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THE GROWTH OF *PLEUROTUS OSTREATUS* ON LIGNOCELLULOSIC MATERIALS

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CRECIMIENTO DE *PLEUROTUS OSTREATUS* SOBRE MATERIALES LIGNOCELULOSICOS

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SUMMARY

The cultivation of edible xylogenous fungi is a process of biodegradation of lignocellulosic wastes with production of fruiting bodies, animal feed and/or other useful substances such as hormones and enzymes. This process can be optimized by understanding the metabolism of the species and special features of the substrate. Biochemical changes during the growth of *Pleurotus ostreatus* strains on different lignocellulosic materials are described, as well as the morphological characters of mycelial growth on these substrates. These results may be used for screening prospective strains of *P. ostreatus* with the aim of biodegradation of wastes and monitoring of the cultivation process.

Key words: *Pleurotus ostreatus*, biodegradation, lignocellulosic substrates, cellulose, lignin, enzyme activity.

RESUMEN

El cultivo de hongos comestibles xilógenos es un proceso de biodegradación de los desechos lignocelulósicos, con producción de cuerpos fructíferos, alimento animal y otras substancias útiles como las hormonas y enzimas. Este proceso puede optimi-

zarse si se tiene un conocimiento profundo del metabolismo del organismo estudiado, así como de las características especiales del substrato. Se describen los cambios bioquímicos durante el crecimiento de cepas de *Pleurotus ostreatus* sobre diferentes materiales lignocelulósicos y los caracteres morfológicos del crecimiento micelial sobre dichos substratos. Estos resultados pueden ser utilizados en la selección de cepas potenciales de *P. ostreatus* para la biodegradación de desechos y monitoreo del proceso de cultivo.

Palabras clave: *Pleurotus ostreatus*, biodegradación, substratos lignocelulósicos, celulosa, lignina, actividad enzimática.

INTRODUCTION

Pleurotus ostreatus is a prospective source of valuable food protein (mycelium and fruiting bodies), and an organism performing effective bioconversion of different lignocellulosic materials (Zadrazil, 1978; Bis'ko and Dudka, 1987). The aim of this work was to study morphological and biochemical aspects of *P. ostreatus* strains growing on three types of lignocellulosic material (wheat straw, flax residue and sunflower residue).

MATERIALS AND METHODS

The studied strains of *Pleurotus ostreatus* (Jacq. ex Fr.)Kumm. were obtained from the culture collection of the N. G. Kholodny Institute of Botany, Kiev. The strain 483 was isolated from fruiting bodies collected from *Fagus sylvatica* L., in the Transcarpathian district, Ukraine, in 1976; strain 517 was isolated from fruiting bodies collected from *F. sylvatica* L., in the Lvov district, Ukraine, in 1977; strain 518 was isolated from fruiting bodies collected from *F. sylvatica* L., in the Krasnodon Territory, RSFSR, in 1977.

The substrates used in this study (wheat straw, flax residue and sunflower residue) were reduced to fragments of 15-20 mm, moistened with tap water up to 75 %, and autoclaved for 40 min at 200°C in glass flasks. The sterile substrates were inoculated in four replicates with a lump of mycelium taken from the surface of beer wort agar cultures. Uninoculated substrates were used as control. The flasks were closed with a cotton plug and the cultures grown at 28°C. The growth phase took 14-16 days for all substrates. Fructification was observed at 15-18°C under laboratory illumination. The length of

fructification was assessed to be of two months, after initiation of fruiting conditions. Strains fruited three times during this period. Samples of the substrate, after 14-16 days of mycelial growth and after fruiting (60 days), were dried to a constant weight and the losses determined. The content of ash was determined in the substrates pre-inoculation, following mycelial growth and after fructification (Pleshkov, 1976). The content of total nitrogen according to Kjeldahl's method (Pleshkov, 1976), cellulose (Kurshner and Hanek, 1974), and lignin (Obolenskaja *et al.*, 1955) were also determined during the three periods listed above. Content of crude protein was calculated as total nitrogen x 6.25.

After mycelial growth or fruiting body formation the composition of substances were determined by the following formula: $\frac{Ax100}{100+B}$

where A= Content of cellulose, lignin or N.
B= Losses of substrate weight after mycelial growth or fruiting.

