Review on Agriculture and Rural Development 2014. vol. 3 (1) ISSN 2063-4803

DEGRADATION OF PHENOL DERIVATIVES BY A *PHANEROCHAETE CHRYSOSPORIUM* STRAIN

CSABA VÁGVÖLGYI[1], MÓNIKA VÖRÖS[1], BETTINA BÓKA[1], MIRA PUCAREVIĆ[2], LJUBINKO JOVANOVIĆ[2], DEJANA PANKOVIĆ[2], LÁSZLÓ MANCZINGER[1]

[1]University of Szeged, Faculty of Science and Informatics, Department of Microbiology, 6726, Szeged, Közép fasor 52., Hungary
csaba@bio.u-szeged.hu
[2]University EDUCONS, Vojvode Putnika BB 21208 Sremska Kamenica, Serbia,
dejana.pankovic@educons.edu.rs

ABSTRACT
The white rot fungus *Phanerochaete chrysosporium* has significant pollutant-degrading capabilities with its oxidoreductases. The lignin-degrading enzyme system of this fungus also allows the breakdown of different organic pollutants (xenobiotics). The most significant enzymes in these processes are the lignin peroxidase and the manganese peroxidase. In this study, *Phanerochaete chrysosporium* strains were isolated from various Hungarian habitats. It was revealed, that one of these strains (Pha78) seems to be very promising for such remediation purposes. The degradation of different phenol derivatives by this isolate was investigated both in lignin peroxidase and manganese peroxidase inductive media. The starting concentrations of phenol derivative compounds were 20 mg l^{-1}. The amounts of the phenolic compounds in the media were measured by the colorimetric aminoantipyrine method. The tested *Phanerochaete* strain was able to degrade different phenol derivatives efficiently, especially in manganese peroxidase-inductive medium.

Keywords: bioremediation, phenol derivatives, *Phanerochaete chrysosporium*, pollution

INTRODUCTION

Agricultural and industrial activities release vast amount of different xenobiotics in the environment. Some of these compounds are classified as dangerous because they could be directly toxic to different organisms and/or they accumulate in the food chain. Beside the aniline and phenol derivatives, some filamentous fungi are capable for the degradation of various PAH (*Polycyclic Aromatic Hydrocarbon*) and POP (*Persistent Organic Pollutant*) compounds. *Phanerochaete chrysosporium*, a basidiomycetous filamentous fungus, is belonging to this group of microbes where this phenomenon is confirmed. *P. chrysosporium* is a white rot fungus which has a highly efficient lignin degrading enzyme system. With these enzyme systems the fungus can also break down different xenobiotic pollutants. In these types of degradation processes, the lignin peroxidase and the manganese peroxidase have great significance.
There are data in the literature, that when these enzymes are used alone or in combinations they target and could degrade pesticides, polycyclic aromatic compounds, chlorinated aromatic compounds, paints and many other xenobiotics (CAMERON ET AL., 2000). Based on this observation, the aim of our present study was to isolate strains of *P. chrysosporium* capable for efficient degradation of a variety of aromatic pollutants and pesticides.

MATERIAL AND METHOD

Isolation of strains

A new type of selective medium was used for the isolation of *Phanerochaete* strains. This contained peptone (0.5%), KH_2PO_4 (0.1%), glucose (1%), $MgSO_4$ (0.05%) and agar (2%) supplemented with 500 µl 5% rose bengal, 1 ml 0,2% dichloran, 100 mg streptomycin and 15 mg carbendazim after autoclaving. The isolates collected from this selective medium and one *P. chrysosporium* strain (DSM 9620) purchased from the *German Collection of Microorganisms and Cell Cultures* (DSMZ) were examined.

Molecular identification of strains

Molecular identification of the isolates was carried out with gene specific PCR and DNA sequencing. The presence of ligninase H8 gene was examined by specific PCR (JOHNSTON AND AUST, 1994) and the ITS regions of the isolates were amplified by ITS4 and ITS5 primers (WHITE ET AL., 1990) and sequenced.

