

Table 1: Change in the percentage loss in viscosity during enzymatic hydrolysis

Time in minutes	Healthy wood (H)	Infected wood (If)	Pileus (P)	Stipes(S)
0	0	0	0	0
10	27.69	23.56	16.97	19.30
20	27.69	23.56	9.33	16.97
30	27.69	21.13	9.33	16.97
40	25.13	17.26	9.33	7.32
50	17.00	10.56	9.33	7.32
60	17.00	10.56	9.33	7.32
70	17.00	10.56	9.33	7.32

Table 2: Increase in free carboxyl groups in 1% pectin by the activity of pectin methyl esterase (PME) in 30 minutes

Samples	Volume of 0.02 N NaOH utilised (ml)
Healthy (H)	0.50 ml
Infected (If)	0.66 ml
Pileus (P)	0.30 ml
Stipes (S)	0.22 ml
Control	0.20 ml

(PG) activity than the pileus where the consumption of sodium hydroxide by pileus was found more than that of stipes showing more production of polymethyl esterase (PME) activity. The production of low enzyme activity of PG and PME by the fungus - *Clitocybe multiceps* might have been due to the presence of inadequate amount of pectic substances in the fungal body (as has been noticed in the estimation of pectin). Due to the production of low cell wall degrading enzyme activity, the degradation of host wood tissues by *Clitocybe* hyphae was found slow. Though pectic enzyme production by a fully grown *Clitocybe multiceps* was low, the damage caused by it was very severe in the infected region. This severe damage of the wood tissues may be explained by the fact that the production of pectic enzyme by the intercellular hyphae are more than the one, which develops intracellularly. These conditions clearly explains the involvement of pectic enzymes in the cell wall degradation of the mango wood by the wood rotting fungus - *Clitocybe multiceps*.

ACKNOWLEDGEMENTS

Authors are thankful to the Head, P.G. Department of Botany, D. M. College of Science, Dhanamanjuri University, Imphal and Head, Department of Life Sciences, Manipur University, Canchipur for encouragement and laboratory facility.

REFERENCES

Albersheim, P., Jones, T. M. and English, P. D. 1969. Biochemistry of the cell wall in relation to infective processes. *Annu. Rev. Phytopathol.* **7:** 171-194.

Bateman, D. F. 1963. The macerating enzyme of *Rhizoctonia solani*. *Phytopathol.* **53:** 1178-1186.

Basham, H. G. and Batman, D. F. 1975. *Physiol. Pl. Pathol.* **5:** 249-261.

Batman, D. E. and Miller, R. L. 1966. Pectic enzymes in tissue degradation. *Ann. Rev. Phytopathol.* **4:** 119-146.

Batman, D. F. and Basham, H. G. 1976. Degradation of Plant cell walls and membranes by microbial enzymes. In Physiological Plant Pathology : (eds. R. Heitefuss and P.H. Williams). Springer -Verlag, Heidelberg & New York. **4:** 316-355.

Brown, W. 1965. Toxins and cell wall dissolving enzymes in relation to plant disease. *Annu. Rev. Phytopathol.* **3:** 1-23.

Chakrabarty, R., Acharya, G. C. and Sarma, T. C. 2014. Anti-fungal activity of some plant extracts against *Ganoderma lucidum* (Curtis Ex. Fr.) Karst., causing basal stem rot- a slow decline disease in arecanut. *The Bioscan*. **9(1):** 271-281.

Cole, M. and Wood, R. K. S. 1961. Pectic enzymes phenolic substances in Apples rotted by fungi. *Ann. Botany*. **25:** 435-452.

Collmer, A. C. and Keen, N. T. 2003. The role of pectic enzymes in plant pathogenesis. *Annual Review of Phytopathology*. **24(1):** 383-409.

Cooper, R. M. 1983. The mechanisms and significance of enzymatic breakdown of host cell walls by parasites. In Biochemical Plant Pathology, J.A Callow, ed (New York: John Wiley and Sons). pp. 101-135.

Dean, R. A. and Timberlake, W. E. 1989. Production of cell wall degrading enzymes by *Aspergillus nidulans* : A model system for fungal pathogenesis of plants. *The Plant Cell*. Vol.-1, American Society of Plant Physiologists, March, 1989. pp. 265-273.

