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Biotic Control of Atrazine Degradation in Soils: Bacterial Functional Diversity of Degradation and Macrofauna Effects

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Earthworms, efficient soil engineers

- organic matter reorganization

litter burying

organic matter fragmentation

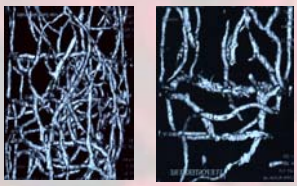
- soil structuration

biostructures: casts and burrows

- Close association with soil microflora:

bacterial and fungi community structuration

protozoa stimulation



3D tomography images of burrow networks
(Jegou et al., 1998)

casts

Soil macrofauna and microorganisms interactions : Intimate and complex interactions

SOIL

heterogeneous environment, nutrient-limited

rare favourable micro-habitats

Macrofauna

·limited metabolic capacity

BUT

• close relationship with OM

• high burrowing capacity

Microorganisms

• numerous and diverse

·high catabolic capacity

BUT

• no or limited mobility

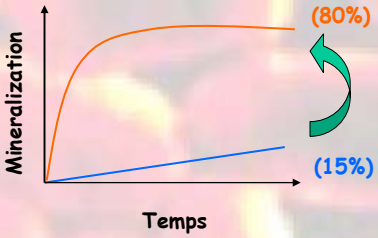
• dormancy

• low activity *in fine*

Physical desorganisation
Soil and OM ingestion

Time-space coincidence
Microorganisms /ressources bring into contact

Herbicide degradation in soil: the case of the model atrazine



repeated treatments

➢ Some soil bacteria are adapted to atrazine treatment and use it as C and/or N sources

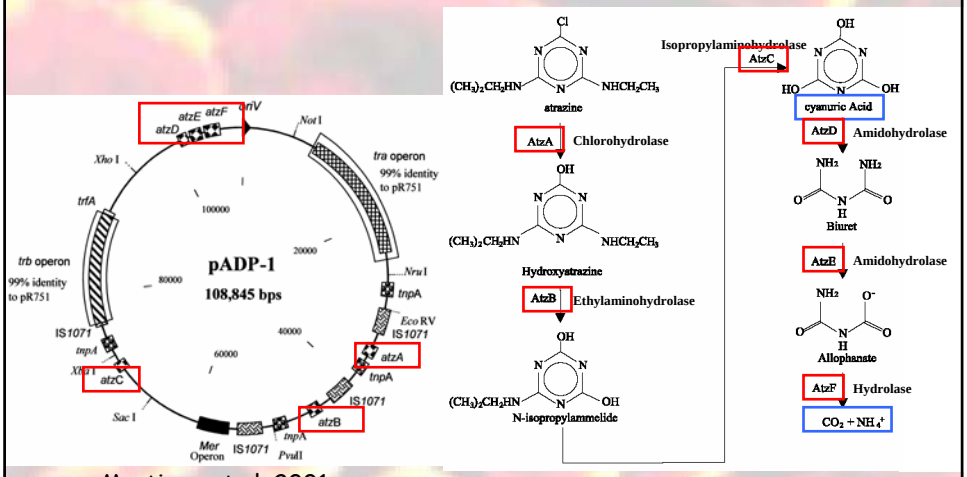
⟶ atrazine degradation

➢ Isolation of these bacteria by culture selection

⟶ different pathways of biodegradation exist

Rapid mineralization pathway

In *Pseudomonas* sp. strain ADP the enzymes involved are encoded by 6 genes located on a single plasmid pADP-1.



