Available online @ www.actasatech.com

acta **SATECH 3(2): 108-112 (2010)**

Enzymatic activity of some fungal isolates from decaying bamboo leaves

[+ **] **O. O. Afolabi,** [*] **Fagade O. E., and** [*] **Ogunjobi A. A.**

[*] *Environmental Microbiology and Biotechnology Unit,*
Department of Botany and Microbiology, University of Ibadan
[**] *Department of Fish Technology and Biotechnology,*
Nigerian Institute for Oceanography and Marine Research, Victoria Island, Lagos.

[+] **Corresponding author** :< olaitan_afolabi@yahoo.com>

ABSTRACT

Decaying bamboo leaf samples were collected from two streams within the University of Ibadan, Ibadan, Nigeria. Fungal isolates were obtained from the leaves through direct plating, leaf washing and baiting techniques. A total of forty fungal isolates were obtained, they were identified as Aspergillus, Fusarium, Mucor, Rhizopus, Trichoderma and some unidentified genera. The fungal isolates were assayed for the presence of cellulase and lignase activity. All the 13 isolates used (which were representatives of all fungi obtained) showed the presence of cellulase, lignin peroxidase and manganese peroxidase. The study showed that aquatic fungi produce a wide range of enzymes necessary for the breakdown of plant tissues which are the major source of carbon in streams.

Keywords: *Decaying leaves, fungi, cellulase, lignin peroxidase and manganese peroxidase.*

INTRODUCTION

A pioneering study of leaf decay in streams showed that fungi were more active than bacteria during the early stages of decay. Fungi therefore act as an intermediate trophic level between fallen leaves (the dominant source of food in most small streams) and leaf-eating invertebrates (Barlocher, 1985; 1992; Suberkropp, 1992). Fungal biomass, sporulation, enzymatic activities and community structures are all influenced by leaf composition and by environmental factors such as water temperature and chemistry, which often fluctuate during extended periods of leaf decay (Suberkropp and Chauvet, 1995; Sridhar and Barlocher, 1997). Most of the fungal biomass on decaying leaves consist of vegetative (non – reproducing) hyphae that cannot be identified through conventional microscopy (Nikolcheva *et al.*, 2003).Aquatic hyphomycetes, an artificial phylogenetically, heterogenous group of true fungi; are fungi dominating leaf decomposition in streams (Webster, 1992). They are anamorphs of Ascomycota and Basidiomycota (Webster, 1992, Alexopoulos *et al.*, 1996,). They comprise fungi that produce conidia exclusively in aquatic environment or in the interstitial water among soil particles. Their preferred habitats are streams with clear, clean, well-aerated water, with moderate turbulence and also reservoirs and lakes with different kinds or levels of pollution. The conidia may be trapped in foam, dispersed in water, floating on water surface or associated to organic decomposing substrates such as leaf litter and twigs (lngold, 1975). They occur mainly in lotic systems and play a crucial role in the trophic chain (Schoenlein-Crusius and Piccolo Grandi, 2003). They are predominant in leaf decomposition process in aquatic environment and there is evidence that they are able to degrade several plant cell polymers such as cellulose, hemicelluloses and pectin (Chamier, 1985). Hasija and Singhal (1991) and Marvanova (1997) both reported that they probably degrade lignin. They produce amylase, cellulase, pectinase, protease, pyrocathecol oxidase, triacylglycerol lipase and xylanase (Chandrashekar and Kaveriappa, 1991; Suberkropp, 1992; Marvanova, 1997). These enzymes may cause breakdown of leaf tissues and increase the palatability of the leaves to leaf-eating invertebrates (Barlocher, 1992; Suberkropp, 1992). Aquatic

hyphomycetes have been reported to produce a wide range of enzymes responsible for the breakdown of leaves in water. Bamboo leaves that sometimes occur in aquatic habitats comprise mainly of cellulose, hemicelluloses and lignin.

This work was therefore aimed at testing for cellulase and lignase activity in fungi isolated from decaying bamboo leaves in water.

MATERIALS AND METHODS

Sample collection

Submerged decaying bamboo leaves were collected from two streams within the University of Ibadan, Nigeria. Ten leaf samples each were collected from the base of the streams in sterile polythene and taken to the laboratory.

