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The Effect of Growth Conditions and Genetic Background on Laccase Production by the Fungus *Pleurotus ostreatus**

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ABSTRACT. The measurement of growth rate of three isolates – P14, P15 and P19 (obtained after regeneration of protoplasts of the control strain) with significantly higher (P14, P19) or lower (P15) activity of laccase than the control strain (*P. ostreatus* florida F6) revealed similar patterns for P14 and P19; P15 had a higher growth rate and the highest rate was observed in the control strain. The rate of mineralization of ^{14}C-lignin on SSF (perlite) by strains F6 and P15 was almost the same and it was higher than that of P14 and P19 strains. The enzyme pattern of the individual isolates, studied by gel electrophoresis and activity staining of laccase, did not show any difference in isozyme patterns of the isolates, but there were differences in the level of gene expression.

Recently, great effort has been made to understand the biochemistry and genetics of the lignin degradative system. In nature the white-rot fungi (*Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *Lentinus edodes etc.*) are primarily responsible for lignin biodegradation. They can degrade lignin more extensively and rapidly than any other known group of organisms (Eriksson *et al.* 1990). Under ligninolytic conditions, these fungi produce two different types of extracellular lignin-modifying enzymes: phenol oxidases (laccase) and peroxidases (lignin peroxidase, Mn-dependent lignin peroxidase). This paper deals with the effect of growth conditions and genetic background on laccase production in the fungus *P. ostreatus*.

MATERIAL AND METHODS

Fungal strains and cultivation. Pleurotus ostreatus florida F6 (wild type control) from the *Hebrew University of Jerusalem* and *P. ostreatus* isolates P14, P15 and P19 (prepared by protoplasting from strain F6 by the authors) were used. The isolates were grown in BSM medium on perlite using a solid state fermentation (SSF) method (Kerem and Hadar 1993). BSM medium contained (g/L): glucose 5.0, asparagin 0.6, K_2HPO_4 1.0, KCl 0.5, yeast extract 0.5, $MgSO_4 \cdot 7H_2O$ 0.5, $FeSO_4$ 0.01, $Mn(CH_3COO)_2$ 0.008, $ZnNO_3$ 0.003, $Ca(NO_3)_2 \cdot 4H_2O$ 0.006 and $CuSO_4 \cdot 5H_2O$ 0.003; pH 5.5. Kirk's medium was prepared according to Tien and Kirk (1988). The growth rate was measured as the respiratory rate (release of accumulated CO_2 was estimated by gas chromatograph).

Lignin biodegradation. The rate and level of mineralization of ^{14}C-lignin was taken as a criterion of the lignin biodegradation process. The solid-state fermentation system was based on perlite inoculated with homogenized fungal mycelium with addition of ^{14}C-lignin. The overall mineralization took place for 65 d and the released $^{14}CO_2$ was measured.

Gel electrophoresis. The enzyme pattern of the individual isolates was studied using gel electrophoresis (Laemmli 1976) and activity staining of laccase. The electrophoresis was run on gel (12 % of non-denaturing PAGE) with applied protein samples (20 μg) from each isolate. The gel was then stained with 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) to detect laccase activity.

Enzyme assay. Activity of laccase was determined spectrophotometrically according to Bourbonnais and Paice (1990) by monitoring the oxidation of ABTS, *i.e.* the absorbance increase at 425 nm. The cultures were grown on BSM or Kirk's medium.

RESULTS AND DISCUSSION

Continuing our previous study on the effect of inoculation and concentration of nitrogen in the culture medium on the activity of ligninolytic enzymes (Voláková *et al.* 1996), we followed the effect of growth conditions and genetic background on laccase production in three isolates of *P. ostreatus* – P14,

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P15 and P19 (obtained after regeneration of protoplasts of the control strain) with significantly higher (P14 – 352 %, P19 – 555 %) or lower (P15 – 34 %) activity of laccase than the control strain (*P. ostreatus* florida F6).

The growth rate of fungi was measured using their respiratory rate (accumulation of CO_2). The results revealed similar growth patterns for P14 and P19 isolates; on the other hand, the growth rate of P15 was higher and the highest growth rate was observed in the control *P. ostreatus* strain F6 (Fig. 1).

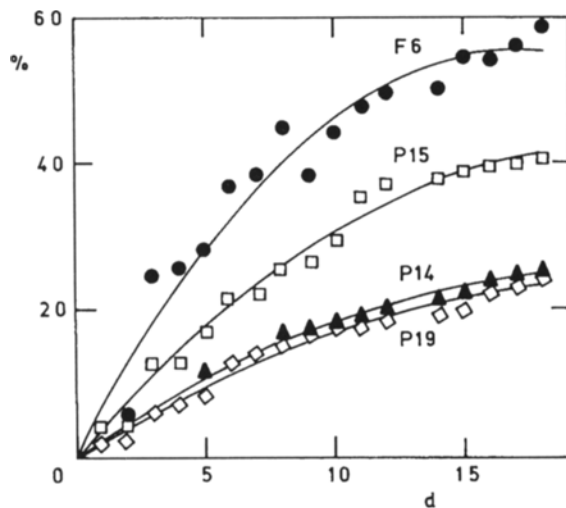

Fig. 1. Accumulated CO_2 release (%) by *Pleurotus ostreatus* strains F6, P14, P15 and P19 during the SSF cultivation in 500-mL flasks on perlite.

