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Characterization of a bacterial consortium mineralizing isoproturon (IPU)

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Introduction

Isoproturon (3-(4-isopropylphenyl)-1,1-dimethylurea) is among the most extensively used herbicides in conventional agriculture in Europe for pre- and post-emergence control of many broadleaf weeds in spring and winter wheat, barley and winter rye (Fournier *et al*., 1975) . Ecotoxicological data have suggested that IPU, and some of its main metabolites are carcinogen and harmful to aquatic invertebrates, fresh water algae and microbial activities. Several studies dedicated to IPU degradation showed that microbial degradation is the primary mechanism of IPU dissipation as well as other phenylurea herbicides (Fournier et al., 1975; Cox *et al*., 1996; Pieuchot *et al*., 1996). The present study was carried out to characterize a bacterial consortium, isolated from French agricultural soils, for IPU mineralization and also for determining the isoproturon degradation pathway.

Materials and methods

The IPU degrading bacterial consortium was isolated from the soil by conducting the enrichment cultures. The degradation capability of the consortium for IPU, diuron, linuron, monolinuron and chlortoluron was studied by HPLC analysis and mineralization kinetics for IPU was determined by using ^{14}C ring-labelled IPU and then analysing for radioactivity content by liquid scintillation counting. Six bacterial strains were isolated by serial dilutions and continuous inoculations on MS-IPU medium plates. Phylogenetic identification of the isolated bacterial strains was carried out by amplifying the 16S rDNA by PCR and then sequencing it. All sequences were compared to known sequences found in Gene-Bank database using Blast (http://www.ncbi.nlm.nih.gov/BLAST/). Multiple alignments of 16S rDNA sequences were realised using the ClustalX programme and the phylogenetic tree was elaborated using the NJ plot programme.

Results and discussion

The bacterial consortium had the capability to degrade IPU but not other phenylurea herbicides (Fig. 1) thus suggesting that the genes for degradation might be specific for IPU and that the bacterial consortium harbours specific catabolic pathway for IPU.

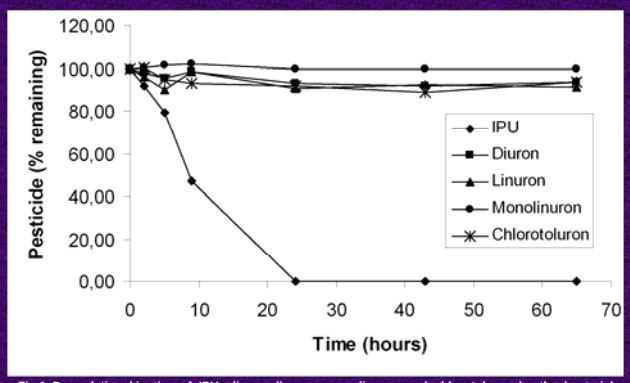

Fig.1 Degradation kinetics of IPU, diuron, linuron, monolinuron and chlorotoluron by the bacterial consortium inoculated in knapp buffer at 28 °C under agitataion (125 rpm).

The bacterial consortium had the ability to mineralise IPU at high rate (Fig. 2). Over an incubation period of 32 hours, the consortium mineralised about 35% of the initially added ^{14}C ring-labelled IPU. About 35% of the radioactivity was incorporated in the microbial biomass and about 5% remained in the cultural medium.

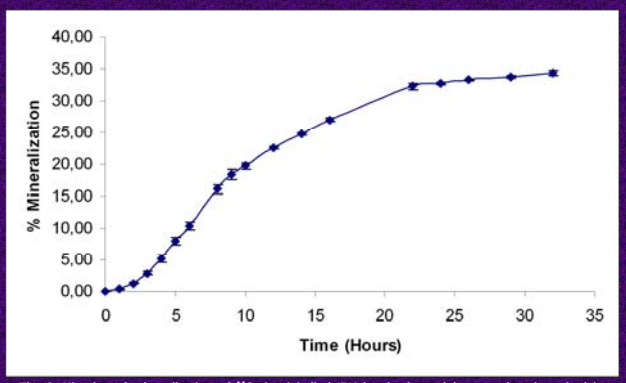

Fig. 2. Kinetics of mineralization of ^{14}C ring-labelled IPU by the bacterial consortium inoculted in Knapp buffer at 28°C under agitation (125 rpm).

By UPLC analysis of the samples collected during the IPU mineralization, we were able to detect the transitory accumulation and degradation of one IPU metabolite i.e. Monodemethylisoproturon (3-(4-isopropylphenyl)-1-methylurea). Thus, it was hypothesized that IPU mineralisation could have started by demethylation (Fig. 3).

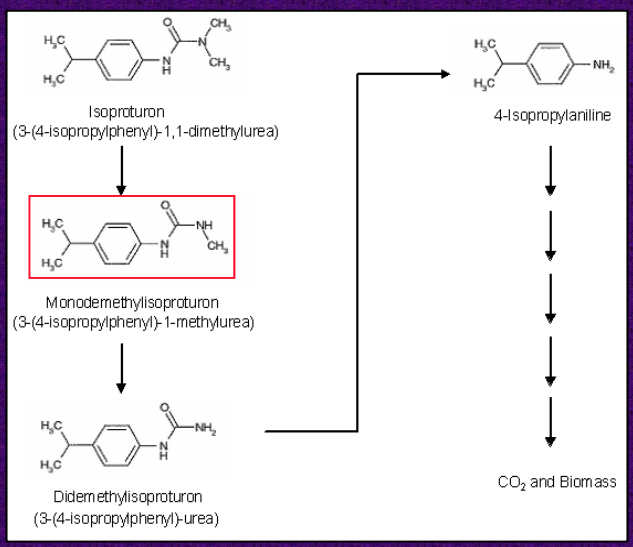

Fig. 3. Proposed metabolic pathway of isoproturon degradation according to Sorensen *et al*. (2001).

Six bacterial strains were isolated and characterised for IPU degradation from the consortium but none of them was found to be able to degrade IPU. Multiple alignment of these isolates was carried out in relation to the bacterial strains already known to degrade IPU (Fig. 4).

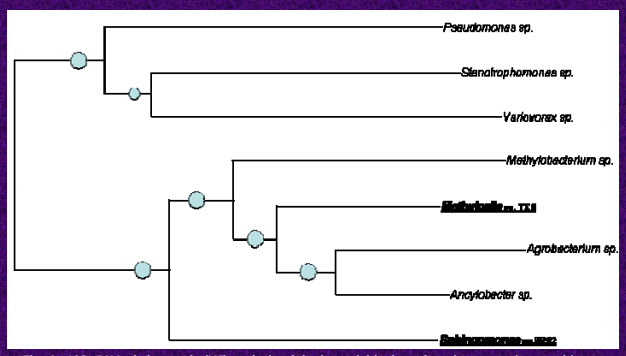

Fig. 4. 16S rDNA phylogenetic (NJ) analysis of the bacterial isolates fromthe consortium and known bacterial strains (underlined) already known to mineralize IPU completely.

Perspectives

Further research will aim at isolating the pure bacterial isolates able to degrade isoproturon, finding the further steps of IPU metabolic pathway and also aiming at identifying the genes coding the enzymes involved in isoproturon mineralization.

References

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