



## BIOCHEMICAL ANALYSIS AND CELLULAR ULTRASTRUCTURE OF THE MYCELIAL AND REPRODUCTIVE PHASES OF *PLEUROTUS OSTREATUS*

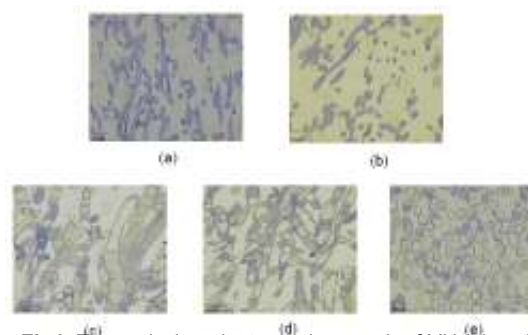
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**Key words:** *Pleurotus ostreatus*, cellular ultrastructure, lignocellulosic residues

**Introduction.** *Pleurotus ostreatus* is a fungus able to degrade lignocellulosic substrates<sup>1</sup>. This organism had two phases of growth; vegetative and reproductive. It has been reported that the wall of hyphae from the central zone of the colony that corresponds to mature zone (MZ) was around twice the thickness of the wall from the periphery of the colony or young zone (YZ). It was also observed that the content of intracellular protein and glycogen from YZ was higher than that observed in MZ<sup>2</sup>. In this work enzymatic activities, content of glucans and glycogen, diameter of the hyphae and thickness of the cell wall were evaluated in the two phases of growth of *P. ostreatus*.

**Methodology.** *P. ostreatus* was grown on petri dishes containing dextrose potato agar (DPA) and MH was separated from YZ using a scalpel<sup>2</sup>. Fruit bodies of *P. ostreatus* of 0.5, 1.0, 2.0 and 4.0 mm tall were obtained in crystal trays containing APD. Activities of laccase, protease and glucanase, and content of glycogen and glucans were determined. In the vegetative phase and in the fruit bodies of the different stages of growth the diameter of the hyphae and thickness of the cell wall were evaluated<sup>3</sup>.

**Results.** MZ and YZ showed the highest activity of laccases, proteases and cellulases. The fruit bodies showed higher content of *R*-glucans and *S*-glucans than the vegetative zone. YZ and the fruit body of 0.5 mm had the highest content of glycogen (**Table 1**). The thickness of the cell wall and the diameter of the hyphae were different in mature and peripheral zone of the colony (**Fig. 1**).



**Fig1.** Transmission electron micrograph of YH (a) and MH (b) of a colony of *P. ostreatus*, and from the base (c), stem (d) and pileus (e) of a fruit body 4 mm tall of *P. ostreatus*. Scale = 100  $\mu$ m.

**Table 1.** Enzymatic activities and content of glucans and glycogen of the vegetative phase (YZ and MZ) and of fruit bodies 0.5, 1, 2 and 4 mm tall of *P. ostreatus*.

Biochemical parameters	Phases of growth					
	Vegetative phase		Reproductive phase			
	YZ	MZ	0.5 mm	1 mm	2 mm	4 mm
Laccases (U/gX)	17.645 $\pm 0.334$	16.108 $\pm 0.670$	0.830 $\pm 0.017$	5.577 $\pm 0.368$	4.754 $\pm 0.200$	4.619 $\pm 0.085$
Proteases (U/gX)	4.056 $\pm 0.274$	7.018 $\pm 0.727$	0	1.135 $\pm 0.629$	0	1.911 $\pm 0.147$
Glucanases (U/gX)	0.366 $\pm 0.020$	0.489 $\pm 0.016$	0.830 $\pm 0.017$	0	0.013 $\pm 0.002$	0.133 $\pm 0.079$
Endoocellulases (U/gX)	0.126 $\pm 0.040$	0.443 $\pm 0.080$	0	0	0	0
Chitinases (U/gX)	0.008 $\pm 0.002$	0.006 $\pm 0.001$	0.054 $\pm 0.003$	0.039 $\pm 0.004$	0.031 $\pm 0.006$	0.132 $\pm 0.032$
Glycogen (U/gX)	0.981 $\pm 0.057$	0.297 $\pm 0.040$	0.843 $\pm 0.058$	0.838 $\pm 0.060$	0.956 $\pm 0.070$	1.297 $\pm 0.112$
S-Glucans (U/gX)	0.988 $\pm 0.062$	1.077 $\pm 0.421$	1.146 $\pm 0.384$	0.542 $\pm 0.012$	9.004 $\pm 0.478$	14.856 $\pm 2.325$
R-Glucans (U/gX)	2.406 $\pm 0.177$	9.593 $\pm 0.508$	0.415 $\pm 0.045$	0.451 $\pm 0.017$	7.120 $\pm 1.336$	31.528 $\pm 4.547$

**Conclusions.** Laccases and proteases activity play and important role in the invasion of the substrate and in the reproductive phase of the fungi, respectively. Fruit body of 4 mm tall had the highest content of glucans since it had the highest amount of mature hypha.

**Acknowledgements.** Suárez Segundo José Luis, wishes to thank CONACYT for the scholarship granted to carry out his master studies.

### References.

1. Sánchez C. (2009). *Biotechnol Adv.* Vol. (27.): 185–194.
2. Sánchez C, Téllez M, Díaz G and Moore D. (2004). *Lett Appl Microbiol.* Vol. (38.) (6): 483-487.
3. Sánchez, C. (2004). *Appl Microbiol Biotechnol.* Vol. (64.) (5):691-4