Preparation of Media

Synthetic and semi – synthetic routine media were used for the isolation which include Potato dextrose agar (PDA), Starch agar, and Malt extract agar (MEA). The media were prepared according to manufacturers' instructions.

Isolation Technique

The two methods used for fungal isolation include: direct inoculation and baiting methods.

Direct inoculation: The leaves were washed to remove surface mud and debris. The leaves were then surface sterilized with 1% Mercuric chloride ($HgCl_2$) and rinsed with distilled water. They were then cut into equal segments of about $1cm^2$ each with sterile scalpel. Four segments were plated per Petri dish of the different media used.

Baiting: The leaves were washed under tap water to remove surface mud and debris. They were then cut into equal segments of about $1cm^2$ each. Four segments were incubated per plate. Some segments were incubated in sterile stream water, while some were incubated in unsterilized stream water and baited with hemp, corn, guinea corn, and crotalaria seeds for five to seven days. Leaves with germinating conidia and mycelia were then transferred unto agar plates (Milanez, 1984). The plates were incubated at 30^0C for five days.

Enzymes Assay

The enzymes responsible for degradation were determined by assaying for cellulase, lignin peroxidase and manganese peroxidase activity in the degradation media.A basal medium was prepared according to the method of Shaw and Quejesky, (1979).

The fungal isolates were grown in 10ml of basal medium containing cellulose as the sole carbon source for the determination of cellulase activity while corn cob (a ligno-cellulosic material) was the sole carbon source for lignase activity. The fungi were grown at 30^0 C for seven days. The enzyme filtrate was obtained by filtering through Whatman No.1 filter paper to separate mycelia mat and culture filtrate.Filter paper activity (FPA) for total cellulase activity in the culture filtrate was determined according to the method of Mandels *et al.* (1976). One unit of filter paper (FPU) activity was defined as the amount of enzyme releasing 1 µmole of reducing sugar from filter paper per ml per minute. The reducing sugar released was estimated by the dinitrosalicylic acid (DNSA) method (Miller, 1959). The absorbance was read at 540nm with a Jenway 6051 colorimeter.Presence of lignases was carried out by assaying for lignin peroxidase and manganese peroxidase activities. Lignin peroxidase activity was done using the method of Berridge (1955). Manganese peroxidase activity was carried out with the method of Varies *et al.* (1995). The absorbance of sample as well as standard was measured with a Cecil 600 spectrophotometer at a wave length of 485nm after 1 minute.

One unit of lignase activity was expressed as the amount of enzyme which catalysed an absorbance change of 0.01 in 1 minute at a wave length of 485nm.

RESULTS

Fungal isolates: A total of forty isolates were obtained. The compendium of soil fungi (Domsch *et al.*, 1980) and Dr Fungus (www.drfungus.com) was then used for the identification. The fungi isolated were identified to belong to the genus: *Aspergillus*, *Rhizopus*, *Fusarium* and *Trichoderma* while some isolates could not be identified.Thirteen isolates (*Aspergillus flavus***,** *Trichoderma viride*, *Trichoderma sp*, *Rhizopus stolonifer*, *Aspergillus niger*, *Trichoderma viride*, unidentified fungus 3, *Fusarium sp*, *Aspergillus sp*, unidentified fungus 2, *Mucor sp*, Unidentified fungus 1, and *Trichoderma sp* which were representative of the different fungi isolated were used for the assay to determine the enzyme activity.

Cellulase and lignase activity: The cellulase activity of the different fungal isolates per min is shown in Figure 1.The unidentified fungus 2(D_2) had the highest cellulase activity while *Aspergillus niger*(C_7)

had the least cellulase activity. *Rhizopus stolonifer(*B_{17}) had the highest lignin peroxidase activity while Trichoderma viride (C_5) had the least. *Aspergillus sp* (B_{10}), had the highest manganese peroxidase activity while *Mucor sp* (B_{13}) had the least activity. Lignin peroxidase and manganese peroxidase activity per minute of the fungal isolates are as shown in Table 1 and 2.

