

# Potential uses of genomic investigations for natural remediation of chlorinated pollutants

Equipe Adaptations et Interactions Microbiennes dans l'Environnement  
Université de Strasbourg, UMR 7156 CNRS

## Permanent scientists

**Stéphane Vuilleumier, PR**

Françoise Bringel, CR1 CNRS

Thierry Nadalig, MC

## Technicians

Christelle Gruffaz

Yousra Louhichi

## PhD students

Christian Penny (2009)

Sandro Roselli (2009)

Emilie Muller

Muhammad Farhan

## Master M2 students

Santiago Guerrero

Louise Ott



[vuilleumier@unistra.fr](mailto:vuilleumier@unistra.fr)

# Potential uses of genomic investigations for natural remediation of chlorinated pollutants

- A brief general introduction
  - Organohalogen pollutants: The situation in France
  - Remediation: Bioremediation and the rest
  - Dehalogenases
- Genomics: state of the art
  - Whole-genome sequencing projects
  - Environmentally-relevant sequencing projects
  - Microbial halogenated C1 metabolism: a minimal model
  - From dehalogenase sequence to function
- Perspectives: Metagenomics
  - Soil: the ultimate frontier?

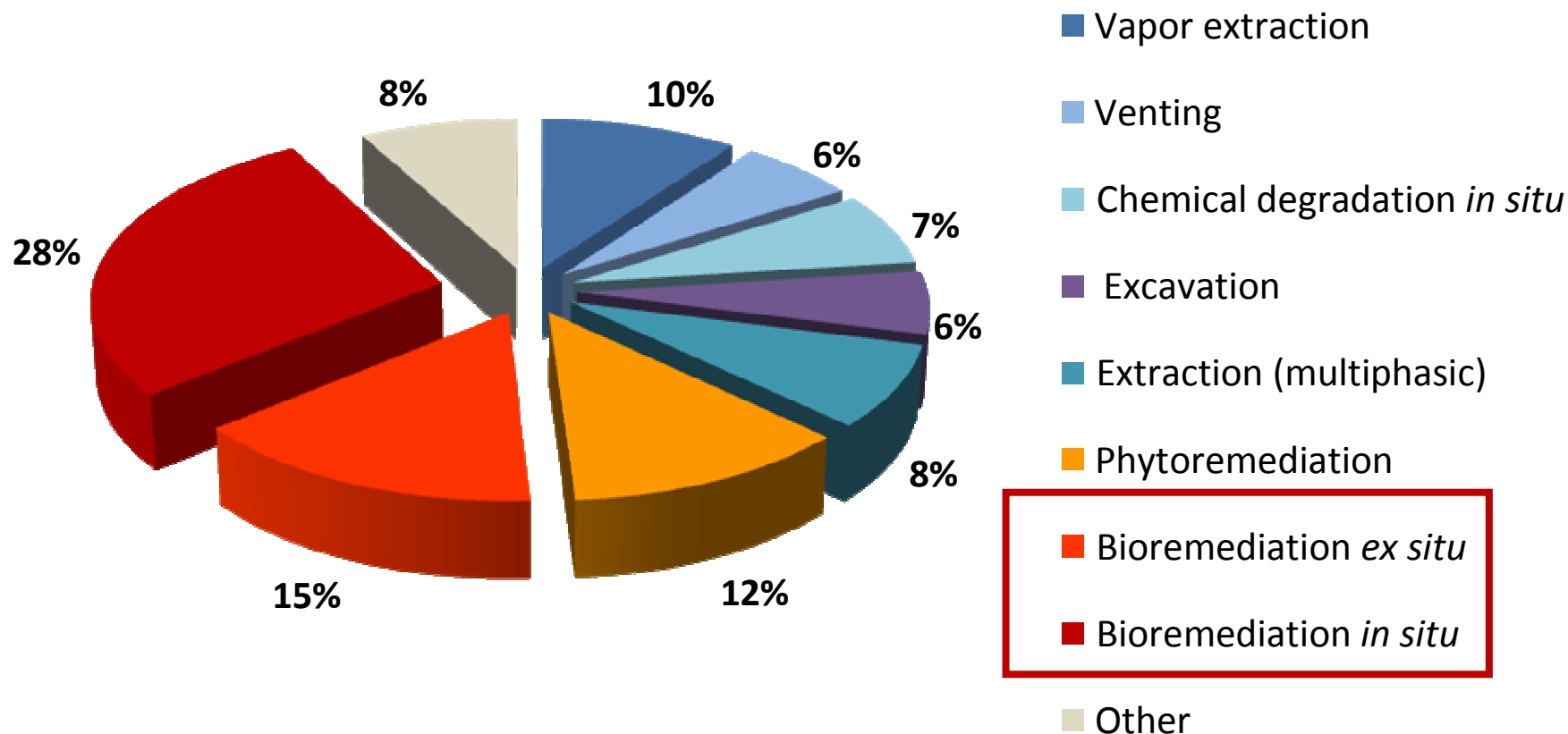
# Why bother about environmental pollution?

