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Biodelignification of wheat straw by different fungal associations

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Abstract

Seven strains of fungi were tested individually as well as in different combinations to determine their lignin degrading ability using wheat straw as natural substrate. When tested individually *Phanerochaete chrysosporium* caused a maximum loss in total organic matter (26.45%) as well as in the lignin component (28.93%). The associations between different groups: white-rot plus white-rot, white-rot plus brown-rot and white-rot plus soft-rot fungi revealed that in certain combinations the ligninolysis was enhanced to variable extent. *Deadalea flavida* plus *P. chrysosporium* was the best association to bring about a lignin loss of 36.27%.

Introduction

The importance of biological delignification has been amply demonstrated frequently in various fields of biotechnology. Fungi hold a great potential in the paper industry to delignify wood chips, not only saving chemical investment but reducing pollution hazards (Eriksson & Vallander 1982; Kirkpatrick et al. 1989). Ligninolytic strains not only prevent pollution but have also been used for the bioconversion of industrial effluents into useful energy yielding chemicals (Eaton et al. 1980; Hammel 1989). With the removal of lignin barrier cellulose becomes easily accessible for bioconversion. Delignification of forage crop residues enhances their digestibility and also improves their nutritive value (Zadrazil & Brunnert 1980; Reid 1989).

White-rot fungi are most important in delignification and of these *Phanerochaete chrysosporium* has attracted the maximum interest of various workers. Brown-rot and soft-rot fungi, though they degrade/modify lignin, but to a limited extent only because they lack the complete array of ligninolytic enzymes (Kirk & Highley 1973; Eslyn et al. 1975). Most studies have been carried out by using monocultures though a few workers have shown that synthetic lignins or their model compounds are best degraded by consortia (Sundman & Nase 1972; Federle & Vestal 1980). The present investigation reports the degradative ability of certain white-rot, brown-rot and soft-rot fungi individually and in association with each other during semi-solid degradation of wheat straw.

Materials and methods

Fungi

The fungal strains were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh and maintained at 4° C on yeast glucose agar medium. *P. chrysosporium* BKM-F-1767 white-rot fungus was, however, received as a gift from Prof. Jeffries, USDA, USA. The fungi belonged to three different physiological groups: white-rot (WR), brown-rot (BR) and soft-rot (SR) fungi. These included *Daedalea flavida* (WR, MTCC 145) *Pyncnoporus sanguineus* (WR, MTCC 137) *Polyporus palustris* (BR, MTCC 169) *Postia placenta* (BR, MTCC 144) *Chaetomium globosum* (SR, MTCC 155) and *Fusarium moniliforme* (SR, MTCC 156).

Substrate

Wheat straw used as a natural substrate was obtained from the local market. It was chopped to fine fibres of 0.5–2.0 cm length and was air dried after thoroughly washing with running water.

Experimental set-up

The fungi were tested for their ability to degrade wheat straw as determined by loss in total organic matter, and loss in the lignin component. The degradation was carried out using individual cultures and in different combinations as described in the text.

Two grams (dry weight) of substrate was placed in 250 ml conical flasks. Triplicate flasks were kept for each organism or the combination of organisms. The substrate was moistened with 10 ml of 0.5% malt extract (pH 4.8–5.0) and sterilized by autoclaving at 121° C for 15 min. Each flask was inoculated with desired fungus/fungi. Two fungal discs (8 mm diam) of the inoculum grown for 7 days on yeast glucose agar plates were added for monocultures and two discs of each fungus were added for mixed cultures. These were then incubated at 25° C as stationary cultures. Blank agar discs were used in the control. The flasks were removed after 30 days of incubation and processed by adding 10 ml of acetate buffer (10 mM pH 5.0). The flasks were put on a rotary shaker for 20 min. The contents were filtered through preweighed Whatman filter paper No. 1. Residual biomass collected on filter paper was dried at 80° C to constant weight. Loss in total organic matter was calculated from the difference in weight between the blank and the inoculated flask. Lignin was estimated according to Kirk & Obst (1988). Percent lignin loss is based on the difference in amount of lignin in the blank and the inoculated substrate.

Results and discussion

The different fungi grew luxuriantly on wheat straw in both monocultures and mixed cultures except for *P. chrysosporium* which apparently suppressed the growth of fungi growing in its presence to a variable extent. Only a slight growth, varying from 12–22 mg was observed when different fungi were grown individually on 10 ml of 0.5% malt extract alone.

White-rot fungi were the best degraders in terms of both total weight loss and lignin loss when tested individually. *P. chrysosporium* was the most efficient giving a total weight loss of 26.45% and a lignin loss of 28.93% (Table 1). The brown-rot fungus *P. palustris* and the soft-rot fungi *C. globosum* and *F. moniliforme* were moderate decomposers (Table 1). *Postia placenta* was the poorest degrader and caused a total weight loss of 1.48% without any loss in lignin. The above observations are in agreement with other reports (Kirk & Farrell 1987; Reid 1989). The white-rot fungi used in the present studies seems to be better decomposers than a much studied ligninolytic strain of *C. versicolor* (Reid 1989) which gave a lignin loss of only 13.63% and a weight loss of 17.33% in wheat straw during the same period (Arora & Sandhu 1986).