Table 1: Lignin peroxidase activity of fungal isolates

Isolate	Change in Absorbance	Lignin peroxidase Activity/min (U/min)
C_{10}	0.25	25
A_{12}	0.27	27
B_6	0.31	31
C_5	0.04	04
B_{13}	0.16	16
B_{17}	0.34	34
A_4	0.27	27
C_7	0.21	21
B_{10}	0.17	17
B_{16}	0.24	24
A_9	0.18	18
D_2	0.20	20
C_{11}	0.29	29

KEY: C_{10}: *Aspergillus flavus***,** C_5: *Trichoderma viride,* A_4: *Trichoderma sp,* B_{17}: *Rhizopus stolonifer,* C_7: *Aspergillus niger,* A_9: *Trichoderma viride,* B_{16}: Unidentified fungus 3, B_6: *Fusarium sp,* B_{10}: *Aspergillus sp,* D_2: Unidentified fungus 2, B_{13}: *Mucor sp,* A_{12}: Unidentified fungus 1, C_{11}: *Trichoderma sp*

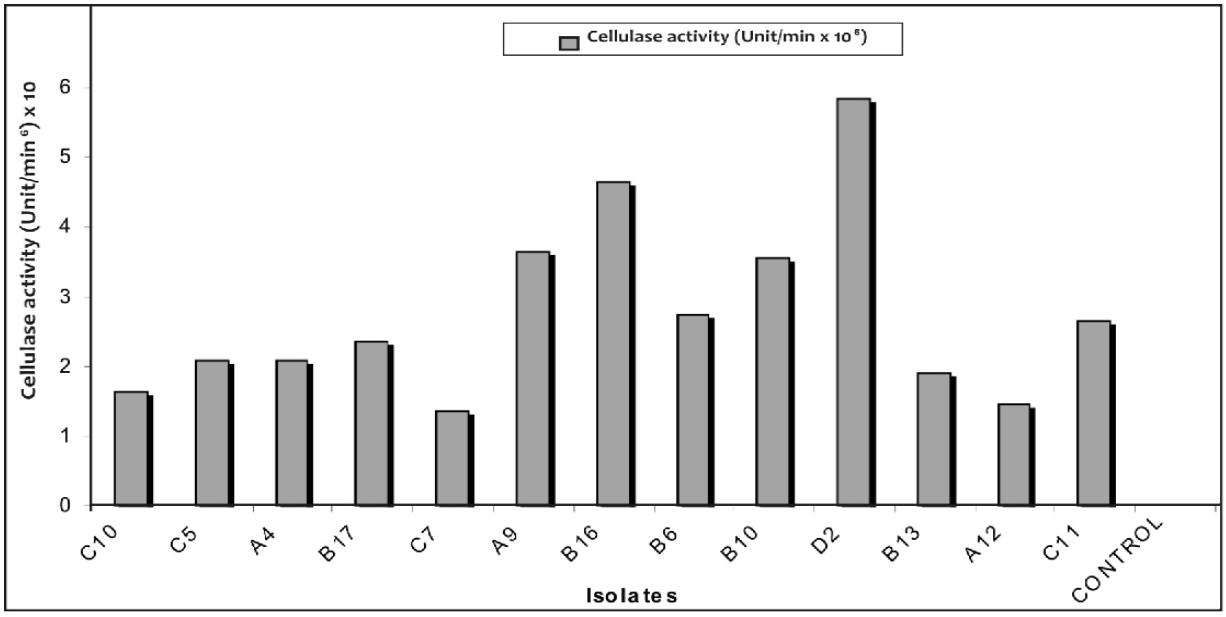

Figure 1. Cellulase activity of different fungi isolated from decaying bamboo leaves

KEY: C_{10} : *Aspergillus flavus***,** C_5: *Trichoderma viride*, A_4: *Trichoderma sp*, B_{17}: *Rhizopus stolonifer*, C_7: *Aspergillus niger*, A_9: *Trichoderma viride*, B_{16}: Unidentified fungus 3, B_6: *Fusarium sp*, B_{10}: *Aspergillus sp*, D_2: Unidentified fungus 2, B_{13}: *Mucor sp*, A_{12}: Unidentified fungus 1, C_{11}: *Trichoderma sp*

