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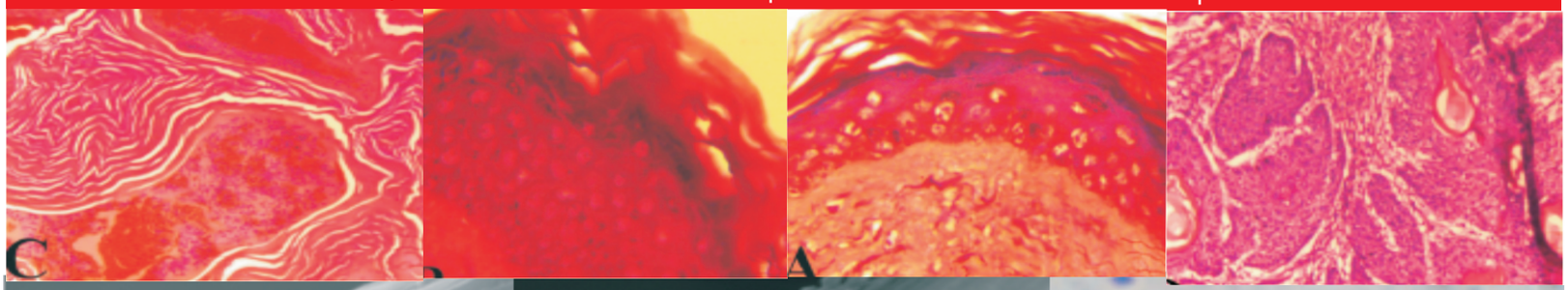

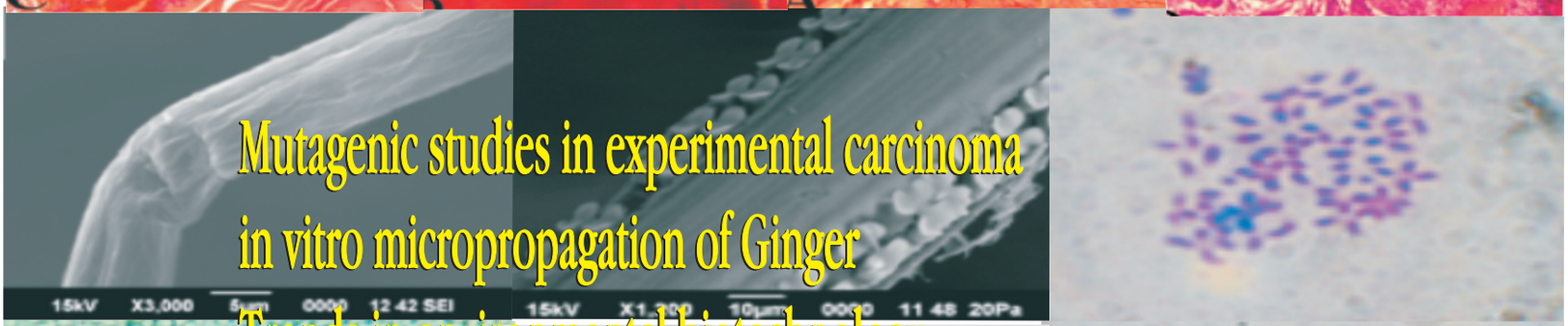

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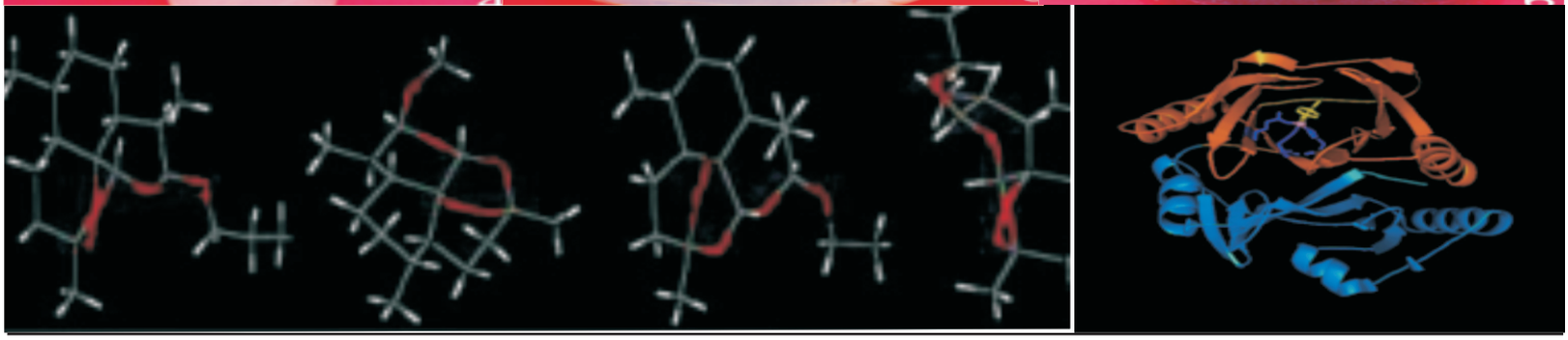