Martinez et al. 2001

Questions

Biotic control of atrazine degradation in soils

1. To what extent earthworms impact on atrazine degradation and atrazine-degrading bacterial abundance?

Bioturbation effect on bacterial atrazine-degrading potential

2. Which bacterial communities degrade atrazine and do earthworms modify their composition and their diversity?

Bioturbation effect on atrazine-degrading bacterial consortium diversity

Field of interest: assessment of the capacity of field margin in pollution control by biodegradation

1. Bioturbation effect on bacterial atrazine-degrading potential

Hypotheses

a. Earthworms might enhance soil bacterial abundance in their biostructures

b. Earthworms should specifically impact the activity of atrazine-degrading bacteria

Work done in collaboration with UMR Microbiologie et Géochimie des Sols, INRA Dijon, Fabrice Martin-Laurent

Materials & methods 1

Experimental design: soil microcosms

Lumbricus terrestris

- With or without atrazine-degrading bacterial inoculums: *Pseudomonas* sp. strain ADP (*atzA*, *B*, *C*, *D*, *E* and *F*) or *Chelatobacter heintzii* (*atzA*, *B* and *C*)
  Inoculation rate: 1.6 10^7 cfu/g of soil
- With or without *L. terrestris*
- Atrazine treatment (1.5 $mg.kg^{-1}$)

Materials & methods 2

Molecular analyses and flux measurements

Sampling of soil microsites :

control soil, midgut, hindgut, casts, burrow-linings

DNA extractions

qPCR

16S rRNA and *atzA*

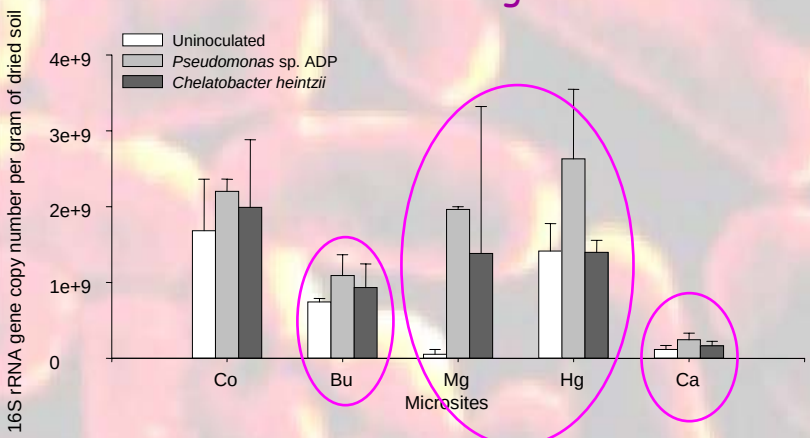
Soil indigenous bacterial community size

Atrazine-degrading bacterial community size

Kinetics of ^{14}C-ring labelled atrazine mineralization

Results 1

Abundance of 16S rRNA gene in soil microsites

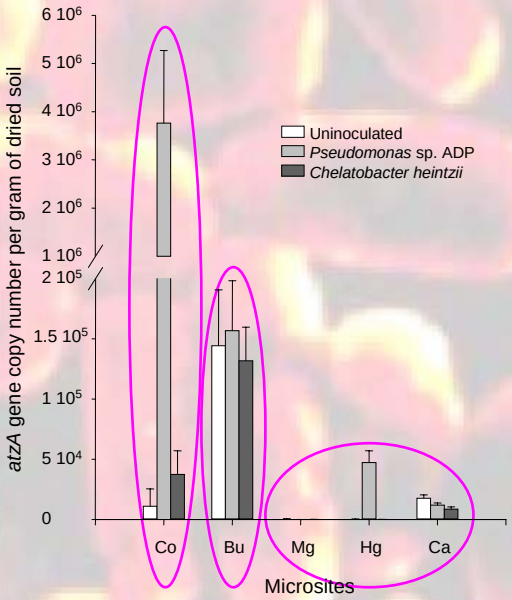


- whatever the treatment the 16S rRNA gene number decreased in burrow-linings and in casts in which it is very low
- A high variability is observed in guts

