Poster presentations A1-A50, B1-B49

Pascale Besse-Hoggan, Université Blaise Pascal-Clermont II, France

A2. Understanding the microbiological and physicochemical influence of alperujo vermicompost on diuron behaviour and soil quality.
Jean Manuel CastilloDiaz, CSIC Granada, Spain

Isabelle Batisson, CNRS/Clermont-Ferrand University, France

A4. Transport and degradation of pesticides in a biopurification system under variable flux.
Tineke De Wilde, Ghent University, Belgium

A5. Effect of DOM quality and quantity on linuron degradation by a multi-species bacterial community in surface water.
Benjamin Horemans, Catholic University of Leuven, Belgium

A6. The non-target effects of azoxystrobin on the structure and function of microbial communities.
Christopher C. Howell, University of Warwick, UK

Sabir Hussain, INRA/Université de Bourgogne, France

A8. Sand-filter bio-augmentation of the pesticide metabolite, BAM, as a tool to clean-up drinking water contaminated by low concentrations.
Ole Jacobsen, GEUS, Denmark

Antonio López Piñeiro, Universidad de Extremadura, Spain

A10. Interaction of cation complexed atrazine and glyphosate herbicides with soil - A theoretical study.
Fabienne Bessac, Université de Toulouse, France

A11. Correction procedure for adsorption coefficients from batch studies based on measured recovery.
Jos Boesten, WUR-Altterra, The Netherlands

A12. Effect of olive mill waste used as soil amendment on diuron olive grove persistence.
Lucía Cox, CSIC Valencia, Spain

A13. Examination of the uptake of pesticides by plants grown in various soils.
Attila Kiss, EGERFOOD Knowledge Centre, Hungary

Ulrich Menke, Bayer CropScience, Germany

A15. Total and soil pore water concentration of pesticides in artificial soils in terrestrial ecotoxicological studies.
Holger Penning, BASF, Germany
Genomic approach to search for an IPU degrading gene: catA as a possible gene target responsible for 4- isopropyl aniline degradation

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Introduction

The phenylurea herbicide isoproturon [IPU] is used for pre- and post-emergence control of many broad-leaved weeds in different crops including winter wheat. Ecotoxicological data have suggested that IPU, and some of its metabolites, are carcinogenic and harmful to living organisms. So, there is a serious need to degrade IPU into simple harmless or less harmful residues. IPU degradation can take place by biotic as well as abiotic processes amongst which microbial degradation is recognized as a primary mechanism. Different bacterial strains having varied IPU degrading capabilities have been isolated from different soils and an IPU-degradation pathway has been proposed (for review see Sorensen et al., 2003). However the genes coding the enzymes responsible for IPU degradation still remain to be identified. The present study was carried out to determine the degradation capabilities and degradation pathway of an IPU-mineralizing bacterial consortium.

Materials and methods

The consortium was isolated from a French agricultural soil by conducting selective enrichment cultures using MS medium (El-Sebai et al., 2004). The capacity of the consortium to degrade IPU, diuron, linuron, monolinuron and chlorotoluron was estimated using HPLC analysis, whereas the metabolites formed during the IPU degradation were studied by ultra performance liquid chromatography (UPLC). IPU mineralization kinetics of the consortium were determined by radiospirometry using ¹⁴C ring-labelled IPU. The culturable members of the consortium were isolated by successive plating as reported elsewhere (Sorensen et al., 2001).

Genomic DNA of the consortium and of the isolated bacterial strains was extracted using QIAGEN Genomic DNA Isolation Kit (QIAGEN, France). 16S rDNA fragment from the genomic DNA was amplified by PCR using 27f (5’-AGA GTT TGA TCH TGG CTC AG-3’) and 1492r (5’-TAC GGH TAC CTT GTT ACG ACT T-3’) primers and then partially sequenced. All the sequenced were compared to known sequences in the GenBank database (http://www.ncbi.nlm.nih.gov/BLAST/).

A genomic DNA clone library was established by using CopyControl™ BAC Cloning Kit (EPICENTRE, France). PCR based screening of the BAC genomic library was carried out for catA sequence coding for catechol degrading 1,2-dioxygenase by using the catAf 5’-CCA TTG AAG GGC CGC TCT ATGT-3’ and catAr 5’-ACC GAA RTT GAT CTG CGT (G,C) GTCA-3’ primers (Sun et al., 2009).

Results and discussion

Degradation capabilities of the bacterial consortium

Over an incubation period of 48 hours, the consortium mineralised about 36% of the initially applied ¹⁴C-ring labelled IPU. UPLC analyses revealed the transitory accumulation and degradation of three IPU metabolites i.e. MDIPU (3-(4-isopropylphenyl)-1-methylurea), DDIPU (3-(4-isopropylphenyl)-urea) and 4-IA (4-
isopropylamine). We thus hypothesized that IPU mineralization could have been initiated by two successive demethylations followed by the cleavage of the urea side chain and ultimately the mineralization of the phenyl ring. This is in accordance with the IPU-metabolic pathway proposed for Sphingomonas sp. SRS2 (Sorensen et al., 2001). Furthermore, this consortium had the ability to degrade only IPU and no other phenylurea herbicides, suggesting that it might be harbouring the enzymatic capacities highly specific for IPU.

Phylogenetic characterization of the consortium

Based on 16S rDNA fingerprinting, only six different clone families were identified. When the sequences of the six clone families were compared to the known sequences, four were found to be closely related to the genus Sphingomonas (>98% similarity for each one) and two other families belonged to the genera Pseudomonas and Agrobacterium with one of the Sphingomonas strains presented as a dominant member (> 80% abundance) of the bacterial consortium. By repeated plating of the consortium on different types of media, several members were isolated and identified as belonging to six different phyotypes close to (> 98% similarity for each one) Ancylobacter, Pseudomonas, Stenotrophomonas, Methylobacterium, Variorovax and Agrobacterium genera. Unfortunately, none of these isolates were able to degrade IPU or its known metabolites.

Screening of BAC genomic library for catechol degrading gene

In order to search for the genes coding for IPU degrading enzymes, a BAC genomic DNA clone library was established by picking about 3000 recombinant clones containing the genomic DNA fragments of 15-25 kb in size. catA PCR screening of the genomic library led to the isolation of six BACs. Those BACs are suspected to harbor the catechol degrading 1,2-dioxygenase gene which may be involved in the degradation of aniline derivatives, including 4-IA. Catechol, which is considered as a key intermediate during the phenyl-ring cleavage of most of the aromatic hydrocarbons, has already been detected as a metabolite during IPU degradation by Sphingobium strains YBL1, YBL2 and YBL3 (Sun et al., 2009).

Perspectives

Further work will aim at fully sequencing the six BACs presenting the catA gene to search for surrounding genes which may contain other IPU-degrading genes. In addition, functional genomics will be applied for studying the activity of catA BACs on the catechol and different aniline derivatives including 4-IA.

References


