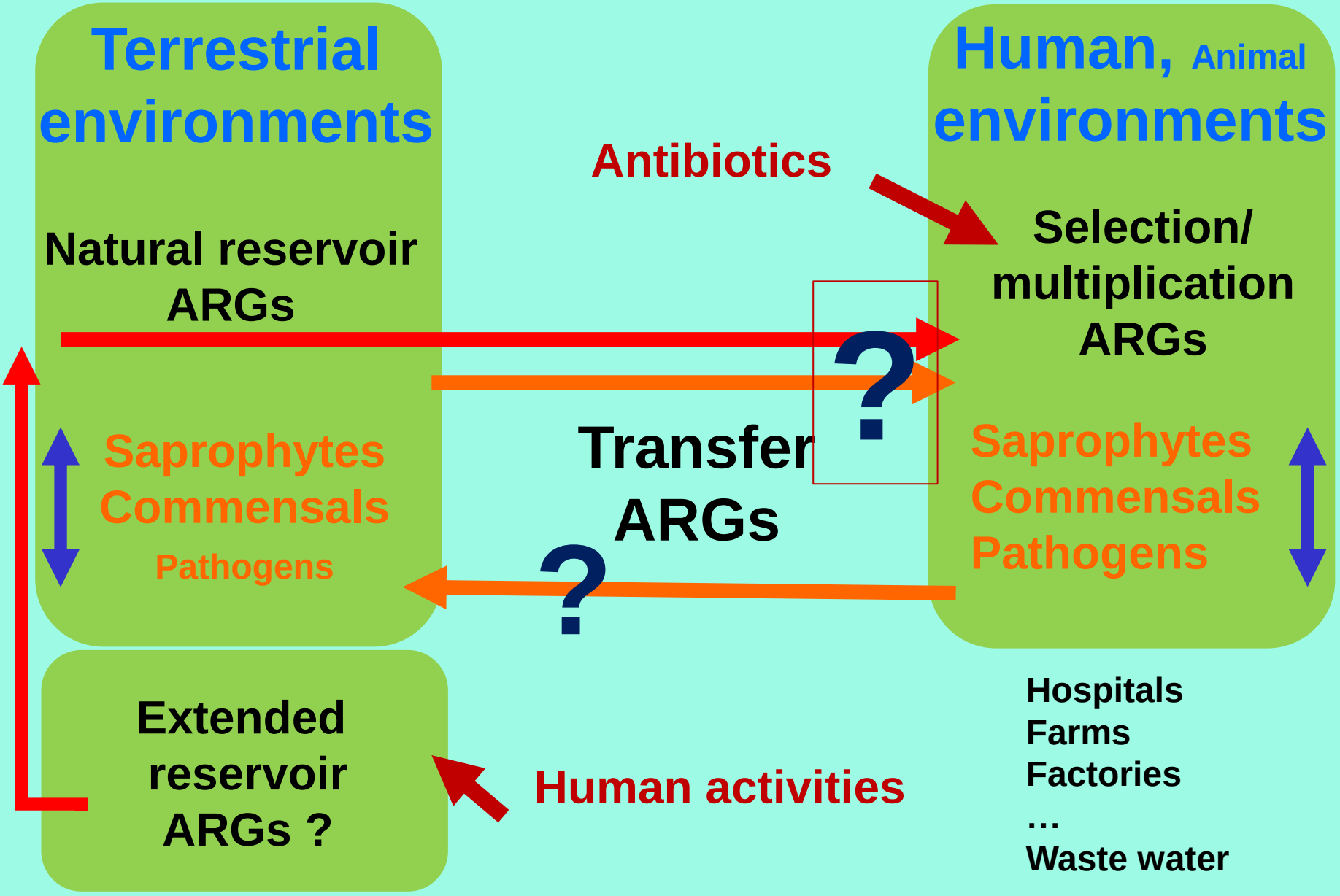
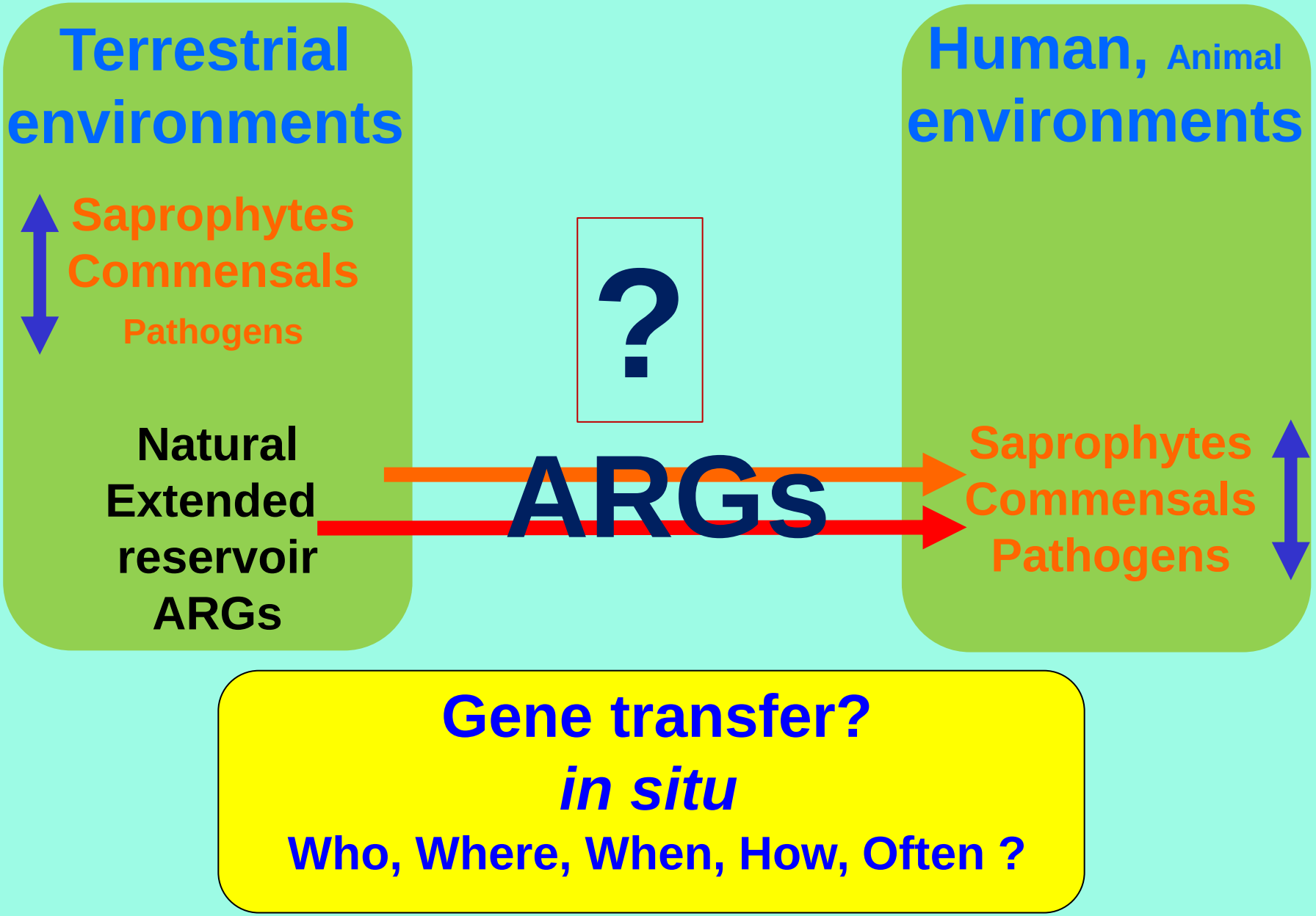
Dynamics of the soil resistome

Fate, Transport,Transfer of DNA (including ARGs) in Terrestrial Environments

Adebiotech - MBIO 2018

Environmental Microbial Genomics Group
Laboratoire Ampère . Ecole Centrale de Lyon . Université de Lyon

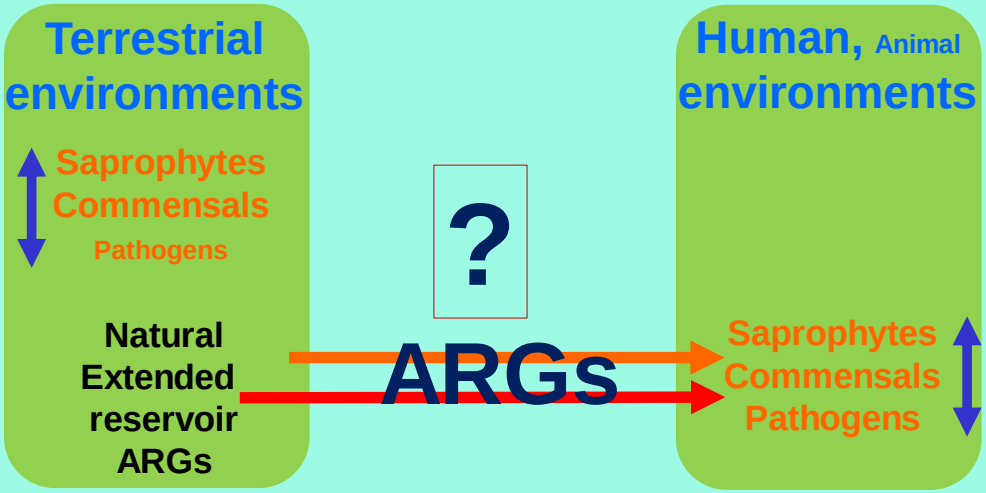

Terrestrial environments
Natural reservoir ARGs
Saprophytes
Commensals
Pathogens
Extended reservoir ARGs ?
Antibiotics
Selection/ multiplication ARGs
Transfer ARGs
?
?
Human, Animal environments
Saprophytes
Commensals
Pathogens
Hospitals
Farms
Factories
…
Waste water
Human activities



Gene transfer?

in situ

Who, Where, When, How, Often ?

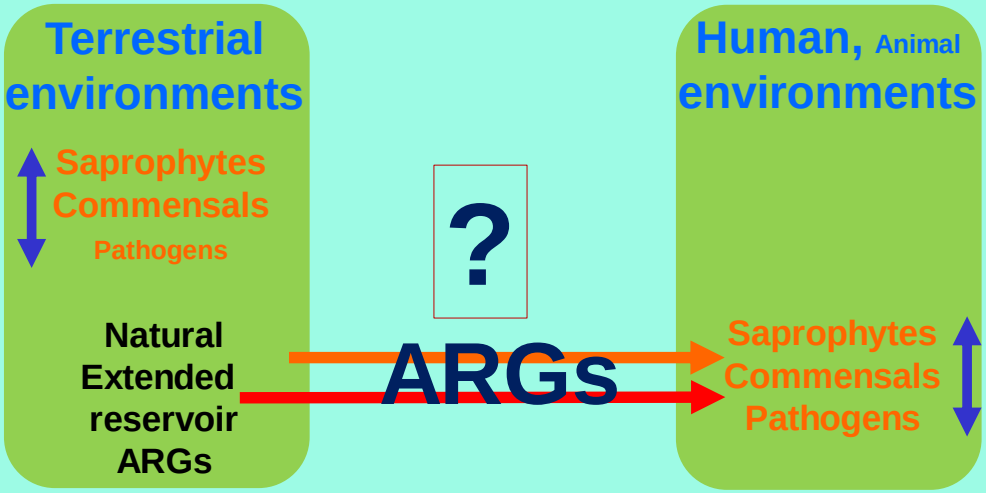


Gene transfer?

in situ

Who, Where, When, How, Often ?

- **Difficult to assess**
- **ARGs widely spread in terrestrial environments (reservoir)**
- **Difficult to discriminate between donors and recombinants**



Gene transfer?

in situ

Who, Where, When, How, Often ?

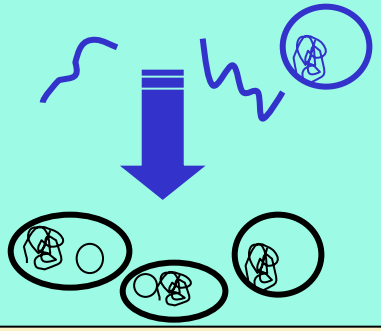
Tools:

GMP

GMP DNA

Possibility of Gene transfer from GENETICALLY MODIFIED PLANT (GMP) to Bacteria

Release of recombinant DNA



Indigenous microflora

HORIZONTAL GENE TRANSFER

The frequent incorporation of procaryote-derived antibiotic resistance gene in GMP has raised questions about the possible transfer of AR genes to :

- **other plant species (pollen)**
- **indigenous microbes in soil**
- **plant-associated bacteria**

NATURAL TRANSFORMATION

ENVIRONMENTAL AND GENETIC BARRIERS AGAINST NATURAL TRANSFORMATION OF BACTERIA IN THE ENVIRONMENT

◆ DNA bioavailability

- Release of « foreign » DNA close to bacteria or DNA transport.
- Protection against degradation by nucleases, chemical modifications.