>4200 officially listed sites in France (>660 with halogenated contaminants)  
*In total, more than 500'000 disused industrial sites in the country*



<http://basol.ecologie.gouv.fr>  
(<http://basias.brgm.fr>)

# Remediation methods : the biological option



Pandey *et al.* (2009)

Integrative approaches for assessing the ecological sustainability of *in situ* bioremediation.

*FEMS Microbiol. Rev.* **33**, 324-375.

# Enzymes and pathways

UNIVERSITY OF MINNESOTA  
BIOCATALYSIS / BIODEGRADATION DATABASE

Home  
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Pathway Prediction System | PredictBT Workshops | Biochemical Periodic Tables

*Microbial biocatalytic reactions and biodegradation pathways.*

[EBI mirror](#) | [KEGG mirror](#)

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- [What's New?](#) Last updated February 23, 2010.
- [Search](#) the UM-BBD for compound, enzyme, microorganism, pathway, or BT rule name; chemical formula; chemical structure; CAS Registry Number; or EC code.
- Pathways and Metapathways in the UM-BBD

- Select a Pathway -

- Lists of [192 pathways](#); [1313 reactions](#); [1219 compounds](#); [854 enzymes](#); [500 microorganism entries](#); [265 biotransformation rules](#); [50 organic functional groups](#); [76 reactions of naphthalene 1,2-dioxygenase](#); [109 reactions of toluene dioxygenase](#); [Graphical UM-BBD Overview](#); and [Other Graphics](#) (Metapathway and Pathway Maps and Reaction Mechanisms).
- *cite using:*  
Gao J, Ellis LBM, Wackett LP (2010) "The University of Minnesota Biocatalysis/Biodegradation Database: improving public access" *Nucleic Acids Research* **38**: D488-D491. [Abstract](#) | [Full Text](#)
- For more information [join our email list](#). Comments related to the database are appreciated; [contact us](#).

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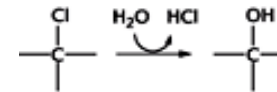
<http://umbdd.msi.umn.edu>

# Bioremediation of chlorinated pollutants: Dehalogenases

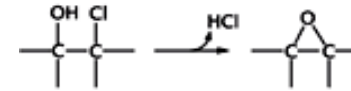


- hydrolytic
- thiolytic
- dehydrodehalogenation
- Methyl group transfer
- oxydative
- reductive
- respiratory

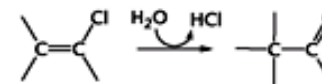
Hydrolytic dehalogenation



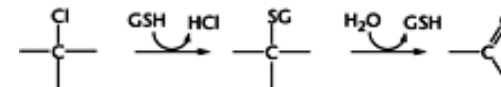
Lyase-catalysed dehalogenation



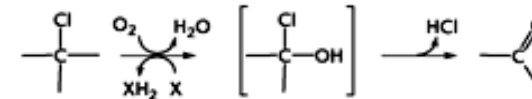
Hydratase-catalysed dehalogenation



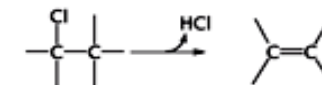
Glutathione S-transferase-dependent dehalogenation



