Functional metagenomics on biobed systems: Evolutionary mechanisms of known and isolation of novel pesticide biocatalytic enzymes

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Introduction

Biobeds are on-farm systems used for the depuration of pesticide-contaminated effluents. They are packed with an organic substrate which supports an active microbial community able to degrade a range of pesticides. **Our hypothesis is that the exposure of the biobed's microbial community to a wide range of pesticides will trigger the evolution of novel catabolic mechanisms and effective enzymes for the degradation of the range of recalcitrant pesticides discharged in biobed systems.** Hence biobeds is expected to be a rich pool of novel pesticide catabolic enzymes which could be exploited in applications in medicine, agriculture and environmental clean-up strategies

Main Aim

To isolate novel pesticide catabolic enzymes and to explore the evolution of known pesticide catabolic enzymes through **functional metagenomics**

Materials and Methods

Biomixture: A sample from INRA's farm biobed, Dijon was used. It showed high mineralization capacity for isoproturon (Figure 1a), presence of the *pdmAB* gene (encoding a demethylating monoxygenase involved in the degradation of phenylureas) and high exposure to a range of pesticide groups (Figure 1b)

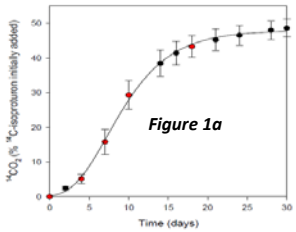


Figure 1a

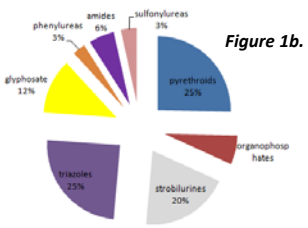
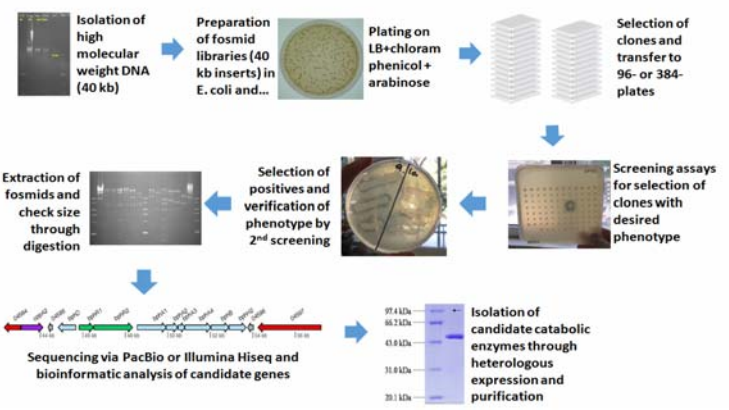


Figure 1b.

Pipeline for the **functional metagenomic** analysis of the biobed material (Figure 2)



Fosmid library screening tests

20000 fosmid clones were screened with **phenotypic assays** for novel:

1. **Esterases** and **carboxyesterases,** known to be involved in the hydrolysis of pyrethroids (25%) and organophosphates (6%) with, **a-napthyl acetate**, **tributyrin** and **mangenta caprylate** hydrolysis assays
2. **Monoxygenases** known to be involved in the transformation of phenylureas (3%) and potentialy triazoles (25%) with the **indole oxidation** test

20000 fosmid clones were **PCR-screened for *pdmAB* genes** (primers Gu et al., 2013) **using the pool-to-pool screening method**

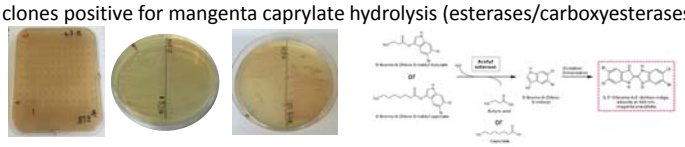
Why looking for pdmAB?: To identify the origin and localization of the *pdmAB* genes (plasmid-encoded) without the bias of cultivation-isolation, to get insights into the mechanism driving their environmental dispersal (mobilization of plasmids or HGT) and to explore if the exposure of the bacterial community to a range of phenylureas in the biobed system studied has triggered the evolution of *pdmAB* with altered specificity for phenylurea herbicides

