THALES Project No.65/1182

Research on the potential of selected ligninolytic fungi to degrade toxic substances

Research Team

Fotis Rigas (*Project Supervisor*), *Assistant Professor, National Technical University of Athens, Greece*

Vasiliki Dritsa (*Principal Investigator*), *National Technical University of Athens, Greece*

Roger Marchant *(Collaborator), Professor, University of Ulster, N. Ireland*

Elizabeth Avramides *(Collaborator*), *Reasearcher, National Agricultural Research Foundation, Greece,*

Evagellia. Kapsanaki-Gotsi *(Collaborator), Lecturer, National Kapodistrian University of Athens, Greece*

Zapi Gonou-Zagou *(Collaborator), Scientific contributor, National Kapodistrian University of Athens, Greece*

Spyros Sklavounos, *(Collaborator), National Technical University of Athens, Greece*

Ioannis Xatzidakis *(Undergraduate student) National Technical University of Athens, Greece*

Arsenios Sakellariou (*Undergraduate student) National Technical University of Athens, Greece*

Abstract

This research project attained the isolation and characterization of certain fungal strains, which proved to be capable to degrade lignin (white rot fungi), intending to using them in bioremediation applications. The fungi were prescreened for their ligninolytic potential with the aid of a polymeric dye and tested for their tolerance towards pentachlorophenol (PCP). The activities of the produced enzymes were estimated in some strains. Finally, the ability of fungi to degrade lindane in liquid cultures was tested.

Introduction

White rot fungi have been reported as the most effective degraders of a wide variety of hazardous environmental pollutants. The enormous structural diversity of pollutants that are degraded by these fungi has made their potential use for bioremediation extremely intriguing. Recalcitrant compounds such as chlorinated pesticides, polyaromatic hydrocarbons, polychlorinated biphenyls, nitroaromatic explosives are degraded to carbon dioxide by white rot fungi [1, 4].

The degradation of lignin and pollutants by white rot fungi depends on the production and secretion of a group of enzymes, such as lignin peroxidase (LiP), manganese

peroxidase (MnP), laccase (Lac). They have developed a unique, nonspecific degradation system that functions in the extracellular environment.

Samples of Basidiomycetes were isolated from the mountains of Greece or afforded by the Department of Biology, University of Athens (Table 1). The strains were prescreened for their ligninolytic activity using decolourisation of Poly R-478, a polymeric dye with violet colour. The chemical monomer of the dye consists of aromatic rings, quinones and a side chain with amines and sulfonated groups. As a result, it can be used as a surrogate toxicant for the detection of ligninolytic enzymes. The colour of this model-dye changes to yellow (colour of degradation products) with the action of ligninolytic enzymes [2, 3].

The biodegradation of Poly R-478 was also determined in a liquid medium by five selected strains. In this case two mechanisms took place, the dye degradation and its adsorption by the fungus mycelium.

The tolerance towards a pesticide was tested on solid media and the growth rate was determined in four concentrations of pentachlrorophenol (PCP).

The enzymes responsible for lignin degradation are LiP, MnP and Lac. The activities of these enzymes were determined in a liquid medium and the day of their maximum production was marked.

Finally, the most promising strain was used in a liquid culture containing another organochlorine pesticide, namely lindane, and its degradation capability was defined in two different temperatures. Methods of extraction and determination of the pesticide were also developed.

Strain	Locality	Substate
Polyporus sp.1	Attiki	On twigs
Polyporus sp.2	Attiki	On twigs
Polyporus brumalis	Attiki	On twigs
Polyporus ciliatus	Attiki	On twigs
Pleurotus dryinus	Arkadia	On wood / *Quercus sp.*
Pholiota squarrosa	Evritania	On wood / *Abies cephalonica*
Polyporus meridionalis	Attiki	On twigs / *maquis vegetation*
Pholiota aurivella	Attiki	On dead trunk /*Abies cephalonica*
Omphalotus olearius	Messinia	Roots or trunk / *Fagus moesiaca*
Armilarria galica	Magnisia	On trunk / *Olea europea*
Armilarria mellea	Arkadia	Roots / *Prunus avium*
Pleurotus ostreatus sp.1	Attiki	On trunk / *Abies cephalonica*
Pleurotus ostreatus sp.2	Korinthia	Commercial compost

Table 1. Fungal strains used in this project.

Material and methods

1. Strains

All fungal strains were obtained from sterile tissue of fresh basidiocarps collected in Greece and inoculated on agar medium.

