Potential of filamentous fungi to degrade recalcitrant xenobiotics in polluted soils

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Potentiel des champignons filamenteux à dégrader des xénobiotiques récalcitrants dans les sols pollués

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Filamentous fungi were screened for biodegradation of organic xenobiotics in soils. These xenobiotics included pesticides, and industrial pollutants such as polycyclic aromatic hydrocarbons. Experiments were performed on wet sand spiked with pollutants. Several strains exhibited a great potential to degrade these chemicals.
By using liquid fungal inocula, or solid inocula consisting of fungi pregrown on organic substrates, we obtained both a fungal growth and an activity in non-sterilized soils. The relationships between physicochemical parameters of soils and the degradation of xenobiotics are currently investigated. At the same time, the enzymatic systems involved in the transformation of pollutants are characterized and purified if necessary. For example, we have studied the enzymology of microsomal mixed-function oxidases (P450s) as well as extracellular oxidoreductases (peroxidases, laccases,...).
Taken together, our results confirm that filamentous fungi can be used for polluted soil bioremediation by using a bioaugmentation approach.
Biotechnological developments will allow us to to obtain and stabilize strains able to overproduce selected enzymatic systems. Degradation experiments on larger scale pilots are constituting the final step for process optimization before application on polluted site.

Key words : filamentous fungi, degradation, xenobiotic, soil
Mots clés : champignons filamenteux, dégradation, xénobiotique, sol

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INTRODUCTION

In many countries, there are increasing problems with soils contaminated by xenobiotics as consequences of human activities. These chemicals are mainly biocides, such as pesticides or chlorinated phenols, but also industrial pollutants (polycyclic aromatic hydrocarbons: PAHs). Biological treatments offer economic and environmentally acceptable solutions when compared to physico-chemical processes, and to encapsulation or landfilling.

Early bioremediation processes (biostimulation) generally involved the enhancement of the activity of degradative organisrns already present in the soil, in most cases bacteria. Unfortunately, bacteria are often unable to degrade substituted molecules also showing low water solubility and availability.

For many years, various researchers have been interested in the development of fungal technology for the biodegradation of the pollutants. Filamentous fungi, including zygomycetes, ascomycetes and basidiomycetes (especially white rot fungi) have been shown to be potentially useful to degrade recalcitrant xenobiotics, at the least in liquid cultures [1]. Moreover, they could be inoculated into polluted soils (bioaugmentation approach).

The Phytopharmacy and Chernical Mediators Unit (INRA Versailles) and the Laboratory of Biotechnology of Filamentous Fungi (INRA Marseille) cooperate to study the degradation of aromatic compounds such as agrochemicals in aqueous systems, but also in soils. Three years ago, INRA has been associated with Krebs corporation (Eurisys network) to remediate polluted industrial soils.

POTENTIAL AND ADVANTAGES OF FUNGI OVER BACTERIA:

Fungi offered many advantages in bioremediation over bacteria:
(1) a fungal inoculum may be able to withstand competition from the native microflora and to colonize the soil (especially when pregrown on a specific and selective substrate)
(2) in general, concentrations of pollutants are too high for microbial processing and the pre-growth offers protection against toxic effects of the chemicals
(3) degradation of the chemicals is not dependant on their concentations since the process is cometabolic and appears mostly as a consequence of growing (nutrient) conditions
(4) fungi possess a range of enzymatic systems (most of them being exocellular and nonspecific) and degrade a wide range of chemicals
(5) the nature of the enzymatic system *(i.e.* the ligninolytic system for white rot fungi) illiminated the problem of steric hindrance since the enzyme oxidize mediators active on the xenobiotics
(6) the fungal strains used are non-pathogenic naturally occuring isolates which have not been genetically manipulated to produce unusual enzymes, and several enzymatic systems can be surexpressed using well defined culture conditions (especially in bioreactors)
(7) The process is environmentally acceptable since toxic leacheates or compounds are not released, and the introduced fungi gradually decline.

DEGRADATION OF PESTICIDES IN AGRICULTURAL SOILS

The white rot fungus *Phanerochaete chrysosporium* has been used to degrade lindane and atrazine in agricultural soils. A solution of growth medium [2] was added to 25 g (equivalent dry soil) silt loam to achieve 35% (wt/wt) soil moisture content. After inoculation of spores in the non sterile soil, a limited amount of fungal growth was detected, since maximal amount of 13.9 mg dry weight (calculated from soil ergosterol content) was noted after a 2-week period. Then, fungal biomass decreased during the following 7 weeks. Results were similar in soils containing 0.7 ppm lindane or 0.6 ppm atrazine, each spiked with radiochemical.

Lindane mineralization due to the indigenous microflora amounted for 21.6% of the initial radioactivity, after 9-week incubations. It reached 49.1% in soils following fungal inoculation. Results indicated that *P. chrysosporium* modified lindane degradation pathway by increasing the conversion of volatile intermediates to carbon dioxide [3].
In similar experiments, atrazine degradation and mineralization due to the indigenous microflora were unaffected by the presence of the fungus.

DEGRADATION OF PAHs IN INDUSTRIAL SOILS

In a first time, 15 strains of filamentous fungi have been screened for the degradation of phenanthrene and benzo[a]pyrene applied in mixture (10 ppm of each) on 35 g wet sand also supplemented with culture medium. Two of them have been shown to be very efficient in carrying out the breakdown of both PAHs [4], as only 1.0 and 8.9%, 6.1 and 26.4% of initial phenanthrene and benzo[a]pyrene amounts remained after 4-week incubations with the fungi, respectively.

Secondly, we developed pelleted solid substrates formed from agricultural by-products. These substrates coated with a sodium alginate of mycelial fragments were incubated until overgrown by the mycelia of selected strains before inoculation into the soil. When calculated from soil ergosterol content, these inocula provided high amounts of fungal biomass (20-30 mg/g soil, dry weight) in the soil [5]. Experiments also showed that the two selected strains were able to grow in a non-sterile soil from a real industrial site containing around 2000 ppm PAHs and 200 ppm cyanides. Nevertheless, long course experiments showed that the fungal biomass decreased with respect to time, and that this decrease could be reversed in part by adding of nutrients.

In these conditions, white rot fungi also produced exocellular peroxidases and laccases, as revealed by activity and Western blot of enzymes in soil extracts. Laccase has been shown to oxidize PAHs [6].

To date, PAH degradation in industrial soils in the presence of strains powerful in liquid media remains low.

DEGRADATION OF POLLUTANTS IN BIOREACTORS

We also investigated a technique to produce important enzyme amounts in large scale. This new method of production could be useful for residual pollutant removal from leachates or effluents produced during soil bioremediation processes. We developed an innovative biofilm reactor system with an immobilization carrier made of nylon wet positioned in a bubble-column reactor [7]. This type of immobilization allows a greater contact area with the culture medium and therefore a more efficient mass transfer. Moreover, the INRA patented *P. chrysosporium* strain I-1512 was used for manganese-dependent peroxidase hyper secretion with yields of 900 U/day/l.

CONCLUSIONS

Taken together with results from other laboratories, our results confirm that filamentous fungi can be used for polluted soil bioremediation by using a bioaugmentation approach. As soon as PAH degradation will be increased in the laboratory, large-scale experiments will be conducted by inoculation of filamentous fungi in batch of 1-2 ton industrial soils.

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