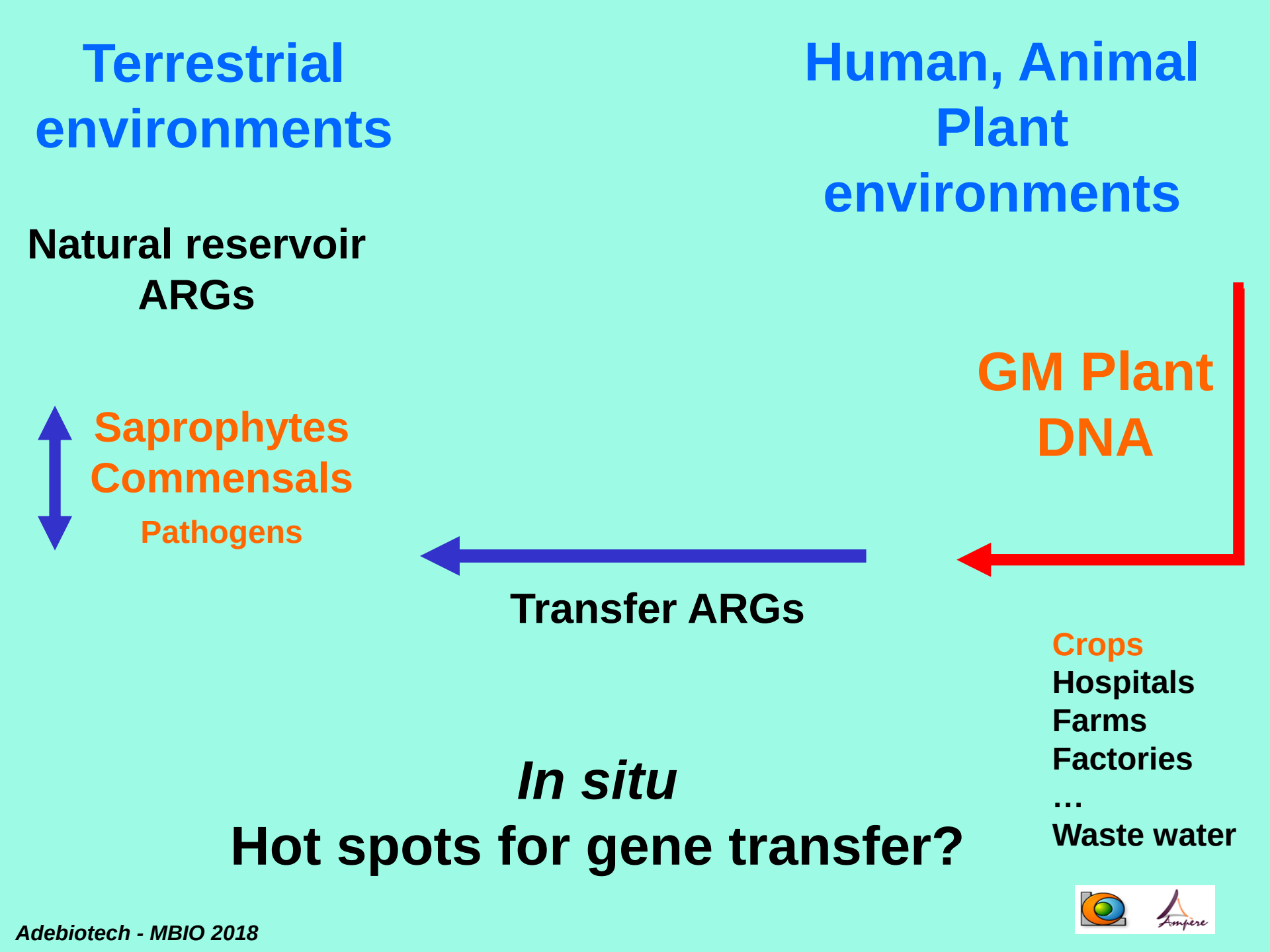
◆ Presence of putative recipient bacteria close to available DNA

- Natural transformable bacteria
- Development of a competent state through active metabolism

◆ DNA integration by bacteria

- Uptake of the « foreign » DNA
- Presence of homologous sequences for recombination
- Expression of « foreign » DNA

Who, Where, When, How, Often ?


Terrestrial environments
Human, Animal Plant environments
Natural reservoir ARGs
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Saprophytes
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Crops
Hospitals
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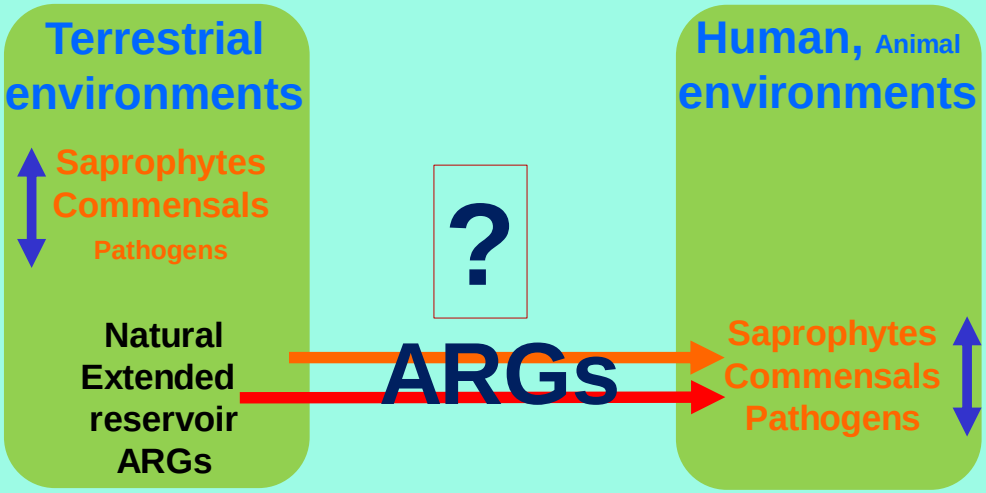
…
Waste water
In situ
Hot spots for gene transfer?



Gene transfer?

in situ

Who, Where, When, How, Often ?

Hot spots for gene transfer?

Transplastomic Tobacco

Transgenic plants where the chloroplast genome contains the transgene (***aadA*** gene conferring resistance to spectinomycin and streptomycin).

Collab Alain Saillant, RhônePoulenc/Bayer Crop science

Novel characteristics:

- Reduced probability of transgene transfer through pollen dispersal;
- Increased copies of transgenes;
- Increased likelihood of homology between chloroplastic and bacterial genomes

PLANT NECROSIS TISSUES :

Phytopathogen *Ralstonia solanacearum* :

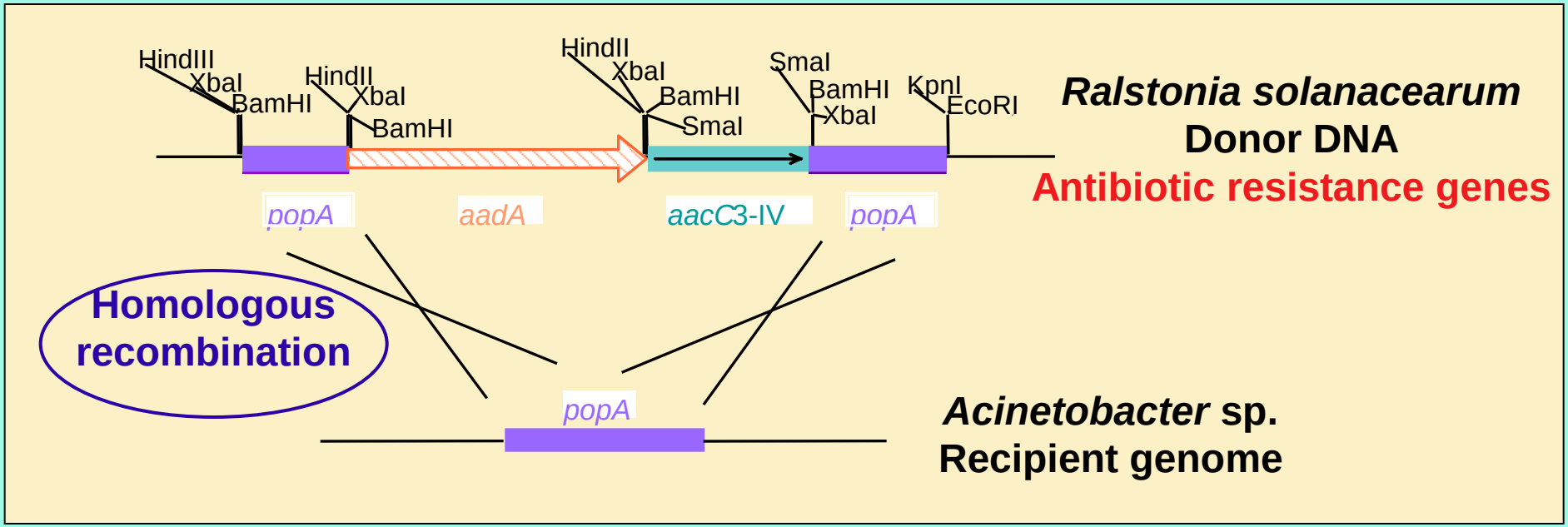
- Infection of Solanaceous plants (tobacco, tomato…)
- Extensive multiplication in plant vascular tissues
- Plant wilting, tissue necrosis, plant cell lysis
- Nutrients and plant DNA release

Co-infection by opportunistic micro-organisms
High bacterial densities
Close contact between active bacteria and plant DNA

A « hot-spot » to study gene transfers from GMP to plant-associated bacteria (and between these bacteria) ?

Gene transfer (transformation) *in planta* between *RALSTONIA* & *ACINETOBACTER*

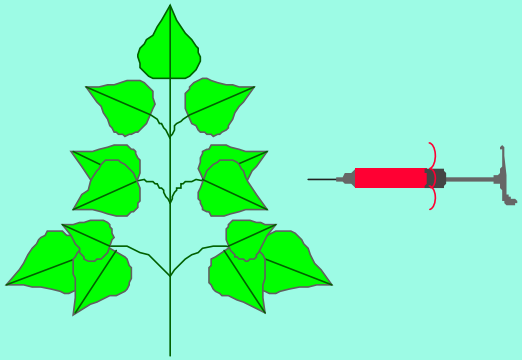
- Development of a competent state by *Acinetobacter* sp.
- Release and persistence of *R. solanacearum* genomic DNA
- homologous sequences for genetic recombination



Detection of transformants ?

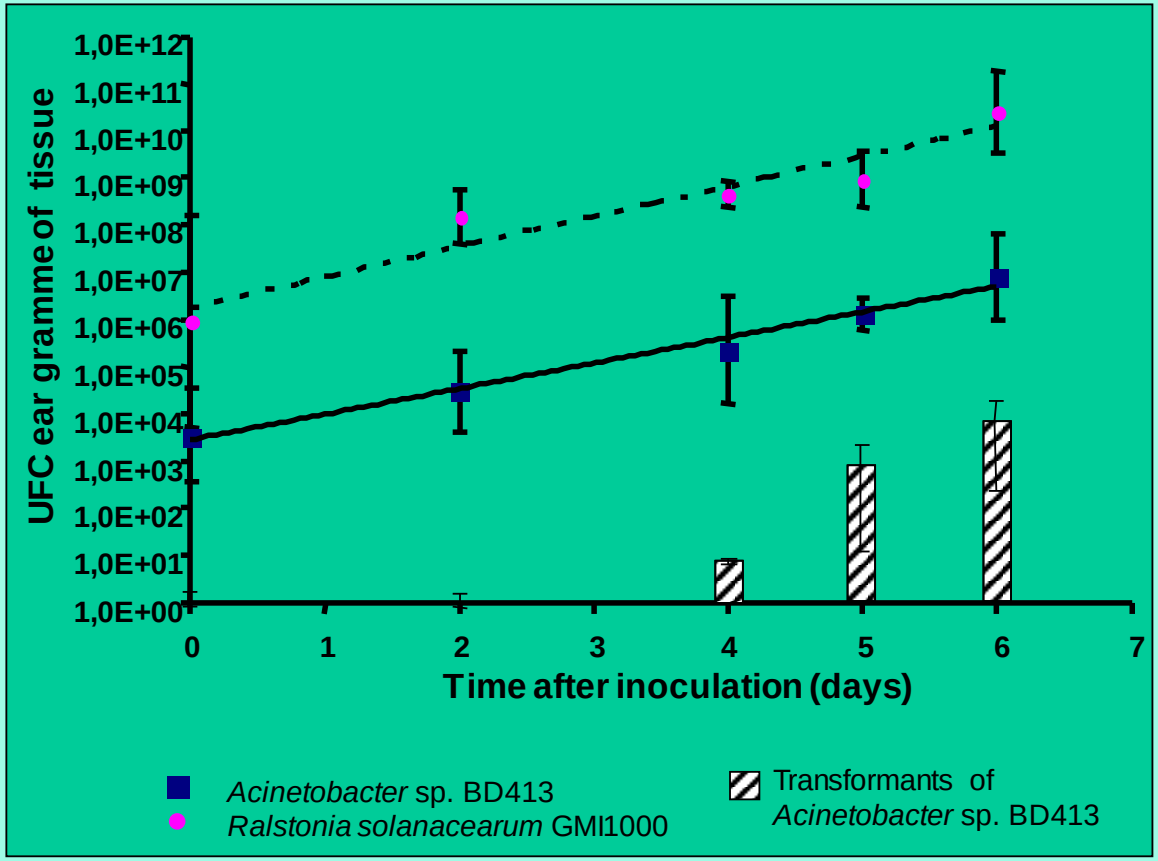
Integration of antibiotic resistance genes by *Acinetobacter* sp. cells ?

***R. solanacearum* and *Acinetobacter* sp. - infected plant**



Plasmid DNA inoculated in infected-plant tissues
(antibiotic resistance genes)

TRANSFORMANTS OF *ACINETOBACTER* ?

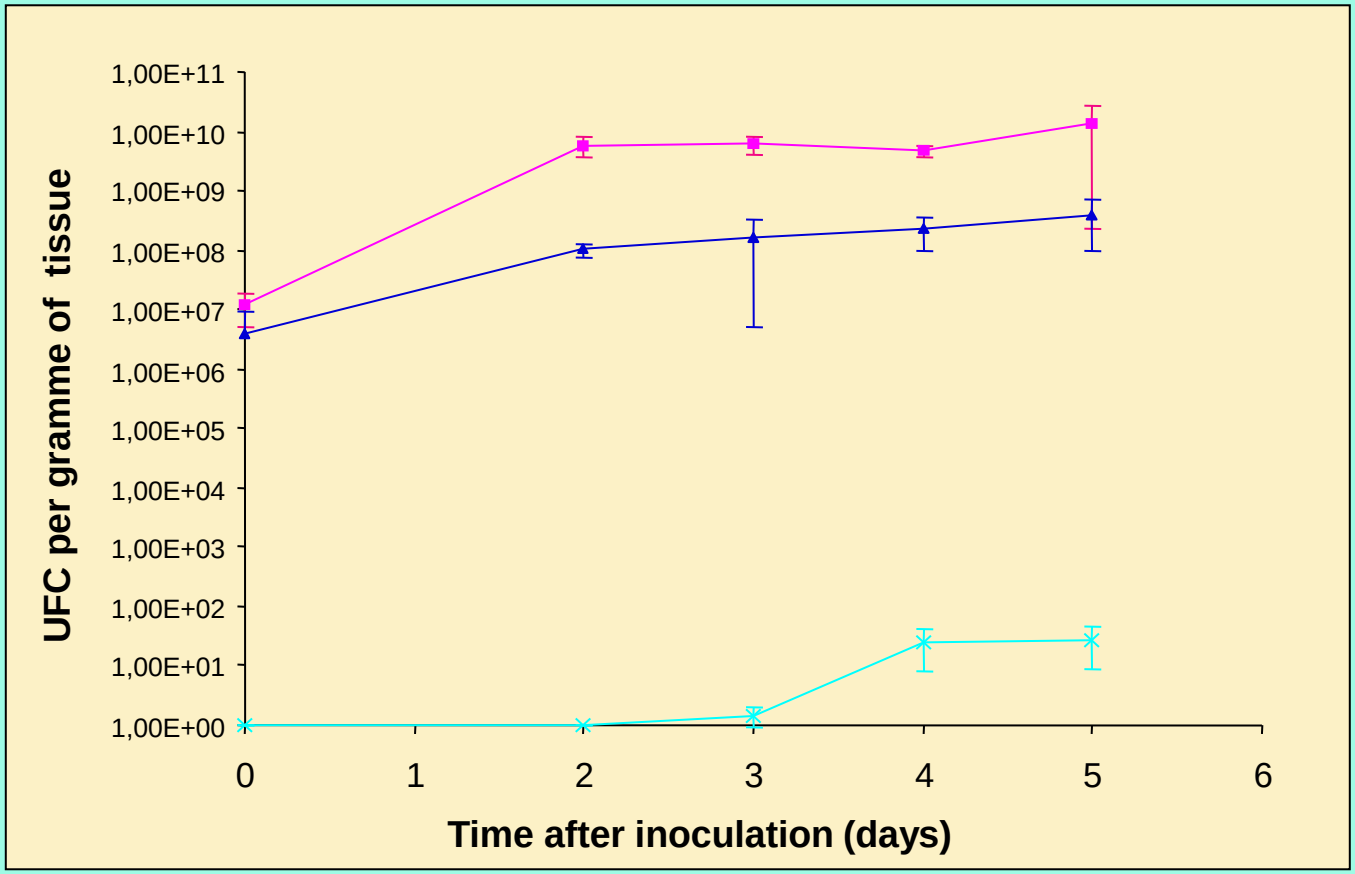


***Acinetobacter* sp:**

- **Stability and availability of inoculated plasmid DNA in necrosis tissue**
- **Plasmid DNA uptake during plant colonization, detection of transformants**