Biopulping of Paddy Straw by *Pleurotus eous*

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Abstract

Biopulping being less energy intensive, inexpensive and causing lesser pollution, can be a viable alternative to chemical and mechanical pulping in paper and pulp industry. In view of shrinking forest reserves, agricultural residues are considered as an alternative raw material for making paper and board. By suitable treatment agriwaste can be converted into substrate for mushroom cultivation. Mushrooms of *Pleurotus sp.* can preferentially remove lignin from agriwaste with limited degradation to cellulose. The present study examines utilization of *Pleurotus eous* for biopulping of paddy straw by solid substrate fermentation. SMS, the mushroom growing medium that results from cultivation process, is a good source of fibre and can be pulped easily. Ligninases present in SMS were able to reduce lignin content to nearly half the initial amount by 21st day of cultivation. Highest cellulose content (% dry weight) was observed on 21st day, while cellulase production commenced from 28th day of cultivation. SEM images revealed that SMS fibres are still associated with non-cellulosic materials when compared to chemically (20% w/v NaOH) extracted fibres.

Keywords: Biopulping; Pleurotus; Spent Mushroom Substrate; Ligninases; Cellulose

Abbreviations: EDS- energy dispersive spectrometer ; LiP- lignin peroxidase; MnP- manganese peroxidase; %- percentage SMS- spent mushroom substrate; SEI- secondary electron image ; w/v- weight per volume; SEM – scanning electron microscope

Introduction

Pulp and paper can be made from lignocellulosic materials such as wood, agricultural residues or from waste paper. Three main types of pulping are mechanical, chemical and biological (biopulping). About 25% of pulp produced in world is created using mechanical pulping method (www.biopulping.com). It is energy-intensive and yields paper with less strength. These disadvantages limit the use of mechanical pulp in several grades of paper. In many cases,chemical pulp is blended with mechanical pulp to add strength. However, chemical pulping is expensive and produces large amounts of both air and water pollutants.Biopulping, the treatment of plant materials with natural wood decaying fungi, has the potential to solve these problems. This process saves substantial amount of electricity, improves paper quality, reduces environmental impact of pulping, and enhances economic competitiveness. Increasing concern over the deterioration of environment due to deforestation has given impetus to technologies aimed at using agricultural residues as an alternative raw material to wood. Rice is a major crop grown worldwide with an annual productivity around 800 million metric tones, corresponding with large production of rice straw. The straw is removed from the field by burning, which is a common practice all over the world. The impact of open field burning of paddy straw on air quality has led to legislation which will help in future to check this practice and save the environment (Wati *et al.,* 2007). Practical utilization of cellulose in paddy straw is limited because of its high lignin content. Fibres from paddy straw are short and weak when compared to wood fibre. On their own, they cannot provide the technical properties demanded by modern pulp and paper manufacturers, nor can they meet the demands of the printing and packaging industries. But straw fibres could supplement wood fibre in some of the less demanding grades of paper and packaging, such as corrugated section of a cardboard box, and when used in small percentages, could be incorporated into higher quality applications. The focus of this study is on the potential utilization of edible mushrooms of *Pleurotus sp*. for biopulping of paddy straw. *Pleurotus sp.* can preferentially remove lignin, with limited degradation to cellulose. Biopulping of paddy straw is described with the help of enzyme assays, chemical analyses and scanning electron microscopy.