2. Growth media

The **growth media** used were: PDA (Potato Dextose Agar), CM (Complete Medium), MA (Malt Agar). **Decolourisation** medium: BM (Basal Medium with the addition of 0.01%w/w PolyR-478). Medium for the production of **enzymes**: Kirk's medium. **Tolerance** test: PDA with the addition of 5, 10, 15 and 20 ppm PCP. Medium for the **degradatio**n of Lindane: BM with the addttion 3 ppm Lindane. The cultures were incubated at 25 o C, in darkness.

Strain	Mean Growth Rate (mm/d)		Pattern/ Day of decolourisation
	PDA	BM	
Pleurotus dryinus	0.89 ± 0.09	0.95 ± 0.04	Radial/ 6
Pholiota squarrosa	1.15 ± 0.04	1.32 ± 0.17	Radial / 8
Polyporus meridionalis	3.43 ± 0.09	2.44 ± 0.18	Diffused spots / 11
Polyporus sp.1	2.29 ± 0.069	2.51 ± 0.051	-
Polyporus sp.2	2.13 ± 0.078	1.99 ± 0.079	Radial / 5
Polyporus brumalis	4.52 ± 0.11	3.43 ± 0.83	Radial / 7
Polyporus ciliatus	3.33 ± 0.078	1.99 ± 0.051	Diffused spots / 7
Pholiota aurivella	1.63 ± 0.34	2.12 ± 0.76	Radial / 5
Omphalotus olearius	3.49 ± 0.45		-
Armillaria gallica	0.92 ± 0.41	1.18 ± 0.13	Diffused spots / 13
Armillaria mellea	0.91 ± 0.34		-
Pleurotus ostreatus sp.1	6.21 ± 0.28	2.07 ± 0.00	Radial / 6
Pleurotus ostreatus sp.2	6.28 ± 0.03	3.25 ± 0.55	Radial / 6

Table 2. Growth rates in PDA and BM and decolourisation patterns.

Results

As shown in Table 2, the fungus *Pl. ostreatus sp.2* presents the highest growth rate when cultivated on Petri dishes, 6.28 mm/d in PDA. The lowest growth rate was observed for the fungus *Pl. dryinus* (0.89 mm/d).

The cultures in the presence of the dye showed a similar pattern for most strains. They showed radial zones, diffused spots or no sign of decolourisation [5].The strains that effectively decolourised the BM medium were *Pl. ostreatus sp.1* and *sp.2*, *Ph. Aurivella* (in 6 and 5 days, respectively).

In stationary liquid medium cultivation, *Ph. aurivella* showed the highest removal of the dye (34% mg dye removed/initial mg dye) in a period of 17 days, in comparison to *Pl. ostreatus sp.1*, which possessed a removal efficiency of 21% (Table 3). *Polyporus ciliatus* and *brumalis* showed similar degradation efficiency [6].

Strain	Degradation Yield	Biosorption Yield	Biomass (g)
Polyporus sp.2	24.57 + 0.59	5.33 + 1.19	0.144+ 0.032
Pol. brumalis	14.86 + 1.26	6.15 + 0.85	0.101 + 0.020
Pol. ciliatus	18.74 + 0.46	5.85 + 1.85	0.128 +0.020
Pl. ostreatus sp.1	20.62 + 2.03	5.04+ 1.14	0.064+ 0.002
Ph. aurivella	30.14 + 2.55	3.85 + 0.19	0.064+ 0.006

Table 3. Decolourisation of Poly R-478 in liquid cultures.

The four concentrations of PCP were not toxic to cultures and the growth rates of the strains *Pl. osteatus sp. 1* and *Ph. aurivella* showed no significant differences with regard to PCP content (Figure 1).

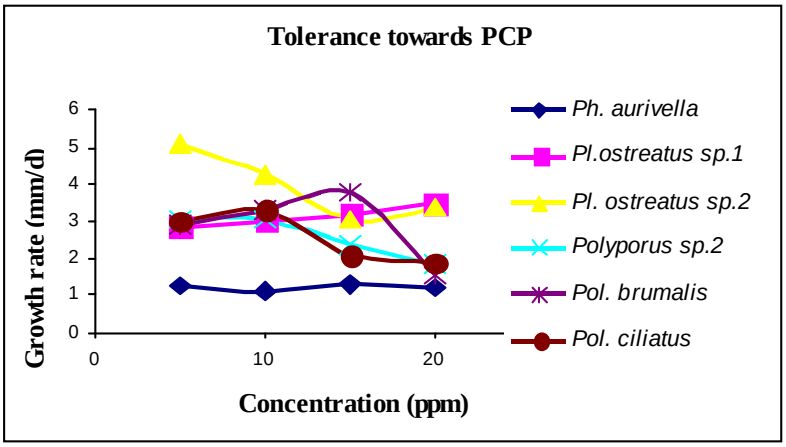

Figure 1. Growth rates of selected strains in four concentrations of PCP (5, 10, 15 and 20 ppm).