DEVELOP A COMPETENT STATE *IN PLANTA*

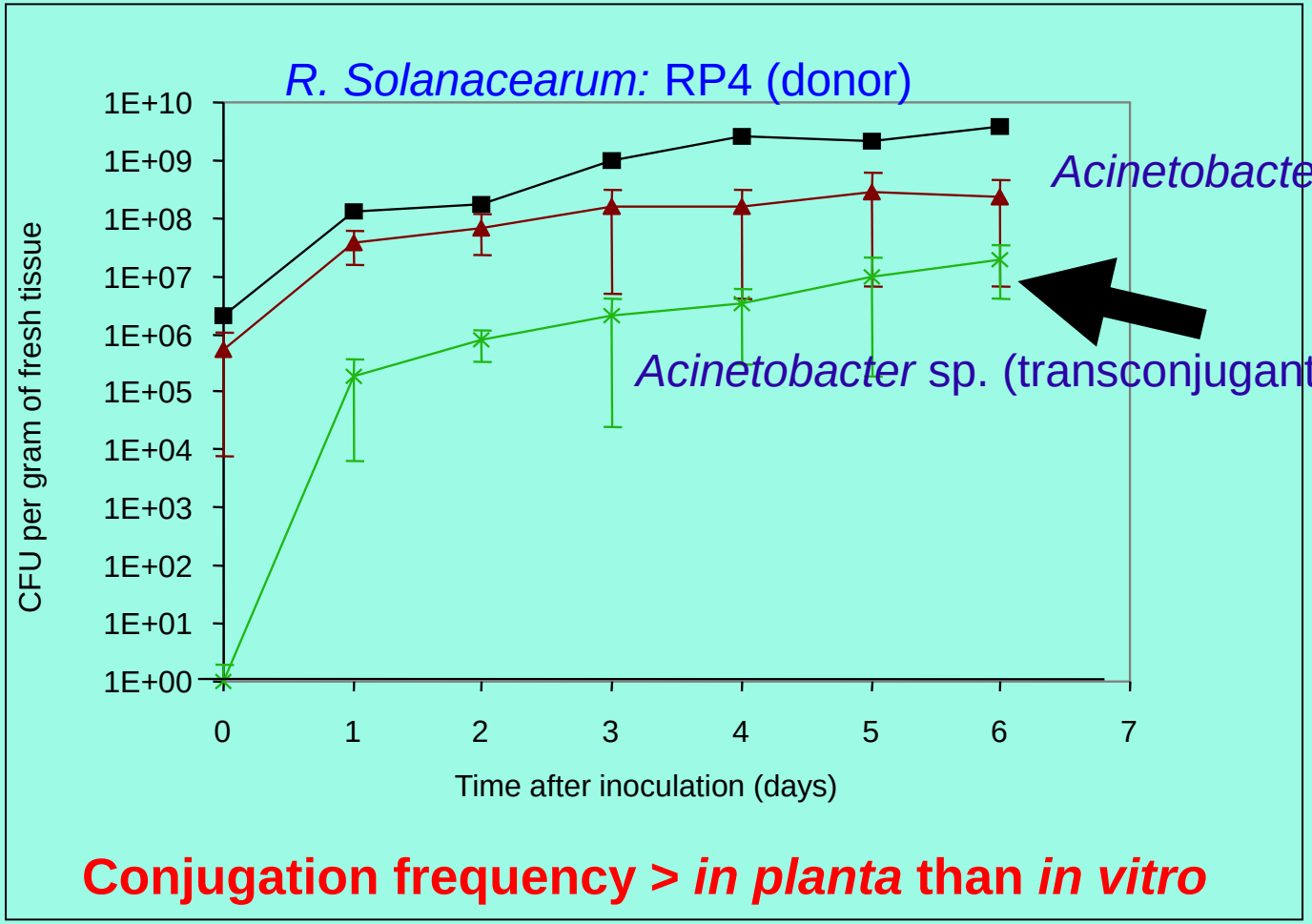
in planta gene transfer (transformation) between *RALSTONIA* & *ACINETOBACTER*



Interspecies gene transfer *in planta*.

Combination of favourable environmental and genetic conditions.

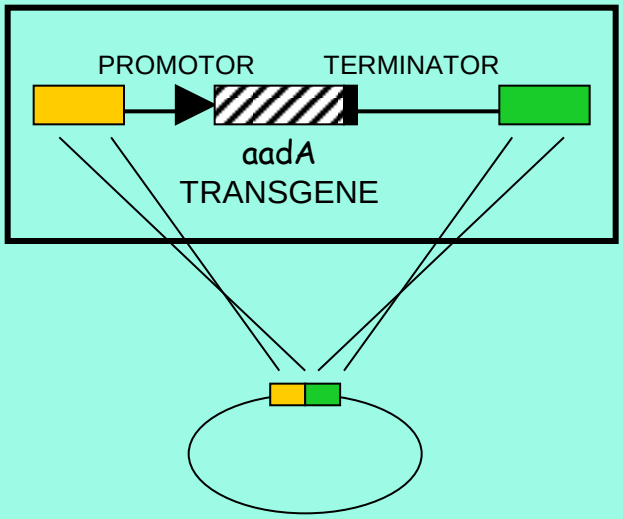
in planta gene transfer (conjugation) between *RALSTONIA* & *ACINETOBACTER*



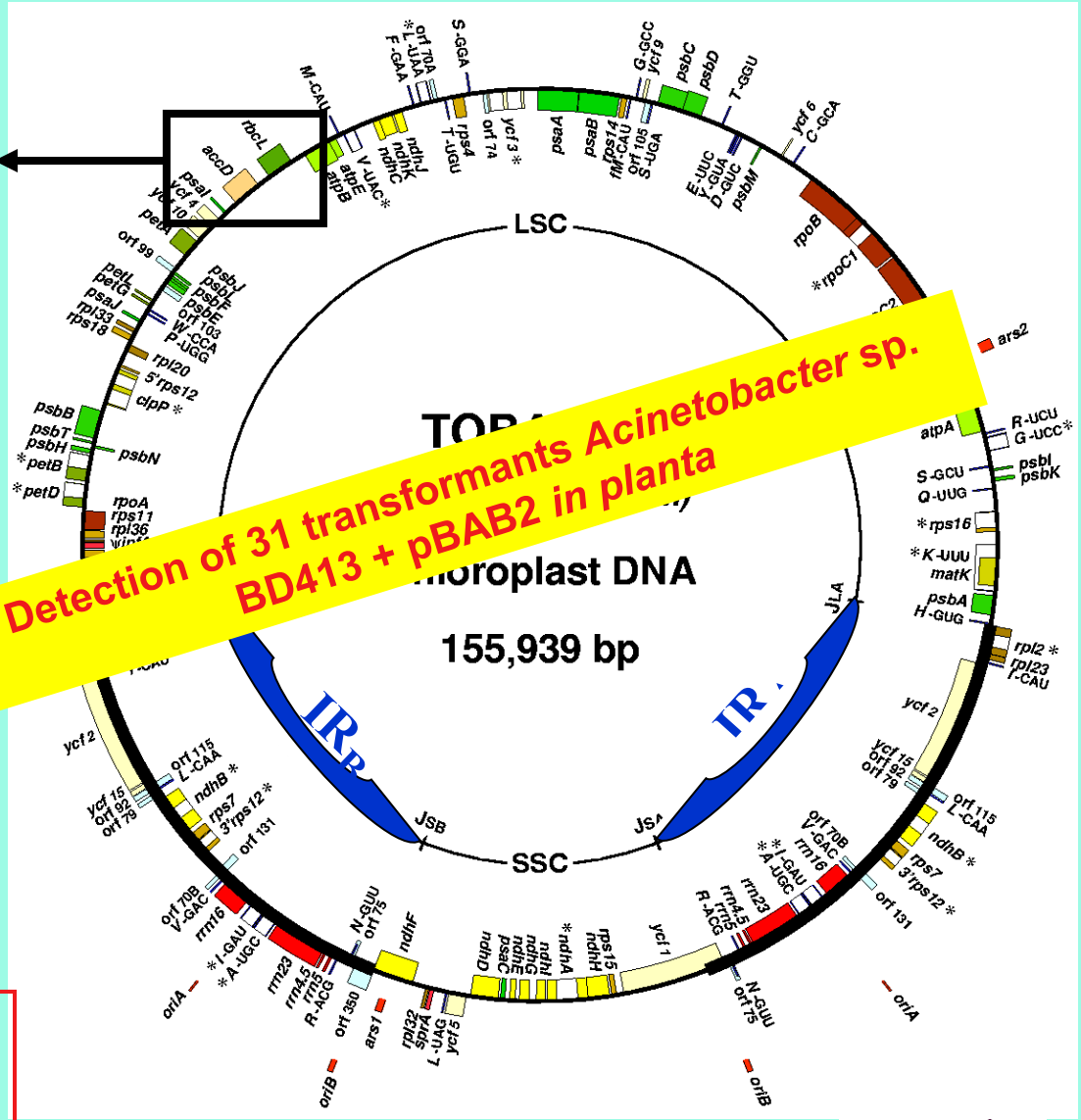
Conjugation frequency > *in planta* than *in vitro*

in planta gene transfer (transformation) between *GMP* & *ACINETOBACTER*

DONOR PLANT DNA



RECIPIENT DNA (plasmid)
***ACINETOBACTER* SP.**
pBAB2



Detection of 31 transformants Acinetobacter sp. BD413 + pBAB2 *in planta*

INTEGRATION OF THE TRANSGENE INTO *ACINETOBACTER* SP. VIA HOMOLOGOUS RECOMBINATION

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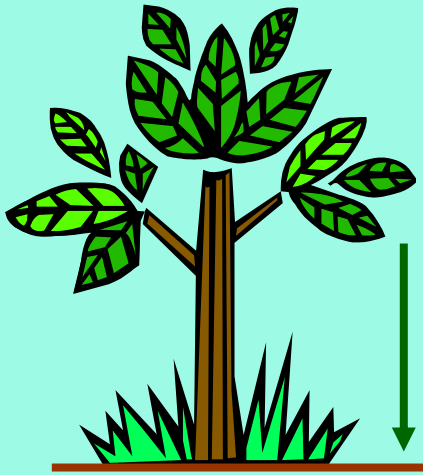
Hot-spots

Necrotic plant tissues = High bacteria cell densities
Metabolically active bacteria
Natural transformation, conjugation (at least)
High transfer frequency *(bacteria to bacteria and plant to bacteria)*.

Gene transfer?
in situ
Where, How, Often, Who, When, ?



in situ gene transfer? Where?

Residuesphere/detritusphere

Another gene transfer « Hot-spot » ?

Release and persistence of DNA in soil ?

Release of plant DNA

(and short term degradation escape rate)

Simulation of the natural process of decay :

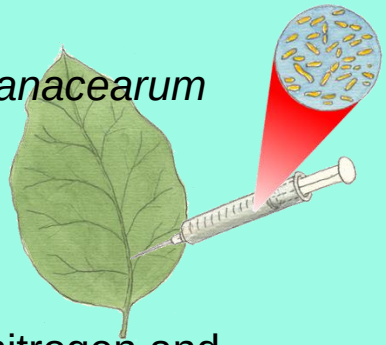
Incubation of leaves with:

Pectinolitic enzymes (*E chrysanthemy)*

Cellulase enzyme (*Trichoderma viride*)

Necrosis of leaves after:

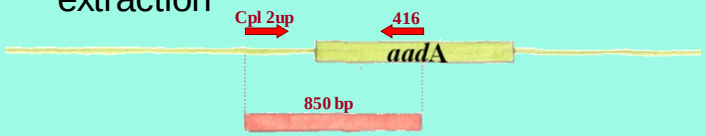
Infection by *R. solanacearum*



Direct exposure of plant DNA to plant Dnases:

Grinding of leaves in liquid nitrogen and exposure at room temperature.

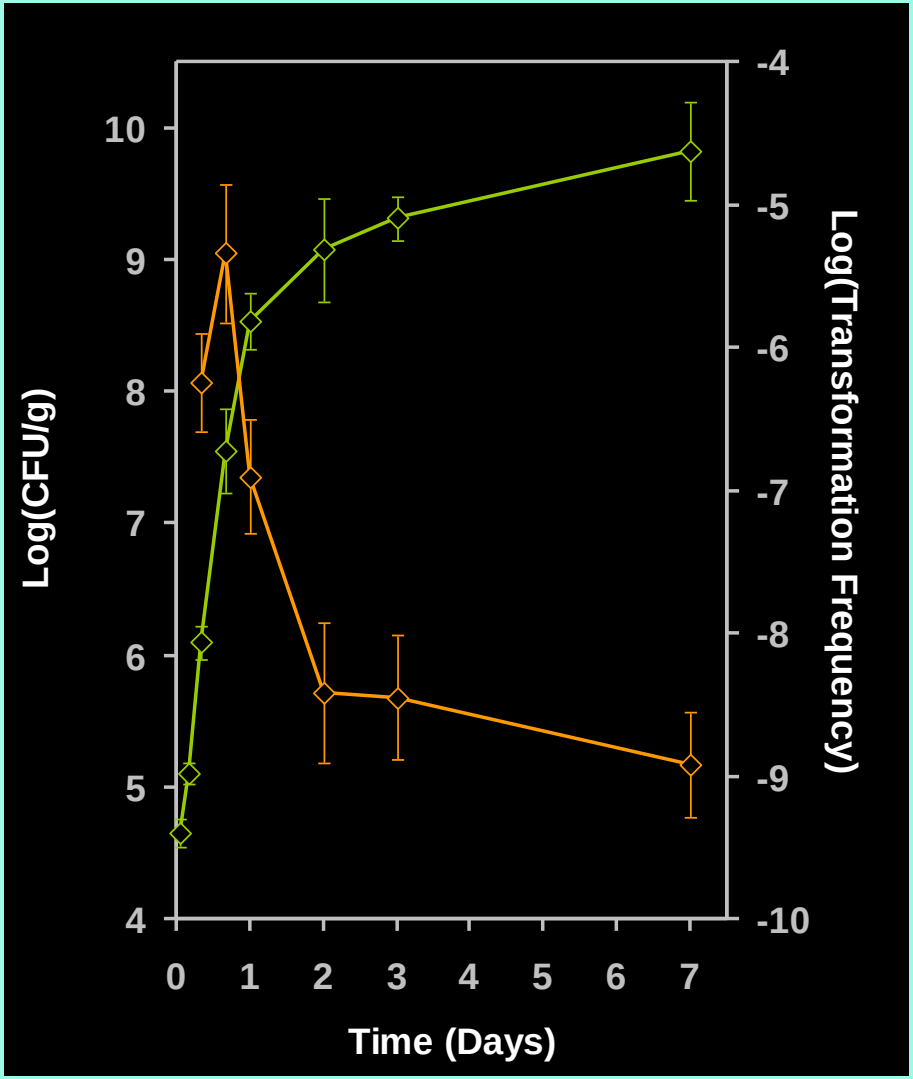
DNA extraction → Agarose gel
UV 260 nm
QPCR



Primers p1531cpl2up and p416 amplified a portion of the plastid and the *aad*A DNA region.

