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INFLUENCE OF ECOLOGICAL FACTORS IN THE BEHAVIOR OF WHITE DECAY FUNGI.

Juan DONOSO GUTIÉRREZ *
Javier GONZÁLEZ MOLINA *
Juan DONOSO PALYI *

Summary

In the last decade they have been carried out, numerous investigations relative to enzymes and involved processes, in the field of the Biotechnology applied to processes of pulping of ligno-celullose materials, as wood.

However, studies on the knowledge and the influence of ecological factors in the behavior of growth of WDF and the generating of enzymes in, are almost nonexistent.

In this work, results obtained are presented over the growth and production of oxidatives enzymes of two fungi species of grateful importance: *Pleurotus ostreatus* and *Ceriporiopsis subvermispora*, under the influence of diverse ecological factors: physical, chemical and biological.

With the purpose of completing this type of monografics studies, without a doubt, fundamental as contributions to the bio-pulping, antecedents are given over the process of lignine decay by the fungous organisms signal, product of an anatomical and morphological study of the affected woody material. In this order, we understand each other, the colonization of the sustrate, the penetration of the hifas and its distribution in the wood.

- ***Chairs Professors of the Wood Science and Technology Laboratory at the University of Chile***
- ***Email: jedg@hotmail.com, jdonoso@uchile.cl***

Introduction:

Mostly the use of biotechnical processes in the pulp and paper industry have been based on the search of mushrooms WDF (mushrooms of white decay), for the obtaining of lignolitics enzymes (1,2,3,4). These enzymes taken place artificially, have been applied fundamentally in processes of pre-digestion of the wood, as well as for pulp bleaching, particularly Kraft (5). Some authors have works in the study of biomechanical pulping with W.R.F. (white-rot fungi; 6). Clonal techniques and molecular analysis of cDNA have been developed, to improve the lignin´s biodegradation (7).

In the work of Highley (8), the biotechnical study of Brown and White-rot Decay is appreciated, with an interesting contribution on the biodegradation processes made up in the cellular wall, with emphasis in lignin.

Even when this interest has wakened up in the last twenty years, in growing form, we can appreciate its effect in the 7th International Conference on Biothecnology in the Pulp and Paper Industry. Vancouver B.C. Canada, June, 1998. In this, 193 papers presented, on embrace studies in biochemical of enzymes and its diverse components. However on *P. radiata* (specie of our interest), there is only one. Shortly, they don't exist in this conference, contributions about ecological factors (9), involved in the development and behavior of the studied mushrooms. Principally, the fungous species are permanently the same (*P. chrysosporium; C. versicolor* and lately *C. subvermispora*). The reached achievements, on the other hand, have been for the most part academic; that is means that those many experiences, have not still been adopted industrially.

We intends in this work, a substantial change in what concerns to the application of mushrooms WDF, in the industry in reference. We have demonstrated that the direct use of cultivations of mushrooms on chips, in that case of *P. radiata*, they achieved promissory results (10). As consequence of this investigation and of other related ones, a project was formulated (FONDEF D97 I 2032), of which is part this contribution. Basically, the ecological factors are studied between those who can have an interaction on the behavior of the mushrooms WDF (White-Decay Fungi), and their stability in field application (biological confrontation), although their lignolitics activities.

The first studies regarding the influence of ecological factors: physical, chemical and biological, were enunciated by Findlay-Cartwright (11). Their behavior was demonstrated by Rypacek (12). In this case several mushrooms WRF has been chosen for this study; preliminary and excellent results of two of them are now presented: *Ceriporiopsis subvermispora* (9-C) and *Pleurotus ostreatus* (9-P). The chosen ecological factors, basically to be governable in land (inoculation in pulps logs of *Pinus radiata*), are: temperature, relative humidity of the air, pH, cultivation substrat, mainly. The variable answers for the optmización of their behavior are: Antibiosis in front of mushrooms competitors in the forest, and production of enzymes (for the moment only measures in their intensity).

Other of ours purposes is to introduce a new and unpublished concept which is the hybridism. This means that we are trying to improve a similar response of the fungi for ours purpose in this case: antibiotical resistance to competitors, and highly productions of lignoliticals enzimes; depending only in the choice of ecological factors, at field level.