Table 1. Degradation of wheat straw by fungal monocultures.

Fungi	Loss in total organic matter (% ± SE)	Lignin loss (% ± SE)
D. flavida	17.45 ± 1.05	16.67 ± 1.56
P. chrysosporium	26.45 ± 2.00	28.93 ± 1.43
P. sanguineus	18.61 ± 1.50	17.16 ± 1.25
P. palustris	12.10 ± 0.95	5.80 ± 1.00
P. placenta	1.48 ± 0.35	0.00
C. globosum	8.12 ± 1.10	7.32 ± 1.00
F. moniliforme	6.91 ± 0.80	5.87 ± 0.75

Apparently the association of various fungi resulted in some enhancement in total weight loss in a few combinations and decline in certain other combinations (Table 2). However, the enhancement was statistically significant ($p = 0.05$) in some of the combinations viz. *P. palustris* plus *P. chrysosporium, C. globosum* plus *D. flavida, F. moniliforme* plus *P. chrysosporium* in comparison to the weight loss caused by the former organisms alone. Similarly, the enhanced weight loss caused by *P. placenta* in association with all the three white-rot fungi was statistically significant in comparison to that caused by it as a monoculture. The maximum weight loss (30.25%) was caused by *P. chrysosporium* plus *F. moniliforme* (Table 2) which was statistically significant at p value of 0.05 in comparison to *F. moniliforme* alone. On the other hand statistically significant suppression in total weight loss was observed only in two cases *D. flavida* plus *P. palustris* and *P. sanguineus* plus *F. moniliforme* wherein the total weight loss caused by these associations was lower than that caused by the former partner as monoculture. The remaining values

Table 2. Degradation of wheat straw by various fungal combinations

Fungal combination	Loss in total organic matter (% ± SE)	Lignin loss % ± SE
White rot + white rot		
D. flavida + *P. chrysosporium*	24.69 ± 1.80	36.27 ± 2.10
D. flavida + *P. sanguineus*	25.43 ± 1.95	29.40 ± 1.80
P. chrysosporium + *P. sanguineus*	24.97 ± 1.60	31.83 ± 2.20
White rot + brown rot		
D. flavida + *P. placenta*	17.57 ± 1.25	19.09 ± 2.0
D. flavida + *P. palustris*	9.65 ± 1.10	13.61 ± 1.50
P. sanguineus + *P. placenta*	19.45 ± 1.40	18.63 ± 1.64
P. sanguineus + *P. palustris*	14.90 ± 1.35	17.56 ± 1.49
P. chrysosporium + *P. placenta*	28.85 ± 2.10	29.40 ± 2.50
P. chrysosporium + *P. palustris*	29.88 ± 1.80	28.90 ± 1.82
White rot + soft rot		
D. flavida + *F. moniliforme*	13.90 ± 1.25	13.15 ± 1.20
D. flavida + *C. globosum*	20.08 ± 1.60	20.57 ± 1.72
P. sanguineus + *F. moniliforme*	5.53 ± 0.45	13.23 ± 1.10
P. sanguineus + *C. globosum*	13.09 ± 1.15	13.70 ± 1.24
P. chrysosporium + *F. moniliforme*	30.25 ± 1.20	28.40 ± 1.48
P. chrysosporium + *C. globosum*	13.09 ± 1.15	32.83 ± 1.50

observed for total weight loss as caused by various associations were statistically insignificant.

Variable responses were observed in the extent of ligninolysis in different combinations tested. White-rot plus white-rot group was the best. The combination *D. flavida* plus *P. chrysosporium* gave the maximum lignin loss 36.27% (Table 2) which was statistically significant ($p = 0.05$) when compared to that of lignin loss as caused by *D. flavida* alone 17.45 ± 1.05% but not significant when compared with that of *P. chrysosporium* alone. The degree of enhancement was better observed in *D. flavida* plus *P. sanguineus* (31.83%), individually they caused a loss of about 17% each. The enhancement in ligninolysis thus observed in this case is statistically significant when compared to the lignin loss as caused by *D. flavida* or *P. sanguineus* alone.

The lignin loss as caused by the association between different soft-rots plus white-rots and brown-rots plus white-rots was significantly higher ($p = 0.05$) in comparison to the lignin loss caused by different brown-rot and soft-rot fungi individually. The enhancement or suppression as caused by the remaining associations was not so significant. No significant enhancement was observed when two brown-rot fungi were grown together (Arora & Hooda, 1992).

The studies corroborate the earlier findings of Sundman & Nase (1972) where lignin degradation was better in mixed cultures. However, their studies pertained to lignin models and were of qualitative nature only. The use of mixed bacterial cultures for better degradation of lignin has also been reported earlier (Crawford & Crawford 1976; Martin & Haider 1981). These studies show that certain associations, particularly between white-rot fungi, can be useful in enhancing the ligninolysis.

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