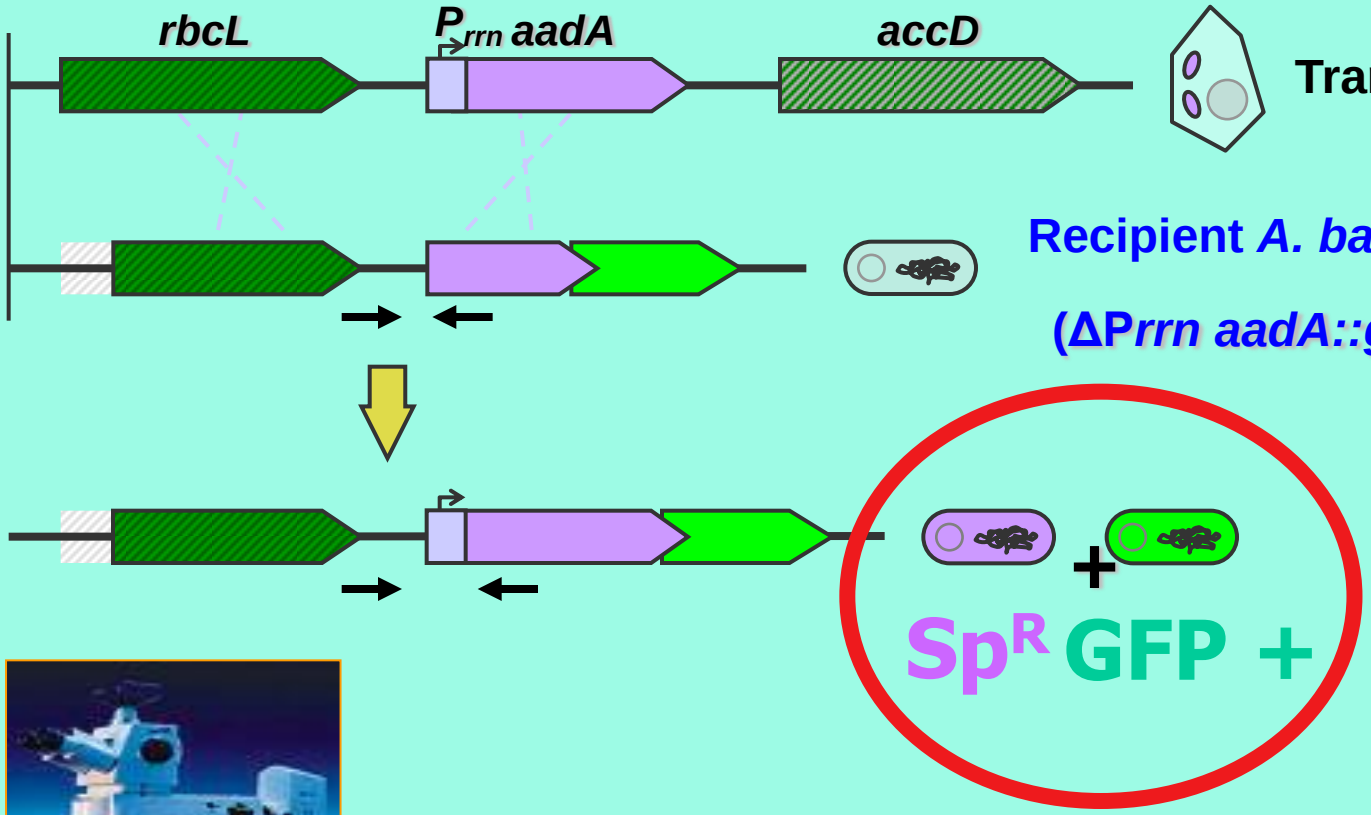
Residuesphere: gene transfer (transformation) between *GMP* & *Acinetobacter*

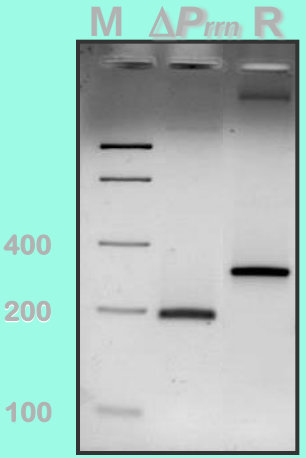
Acinetobacter: growth, competence



Residuesphere: gene transfer (transformation) between *GMP* & *Acinetobacter*

Construction of a "bioreporter" *A. baylyi* BD413 strain



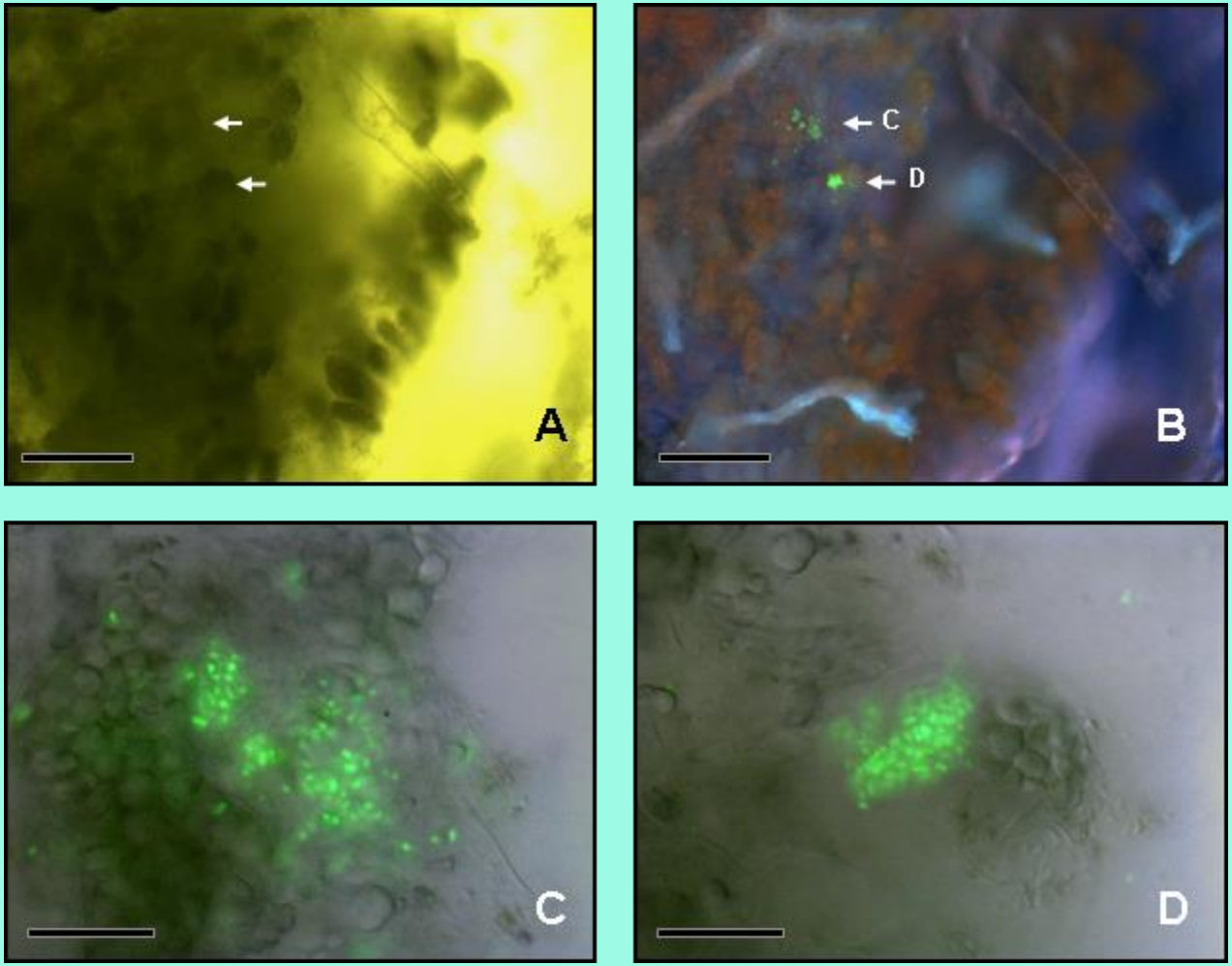


Collaboration A.Rizzi and D.Daffonchio

Epifluorescence: Visualization of HGT events *in situ*.

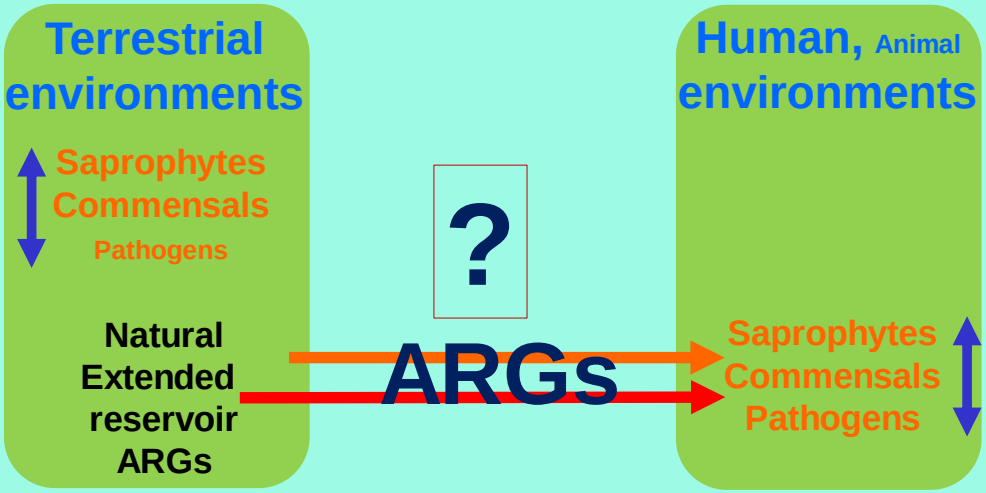
Residuesphere: gene transfer (transformation) between *GMP & Acinetobacter*

Bright-field image, arrows point at the localization of transformants. (A, B: bars = 50 µm).



Epifluorescence micrograph showing transformants(green)chloroplasts (red) and veins (cyan).

Pontiroli, et al . Visual evidence of horizontal gene transfer between plant and bacteria in the phytosphere of transplastomic tobacco. Applied and Environmental Microbiology 2009, 75:3314-3322



Gene transfer?

in situ

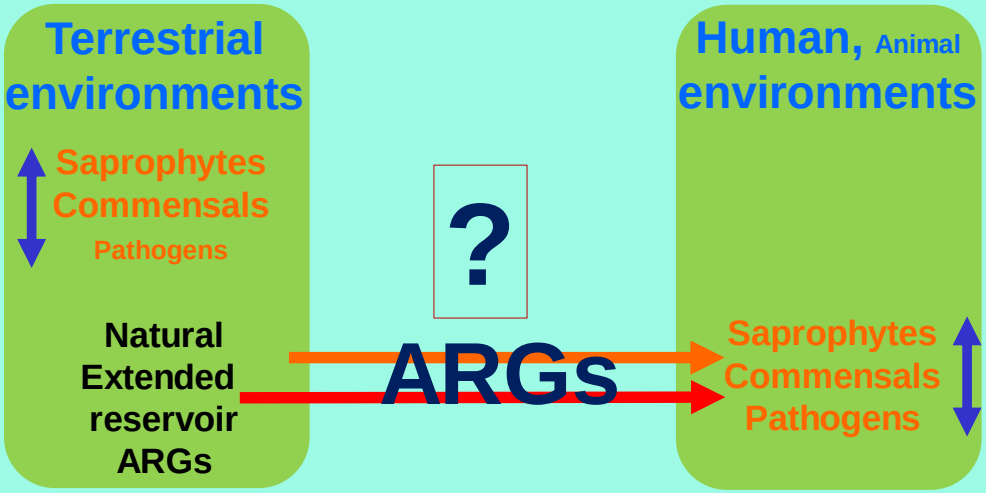
Who, Where, When, How, Often ?

Hot spots for gene transfer?

Tools:

- **GM Plant**
- **GM Plant DNA**
- **Selected bacteria**

Yes… and extremely efficient



Gene transfer?

in situ

Who, Where, When, How, Often ?

Persistence and transport of DNA in the environment?

When? Persistence of DNA.

