

Processes involved in microbial adaptation to pesticide biodegradation; perspectives for improving the Biobed functioning.



IN

Burgundy's vineyards

M. Devers, N. Rouard & *F. Martin-Laurent*





Dijon's mustard

Center for Soil and Environmental Microbiology - CMSE Laboratory for Soil and Environmental Microbiology (UMR MSE)

INRA/Université de Bourgogne, 17 Rue Sully, BP 86510, 21065 DIJON, FRANCE.





Biobed functioning : pesticide dissipation ...



Pesticides and metabolites accumulation/storage: ultra-delayed contaminations ?
Impact on Biobed functioning ?

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« The whole is much more than the sum of its components », Aristotle



The black box concept could define the Biobed-system as a complex of interacting elements, linked by <u>structural and functional relations</u> of interdependence and interaction, between each other, as well as with the entire system.

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How to deal with the Biobed black box?



Estimation of pesticides fluxes

-Quantifying input and output variables (pesticides effluents and pesticides residues,...)



Search for effectors: estimation of microbiological parameters

- microbial density (microbial C biomass, enumeration), activity (respiration, radiorespirometry...), - isolation and characterization of functional microbial populations (C and N cycles, pesticide



Omics' to open the Biobed black box ?





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➢Following repeated application of pesticides, the microflora may adapt to accelerated pesticide biodegradation.

What are the processes involved in microbial adaptation to accelerated biodegradation of pesticides?



Research strategy applied

<u>Aim</u>: Understand the functioning of microbial communities responsible for pesticides biodegradation (structure \Leftrightarrow diversity \Leftrightarrow density \Leftrightarrow function).



Atrazine-degrading community as a model

Herbicide of the s-triazines family (photosynthesis inhibitor)

Applied on corn crop, in vineyard and orchard at 1 kg.ha⁻¹

Forbidden since September 2003, but still contaminating French water resources

Tools available to analyze pesticide-degrading community

DNA/RNA analysis of the global structure of soil microflora

Density/Expression of atz-degrading community

□ The atrazine-degrading genetic potential (*atzA*) transitory increases after adding atrazine to soil.

□ Similarly, the expression of the atrazinedegrading genetic potential transitory increases after adding atrazine to soil, (amplification x 1000 due to transcription).

After adding the herbicide, the atrazine-degrading genetic potential and its expression behave similarly.

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Expression of the *atz*-degrading genetic potential in the *Pseudomonas* sp. ADP population : the lower pathway


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Devers (2004) J. Microbiol. Meth. 56:3-15
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- -The atzDEF genes are expressed at a basal level (lowly),
- The *atzD* and *E* genes are transitory over-expressed following atrazine application,

- An expression gradient is observed from gene *atzD* to gene *atzF* which are organized in operon on pADP1.

Regulation of the expression of atzDEF genes by N

RNA

The *atzDEF* genes are organized in an operon on pADP1 and placed under the regulation of the *atzR* transcription factor (*LysR* family)

Working regulatory model for *atzR* and *atzDEF*.

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Second European Biobed Workshop, Ghent, Belgium

Activity~

Expression of the upper pathway: gene dosage effect...

atzB gene duplication on pADP1 mediated by ISs homologous recombination,

Gene dosage effect leading to the overexpression of *atzB* explains the gain of fitness of the newly evolved population of *Pseudomonas* sp. ADP.

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Devers et al. (2007) Environ. Microb. In press

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Repetitive culture of *Pseudomonas* sp. ADP on MS-atrazine medium yields to the appearance of a newly evolved population growing and mineralizing faster than the original.

Activity~

Complexity of the soil atrazine-degrading community ...

-Lower pathway: *trzD* detected in two members (CA1, CA2)

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presents in ATZ2 and ATZ1, respectively.

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Genetic plasticity of the atrazine-degrading function...

atz genes are: located on conjugative plasmids submitted to conjugation (Devers et al. 2005) surrounded by ISs submitted to gene rearrangement (Devers et al. 2007a).

As a result, *atz* genes have been found on various plasmids and are shared by the atrazine-degrading community (Devers et al. 2007b)

Devers et al. (2005) Pest Manag Sci. 61:870-880, Devers et al. (2007a) Gene, 392:1-6, Devers et al. (2007b) FEMS Microbiol. Lett, 273:78-86.

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Adaptation of soil microflora to atrazine biodegradation

Conjugation

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= HGT leading to the dispersal of catabolic plasmids harboring atz genes among soil microflora.

Genetic rearrangement (IS-mediated transposition)

= Diversification of the genetic location of *atz* genes

These two mechanisms contribute to:

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Increase the host spectra of catabolic plasmids increase the conjugation frequency

The adaptation of the soil microflora to atrazine-ABD

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Conclusions

Proteins

The understanding of the processes responsible for the regulation of a microbial function in soil requires an integrative approach (from the gene to the community) and should be carried out in light of evolution.

For genes (upper atrazine-pathway) not yet regulated:

DNA

- the estimation of the density is correlated with the activity,

-duplication leading to gene dosage effect, up-regulates the function (at population level),

RNA

-Genetic rearrangement and horizontal gene transfer contributes to share atrazine-degrading genetic potential and to improve the function (at the community level).

For genes (lower atrazine-pathway) organized in an operon and finely regulated:

- the estimation of the density is not necessarily correlated with the activity,

- the estimation of the expression of the atrazine-degrading genetic potential will produce a better view of the fine regulation of the function.

Activity

Biobed functioning

-Favor adaptation processes to support metabolic pathways in the Biobed functioning,

-Characterize bacterial adaptation processes occurring in the Biobed as an interesting example of "adaptation under high level of stress (exerting a high selection pressure) with an initial limited biodiversity",

- Search for microbial strains and consortia degrading the principal pesticides in use in European countries to develop inoculants and biostimulants with the aim at improving Biobed functioning.

Biobed functioning – early indicators

- Develop innovative tools to monitor Biobed functioning notably bioindicators in order to establish "decision rules to manage the Biobed",

- Assess the biological risk related to the adaptation of the microflora in response to the important stress caused by repeated pesticide effluents (accelerated biodegraders, multi-resistant pathogenic fungal strains).

Pesticide biodegradation research group

Fabrice Martin-Laurent (DR) Jean-Claude Fournier (IR)

Jérémie Beguet (IE) Marion Devers (IE) Nadine Rouard (TR)

PhD

Séverine Piutti (1999-2001) Talaat El Sebai (2001-2004) Marion Devers (2002-2006) Najoi El Azhari (2004-2007) Sabir Hussain (2007-...) Frédérique Changey (2007-...)

Emilie Vieille (2005...)

Post-Doc Juan-Carlos Lopez-Gutierrez (2004-2005) Amadou Sarr (2005-2007) Anicet Manga (2007-...)

