Isolation of pesticide-degrading bacteria by Most Probable Number (MPN) method using tetrazolium salts

Stauffert, M.¹,², Besse-Hoggan, P.³,⁴, Mallet, C.¹,², Batissan, I.¹,²

1 Clermont Université, Université Blaise Pascal, LMGE, F-63100 Clermont-Ferrand, France; ²-CNRS, UMR 6022, Laboratoire Microorganismes Génomes et Environnement, BP 80206, F-63171 Aubière Cedex, France; ³Clermont Université, Université Blaise Pascal, ICF, F-63000 Clermont-Ferrand, France; ⁴-CNRS, UMR 6024, Institut de Chimie de Clermont-Ferrand, BP 80206, F-63171 Aubière Cedex, France

Pesticides biodegradation capacity developed by soil microorganisms is a major issue limiting contamination and maintaining the resilience of soil. Some studies focused on pesticides-degrading bacteria but little is known about metabolic pathways degradation of these molecules. Hence, isolation of pesticide-degrading microorganisms and characterization of produced metabolites are essential for a better knowledge of pesticides fate in the environment. Assuming the postulate that the probability to highlight different pesticides degradation pathways increases with the number of studied isolates, we used a most probable number (MPN) method in 96-well microplates to isolate aerobic pesticides-degrading bacteria.

**SOIL SAMPLES**

![Biobac system and agricultural soil](image)

**SLURRY 10% (v/w)**

Two sites were selected in Puy-de-Dôme (France)

**MPN PROCEDURE**

Pesticide sole carbon source

**OPTIMAL DILUTION SELECTION for each herbicide**

the highest positive dilution for which a bacterial growth is observed

**ISOLATION IN 96 WELL MICROPLATES**

"HIGH-THROUGHPUT INOCULATION OF OPTIMAL DILUTION"

**CULTURE VOLUME: 1 ml**

**GROWTH OF 227 POSITIVE WELLS**

**BIOBAC SYSTEM: 105**

**AGRICULTURAL SOIL: 122**

**SCREENING OF MICROBIAL CONSORTIA BASED ON HERBICIDES DISSIPATION CAPACITIES: HPLC experiment**

- Culture volume: 250 µl
- Addition of INT after the 7th day of incubation

After five successive subcultures under selective pressure of herbicides, isolates were assayed for their biodegradation capacity.

We observed herbicides dissipation for 62% (65 isolates) and 54% (66 isolates) of isolates obtained respectively from biobac® and agricultural soils.

**MOLECULAR CHARACTERIZATION OF DEGRADING-ISOLATES CONSORTIA by 16S rRNA genes diversity analyses (TTGE)**

**Consortia profiles comparison by MDS analysis**

(Multidimensional scaling: presence / absence analysis)

<table>
<thead>
<tr>
<th>Consortia Profiles number</th>
<th>Different profiles number</th>
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</thead>
<tbody>
<tr>
<td>Mesotrione</td>
<td>23 (43%)</td>
</tr>
<tr>
<td>Nicosulfuron</td>
<td>36 (44%)</td>
</tr>
<tr>
<td>S-metolachlor</td>
<td>24 (87%)</td>
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</tbody>
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No clear bacterial communities separation between sites further analyses are needed