Physical and biological Persistence (long term) of DNA in Soil

Transplastomic Tobacco
(***aadA*** spectinomycin and streptomycin resistance)

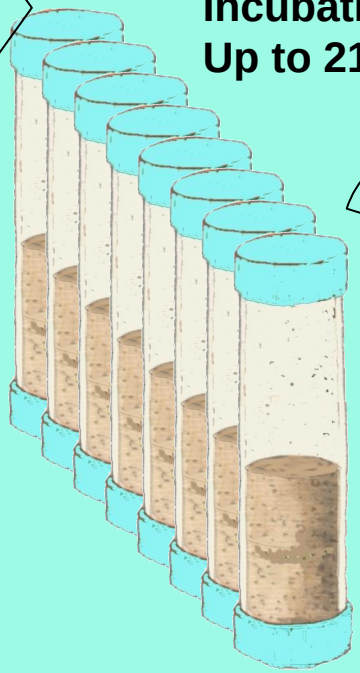
Incubation (RT):
Up to 210 weeks

DNA extraction

Disk: 0.5 g

Disk: 0.05 g

Crushing fresh leaves : 0.5 g



- **Agarose gel**
- **UV 260nm**
- **Real-Time PCR**
- **Bacterial Transformation**

Soil microcosms

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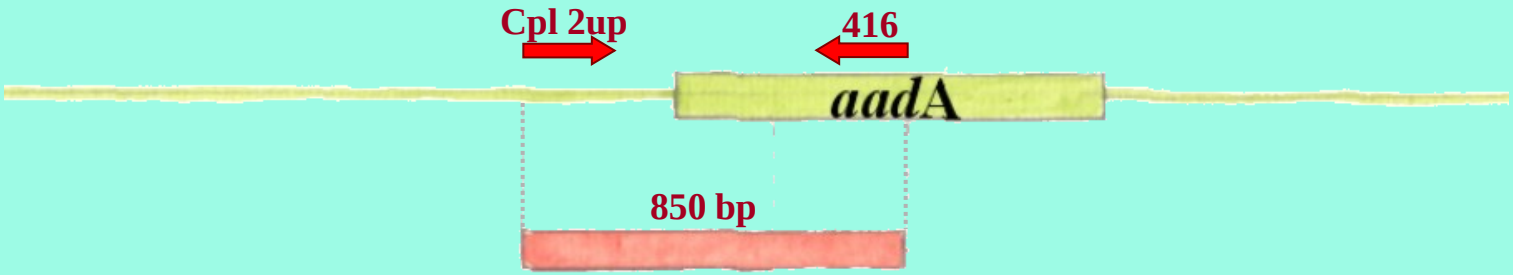
PCR detection of transgene sequences in soil

Transplastomic Tobacco
(***aadA*** spectinomycin and streptomycin resistance)



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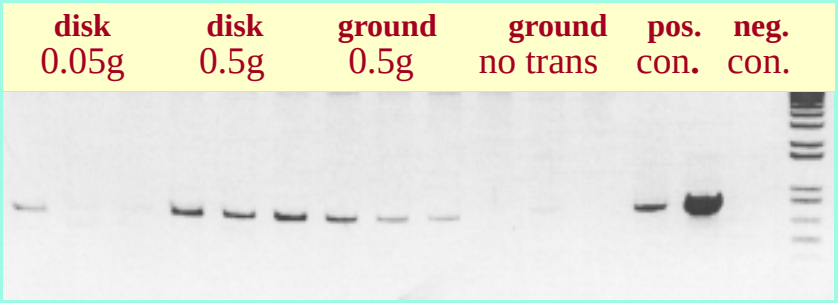
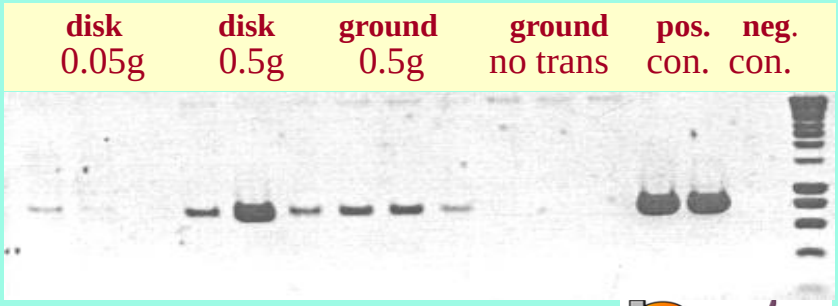
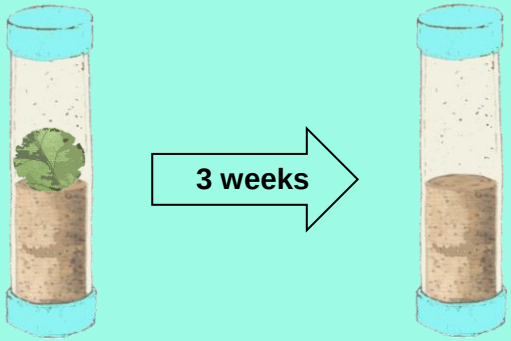
3 week incubated microcosms



210 week incubated microcosms



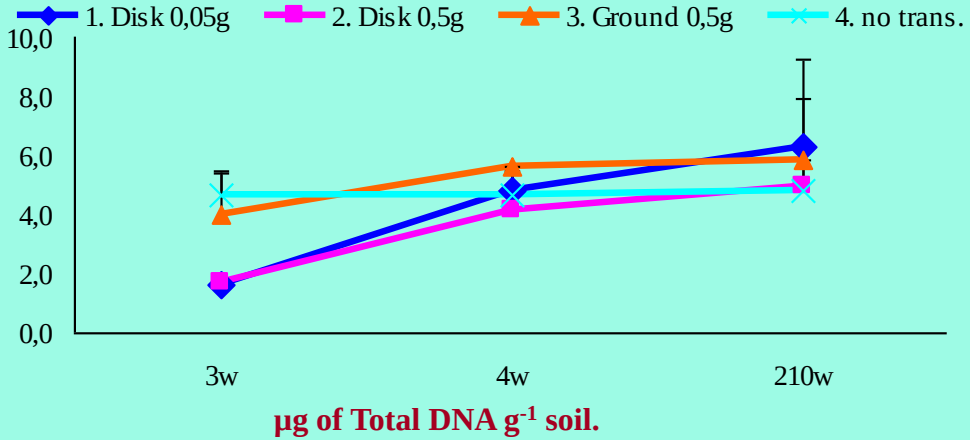
Plant material



Degradation of plant material: <3 weeks (visual estimation)

Physical persistence of DNA

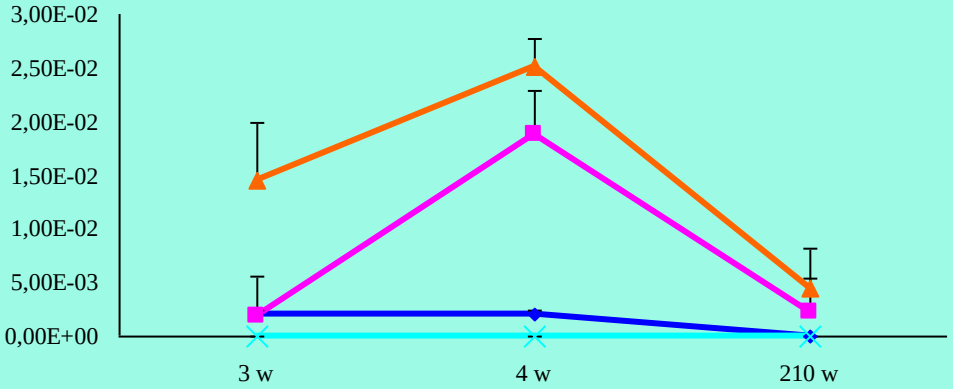
Total DNA



µg of Total DNA g^{-1} soil.

Increase of the amount of total DNA (plant material degradation, bacterial multiplication ?)

Transgenic DNA

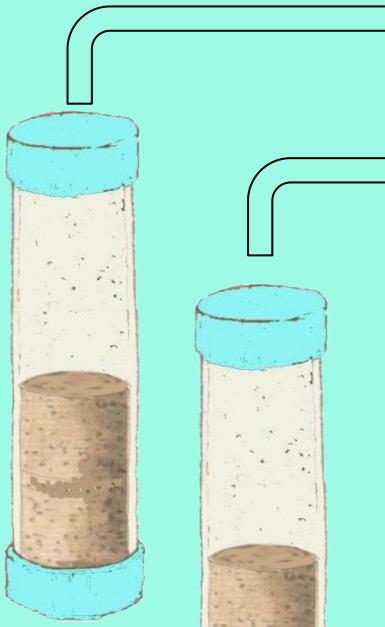


Number of *aad* A gene sequences μg^{-1} total soil DNA.

Initial (4 weeks) increase of the amount of *aadA* sequences (plant material degradation ?)

Regular (long-term) decrease of the amount of *aadA* sequences (DNA degradation, adsorption ?)

Biological potential (transformation) of persisting plant DNA



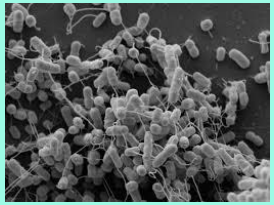
3 weeks

210 weeks

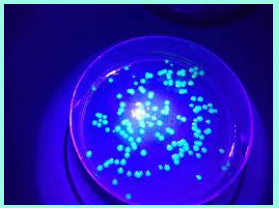
(0.5 g ground leaf)

Total soil DNA extraction

Transformation *Acinetobacter*

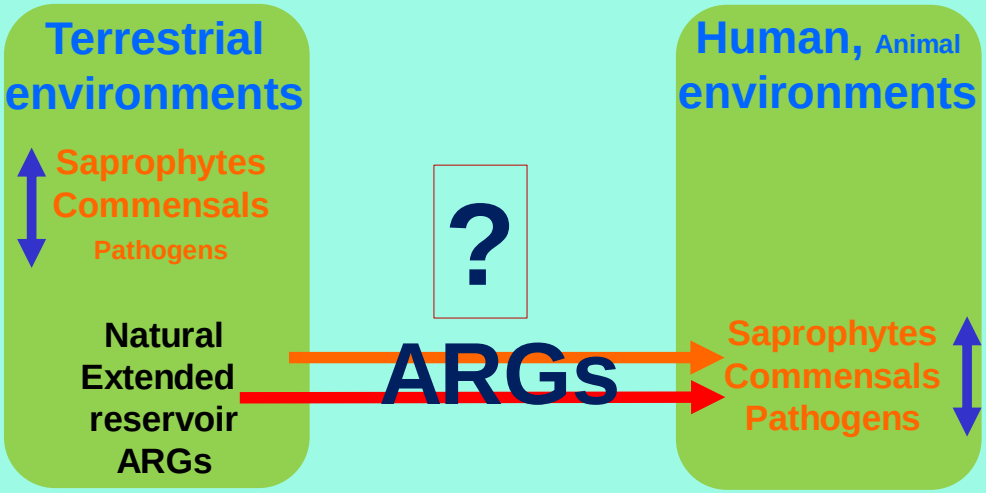


Plating on selective medium



Detection of *Acinetobacter* recombinant (Sm, Spc and fluorescent) clones

Transformation frequency ~10^{-8} cell ml^{-1}



Gene transfer?

in situ

Who, Where, When, How, Often ?

Long term persistence of « active » DNA

Long term persistence of « active » bacteria?

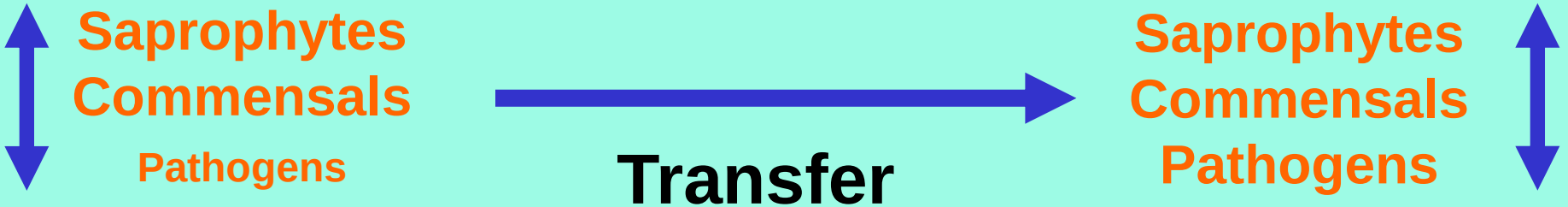
Terrestrial environments

Human, Animal environments

Natural (extended) Reservoir ARGs

***in situ* gene transfer?**

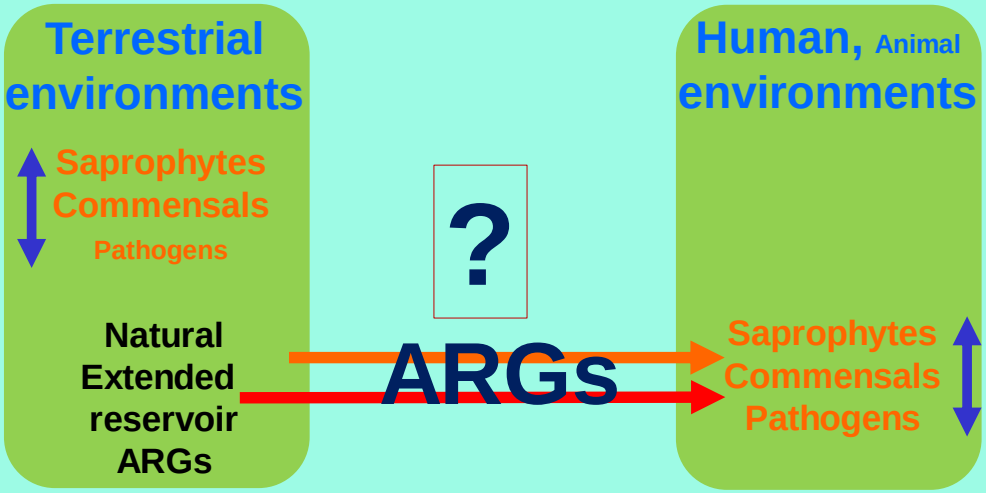
Who, Where, When, How, Often ?



***in situ* gene transfer?**

Who, Where, When, How, Often ?

Transport of DNA in soil and water ?