Table 2: Manganese peroxidase activity of fungal isolates

Isolate	Change in Absorbance	Manganese peroxidase activity/min (U/min)
C_{10}	0.21	21
A_{12}	0.18	18
B_6	0.10	10
C_5	0.24	24
B_{13}	0.11	11
B_{17}	0.27	27
A_4	0.23	23
C_7	0.19	19
B_{10}	0.30	30
B_{16}	0.16	16
A_9	0.25	25
D_2	0.22	22
C_{11}	0.13	13

KEY: C_{10}: *Aspergillus flavus***,** C_5: *Trichoderma viride,* A_4: *Trichoderma sp,* B_{17}: *Rhizopus stolonifer,* C_7: *Aspergillus niger,* A_9: *Trichoderma viride*, B_{16}: Unidentified fungus 3, B_6: *Fusarium sp,* B_{10}: *Aspergillus sp,* D_2: Unidentified fungus 2, B_{13}*: Mucor sp,* A_{12}: Unidentified fungus 1, C_{11}: *Trichoderma sp*

DISCUSSION

The fungal flora isolated can be divided into aquatic and terrestrial fungi. All the identified fungi are terrestrial fungi, which can also be present in the aquatic environment, while the unidentified fungi are probably aquatic fungi. The presence of typical terrestrial fungi may be connected with the initial stages of the decomposition of the leaves on the soil before being washed into the stream. *Aspergillus sp, Rhizopus sp, Fusarium sp*., and *Trichoderma sp.* were isolated from the decaying bamboo leaves. Schoenlein-Crusius *et al.* (1990) has also reportedly isolated *Mucor sp., Rhizopus sp., Fusarium sp.,* and *Trichoderma sp.* from decaying leaves in water. The fungi isolated were able to colonize these leaves, probably as a result of their saprophytic nature and their possession of an efficient enzymatic system to degrade the tough bamboo leaves which are composed mainly of lignin, cellulose and hemicelluloses (Tomalang *et al.*, 1980). This was revealed by the ability of the organisms to elicit the presence of cellulase and lignase activities. Some of the isolated fungi could not be identified as a result of the absence of spores, in line with Nikolcheva *et al.* (2003), that most of the fungal biomass on decaying leaves consist of mycelia that cannot be identified through ordinary microscopy. The production of cellulase and lignase enzymes by the identified and unidentified fungi leads to the breakdown of leaf litter in streams with the end products being reducing sugars. This is very important in elucidating energy flow and nutrient cycling in streams. These products of leaf degradation in streams serve as a source of energy for aquatic microorganisms which are at the base of aquatic food chain, and also serve as food to other aquatic invertebrates. These aquatic microorganisms therefore occupy a very important ecological niche in the aquatic ecosystem.The presence of cellulase and lignase activity is in agreement with Marvanova (1997), that aquatic hyphomycetes produce amylase, cellulase, pectinase and some other enzymes, and that they probably degrade lignin which is primarily the main source of organic matter in aquatic environment (due to the shedding of leaves and other woody materials into streams).The cellulose activity of the *Aspergillus* spp obtained were higher than that of Narasimha et al (2006) which had *Aspergillus niger* that had cellulase activity of 1.01FPU/min. The *Trichoderma sp*. cellulase activity also was not in agreement with the work of Zaldivar *et al.* (2001), which reported a *Trichoderma aureoviride,* whose cellulase activity did not exceed 1.5 FPU/min. Aquatic hyphomycetes are very important in the breakdown of plant materials in streams. They produce a wide range of enzymes needed for degradation of complex molecules into simpler molecules that can be assimilated by aquatic invertebrates; therefore they play an essential role in the aquatic food chain.

CONCLUSION

This work showed that the aquatic environment is blessed with abundant supply of microorganisms waiting to be explored in various areas such as biodegradation, wastes management, *etc.* Cellulase and lignase-producing aquatic fungi can be explored

in the area of cellulosic and ligno-cellulosic wastes management.

REFERENCES

Alexopoulos, C. J., Mims, C. W. Blackwell, M. 1996**.** *Introductory Mycology*, 4th Edition. John Wiley and sons, Inc. New York.

Barlocher, F. 1985.The role of fungi in the nutrition of stream invertebrates. *BotanyJournal of Linn. Society*. **91**:83-94.

Barlocher, F. 1992. *The Ecology of aquatic hyphomycetes.* Springer-Verlag, Berlin,Germany.