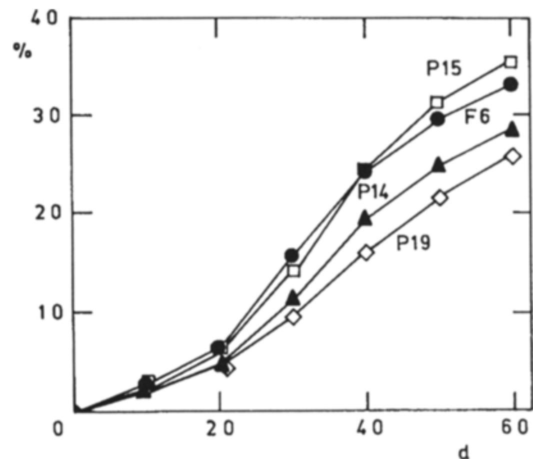

Fig. 2. Mineralization of ^{14}C-lignin during SSF by *Pleurotus ostreatus* strains F6, P14, P15 and P19 grown on perlite. The data are plotted as percent of initial amount of ^{14}C.

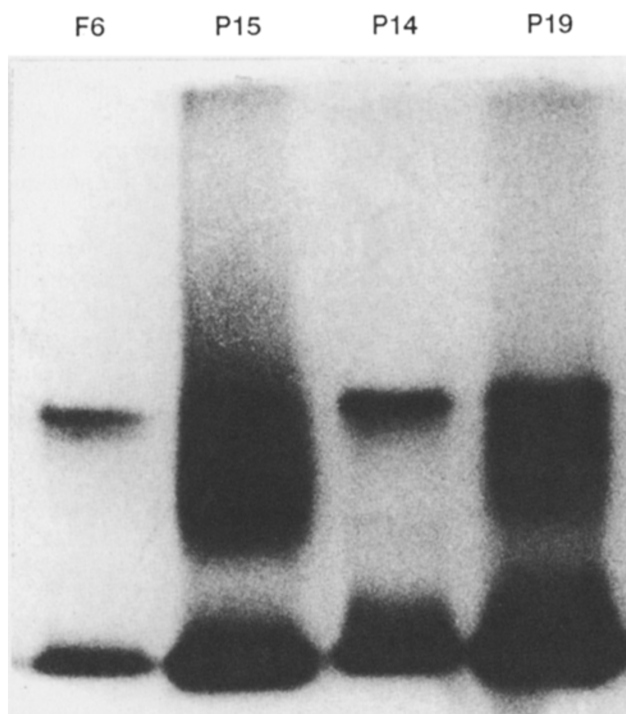

Fig. 3. Enzyme patterns of *Pleurotus ostreatus* strains F6, P14, P15 and P19 on the gel (12 % nondenaturing PAGE, 20 μg protein of each strain was loaded) stained with ABTS (detection of laccase).

Mineralization of ^{14}C-lignin on SSF (perlite) was measured using the amount of the released $^{14}CO_2$. Fig. 2 shows that the mineralization rates of strains F6 and P15 were almost the same and were higher than those of the remaining two isolates P14 and P19.

The enzyme pattern of the individual isolates was estimated using gel electrophoresis and activity staining of laccase. No difference was found in isozyme patterns of the isolates, but it was found in the level of gene expression (Fig. 3).

Table I. Induction of laccase activity by addition of different inducers[a] to fungal cultures of various age in percent activity without inducer

Strain	Lignin			Cotton extract			2,5-Xylidine	
	5th day	9th day	20th day	4th day	9th day	13th day	5th day	9th day
F6	567	156	375	2000	2200	200	0	1300
P15	350	400	650	117	475	0	433	118[b]

[a]Soluble lignin – 100 mg per flask, cotton straw extract in ethyl acetate – 21 mg/mL, or 2,5-xylidine – 2 mmol/L.
[b]10th day.

More detailed studies were performed with isolate P15 and control strain F6. Addition of different inducers – soluble lignin, CSE (cotton straw extract) in ethyl acetate and 2,5-xylidine – was tested for the influence on the laccase formation in *P. ostreatus* strains F6 and P15 (Table I). Again it was confirmed that these strains do not differ in the isoenzyme patterns but in the level of the gene expression of the enzymes. All the inducers were able to increase the laccase production in *P. ostreatus* strains F6 and P15. The addition of lignin induced laccase activity whenever it was applied.

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