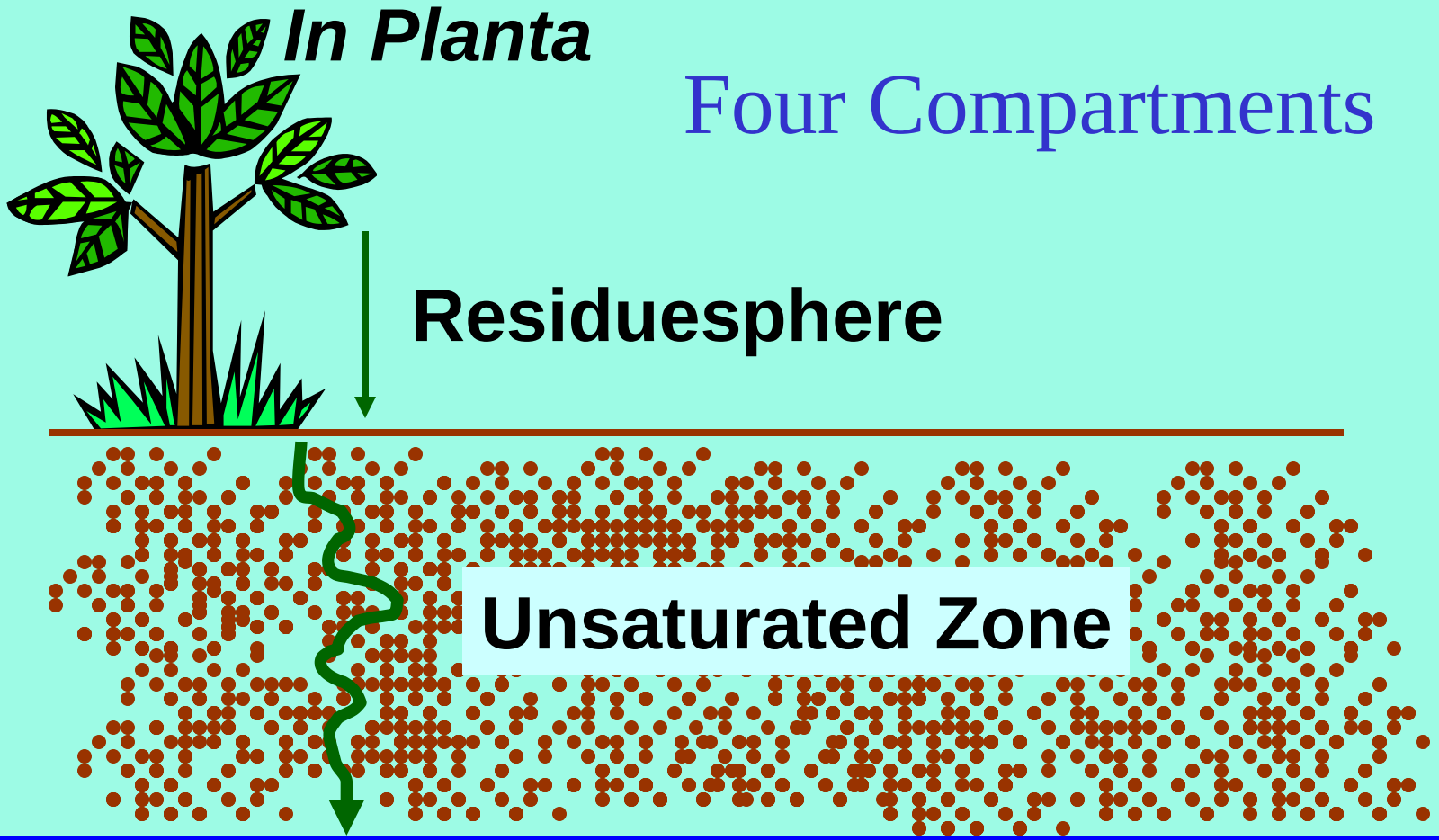
Gene transfer?

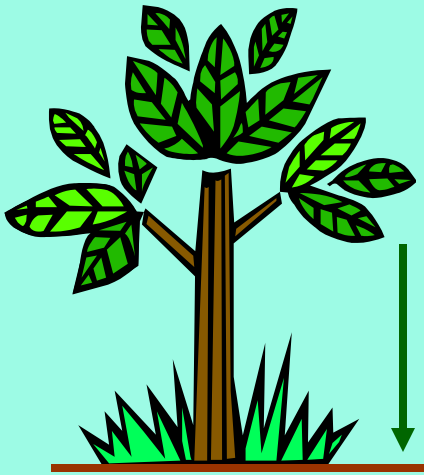
in situ

Who, Where, When, How, Often ?

Persistence and transport of DNA in the environment?

Four Compartments

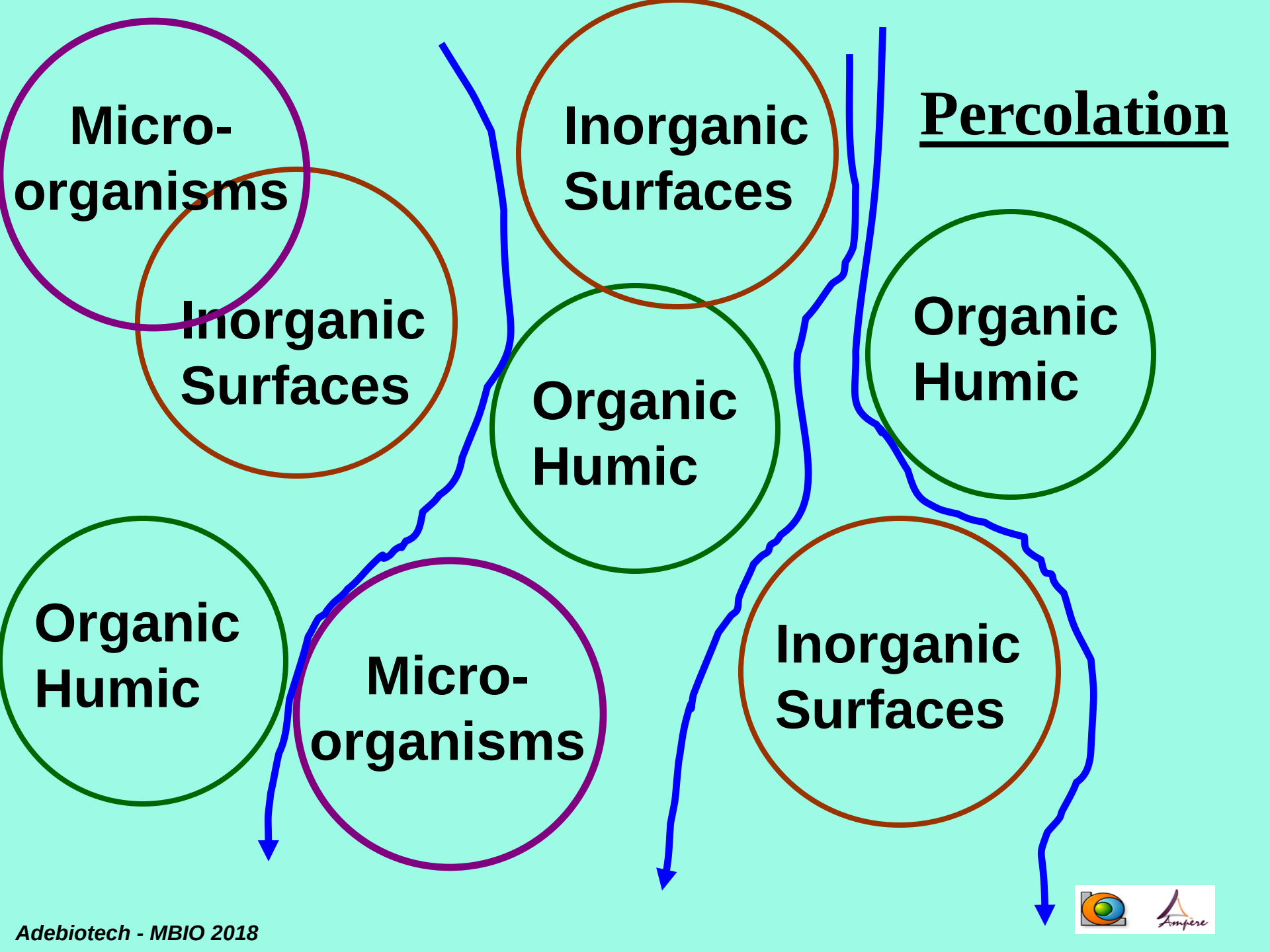




Second Compartment

Residuesphere

Unsaturated Zone


Micro-organisms
Inorganic Surfaces
Organic Humic
Inorganic Surfaces
Organic Humic
Organic Humic
Micro-organisms
Inorganic Surfaces
Percolation

Factors affecting DNA Persistence and transport in Soil

Temperature

Soil characteristics

- **structure**
- **humidity**
- **clay content**
- **organic content**
- **aggregates**
- **pH**

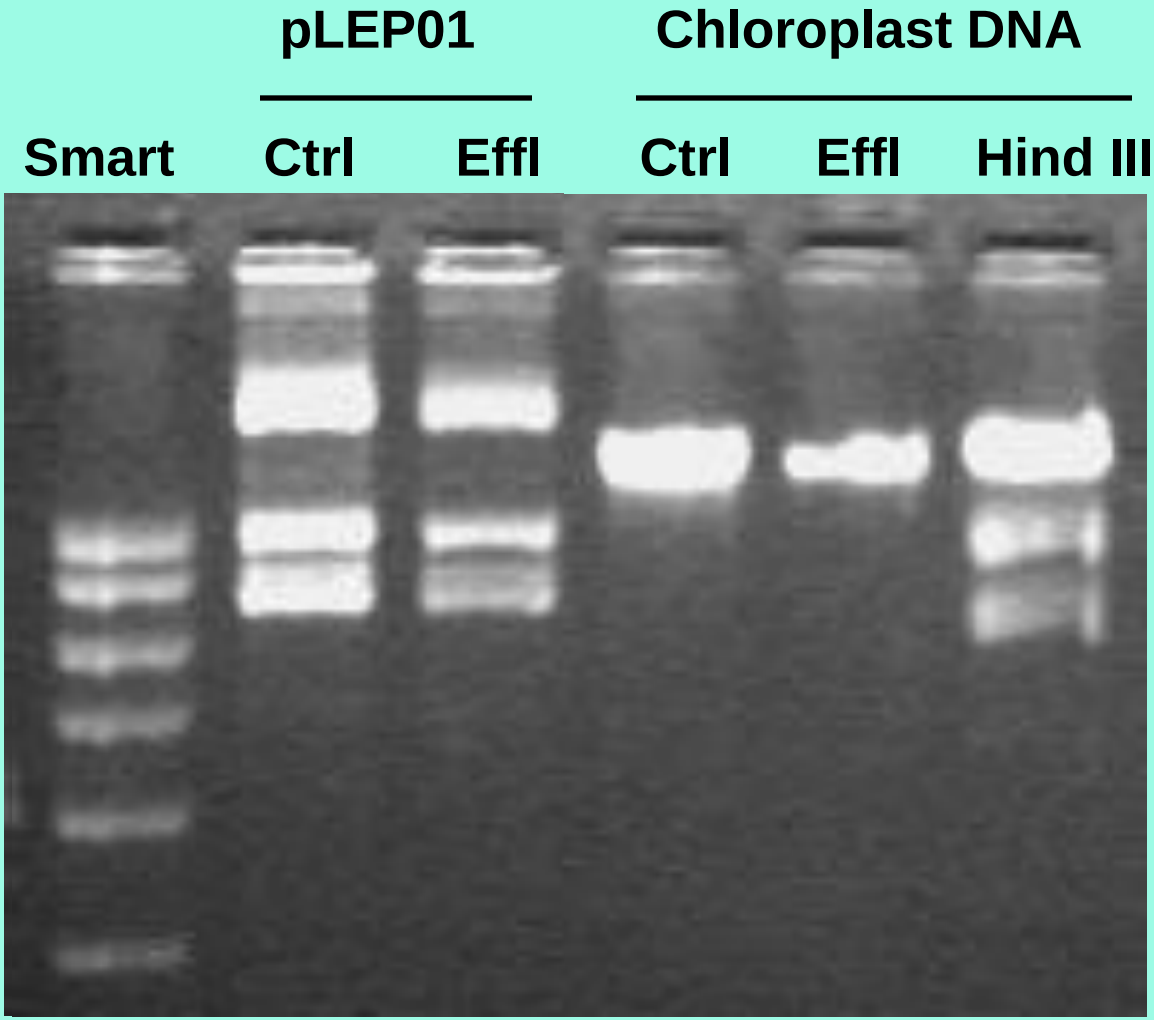
DNA characteristics

- **Form**
- **Protection**
  - **proteins,etc**

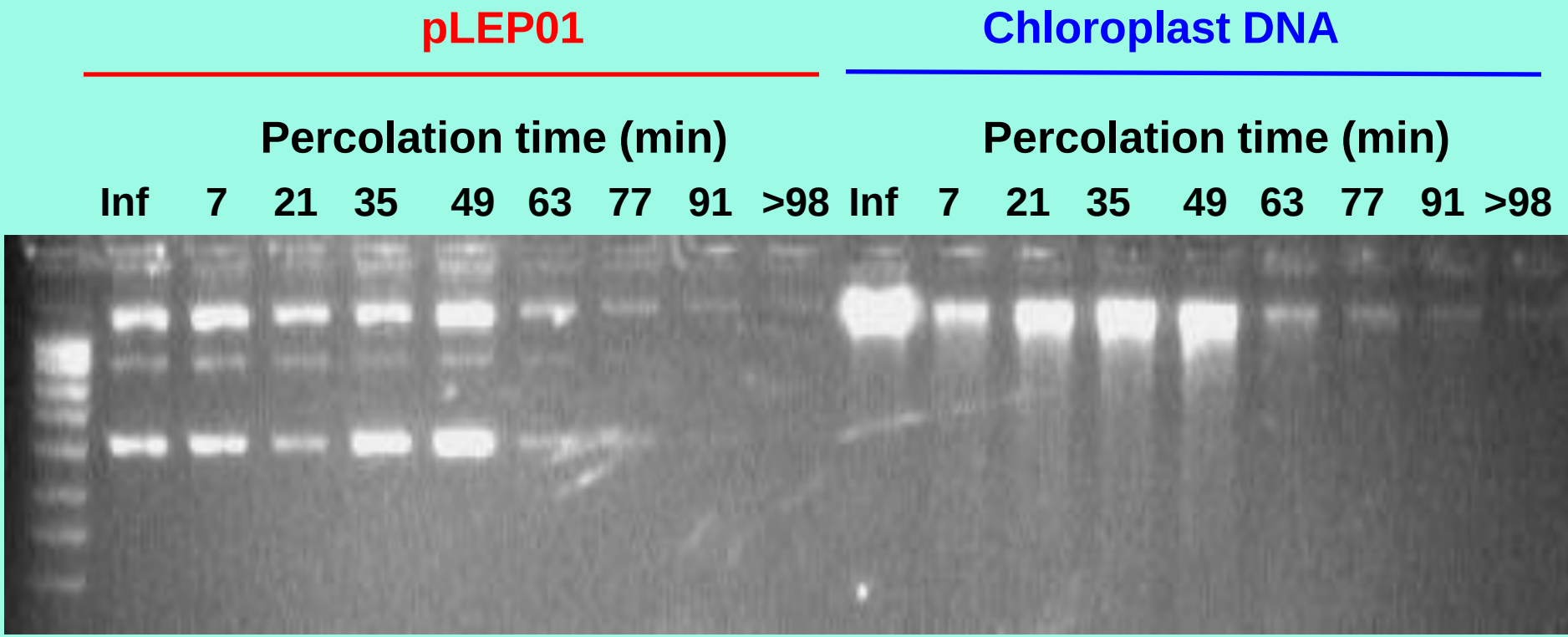
Soil Column set-up

- Double jacketed for temperature control
- Teflon tubing
- Perastaltic pump
- Length 40 cm
- Diameter 4 cm