Berridge, N. J. 1955. *Methods in Enzymology* 1. Academic Press pp 185-192.

Chamier, C.A.1985.Cell-wall degrading enzymes of aquatic hyphomycetes: A review. *Botany Journal of Linnean Society* 91:67-81.

Chandrashekar, K. R. and Kaveriappa, K. M. 1991. Production of extra-cellular cellulase by *Lunulospora curvular* and *Flagellospora penicilloides*. Ibid, **33**:55- 58.

Domsch, K. H., Gams, W., Traute-Heidi, 1980. Compendium of Soil Fungi vol**1**. Anderson Academic Press (London) Ltd.

Hasija, S. K. and Singhal, P. K. 1991. Degradation of plant litters by Aquatic Hyphomycetes. In: Arora, D. K., Rai, B., Mukerji, K. G., Knudsen, G. (ed). Handbook of Applied Mycology: soils and plants. Marcel Dekker New York, pp 481- 505.

Ingold, C. T**.** 1975. *An illustrated guide to Aquatic and Water-borne Hyphomycetes* (Fungi Imperfecti) *with notes on their Biology*. Freshwater Biological Association,Ambleside. pp96.
Http// www.drfungus.com

Mandels, M., Andreotii, and Roche, C.1976. Measurement of Saccharilytic cellulase. *Biotechnology and Bioengineering Symposium*. **6**: 21-23.

Marvanova, L. 1997. Freshwater Hyphomycetes: a survey with remarks on tropical taxa. In: Janardhanan, K.K., Rajendran, C., Natarajan, K. and Hawksworth, D L. (Eds). *Tropical Mycology*, Science Publishers Inc.1997. Pp169-226.

Milanez, A. I. 1984. Fungos zoosporicos do Estado de Sao Paulo.II. Chytridiomycetes da regiaooeste. *Rickia* 11**:**115-127.

Miller, G.L. (1959) Use of dinitrosalicylic acid reagent for determination of reducingsugar. *Analytical Chemistry.* 31, 426-428.

Nikolcheva, L. G., Cockshutt, A. and Barlocher, F. 2003. Diversity of freshwater fungi on decaying leaves-comparing traditional and molecular approaches. *Applied and Environmental Microbiology* 69:2548-2554.

Schoenlein-Crusius, I.H., and R.A. Piccolo Grandi, 2003. The diversity of aquatic hyphomycetes in South America. *Brazilian Journal of Microbiology*. 34:183- 193.

Schoenlein-Crusius, I.H., Pires, C.L., Milanez, A.I. (1990). Sucessao fungica em folhasde *Quercus robur* L. (carvalho) submerses em um lago situado no municipio de Itapecericada Serra, SP. *Review of Microbiology*. **21**: 61-67.

Shaw, T.V. and Quejesky, H. 1979. Characterisation of the growth and cellulolytic activities of a *Trichoderma* sp. soil isolate. *Developments in Industrial Microbiology* 20: 553- 562.

Sridhar, K.R. and Barlocher, F. 1997.Water chemistry and sporulation by aquatic hyphomycetes. *Mycology Research* .101:591-596

Suberkropp, K.1992. Interactions with invertebrates, pp118-134. In: F. Barlocher (Ed).The Ecology of aquatic hyphomycetes. Springer-Verlag, Berlin, GermanySuberkropp, K. and Chauvet, E. 1995. Regulation of leaf breakdown by fungi instreams: influences of water chemistry. *Ecology* **57**:1433-1445

Tomalang, F. N., Lopez, A. R., Semara J. A., Casin R. F. and Espiloy, Z. B. 1980. Properties and utilization of Philippine erect bamboo. International Seminar on Bamboo Research in Asia held in Singapore, May 28-30, 1980: Proceedings edited by Lessard G. and Chouinard A. Singapore: International Research Center and theInternational Union of Forestry Research Organization.Pp266-275.

Varies, T., Kalsi, M. and Hataka, A. 1995. Lignin peroxidases, Manganese peroxidases and other Ligninolytic Enzymes produced by *Phlebia radiate* during solid state-state fermentation of Wheat Straw. *Journal of Applied and Environmental Microbiology*: **61**(10): 3515-3520.