Materials and Methods

Spawn of *Pleurotus eous* used in this study was procured from Kerala Agricultural University, Vellanikkara, and Thrissur. The substrate paddy straw was procured locally. Mushroom cultivation was done as per the procedure outlined by Madhusudhanan *et al.* (2003). Mushrooms were harvested from the 18th day for a period of one week. After the harvest, the SMS was incubated for a further period of 70 days at 28˚C and 57% humidity. For studying the fibre characteristics, the SMS was pulverized in a kitchen blender for 30 s, with sufficient water for forming a pulp. The fibre from SMS was compared to "control" fibre got by cooking paddy straw in 20% (w/v) NaOH for 30 min. The alkali was removed by rinsing the fibre in running water to neutral pH and the fibre pulped by pulverizing in a kitchen blender for 30 s. SEM images of the control and SMS fibres were recorded using SEM-EDS in SEI mode, under low vacuum resolution (4 nm). Cellulose content of fibres is estimated by anthrone method (Updegroff, 1969). The amount of acid insoluble lignin

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was estimated by the Klason method (KCL, 1982). Quantitative assays were done for the extra cellular enzymes present in the SMS of P. eous from 7-70th day of cultivation at 7 day intervals. Assays were done at room temperature and at pH 5.4 (pH of the mushroom bed). Filter paper assay was done for the cellulases (Ghose, 1987). One unit of filter paper activity is defined as the quantity of enzyme releasing 1µg of glucose per milliliter of reaction mixture per minute under standard assay conditions. LiP was assayed as per the procedure outlined by Tien and Kirk (1988). One unit of enzyme activity is defined as the amount of enzyme oxidizing 1µmol of substrate per minute. MnP assay was done according to the method of Kuwahara *et al.* (1984). One unit of enzyme activity is defined as the amount of enzyme oxidizing 1µmol of substrate per minute. Laccase was assayed as per the method of Buswell and Odier (1987). One unit of enzyme activity is defined as the amount of enzyme oxidizing 1mmol of substrate per minute. Assays and estimations were done in triplicate.

Results

Paddy straw fibre from SMS was lighter in colour but coarser than that obtained by treating the straw with alkali (control). From the SEM images it is apparent that the control fibre is finer and smoother but the SMS fibre is still associated with distinct phytoliths and lignin globules (Fig. 1). Growth of the mushrooms on paddy straw decreased the content of lignin (as % dry weight) from 20–10% within 21 days of cultivation (Table 1). Further reduction in lignin content to 8% could be achieved by incubating the SMS for 70 days. In comparison, cooking the paddy straw in 20% (w/v) alkali brought about a ten fold reduction in lignin content. In the SMS, the reduction in lignin content was apparently brought about by the activity of laccase and LiP, since the activity of MnP was low during the assay period (Fig. 2). While LiP activity could be detected by 7 days, laccase activity could be detected only after 14 days of incubation. However, of the three ligninases studied, laccase activity was several folds higher than LiP and MnP. Cellulose content (as % dry weight) increased from 10% in untreated paddy straw to 40% in alkali treated (control) and 32.9% in 21 day old SMS (Table 1). In SMS however, further incubation resulted in a decrease in cellulose content. This decrease could be correlated to an increase in cellulase activity from 28–42 days of incubation (Fig. 3).

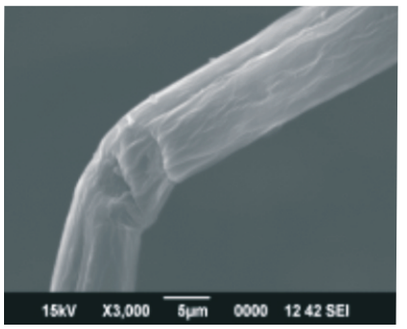

Figure 1a. SEM image of alkali treated control fibre.