negative effect of earthworms on the soil indigenous bacteria

Results 2

Abundance of *atzA* gene in microsites

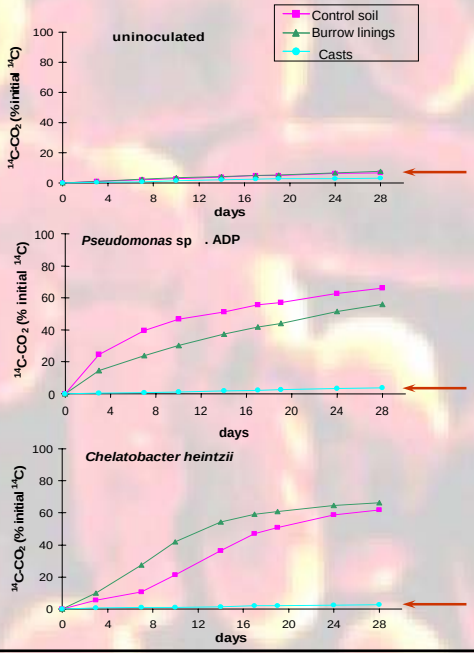


- *C. heintzii* maintained at a low level in soil unlike *Pseudomonas* sp. ADP
- whatever the treatment, *atzA* gene numbers are low in guts and casts but not in burrow-linings
- whatever the treatment, in burrow-linings, *atzA* gene numbers are similarly high

Contrasting earthworm effects on atrazine-degrading bacteria

Results 3

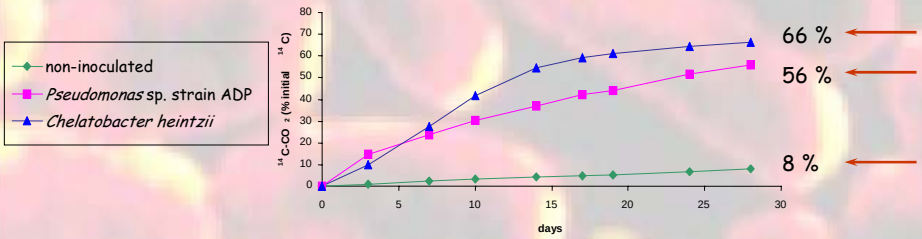
Kinetics of ^{14}C-atrazine mineralization



- Without inoculation the rate of atrazine mineralization was very low (less than 8 % after 28 d)
- In soil inoculated mineralization was significantly enhanced except in casts
- Although *C. heintzii* was at a low level in soil, it still well degrade atrazine

Results 4

Burrow-linings



In burrow-linings, similar *atzA* gene numbers lead to different atrazine mineralization rates :

- gene harboured by inoculated strains → enhanced mineralization
- gene harboured by indigenous bacteria → low mineralization

It might be that indigenous bacteria do not harbour the entire atrazine mineralization pathway

Conclusions

- Despite the poor survival of *C. heintzii* in soil, the small number which survived might be metabolically active and able to enhanced atrazine mineralization.
- Earthworms favor spatial heterogeneity of the atrazine degradation process in soil
- We observed low 16S rRNA and *atzA* gene copies number in casts:

In a limited organic carbon soil, *L. terrestris* seems to use soil bacteria as nutrient source.

- Burrow-linings form a favorable microsite for both indigenous atrazine-degrading bacteria and inoculated atrazine-degrading strain

