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Litter decomposing macrofungi and their lignolytic activity

ANINDYA BISWAS, SATADRU PRAMANIK, DHRUBA DAS AND SUJATA CHAUDHURI
Mycology and Plant Pathology Section, Department of Botany, Kalyani University, Kalyani 741235, Nadia

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Forest litter is an important habitat for several macrofungi including the litter decomposing fungi (LDF). Because LDF include saprophytic macrofungi, nearly all constituents of the litter are open to degradation by these fungi. This work presents a preliminary data on the identification of some litter decomprosing macro-fungi and their lignolytic enzyme activity.

Key words: Litter decomposing macrofungi, humic substances, laccase activity, manganese, peroxidase activity.

INTRODUCTION

Microbial degradation of humic substances and in particular humic acids (HA) is of utmost importance to drive humus turnover that is essential in maintaining the global carbon cycle (Atri *et al.,* 2000). HAs are essential for soil fertility and act as a source of growth promoting substances for plants and other soil organisms (Baldrian,2006). HA degradation has been studied by several workers using lignolytic white rot fungi (WRF) (Bezalel *et al.,* 1997; Blondeau, 1989; Bogan and Lamar, 1996) because of their ability to efficiently degrade lignin, which is one of the main parent materials of HAs (Cooke and Rayner, 1984). However, it remains doubtful whether the WRF are involved in HA degradation in nature, bacause they are mainly restricted to the wood and do not compete well in soil environments (Collins and Dobson, 1997). The ecological group of litter decomposing fungi (LDF) is represented by species closely related to the WRF and inhabits the natural environment of soil and decaying litter (Cox *et al.,* 2001).

LDFs differ from WRF species with respect to their growth substrate, forest litter and soil. Due to the production of lignolytic enzymes, such as laccase and manganese peroxidase (MnP), LDFs oxidize a broad range of substances in the litter. It has been confirmed that both MnP and laccase have equally important role in the degradation process and they are able to depolymerize and mineralize HAs (Dedeyan *et al.,* 2000; Michael *et al.,* 1999). In addition polycyclic aromatic hydrocarbons (PAH), which are ubiquitous environmental pollutants derived from various manmade and natural resources, have been found to be degraded efficiently by WRF (Punnapayak *et al.,* 2008). MnP is implicated as the key enzyme in the degradation process. This, therefore, is the underlying importance and significance of LDFs.

Very little information regarding LDF is available in India. This communication reports few LDFs, among the several collected from the forest litter of Bethuadahari, Nadia district of West Bengal, with significant laccase and MnP activity.

MATERIALS AND METHODS

Collection and identification : Collection was carried out in Bethuadahari forest (0.67 sq. km) of Nadia district in West Bengal during September and October of the running year. Macrofungi growing on dead decaying leaf, woody litter and soil were photographed and collected. The specimens were brought to the laboratory in sealed air-tight polythene bags. Identification was done based on standard keys (Bessey, 1971; Orson *et al.,* 2006; Zoberi; 1976); Frank, 2006).

Media and culture : Attempts were made to obtain cultures of all collected samples. They were grown in modified Potato Dextrose Agar medium. Portions of the hymenial surface were surface sterlized (70% alcohol for 2 minutes), washed thoroughly in sterile water and suspended near the edge on the lid of the agar plates. Plates were incubated in the dark

at 30 ± 10C. In this way tissue cultures were obtained and from these pure cultures were prepared by hyphal tip transfer technique.