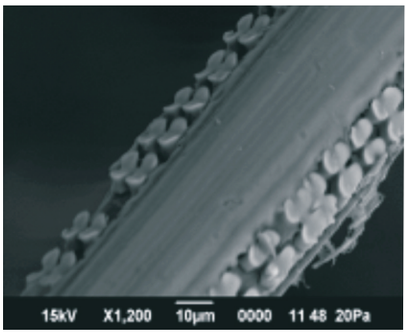

Figure 1b. SEM image of SMS fibre of *Pleurotus eous*

Discussion

Preliminary findings suggest that paddy straw fibres could be extracted from SMS obtained by culturing *P. eous,* obviating the need for chemical treatment for fibre extraction. White-rots of Pleurotus sp. can produce extracellular enzymes to degrade all the major components of plant material i.e. cellulose, hemicelluloses, lignin and pectin at the same time (Eriksson, 1990). However, these enzymes are secreted in different proportions. Many enzymes are involved in the oxidative degradation of lignin; most significant among them are LiP, MnP and laccase. Results of the present work suggest that laccase is the predominating enzyme causing delignification of paddy straw. Cellulose and hemicellulose components of the plant cell walls are intimately associated with the lignin moiety, which presents a barrier to the hydrolyzing enzymes catalyzing the degradation of the polysaccharides. The present study

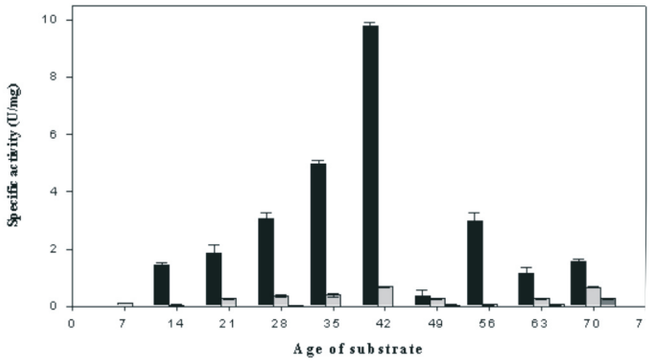

Figure 2. Specific activity of extra cellular ligninases in the SMS of *P. eous*. [Note: Error bars represent ± standard deviation from a triplicate average (Bar 1 - Laccase, Bar 2 - LiP, Bar 3 - MnP)].

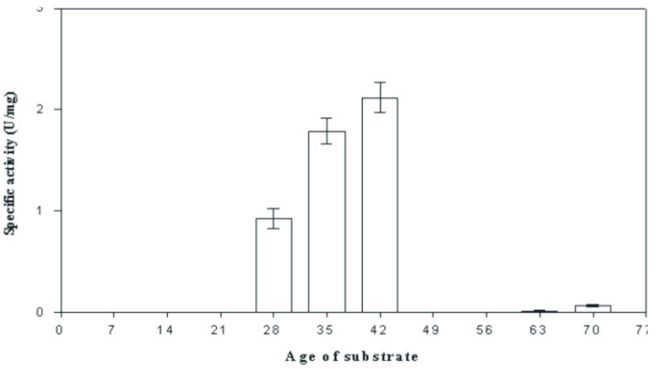

Figure 3. Specific activity of extra cellular Cellulases in the SMS of *P. eous* [Note: Error bars represent ± standard deviation from a triplicate average].

Paddy straw	Age of substrate (days)	Cellulose (%)	Lignin (%)
Untreated		10.04 ± 0.06	20.34 ± 0.43
Alkali treated		40.08 ± 0.00	2.38 ± 0.34
SMS	21	32.92 ± 0.62	10.91 ± 0.41
SMS	49	22.80 ± 0.45	8.75 ± 0.74
SMS	70	19.36 ± 1.24	8.52 ± 0.11

Table 1. Cellulose and lignin content (in % dry weight) in the substrate of *P. eous*

indicates that the ligninases produced by *P. eous* are capable of degrading the polymers of lignin, as the lignin content in the 21 day old SMS was half that in untreated paddy straw. Conventional cultivation practice is to harvest 2-3 flushes of mushrooms by the 24th day, after which the SMS is discarded. Based on the enzyme profile, it appears that cellulose rich fibres could be obtained till the 28th day, as subsequently degradation of cellulose could occur.

Conclusion

Industrial application of biopulping can save chemicals and augment pulp quality. In addition to being entirely ecofriendly, the process described here assures double benefit for the mushroom cultivators – production of edible/medicinal nutritious mushrooms and fibre from the SMS. Also, the process offers a viable solution for solid waste management of spent mushroom substrate.

Acknowledgement

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