Gupta, I. 1979. In vitro and in vivo production of pectolytic and cellulolytic enzymes by four isolates of *Colletitrichum*. In "Physiology of Parasitism" (Eds. Agarwal and Bilgrami) Vol. 7, pp. 197- 207.

Hancock, J. G. and Miller, L. 1965. Association of cellulolytic, proteolytic and Xylolytic enzymes with Southern Anthracnose, Spring black stem and *Stemphylium* leaf spot of *Alfa alfa*. *Phytopathol.* **55:** 356-360.

Keon, J. P. R., Byrde, R. J. W., and Cooper, R. M. 1987. Some aspects of fungal enzymes that degrade plant cell walls. In Fungal Infection of Plants. G. F. Pegg and P. G. Ayres. eds. (Cambridge: Cambridge University Press). pp. 133-157.

Khanna, R. K. and Chandra, S. 1979. Production of pectic and cellulolytic enzymes by isolates of *Alternaria alternata* (FR). Keissler in vitro and in vivo. In "Physiology of Parasitism", (Eds. Agarwal and Bilgrami) Vol.7, pp. 89-99.

Mahadevan, A. 1966. Biochemistry of Infection and Resistance. *Phytopathol*. **57:** 96-99.

Mehta, A. and Mehta, P. 1985. Production of pectolytic and cellulolytic enzymes by *Fusarium oxysporum* and *F. moniliforme* under different cultivation conditions. *Folia Microbiol.* **30:** 42-50.

Mehta, A., Deolia, A., Pathak, S., Rajput, S., Somal, P. and Mehta, P. 2007. Production of pectolytic and cellulolytic enzymes during pathogenesis of fruit rot of *Malus sylvestris* (apple). *The Indian Journal of Botanical Society*. **86(1&2):** 61-64.

Mount, M. S., Bateman, D. F. and Basam, H. G. 1970. Induction of electrolyte loss, tissue maceration and cellular death of potato tissue by endopolygalacturonate transeliminase. *Phytopathol.* **60:** 924-931.

Prasad, S. S. and Sinha, S. K. 1979. Cell wall dissolving enzymes of certain fungi during pathogenesis. In "Physiology of Parasitism". (Eds. Agarwal and Bilgrami) Vol.7, pp. 155-161.

Ramaraj, B. and Vidyasekaran, P. 1982. Possible involvement of pectic enzymes in Betelvine wilt development. *Ind. Phytopathol.* **35(1):** 71-72.

Saharia, D. and Sarma, T. C. 2012. Screening of macro-fungi responsible for post-harvest decay of Bamboo culms in storage. *The Bioscan*. **7(1):** 95-99.

Sharma, R. L. 2000. In vitro and in vivo production of cellulolytic enzyme by *Monilinia* sp. causing brown rot of apple. *Indian J. Mycol. & Plant Pathol.* **30(1):** 31-33

Sharma, R. L. and Kaul, J. L. 1989. Studies of pectic enzyme in the rotting of apple by *Monilinia* sp. *Indian J. Mycol. & Plant Pathol.* **19(1):** 30-36.

Singh, S. N. and Shukla, P. 1999. Variation in the production of enzymes by different isolates of *Myrothecium roridum* caused leaf spot of Mungbean. *Indian Phytopath.* **52(1):** 63-65.

Simbo Aboaba, 2009. The role of pectinase enzyme in the development of soft rot caused by *Pseudomonas fluorescens* in the purple variety of onions (*Allium cepa*). *African Journal of Microbiology Research.* **3(4):** 163-167.

Umabala, K. and Ramarao, P. 1979. Production of pectolytic and cellulolytic enzymes by *Phytopthora palmivora* causing leaf blight of

Colocasia esculanta. In "Physiology of Parasitism". (Eds. Agarwal and Bilgrami) Vol.7, pp. 105-119.

Weinhold, A. R. and Jerome Motta 1973. Initial host response in cotton to infection by *Rhizoctonia solani*. *Phytopathol*. **63:** 157-162.