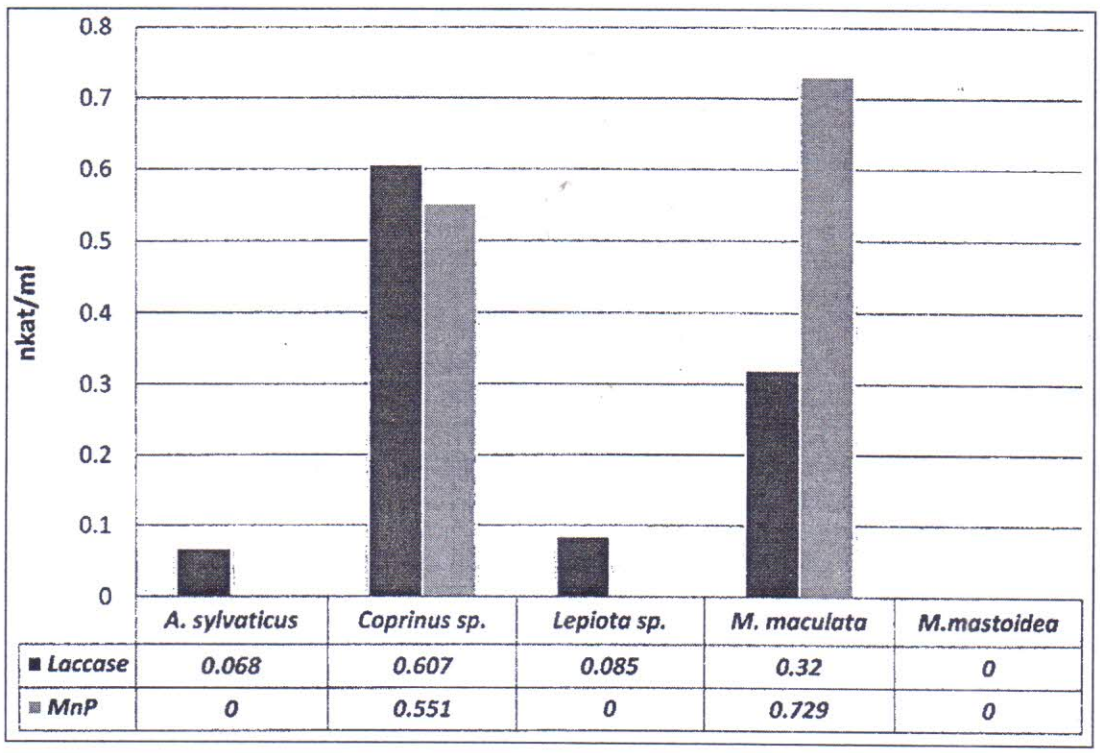
These pure tissue cultures were maintained and regularly sub-cultured. Five macrofungal species, namely *Agaricus sylvaticus* Schaeff, Coprinus sp, *Lepiota* sp. *Mycena maculata* P. Karst. and *Macrolepiota mastoidea* (Fr.) Singer, showing vigorous growth under cultural conditions, were selected for the study.

For enzymatic study, the different fungal species were grown in Kirk Basal Salt (KBS) medium. (Tien and Kirk, 1998), Equal amount of young growing mycelia were transferred to conical flasks containing KBS medium. The flasks were kept in shaker incubator at 28°C for 24 hrs. and then transferred to still incubator at 28°C for different time period.

Enzyme assay : The reaction for laccase assay (Niku-Paavota *et al.,* 1988) was monitored by measuring the change in absorption at 436 nm. MnP assay (Glen and Gold, 1985) reaction was monitored by measuring the change in absorbance at 610 nm, while for LiP activity (Tien and Kirk, 1984) the absorption change was measured at 310 nm. The activity of the different enzymes was expressed in nanokatal/ml (nkat/ml).

RESULTS

None of the five species showed any LiP activity. Out of the five species studied, *M. mastoidea* did not exhibit any lignolytic enzyme activity, while in the other four species, different degrees of laccase and MnP activities were recorded, at different periods of incubation. *Coprinus* sp. and *M. maculata* showed comparatively higher laccase and MnP activities than the other two macrofungi (Fig.1). In the former two species, both these enzyme activity were recorded from the 5th day of incubation and the activity increased on the 7th day. In *Lepiota* sp. only laccase activity could be detected from the 5th day onwards and the degree of activity was lower than that of *Coprinus* sp. and *M. maculata.* In *A. sylvaticus,* no MnP activity was recorded. The detailed enzyme activity of the said species has been presented in Fig.2. However, the laccase enzyme activity was recorded on the 6th day of incubation increased twofold on the 7th day. In *Coprinus* sp. the mean laccase activity was higher than MnP while in *M.maculata* the activity of MnP



	A. sylvaticus	Coprinus sp.	Lepiota sp.	M. maculata	M.mastoidea
Laccase	0.068	0.607	0.085	0.32	0
MnP	0	0.551	0	0.729	0

Fig. 1 : Mean activity of laccase & MnP in different LDF species.

was greater than laccase.

DISCUSSION

Laccase is the most common lignolytic enzyme among the LDF while MnP is produced by only a few (Baldrian, 2006; Steffen *et al.,* 2002). In the present study also only two species were found to produce MnP *in-vitro,*whereas four out of five species were recorded to produce laccase. The MnP producers also showed laccase activity.