Investigation of the *in vitro* xenobiotic-degrading abilities

The degradation of phenol derivatives were investigated in lignin peroxidase (JAGER ET AL., 1985) and manganese peroxidase inductive liquid media. The manganese peroxidase inductive nutrient solution contained 2 g glucose, 10 mM ammonium sulphate, 20 mM potassium sodium tartrate, 2 g KH_2PO_4, 0.5 g $MgSO_4$,10 mg thiamine, 30 mg $MnSO_4$, 1 mg $FeSO_4$, 1 mg $CuSO_4$, and 1 mg $ZnSO_4$ in one liter medium. The starting concentration of phenol derivatives was 20 mg l^{-1} in both media. Fifteen ml of both media were inoculated with 0.5 ml concentrated fungal spore-mycelium suspension of the *P. chrysosporium* Pha78 (= SZMC 20961) strain. The incubation was carried out without shaking at 25 ^{0}C for 7 days. The amounts of the phenolic compounds in the media were measured by the colorimetric aminoantipyrine method (DANNIS, 1951).

RESULTS

DIETRICH AND LAMAR (1990) reported the isolation of *P. chrysosporium* strains from soil by application of a selective media containing benomyl. Our efforts to isolate *P. chrysosporium* in this medium were unsuccessful. However, a new modified medium which contained dichloran, rose bengal and carbendazim was efficiently used for the isolation of *P. chrysosporium* from different Hungarian environmental samples.

Besides a micromorphological investigation, there is a possibility for rapid identification via the detection of the lignin peroxidase H8 gene of *P. chrysosporium* (JOHNSTON AND AUST, 1994). The special primer pair for this gene was used to identify our isolates. Fragments of the proper size (600 bp) were detected in 6 strains after the specific PCR reactions. Besides this sequencing of the ITS region were used for the exact identification of the strains. The sequences were analyzed with the NCBI BLAST service. These results proved that 5 of our strains were *P. chrysosporium* and one was *P. sordida*.

In a preliminary experiment, the degradation of xenobiotics was examined with 4 strains (DSM 9620, SZMC 20959, SZMC 20960 and Pha78) in soil microcosm experiments. The degradation of herbicides (chlortoluron, diuron, isoproturon and linuron) and parabens were measured. The rates of decomposition of inoculated samples were compared to the controls. The degradation of herbicides took place also in the non-sterile control samples, however, the inoculation of the degrading fungus highly improved this process in certain cases. The best degradation of herbicides and parabens was accomplished by the strain SZMC 20961 (*results*

are not shown). The results of the degradation of different phenol derivatives by the strain Pha78 in lignin peroxidase and manganese peroxidase inductive media are presented in *Figure 1* and *2*, respectively. The starting concentration of phenol derivatives was 20 mg l^{-1}. After one week incubation the tested *Phanerochaete* strain was able to degrade different phenol derivatives in the inductive media, first of all in the manganese peroxidase inductive medium (*Figure 2*). The most successful degradation values could be accomplished in the case of phenol, resorcinol, o-cresol and m-cresol.

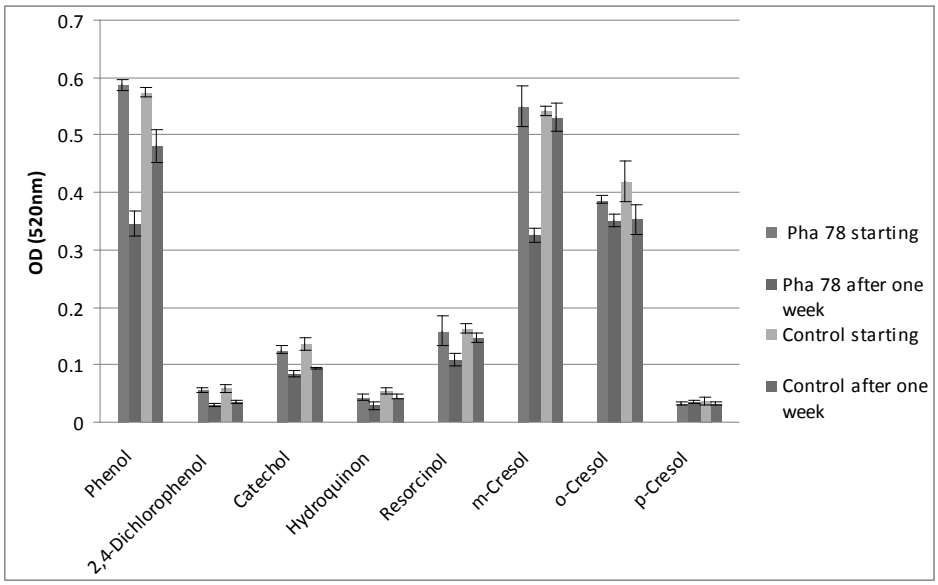

Figure 1. Degradation of distinct phenol derivatives by the *Phanerochaete chrysosporium* Pha78 strain in lignin peroxidase inductive medium