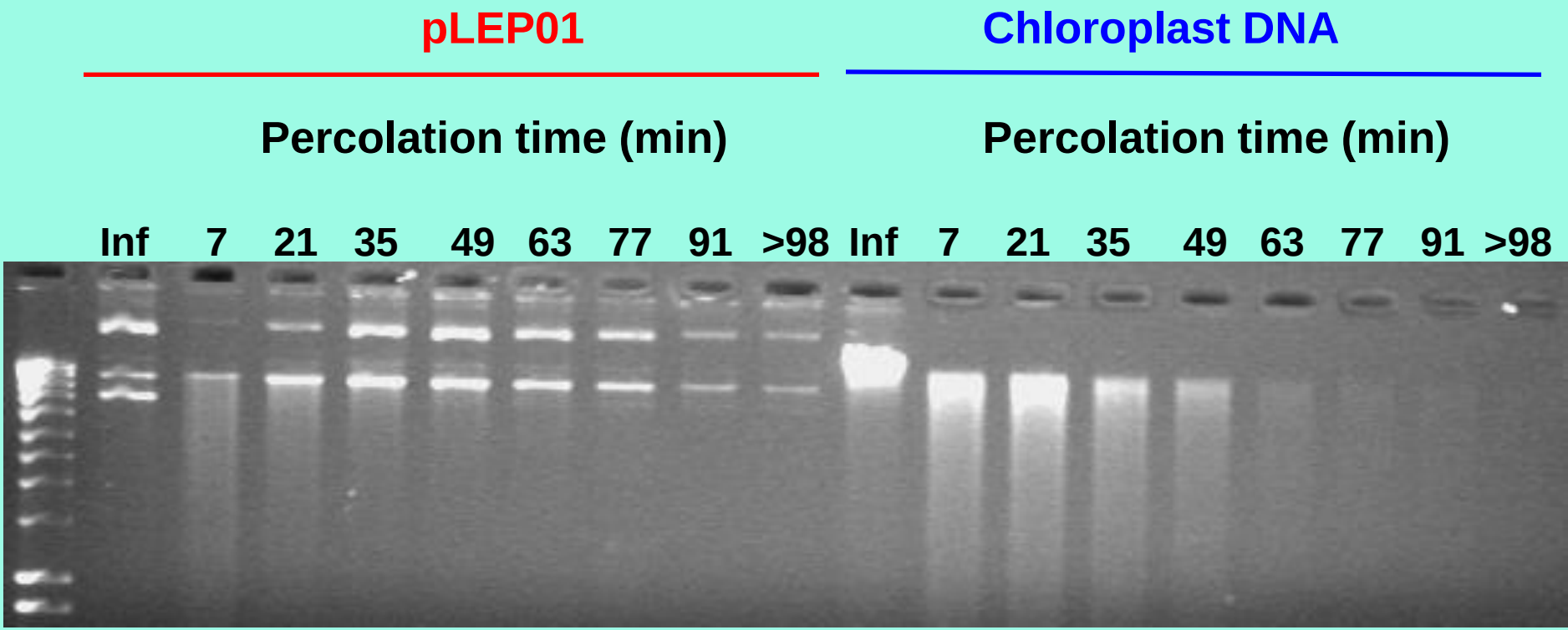
Effluent from unsaturated soil during percolation test (30 min exposure)



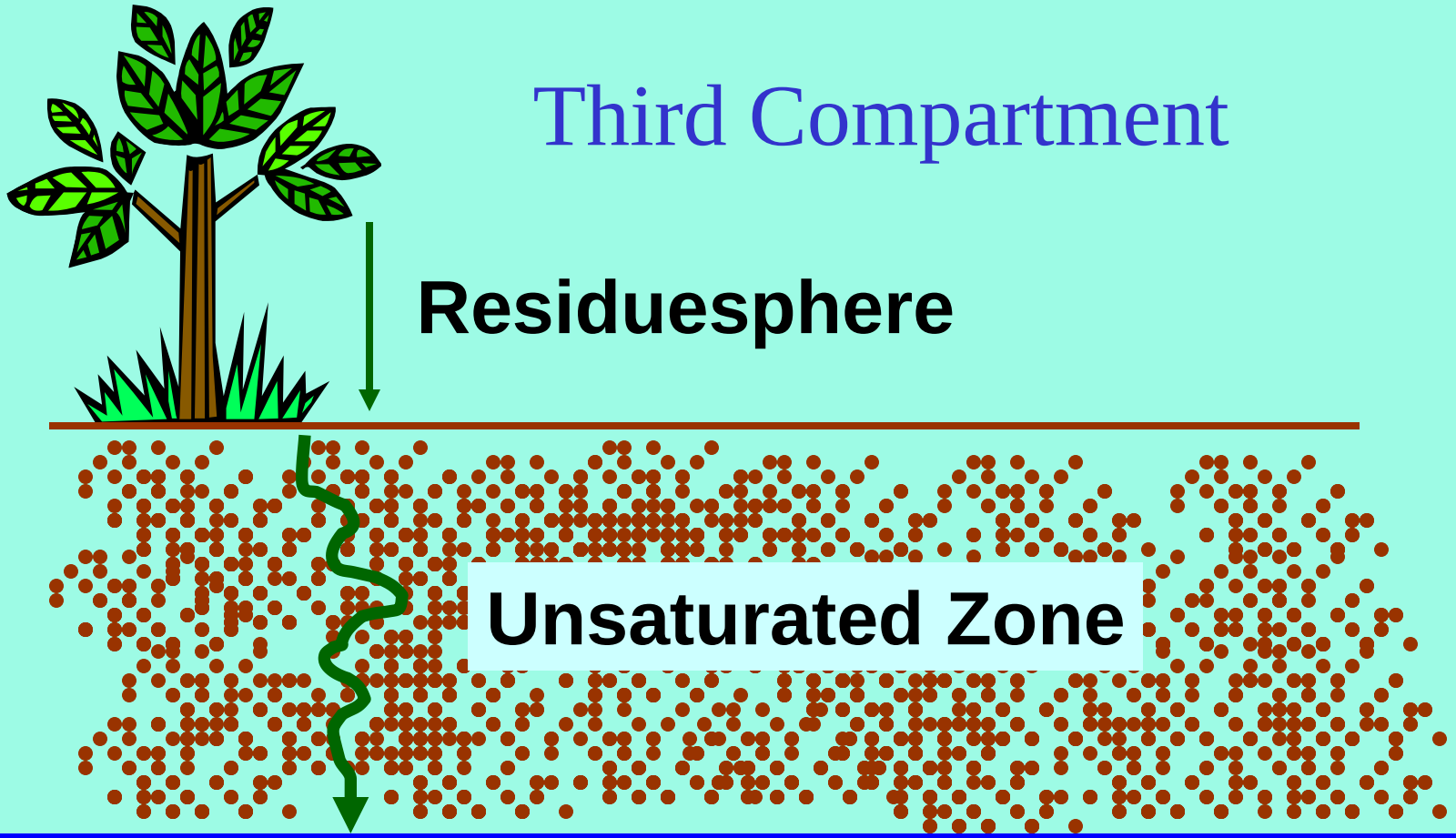
Effluent from unsaturated soil during percolation test (30 min exposure)

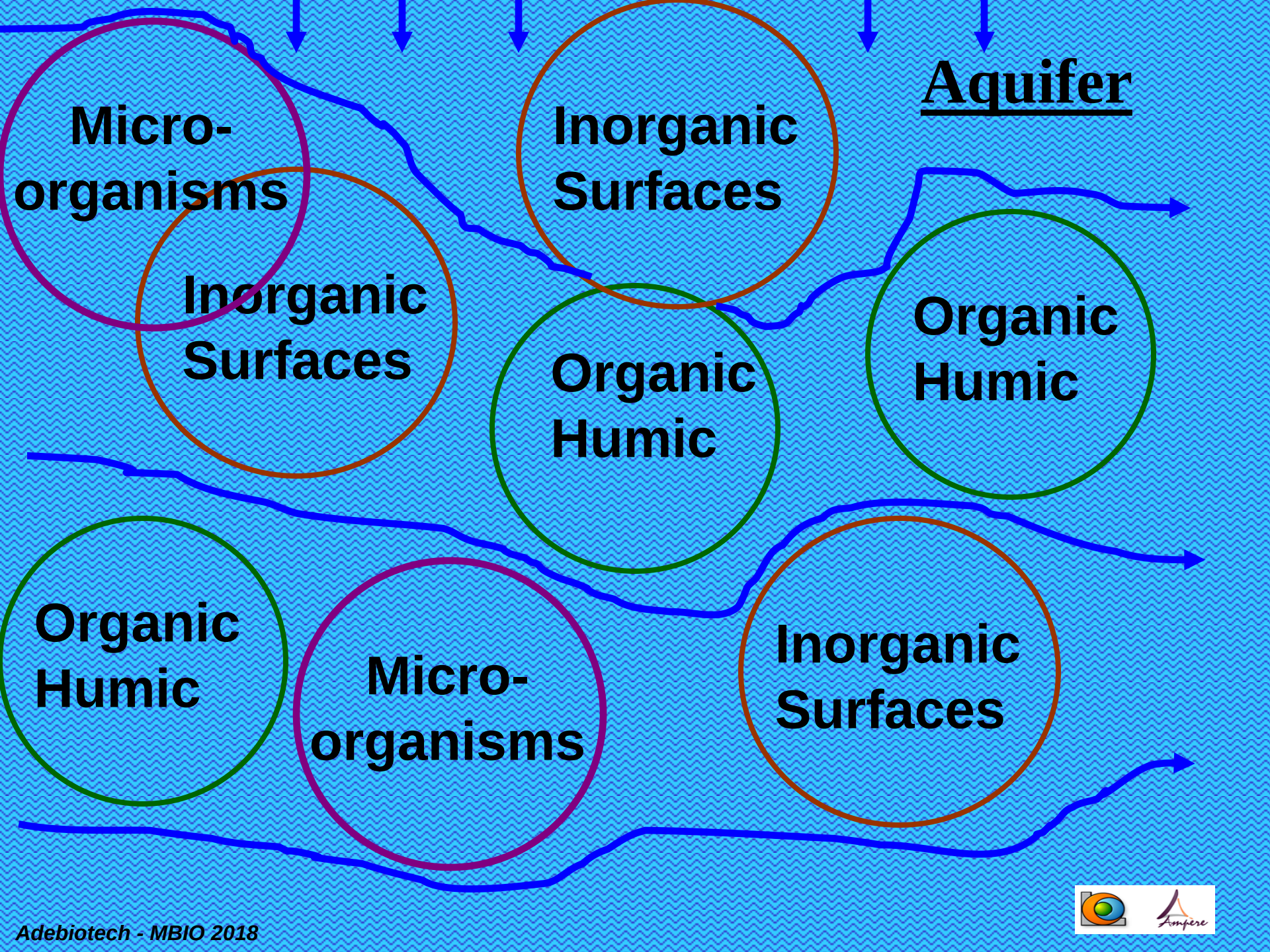


Effluent from unsaturated soil during percolation test (3 hr exposure)