Results – screening of the fosmid library

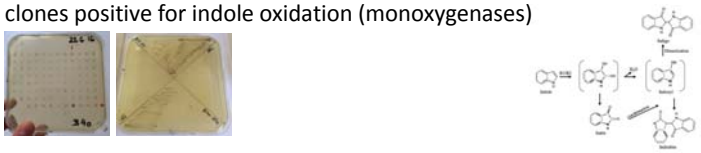
Phenotypic Screening tests ended with 12 positive clones

- 9 clones positive for tribyturin hydrolysis (esterases/hydrolases)

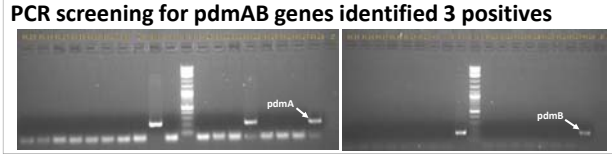
- 3 clones positive for mangenta caprylate hydrolysis (esterases/carboxyesterases)



- 3 clones positive for indole oxidation (monoxygenases)



PCR screening for pdmAB genes identified 3 positives



Results – Sequencing of positive clones

Sequencing of fosmids (insert size 32.8-41.9 kb) identified several repeats (plasmid origin) and genes with **putative catabolic function (esterase, carboxy-esterase, monoxygenase) or involved in ring cleavage pathways (protocatechuate)**

Phenotype	Genes with catabolic functional annotation
Tributyrin	nlhH carboxylesterase
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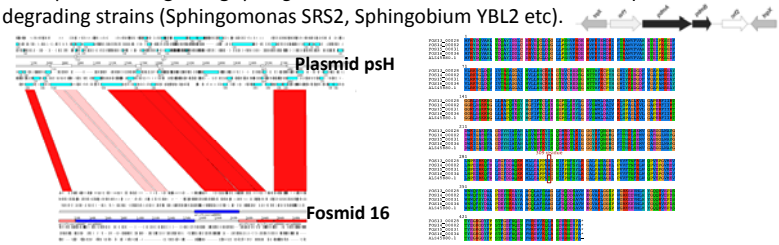
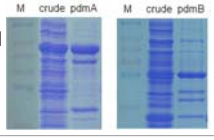
Tributyrin	L-aminopeptidase/D-esterase, 4-carboxymuconolactone decarboxylase, 3-oxoadipate enol-lactonase 3-carboxy-cis-cis-muconate cycloisomerase
Tributyrin	Esterase (estB)
Tributyrin	Esterase (estB)
Tributyrin	Alpha/beta hydrolase fold protein
Indole/mangenta caprylate	Flavin – dependent monoxygenase
Indole/mangenta caprylate	Flavin – dependent monoxygenase
Indole/mangenta caprylate	Phenoxybenzoate dioxygenase subunit beta, 3-oxoadipate enol-lactonase , 2,5-dihydroxypyridine-dioxygenase p-hydroxyphenylacetate 3-hydroxylase reductase

Results – pdmAB story……

Sequencing of *pdmAB*-carrying fosmids (inserts 34 – 46.1 kbs) showed high homology with a region of approximately 4460 bp (**pdmAB cassette**) found in the psH plasmid of an Isoproturon-degrading *Sphingomonas* sH strain and also in other isoproturon-degrading strains (Sphingomonas SRS2, Sphingobium YBL2 etc).



- Alignment of the sequences of the PdmA from the metagenome and the PdmA from psH plasmid showed one a.a. substitution at the 309th residue (Ala to Thr)
- The impact of this polymorphism on PdmAB activity will be tested *in vitro* (via heterologous expression and isolation of the enzymes from the metagenome & *Sphingomonas* sH) in the presence of a relevant phylogenetically reducatase component



Conclusions and future perspectives

- Functional metagenomic analysis revealed a rich pool of novel putative catabolic enzymes (probably of plasmid origin) whose activity against pesticides like pyrethroids, triazoles, phenylureas will be tested *in vitro* after their isolation via heterologous expression
- PdmAB cassette was detected in the metagenome, most probably in plasmids different from the psH of *Sphingomonas* sH suggesting its dispersal via HGT

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