Experimental process:

The first results are presented for the selected mushrooms (9-C and 9-P).

- Experimental fungi:

C. subvermispora; imported from Madison Laboratory (U.S.A.), reproduced with induced sexual bodies in our laboratory; of the induced fruitful bodies, the studied cultivations were obtained; from those corps we have isolated our own strands.

P. ostreatus; obtained in the same way, from cultures belonging to M.N.H.N. Paris, France.

- Study of Temperature and pH, in function of micelium growth:

Petri disc with Agar 2% and Malt 3%, were inoculated with both fungis in separate form and cultivated during ten days. The pH was adjusted later to the sterilization. They stayed during 10 days in cultivation camera. Three temperatures were used: 20°C, 25°C and 30°C ± 1°C three pH: 4.0, 5.5 and 7.0 maintained to 85% of relative humidity ± 1%.

- Study of weight loss:

Based in three differents temperatures, we trial to determinate their effect (temperatures) in the behavior of the weight loss in sapwood of *P. radiata* and *Eucalyptus globulus*, according to French norm NF 41-502 reiterated by Levin (13). They stayed for 30 and 60 days.

- Answer of lignolitics enzymes:

It is based on the proposition of Nobles (14). For this purpose, Petri disc with Tanic acid medium at pH 5.5, where inoculated separatly with both fungis. Their effectiveness is measured at 10 days. In function of the radius of tanic oxidation (brown color) and their intensity (clear-dark), the lignolitic effectiveness is evaluated.

Results and Discussion:

- Temperature versus pH.

In tables N°1 and 2, we can observe the development of the fungis (preliminary results), the behavior expressed in terms of vigor of the micelium growth is evident.

The mushroom 9-P, develop the bigger growth at pH 7.0 and 30°C. This tendency decreased slowly at 25°C, and it is reestablished at the 20°C. We can conclude that the temperature and pH, are innocuous for his development. This is proved the reality in nature; because it is a pleiophagic and cosmopolitan fungi.

9-C, on the other hand, present an increases in growth vigor in function of the drop of temperature increased over acid pH. In accord with ours experiences, this fungi should be used for the pre-digestion of Softwoods at high temperatures and Hardwoods at lowers temperatures.

Pleurotus ostreatus

9-P

Temperature °C	pH	Micelium growth
20 °C	4.0	+
	5.5	++
	7.0	+++
25 °C	4.0	+
	5.5	+++
	7.0	++
30 °C	4.0	+
	5.5	++
	7.0	+++

Ceriporiopsis subvermispora

9-C

Temperature °C	pH	Micelium growth
20 °C	4.0	+++
	5.5	++
	7.0	+
25 °C	4.0	+++
	5.5	++
	7.0	+
30 °C	4.0	+++
	5.5	++
	7.0	+

+: low vigor.
++: medium vigor.
+++: strong vigor.

- Weight loses:

The percent of the Weight loss, is represented in the Table N° 3.

9-P

Days	Temperature °C	Weight loss %	
		P. radiata	*E. globulus*
30	20	4.2	3.0
	25	4.9	3.1
	30	5.8	3.4
60	20	4.9	3.6
	25	5.0	3.8
	30	6.3	4.8

9-C

Days	Temperature °C	Weight loss %	
		P. radiata	*E. globulus*
30	20	3.0	4.2
	25	5.7	4.3
	30	7.8	4.6
60	20	4.1	6.8
	25	6.2	7.2
	30	7.0	8.6

According this table, we can affirm that those who have settled in temperature versus pH. That is means, that fungi 9-P is independent of pH and temperature in relation to the forest species; instead 9-C who change his activity clearly, in function of the pH and temperature.

- Lignolitics enzymes:

In images annexes, we can appreciate the enzymatic activity visually, over tanics medium.

We can observed in those, that the activity of the stump 9-C is quite bigger than 9-P. In a future, we will be able to experiment with hybrids strands that we are now creating for their utilization in the pulp and papers industries.

Conclusions.

The hybridization, is without any doubt a new alternative in the handling of stumps of WDF, for their use in biotechnology applied in pulp and paper industries (10).

It is important to consider the dissimilar behavior, according to the cosmic variables (ecological factors).