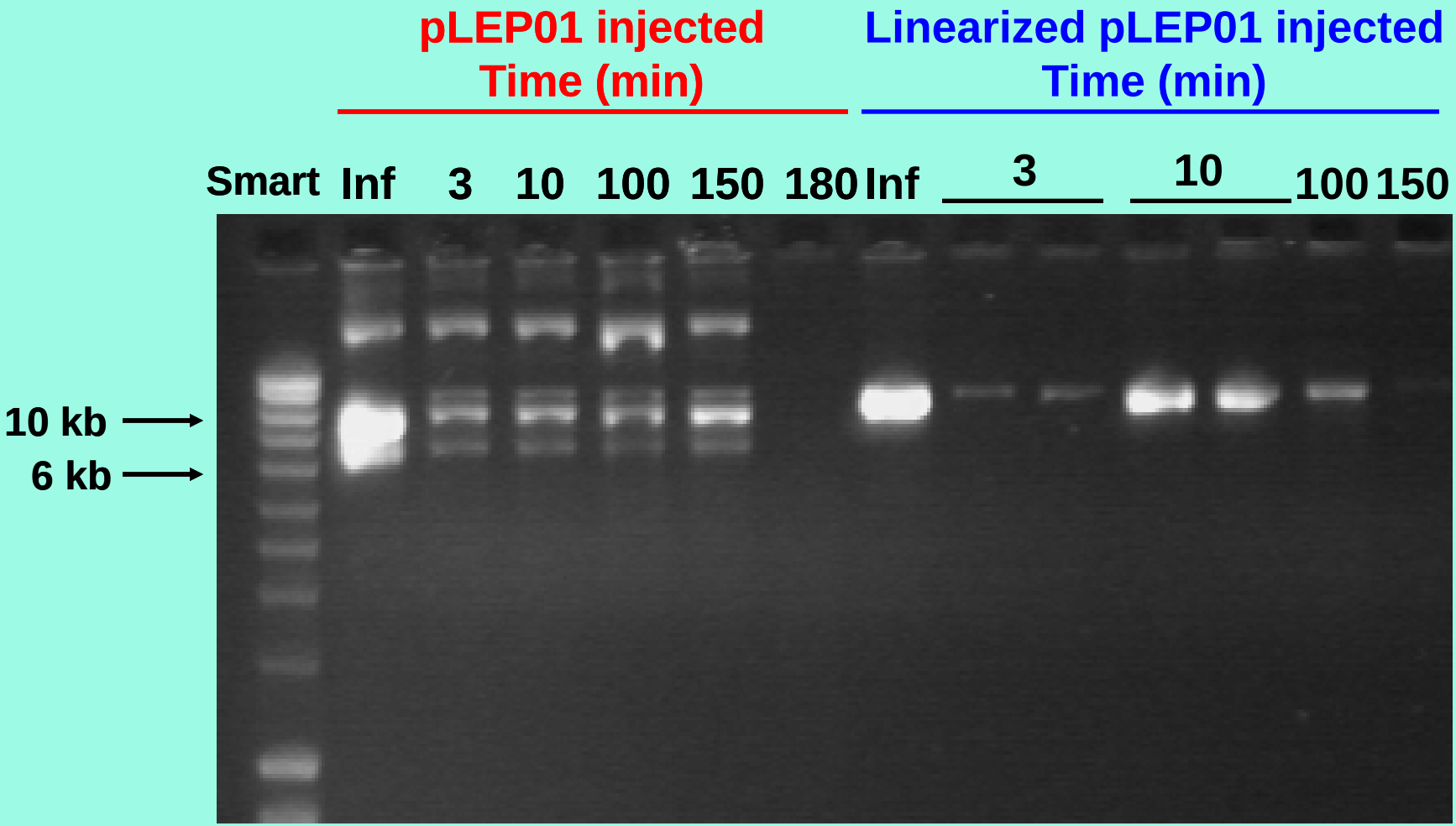


Third Compartment

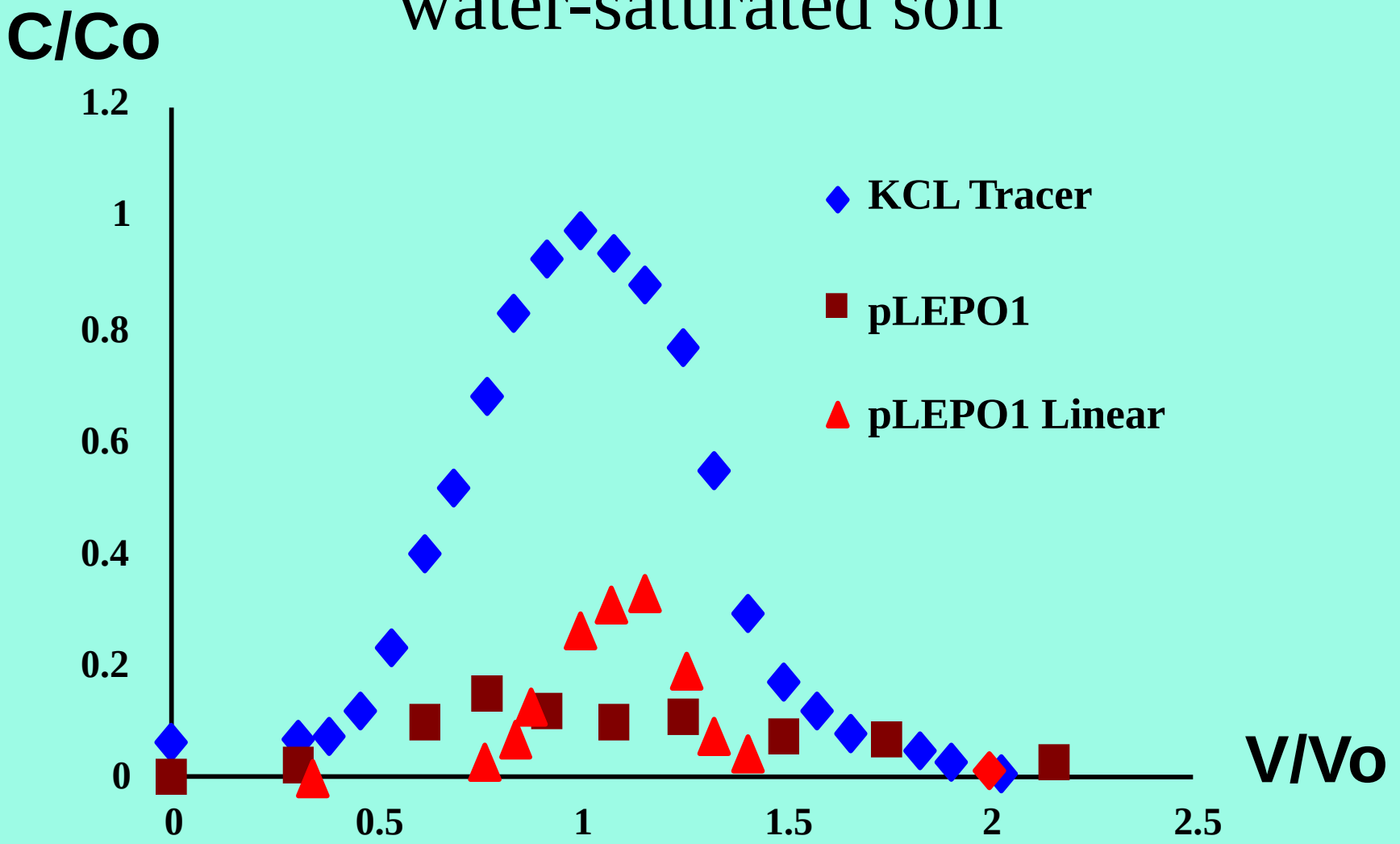



Aquifer
Micro-organisms
Inorganic Surfaces
Inorganic Surfaces
Organic Humic
Organic Humic
Organic Humic
Micro-organisms
Inorganic Surfaces

Agarose gel of saturated soil column influent and effluent



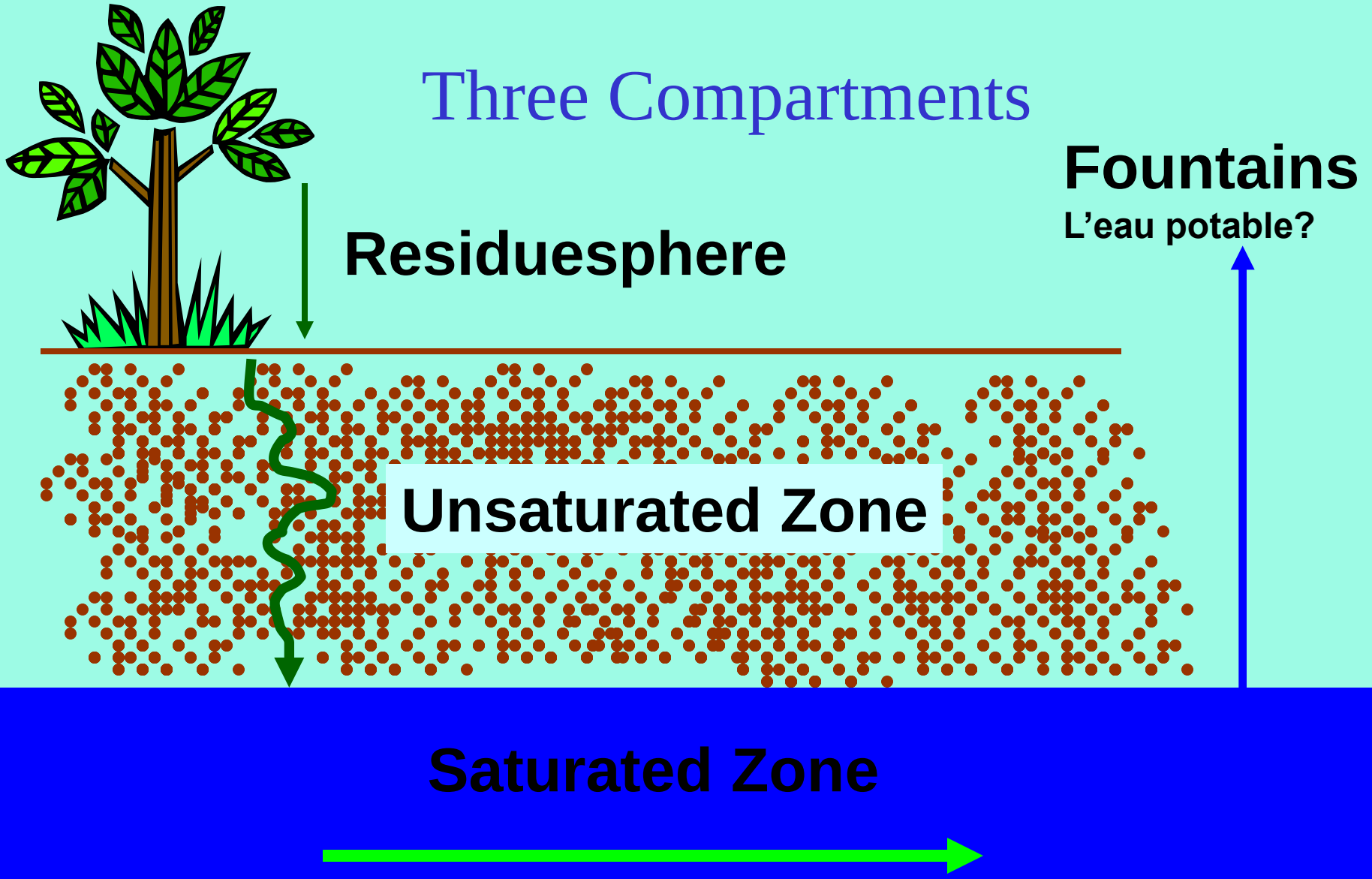
Breakthrough curves in water-saturated soil



Transformation with Column Effluent

Samples	*E. coli*	*Acinetobacter*
pLEP01 control	++++	++++
pLEP01 linearized by SacI	++++	++++
Effluent (3 min) supercoiled/linear	++++/++++	++++/++++
Effluent (10 min)	++++/++++	++++/++++
Effluent (100 min)	++++/++++	++++/++++
Effluent (150 min)	++++/++++	++++/++++
Effluent (180 min)	-	-
After 1 hr with no flow in column	++++	++++
After 3 hr with no flow in column	++++	++++
After 10 hr with no flow in column	+	++
After 24 hr with no flow in column	-	+
After 48 hr with no flow in column	-	-

Three Compartments



Collab. John Poté, Walter Wildi (Forel institute, Univ. Geneva, Switzerland)

RFLP Profile Types

Sample Location	Profile Types Plant DNA	Number tested with RFLP / Number of clones
Groundwater	A, A, A, B	4/30
Fountain 1	C, D	2/30
Fountain 2	D, E, E	3/30
Fountain 3	A, A, A, D	4/30
Fountain 4	A, A, A, A	4/30
Fountain 5	B, B, D, D	4/30
Fountain 6	B, F, G	3/30

Plant DNA Identification

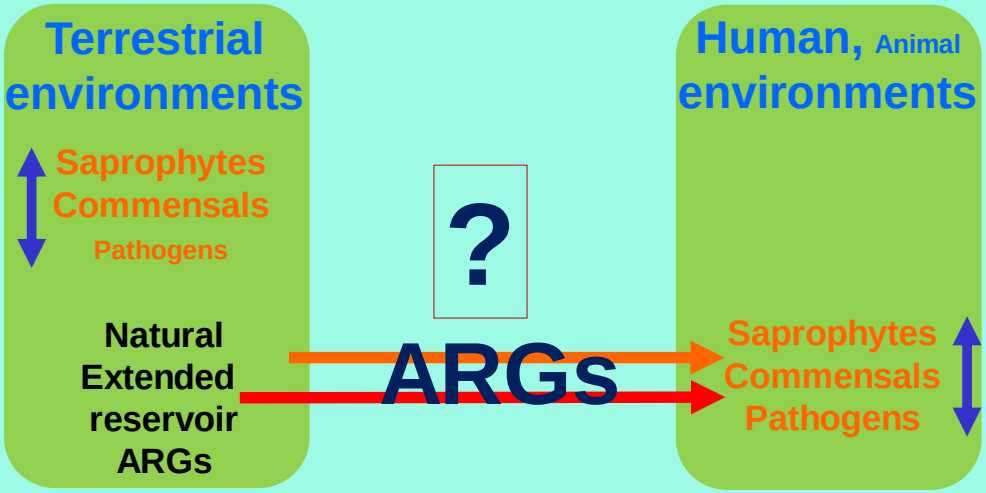
- DNA extracted from fountain and groundwater was cloned and sequenced (18 S).

- Identification of potential source plants:

  Vitis berlandieri (100%)

  Polygonum sp. Soltis (95%)

  Sinapis alba (85%)

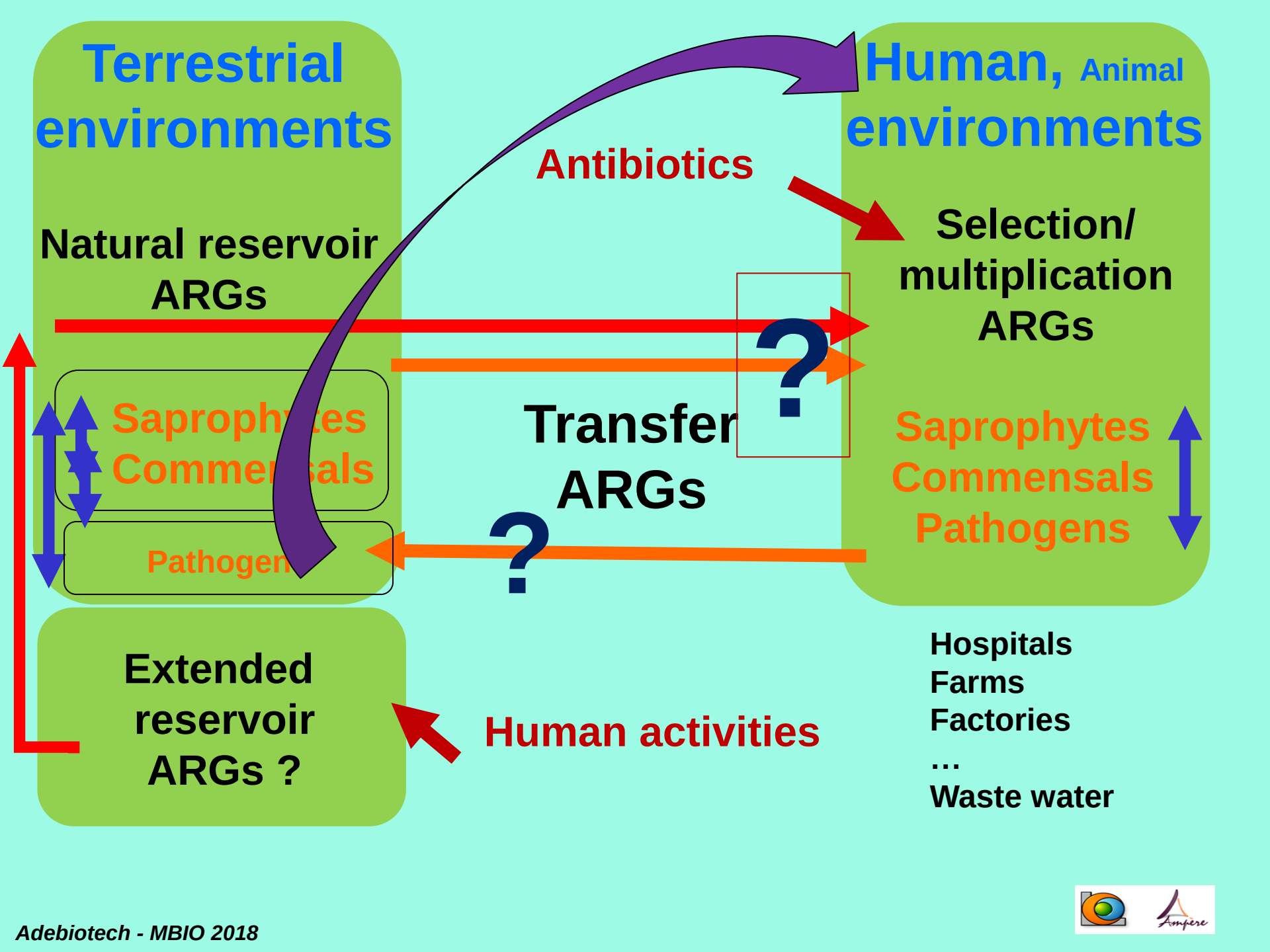


Gene transfer?

in situ

Who, Where, When, How, Often ?

- A measurable fraction of the DNA escapes degradation (persistence)
- DNA can be detected far away from the dead organism (transport)


Terrestrial environments
Natural reservoir ARGs
Saprophytes
Commensals
Pathogen
Extended reservoir ARGs ?
Antibiotics
Transfer ARGs
?
?
Human activities
Human, Animal environments
Selection/ multiplication ARGs
Saprophytes
Commensals
Pathogens
Hospitals
Farms
Factories
…
Waste water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Tim Vogel