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Society of Environmental Toxicologists and Chemists

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Pesticide Microbiology Group

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Institut National de la Recherche Agrochimique

Biotransformation of Pesticides in Soils Inoculated with the White Rot Basidiomycete *Phanerochaete chrysosporium*

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Introduction

For many years, various research groups have been interested in the development of white rot fungus technology for the biodegradation of environmental pollutants. Among these fungi, *Phanerochaete chrysosporium* has been extensively studied (Barr and Aus, 1994). In our laboratory, we showed that this fungus was able to transform atrazine to N-dealkylated metabolites in liquid cultures (Mougin *et al.*, 1994). Also, we have studied lindane breakdown by the fungus, and we have identified the main metabolites produced in the culture medium (Mougin *et al.*, 1994).

In this work, we studied the ability of *P. chrysosporium* to growth in an agricultural soil after inoculation, and to increase the natural degradation of both pesticides.

Experimental

A solution of growth medium (10 ml) including fungal spores (Mougin *et al.*, 1994) was added to 25 g (equivalent dry soil) of a silt loam from Versailles, to achieve 35% (wt/wt) soil moisture content. Thereafter, the soil was supplemented with [ring-UL-¹⁴C]atrazine or [¹⁴C]lindane, each in mixture with unlabeled pesticide, to give final concentrations of 0.6 and 0.7 ppm, respectively.

Fungal growth was estimated from soil ergosterol content. Pesticide mineralization was calculated from ¹⁴CO₂ trapping. ¹⁴C partitioning was established following solvent extraction of the radioactivity, HPLC analysis, and combustion of unextracted radioactivity.

Results and Discussion

After inoculation of spores to a sterilized soil, the fungal biomass increased rapidly during the first week, and then decreased to a lower level during the following 3-weeks, before reaching a plateau. The final biomass value (85.4 mg dry weight) was greater than that obtained in liquid cultures. The biomass in soil was consistent with the proliferation of white mycelium observed throughout the soil experiments. No fungal growth was observed in sterile soils inoculated with culture medium alone. In contrast to this, a limited amount of fungal growth was detected in non sterile soils after inoculation of *P. chrysosporium*, since maximal amount of 13.9 mg dry weight was noted after a 2 weeks period. This could be due

to the presence of a competitive indigenous soil microflora. Fungal biomass in the corresponding controls did not exceed 2.2 mg. Results were similar in soils containing either atrazine, or lindane.

Atrazine transformation was studied in two experiments with different endogenous microflora activity. After a short lag phase, the CO_2 released accounted for 18.3% and 47.9% of the initial radioactivity after 9 weeks, respectively. In both experiments, atrazine mineralization was unaffected by the presence of the fungus. No mineralization occurred in autoclaved soils, with or without *P. chrysosporium*.

Sterilization also increased the amounts of extractable radioactivity from soils with respect to non sterilized soils. In contrast, fungal inoculation had no significant effect on ^{14}C partitioning in soils, where levels of unextractable radioactivity were similar.

Lindane transformation was also studied. In non sterilized soils, insecticide 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. In contrast, the $^{14}\text{CO}_2$ evolved from autoclaved soils was quite negligible.

Only parent compound lindane could be extracted from all soils. Amounts of extracted lindane were lower in non sterilized soils, being the lowest in the presence of the fungus. Conversely, amounts of unextractable radioactivity were high in non sterile soils, with a decrease in the presence of the fungus. This seems to indicate that the increased mineralization is due to a direct effect of *P. chrysosporium* on the parent compound lindane, and also on the levels of unextractable radioactivity in soils.

Conclusions

P. chrysosporium is able to growth in a soil from a liquid inoculum. In some cases, it can increase the degradation of selected pesticides.

Résumé

Le basidiomycète *P. chrysosporium* est capable de se développer dans des sols non stérilisés, à partir d'un inoculum liquide. Dans ce cas, il n'améliore pas la biodégradation de l'atrazine due à la microflore endogène. En revanche, il synergise nettement la minéralisation du lindane, en agissant à la fois directement sur le pesticide, et en diminuant les teneurs du sols en radioactivité non extractible.

References

- Barr D.P. and Aust S.D. 1994. Environ. Sci. Technol. 28:78A-86A.
- Mougin C., Laugero C., Asther M., Dubroca J. Frasse P. and Asther M. 1994. Appl. Environ. Microbiol. 60:705-708.
- Mougin C., Péricaud C., Malosse M., Laugero C. and Asther M. 1996. Pestic. Sci. (in press).

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Introduction

Since many years, various research groups have been interested in the development of white rot fungus technology for biodegradation of environmental pollutants. Among these fungi, *Phanerochaete chrysosporium* has been extensively studied [1]. In our laboratory, we showed that this fungus was able to transform atrazine to N-dealkylated metabolites in liquid cultures [2]. Also, we have studied lindane breakdown by the fungus, and we have identified the main metabolites produced in the culture medium [3].

In this work, we study the ability of *P. chrysosporium* to grow in an agricultural soil after inoculation, and to increase the natural degradation of both pesticides.

Experimental

A solution of growth medium (10 ml) including fungal spores [2] was added to 25 g (equivalent dry soil) of a silt loam from Versailles, to achieve 35% (wt/wt) soil moisture content. Thereafter, the soil was supplemented with [ring-UL-¹⁴C]atrazine or [¹⁴C]lindane, each in mixture with unlabeled pesticide, to give final concentrations of 0.6 and 0.7 ppm, respectively.

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Conclusions

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[1]Barr D.P. and Aust S.D. 1994. Environ. Sci. Technol. 28:78A-86A.

[2]Mougin C., Laugero C., Asther M., Dubroca J. Frasse P. and Asther M. 1994. Appl. Environ. Microbiol. 60:705-708.

[3]Mougin C., Péricaud C., Malosse M., Laugero C. and Asther M. 1996. Pestic. Sci. 47:51-59.

Biotransformation de pesticides dans des sols inoculés par le basidiomycète de la pouriture blanche *Phanerochaete chrysosporium*.

Christian Mougin, Jacqueline Dubroca et Marcel Asther

Le basidiomycète *P. chrysosporium* est capable de se développer dans des sols non stérilisés, à partir d'un inoculum liquide. Dans ce cas, il n'améliore pas la biodégradation de l'atrazine due à la microflore endogène. En revanche, il synergise nettement la minéralisation du lindane, en agissant à la fois directement sur le pesticide, et en diminuant les teneurs du sols en radioactivité non extractible.