PAHs REMOVAL FROM SOIL BY A STRAIN OF ASPERGILLUS NIGER PRODUCING MANGANESE PEROXIDASE

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Key words: PAHs, biodegradation, heterologous expression

Introduction. Polycyclic aromatic hydrocarbons (PAHs) are a class of toxic pollutants that have accumulated in the environment due to a variety of anthropogenic activities, these are mutagenic and carcinogenic, which the importance of their removal from the environment. The ability of white-rot fungi to degrade this compound has been attributed, to the ligninolytic enzymes(1). *P. chrysosporium* is capable of producing these and has therefore become of importance in the bioremediation of contaminated soils, however its efficiency is diminished because soil is not its natural habitat. The use of native microbiota from soil is of considerable interest, however, does not show the ability to efficiently degrade the pollutant, the heterologous expression of peroxidases is an alternative to obtain microorganisms with the desired degrading ability(2).
The aim of the study is to show the effect of *mnp*1 expression under a constitutive strong promoter in *A. niger* strain on the removal of PAHs in contaminated soil.

Methods. *A. niger* SCB2 was used as the recipient in transformation with pGMG-Hyg (expression cassette: *mnp*1 cDNA with constitutive *gpd*A promoter and the *Hyg*B resistance gene for the selection of transformants). Fungal transformation was done by biolistic. Colony transformants were assayed for MnP activity using a modified plate assay method with o-anisidine and which developed a purple halo were selected. Incorporation of *mnp*1 gene was checked through specific amplification in a PCR. The ability of wild-type and transformant strains to remove PAHs was determined in solid-state microcosm system. Sugarcane bagasse was used as a fungal growth support and carbon source. Sterile soil was contaminated with 1500 ppm of PAHs mixture, all cultures were incubated at 30°C for 20 d. Evolution of CO_2 was measured to quantify the heterotrophic activity. PAHs were extracted with microwave extraction (EPA method 3546). PAHs removal was determined by HPLC. The MnP activity was determined using phenol red oxidation.

Results. A total of 8 transformants were isolated for their capacity to grow in Czapek plates with HygB. The result showed a single amplicon of 635 pb fragment observed in agarose gel electrophoresis and no bands were observed for the wild-type stain. Four transformants formed purple halos around the agar disk after 8 d of incubation, indicating extracellular peroxidase activity (fig. 1). The wild-type strain showed no coloration; however, the control strain of *P. chrysosporium* showed a greater purple halo. Two tested strains showed different profiles of CO_2, the transformant strain produced more than the wild-type (fig. 2)

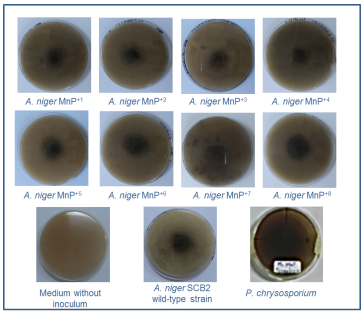

Fig.1 Qualitative determination of MnP activity produced by *A. niger* transformants in Petri dishes using o-anisidine as an indicator.

Both strains presented a decrease in CO_2 production in the presence of PAHs.

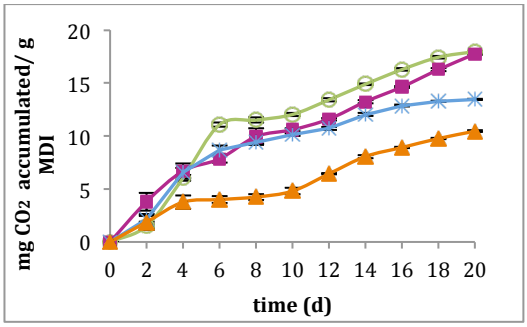

Fig. 2. Microbial activity of both *A. niger* strains in solid culture in microcosm, were: (▲) *A. niger* SCB2 without Phe; (✳) *A. niger* SCB2 with Phe; (■) *A. niger* MnP^{+7} without Phe; (⊖) *A. niger* MnP^{+7} with Phe

The wild-type strain had the lowest PAHs removal capacity (approximately 55%) compared with MnP^{+7} strain, which was able to remove approximately 89% of the initial PAHs (1.5 mg/g IMD) in 20 d.

Conclusions. The increase in the % removal of PAHs by the MnP^{+7} strain in solid culture suggests that it is due to the production of MnP enzyme recombinant. This fact has led to an increased tolerance in plate and solid culture and greater removal efficiency in high PAHs concentrations in solid culture.

Acknowledgements. This work was supported by CONACYT, project CB2008-105643 and IPN project SIP20131157.

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