However, lower activity of laccase, in comparison to other reports of WRF and LDF species (Coll *et al.,* 1993; Pala' ez *et al.*, 1995; Slomczynski *et al,* 1995; Thurston, 1994) may be due to shorter incubation period (7 days). Most of the other reports on lignolytic enzyme activity have generally been recorded after incubation of 21-28 days in culture. Apart from this, media selection may also play important role in the reduced growth rate and subsequent low production of enzyme.

Since *Coprinus* sp. and *M. maculata* produced both MnP and laccase in culture, they appear to be potentially important LDF candidates which could be further exploited for bioremediation study.

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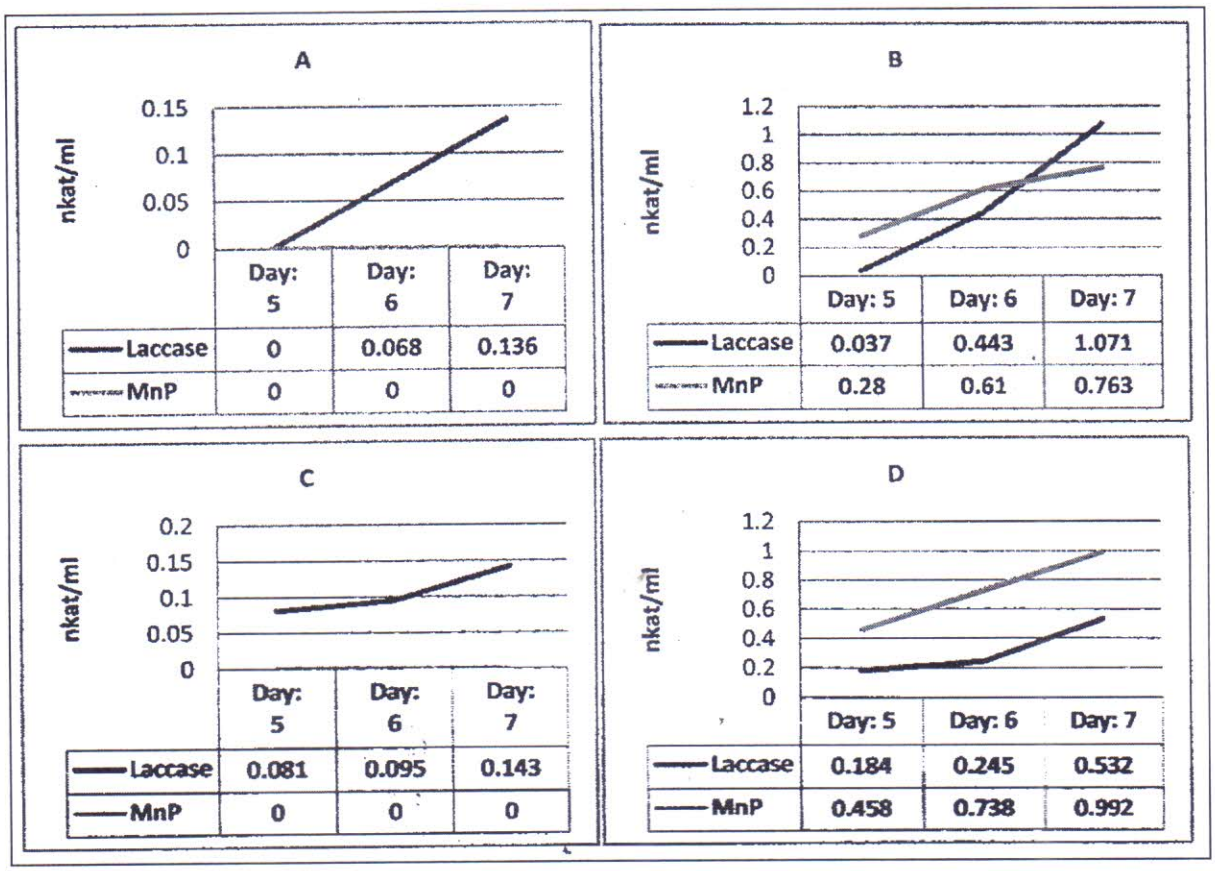

Fig. 2 : Activity of Laccase & MnP at different time Incubation in different LDF species : (A) *A. sylvaticus;* (B) *Coprinus* sp. (C) *Lepiota* sp. and (D) *M. maculata.*

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