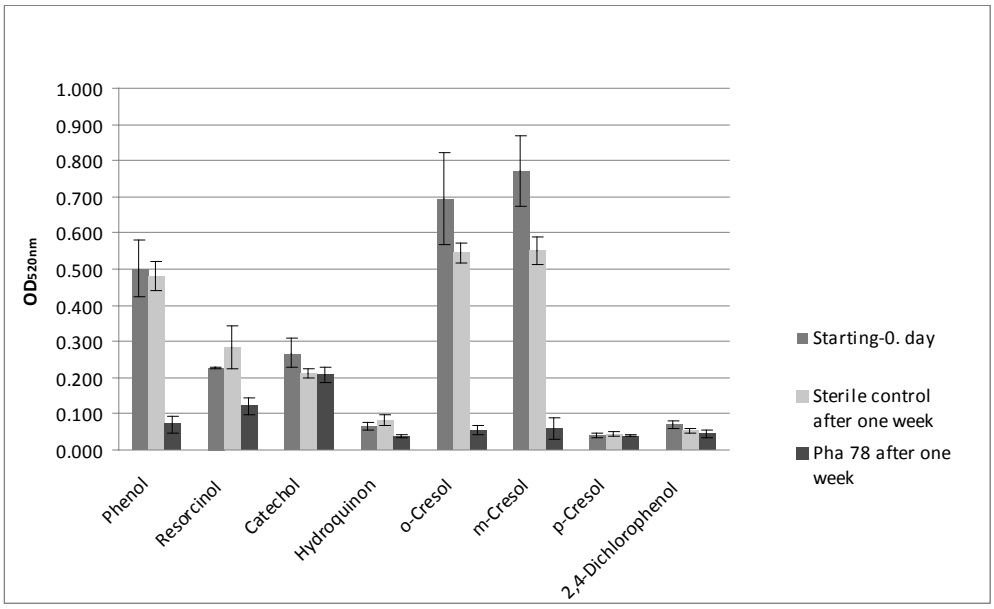

Figure 2. Degradation of distinct phenol derivatives by the *Phanerochaete crysosporium* Pha78 strain in manganese peroxidase inductive medium

CONCLUSIONS

The application of a new selective medium proved very useful to collect *P. chrysosporium* isolates from soil samples. The isolated *P. chrysosporium* strains efficiently degraded various xenobiotics in soil microcosm experiments. One of the isolated *P. chrysosporium* strains Pha78 was able for significant degradation activities even in sandy soil. This strain also degraded efficiently dangerous environment pollutant phenol derivatives.

ACKNOWLEDGEMENTS

This work was supported by the European Union through the Hungary-Serbia IPA Cross-border Co-operation Programme (PHANETRI, HUSRB/1002/214/068) and by the European Union co-funded by the European Social Fund (TÁMOP-4.2.2/B-10/1-2010-0012).

REFERENCES

CAMERON M.D., TIMOFEEVSKI S, AUST S.D., CAMERON, M.D., TIMOFEEVSKI, S., AUST, S.D. (2000): Enzymology of *Phanerochaete chrysosporium* with respect to the degradation of recalcitrant compounds and xenobiotics. Applied Microbiology and Biotechnology, Volume 54. pp. 751–758.

DANNIS M. (1951): Determination of phenols by the amino-antipyrine method. Sewage and Industrial Wastes, Volume 23, pp.1516-1522.

DIETRICH D.M., LAMAR, R.T. (1990): Selective medium for isolating *Phanerochaete chrysosporium* from soil. Applied and Environmental Microbiology, Volume 56. pp. 3088–3092.

JAGER, A.. CROAN C., KIRK, T.K. (1985): Production of ligninases and degradation of lignin in agitated submerged cultures of *Phanerochaete chrysosporium*. Applied and Environmental Microbiology 50, 1274–1278.

JOHNSON C. G., AUST S. D. (1994): Detection of *Phanerochaete chrysosporium* in soil by PCR and restriction enzyme analysis. Applied and Environmental Microbiology, Volume 60. pp. 2350–2354.

WHITE, T.J., BRUNS T., LEE S., TAYLER, J. (1990): Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In PCR protocols: A guide to methods and applications, Innis M. A. – Gelfand D. H. – Sninsky J. J. – White T. J. (eds.) Academic Press, San Diego, California, pp. 315–322.