For determination of extracellular enzyme activity, 50 ml Erlenmeyer flasks were filled with 10 ml of the following medium: KH_2PO_4 (0.06%), $K_2HPO_4.3H_2O$ (0.04%), $MgSO_4$ (0.05%), $NH_4H_2PO_4$ (0.20%), 1% crushed sunflower residue (1-2 mm), distilled water to 100 ml. After sterilization during 30 minutes at 121°C, the Erlenmeyer flasks were inoculated with mycelium disks taken from 7 day old cultures and grown on wort agar medium. The flasks were incubated at 28°C during 3 and 7 days.

Extracellular enzyme activity was determined in the liquid medium. The activity of monophenol-monooxygenase (E.C. 1.14.18.1; mol min^{-1} ml^{-1}), was estimated by the initial rate of benzodin oxidation using the spectrophotometric method (Semichaevskii *et al.*, 1984). The activity of endo-1,4-β–glucanase (E.C. 3.2.1.4; % min^{-1} ml^{-1}) was estimated by the initial rate of viscosity decrease in 0.3% solution of sodium salt of carboxy-methyl cellulose (CMC). The ability of the cellulase complex to saccharify CMC (CMC-activity) and filter paper (FP-activity) was estimated as final glucose equivalent (Rodionova *et al.*, 1966). The mycelial growth of *P. ostreatus* on the substrates was studied by the scanning electron microscopy (SEM). Samples of each substrate with mycelium of *P. ostreatus* were prepared by vapor fixing (2% OsO_4 for 48 h), coated with gold and examined using a JEOL JSM 35-C Scanning Electron Microscope.

Table 1. Changes in substrate composition during mycelial growth of *Pleurotus ostreatus* strains on different lignocellulosic materials.

Substrate	Strain	Indices (% A.D.M.)				Losses (%)	
		Ash	CP	C	L	C	L
Before inoculation							
Wheat straw		2.6	1.2	44.9	27.3	0.0	0.0
Flax residue		1.6	2.6	30.9	39.4	0.0	0.0
Sunflower residue		2.2	4.6	31.2	26.6	0.0	0.0
After mycelial growth							
Wheat straw	518	2.9	1.4	42.5	26.5	3.3	2.9
	483	3.4	1.6	43.7	26.9	2.7	1.5
	517	3.2	1.3	43.9	26.8	2.2	1.8
Flax residue	518	3.3	3.8	29.8	38.0	3.6	3.2
	483	2.0	4.1	29.2	38.2	5.5	3.0
	517	2.1	2.7	28.3	38.4	7.4	2.5
Sunflower residue	518	4.4	5.8	28.7	25.0	8.0	6.0
	483	4.3	5.5	29.1	25.1	6.7	5.6
	517	4.4	8.3	29.2	25.3	6.4	4.9
After fruiting							
Wheat straw	518	3.2	3.0	38.2	23.1	14.9	15.4
	483	3.2	3.0	39.4	23.5	12.2	13.9
	517	3.0	2.6	39.8	23.7	11.4	13.2
Flax residue	518	3.6	4.3	27.1	36.0	12.3	8.6
	483	3.4	4.6	27.4	36.3	11.3	7.9
	517	3.5	3.1	27.9	36.6	9.7	7.1
Sunflower residue	518	6.2	5.1	27.0	20.2	13.5	24.1
	483	6.1	5.2	27.6	21.0	11.5	21.1
	517	5.3	5.9	27.8	20.8	10.9	21.8

A.D.M.= Absolute dry matter.
C= Cellulose.
L= Lignin.
CP= Crude protein (Nx6.25).

RESULTS AND DISCUSSION

Analysis of data given in Table 1 showed that the process of cellulose degradation predominates when compared to lignin, and this was characteristic of the mycelial growth stage of all the studied strains. At the same time, the strains of *P. ostreatus* differed in the utilization of lignin and cellulose in this period. The weight loss of these substances is almost equal for the strain 517 grown on wheat straw and the strain 518 grown on flax residue. However, in the strain 517, the quantity of cellulose utilized from flax residue was almost 3 times higher compared to lignin (Table 1). The degree of cellulose or lignin degradation by *P. ostreatus* depends on many factors, such as enzyme activity, substrate composition, strain genotype and morphogenesis stage (Danilyak *et al.*, 1989). The data obtained in this work show phenotype variability, which characterizes cellulose and lignin degradation by the studied strains. Similar results also showed that the mycelium of *P. ostreatus* strains grown on wood substrates utilizes cellulose more rapidly than lignin (Bis'ko *et al.*, 1983, 1984, 1986).