The enzyme activities were measured on Kirk's medium. Laccase and manganese peroxidase activities were measured, but none of the strains expressed lignin peroxidase. *Pl. ostreatus sp.2* showed a fast increase until a maximum activity for laccase at the 16th day (34.46 U/l). The maximum MnP activity for the same strain was observed at the 12th day (46.3 U/l) (Table 4). The two stains of *Pleurotus ostreatus sp.1* and *sp.2* showed significant differences in the production of laccase (16.5 and 36.5 U/l respectively). Similar levels of MnP were observed for the former strains.

Strain	Maximum activity (U/l)	
	Laccase	Manganese Peroxidase
Polyporus sp.2	21.54 ± 5.41	27.83 ± 2.26
Pol. brumalis	21.17 ± 6.26	43.03 ± 5.45
Pol. ciliatus	32.00 ± 4.07	38.35 ± 5.31
Pl. ostreatus sp.1	16.49 ± 2.34	40.46 ± 12.4
Pl. ostreatus sp.2	34.46 ± 6.28	46.29 ± 3.53

Table 4. Ligninolytic activities of selected strains

Finally, the removal efficiency of the organochlorine pesticide lindane by the fungus *Pl. ostreatus sp.2* reached 64.5 %, at the low level temperature (18^{o}C). The removal of Lindane at 28^{o}C was 56.8 % (Figure 2**).**

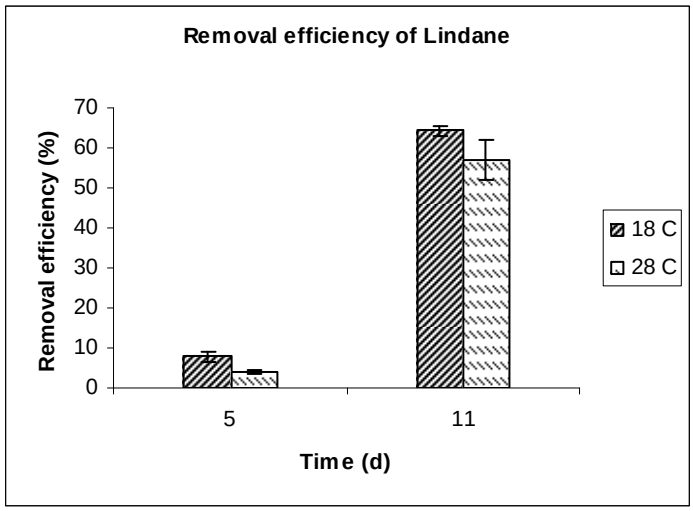

Figure 2. Degradation of lindane in liquid medium by *Pl. ostreatus sp.2.*

.

Conclusions

According to the screening of 13 basidomycetes, it is evident that the fungus *Pl. ostreatus sp.2*, which showed satisfying degradation capacity, can be used in bioremediation applications. This fungus is a candidate to be further studied in solid state cultures and then in contaminated soils.

References

1. Barr DP, and Aust SD.: “Mechanisms white-rot fungi use to degrade pollutants”, *Environ. Sci. Technol.*, **28,** 78–87, 1994

2. Dritsa, V., Rigas, F., Marchant, R., Kapsanaki-Gotsi, E., Gonou-Zagou, Z. and Avramides, L.: “Screening of certain wood rotting fungi for their ability to degrade toxic compounds” *Second European Bioremediation Conference*, Chania, Crete, Greece, June 30 –July 4, 2003

3. Glenn J.K. and Gold M.H.: “Decolorization of several polymeric dyes by the lignin-degrading basidiomycete Phanerochaete chrysosporium”, *Appl. Environ. Microbiol.*, **45**, 1741–1747, 1983

4. .Reddy C.A.: “The potential for white-rot fungi in the treatment of pollutants”, *Curr. Opin. Biotechnol.*, **6,** 320–328,1995

5. Rigas, F., Marchant, R., Dritsa, V., Kapsanaki-Gotsi, E., Gonou-Zagou, Z. and Avramides, E.: “Screening of wood rotting fungi potentially useful for the degradation of organic pollutants”, *Water, Air, and Soil Pollution: Focus* **3,** 201-210, 2003

6. Rigas, F., Dritsa, V., Chatzidakis, J. and Marchant, R.: “Detoxification characteristics of selected Polyporus species for bioremediation applications”, *8th Conference on Environmental Science and Technology***,** Lemnos, Greece, 2003

Acknowledgements

The research team would like to thank the Research Committee of the National Technical University of Athens, for partially financing this project, in the framework of THALES programme.