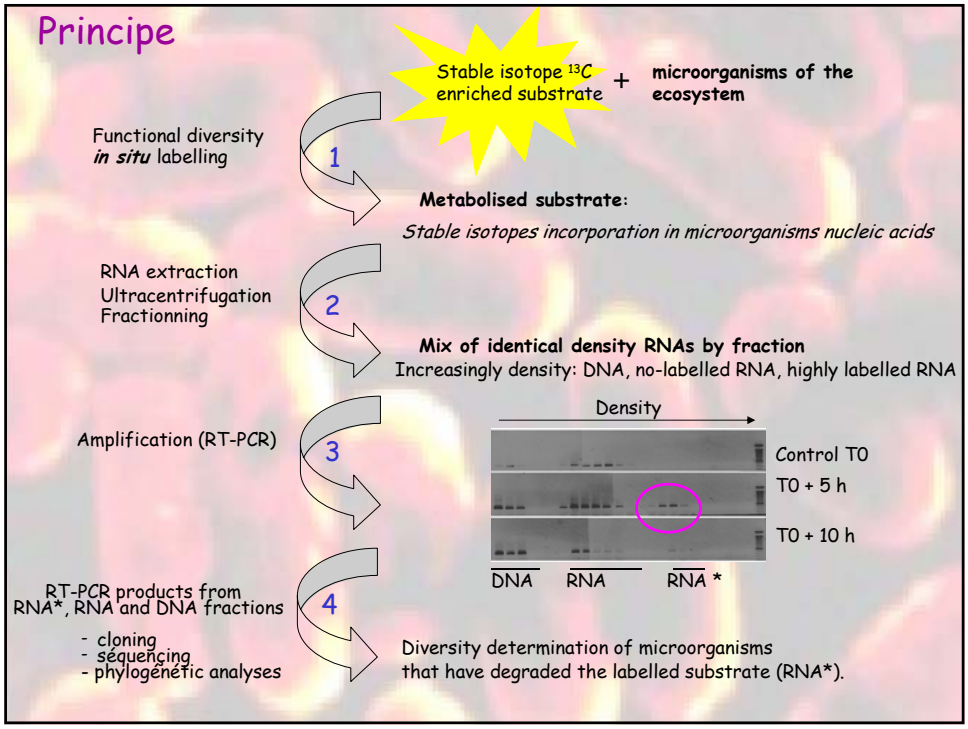
2. Bioturbation effect on atrazine-degrading bacterial consortium

Experimental strategy:

Stable Isotope Probing RNA (SIP-RNA):

Access to the whole genetic pool of bacteria involved in atrazine-degradation **without selection by plating culture**

Principe



SIP-RNA optimisation

Objectives:

SIP-RNA optimisation with soil samples and a substrate easily metabolised by soil bacteria

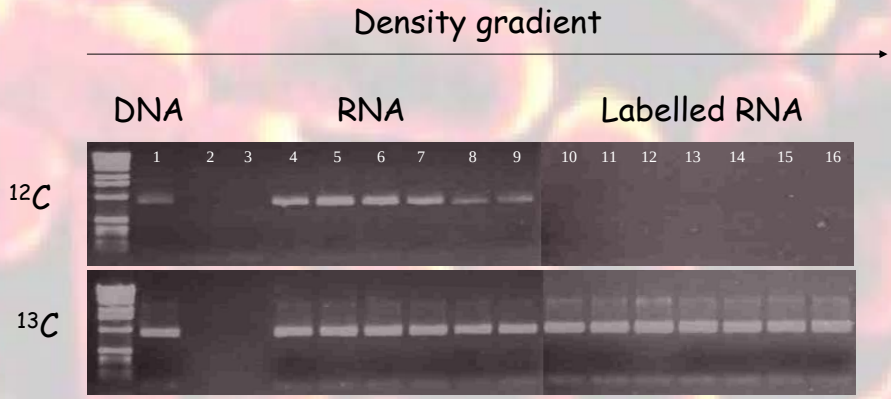
Preliminary experiments with ^{13}C-Glucose

soil + minimum media + ^{13}C-Glucose

RNA extraction
Ultracentrifugation
Fractionning
RT-PCR

Results

RNA extraction 22h following ^{13}C-glucose addition



Further Objectives

SIP-RNA analyses on natural soil samples and after then on earthworms biostructures following ^{13}C-atrazine application

- Atrazine-degrading bacterial consortiums diversity
- Earthworms impact on atrazine-degrading consortiums

Preliminary experiments with ^{14}C-atrazine are currently running to assess incorporation of the carbon derived atrazine into bacterial RNA in control soil, burrow-linings and casts.

Thank you for your attention