Total nitrogen (N), from which the quantity of crude protein was calculated, includes N as a constituent part of chitin too. Nevertheless, N from chitin constitutes about 5% of the dry matter or 7% of the total N in *P. ostreatus* (unpublished data). In the process of mycelial growth, the content of crude protein is increased (Table 1), concurrently with degradation of substrate main components (cellulose and lignin). In sterile substrates, this can be explained by the metabolic activity of growing mycelium, and as a result part of the substrate is decomposed in CO_2 and H_2O (Zadrazil, 1975). Accordingly the relative content of total nitrogen in a substrate increases. It should be noted that the accumulation of crude protein was the highest on flax residue in all trial strains. Higher values of cellulose and lignin losses testify to the effective utilization of this substrate. In these experiments, an increase of crude protein of 12-33% was observed on wheat straw, 4-58% on flax residue, and 20-80% on sunflower residue, if compared with the content in substrates before inoculation. In spite of translocation of nitrogen containing substances to the fruiting bodies, the quantity of crude protein in the spent substrate is observed to be higher than before inoculation.

Losses of cellulose during fruiting, as well as at the mycelial growth stage, are higher than those of lignin for all studied strains of *P. ostreatus*. After fruiting, wheat straw losses of lignin are approximately equal, and in case of

Table 2. Activity of extracellular enzymes in *Pleurotus ostreatus* strains during mycelial growth on sunflower residue.

Strain		A		B		CMC-activity		FP-activity	
	Day	3th	7th	3th	7th	3th	7th	3th	7th
483		0.0	224.0	0.0	177.3	0.0	0.09	0.0	0.0
517		22.0	101.8	94.8	99.9	0.01	0.06	0.05	0.05
518		26.0	70.0	0.0	144.0	0.07	0.35	0.09	0.0

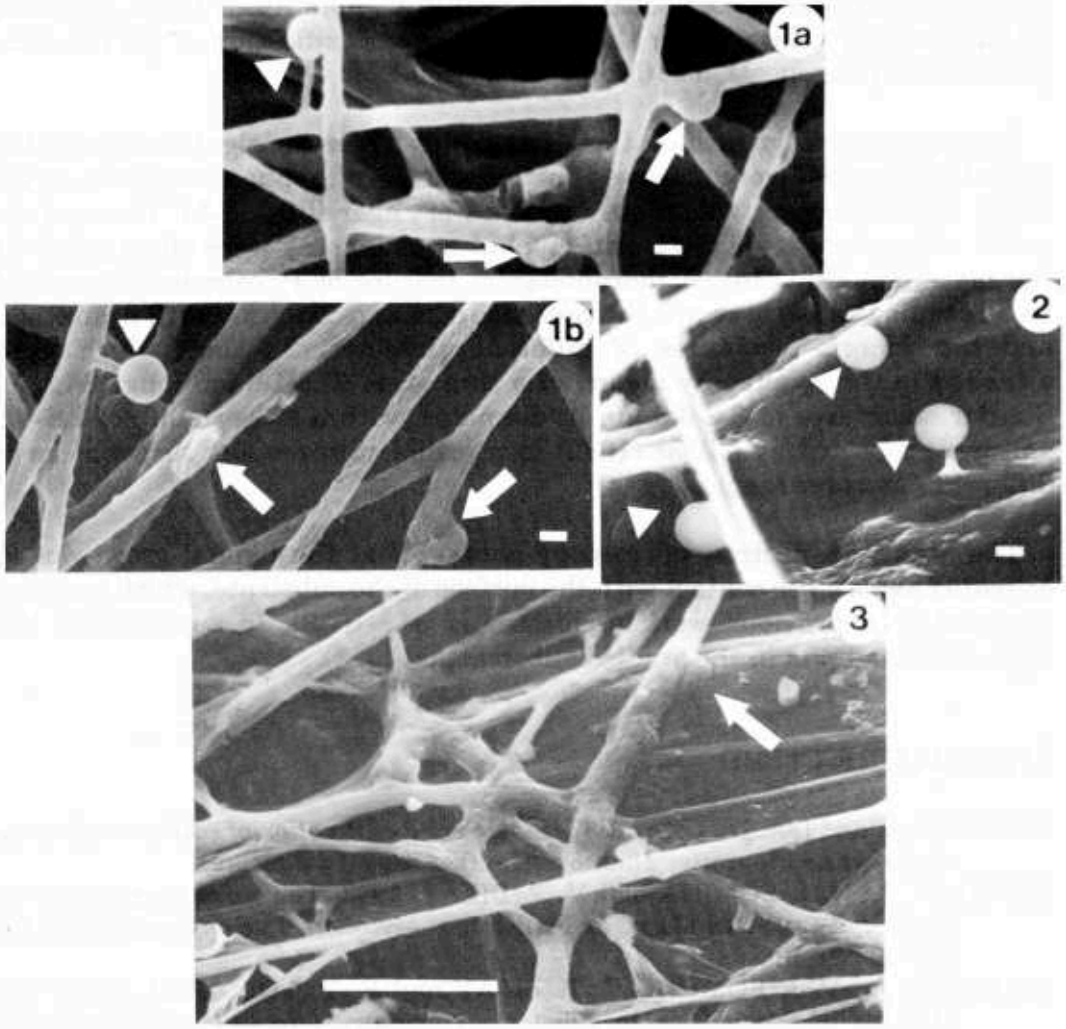
A= Monophenol-monooxygenase x 10^3 (mol min^{-1} ml^{-1}).
B= Endo-1,4-β-glucanase (% min^{-1} ml^{-1}).
CMC= Carboxy-methyl cellulase (% min^{-1} ml^{-1}).
FP= Filter paper (mol min^{-1} ml^{-1}).