A clear tendency is observed, in the use at future, the micelium of WRF for the pre-digestion of timbers (logs or chips), instead of enzimes, based in their facility of management, lower cost, higher activity and stability, between others. But, for those objectives it is absolutment necessary the knowledge of the influence and interaction of ecological factors..

This is a first approach and advance state, of an innovative line of technological development.

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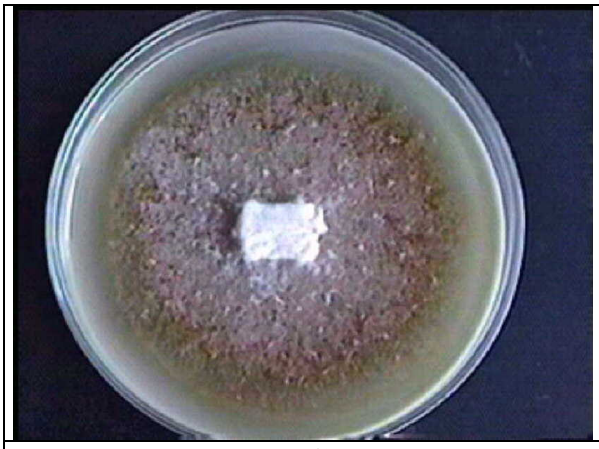
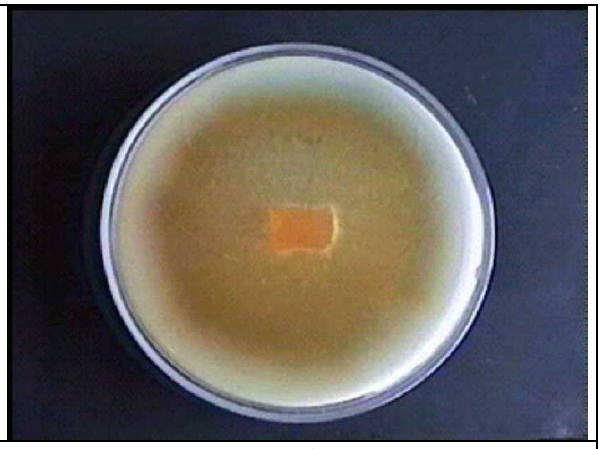
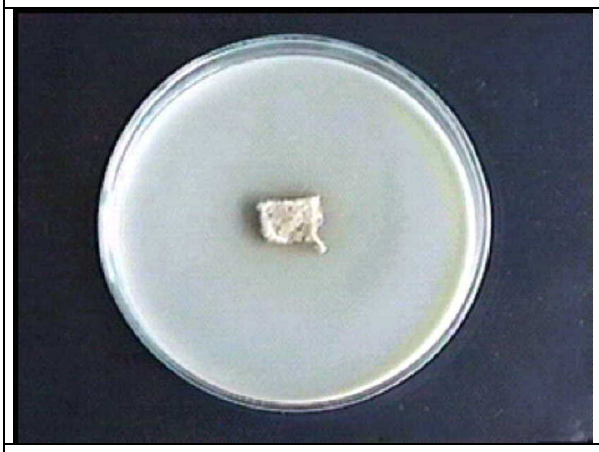
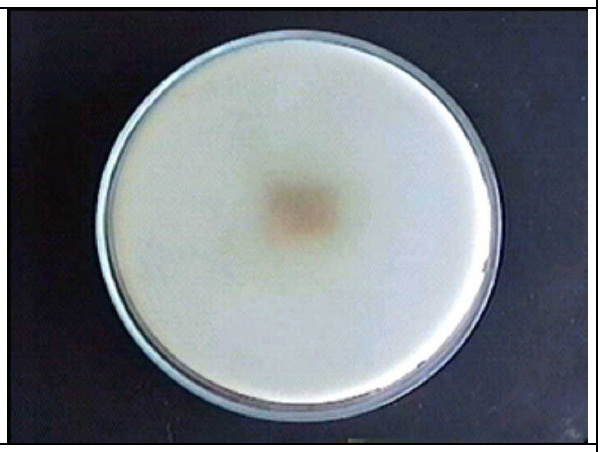
Anexe 1.
ENZIME ACTIVITY

	
9-C	**9-C**
	
9-P	**9-P**