sunflower more higher than cellulose. It can be seen in Table 1 that the degradation process in sunflower residue is more intensive, if compared with the other two substrates.

The enzymatic activity study of *P. ostreatus* growing on sunflower residue showed that the monophenol-monooxygenase activity in strains 517-518, as well as the endo-1,4-β-glucanase activity in the strain 517, are observed three days after inoculation. These strains have considerable differences in enzyme activity. For example, the CMC activity of strain 518 is 4-5 times higher than that of other strains. However, all studied strains of *P. ostreatus* showed activity of monophenol-monooxygenase and endo-1,4-β-glucanase (Table 2).

Earlier studies have proved that cellulase production of *Pleurotus* species outstrip the production of monophenol-monooxygenase (Semichaevskii, 1985; Semichaevskii and Bis'ko, 1987). Activity of monophenol-monooxygenase, endo-1,4-β-glucanase and CMC-activity in the strains 483, 517, and 518 is higher after seven days than at three days after cultivation (Table 2).

It was found that the rate of enzyme activity excluding genotype depends upon conditions and duration of cultivation, availability of oxygen and peculiarities of interaction of mycelium with the substrate. Variability in enzyme activity (Danilyak *et al.*, 1989), and the apparent absence of connection



Figs. 1-3.- Scanning electron micrographs of mycelial growth of *Pleurotus ostreatus* on different substrates. 1a-b: Aerial hyphae during growth on beer wort agar (a), and on lignocellulosic substrate (b). Note clamp connections (arrow) and spheric structures (arrowhead). Bar= 1 μm. 2: Spheric structures (arrowhead) in the mycelial layer on lignocellulosic substrate. Bar= 1 μm. 3: Hyphal growth on the surface of a lignocellulosic substrate. Note clamp connnections (arrow). Bar= 10 μm.

between monophenol-monooxygenase activity and degradation of some substrate components is connected with multifunctionality of these enzymes, which also can take part in the processes of detoxification of phenol compounds, morphogenesis, etc. (Molitoris, 1978).

During mycelial growth on the studied substrates, no significant morphological differences were observed in the texture of hyphae and their structures, in comparison with those on beer wort agar (Fig. 1a-b). The average width of hyphae was 1.0-2.5 μm. Hyphal anastomoses were often noted on the mycelium (Fig. 1a). A morphological feature of *P. ostreatus* mycelium on all studied substrates was the presence of clamp connections (2-3 μm wide) and the spheric structures of different diameter (2-3 μm), which were attached to the hyphae by means of a thin stalk (0.8-1.0 μm long) (Fig. 2). Similar structures of 3.2-6.9 μm in diameter, with a longer stalk of 2.6-4.8 μm, were reported by Hilber (1982) in mycelial cultures and carpophores of different *Pleurotus* species.

The scanning electron microscopy showed that the hyphal growth of *P. ostreatus* had a close contact with the surface of the substrate, which was also reported in a previous work (Schiesser *et al.*, 1989). In some cases, the hyphae formed pits in the studied substrates (Fig. 3).

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