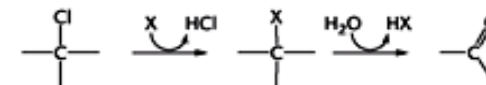
Dehalogenation by oxygenase



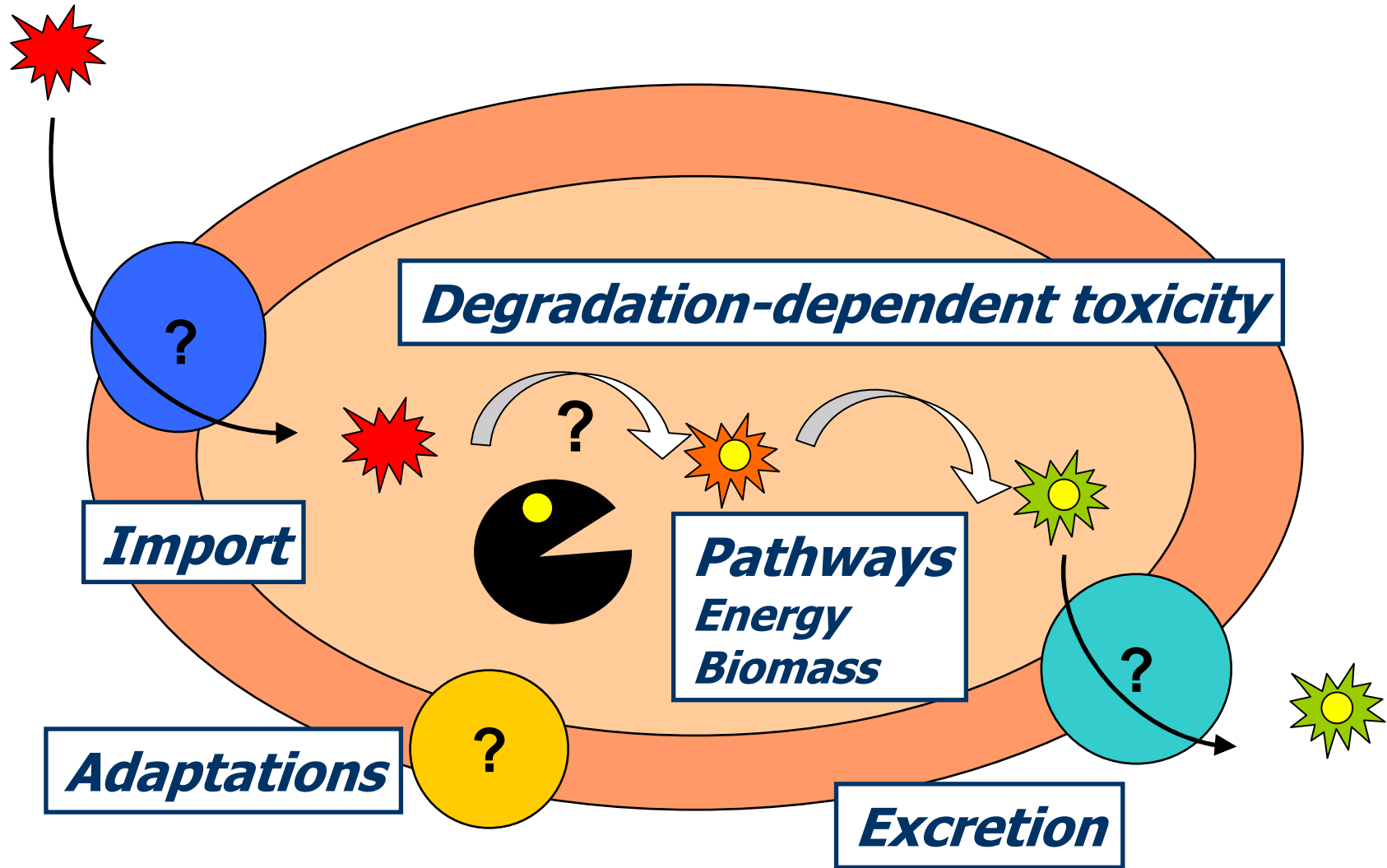
Dehydrohalogenation



Dehalogenation by methyltransferase/dehydrogenase



# Microbial degradation of halogenated pollutants: It's not just the enzymes!

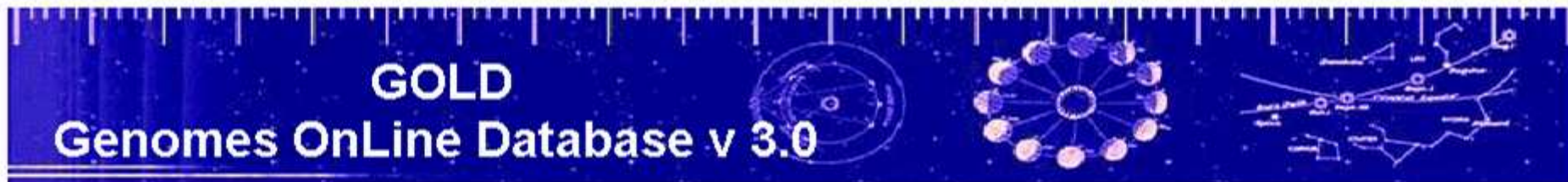



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# Genomic information databases



<b>Contact:</b> <a href="http://Genomesonline">Genomesonline</a>	<b>Last Update:</b> <b>2010-03-12</b>	<b>Location</b> <a href="http://www.genomesonline.org">www.genomesonline.org</a>
<b>1217</b> Published Complete Genomes	<b>Search GOLD: 6926</b> genome projects	<b>208</b> Metagenomes
<b>202</b> Archaeal Ongoing Genomes	<b>3976</b> Bacterial Ongoing Genomes	<b>1323</b> Eukaryotic Ongoing Genomes
 <a href="#">GOLD RSS Feeds</a>	Click to save all data: <input type="button" value="DOWNLOAD"/>	
<a href="#">PROJECT TYPE DISTRIBUTION</a>	<a href="#">SEQUENCING STATUS DISTRIBUTION</a>	<a href="#">PHYLOGENETIC DISTRIBUTION</a>

[www.genomesonline.org](http://www.genomesonline.org)

# Microbial genomes: what about biodegradation ?

**NCBI** (March 2009)

- 3670 microbial genomes, 1087 complete

(December 2009: 3155 total/979 complete)

- Biorem*	101
- Metal	90
- Pollut*	51
- Dehalo*	13
- Dechlor*	12
- Chlorinated	8
- Halogenated	3

Total of  
190 «biodegradation-  
relevant» projects

**«dehalogenation»: 21**

# Genes annotated as dehalogenases in genome databases

## Integrated Microbial Genomes (Joint Genomic Institute, December 2009)

1535 Bacterial, 71 Archaeal, 49 Eucaryota genomes

### «Dehalogenase» annotations

	Types	Total	#genomes/type
Eucaryota	19	32	1.3
Archaea	21	111	3.0
Bacteria	237	3534	7.6

# One-carbon metabolism : a minimal model for microbial dehalogenation goes genomic

***Aerobiosis***

Mineralisation  
(carbon and  
energy source)

Cometabolism



***Anaerobiosis***

Mineralisation  
(carbon and  
energy source)

Cometabolism



# ADEME BIO-DCM project (2010): WWTP biofilter for treatment of gaseous effluents

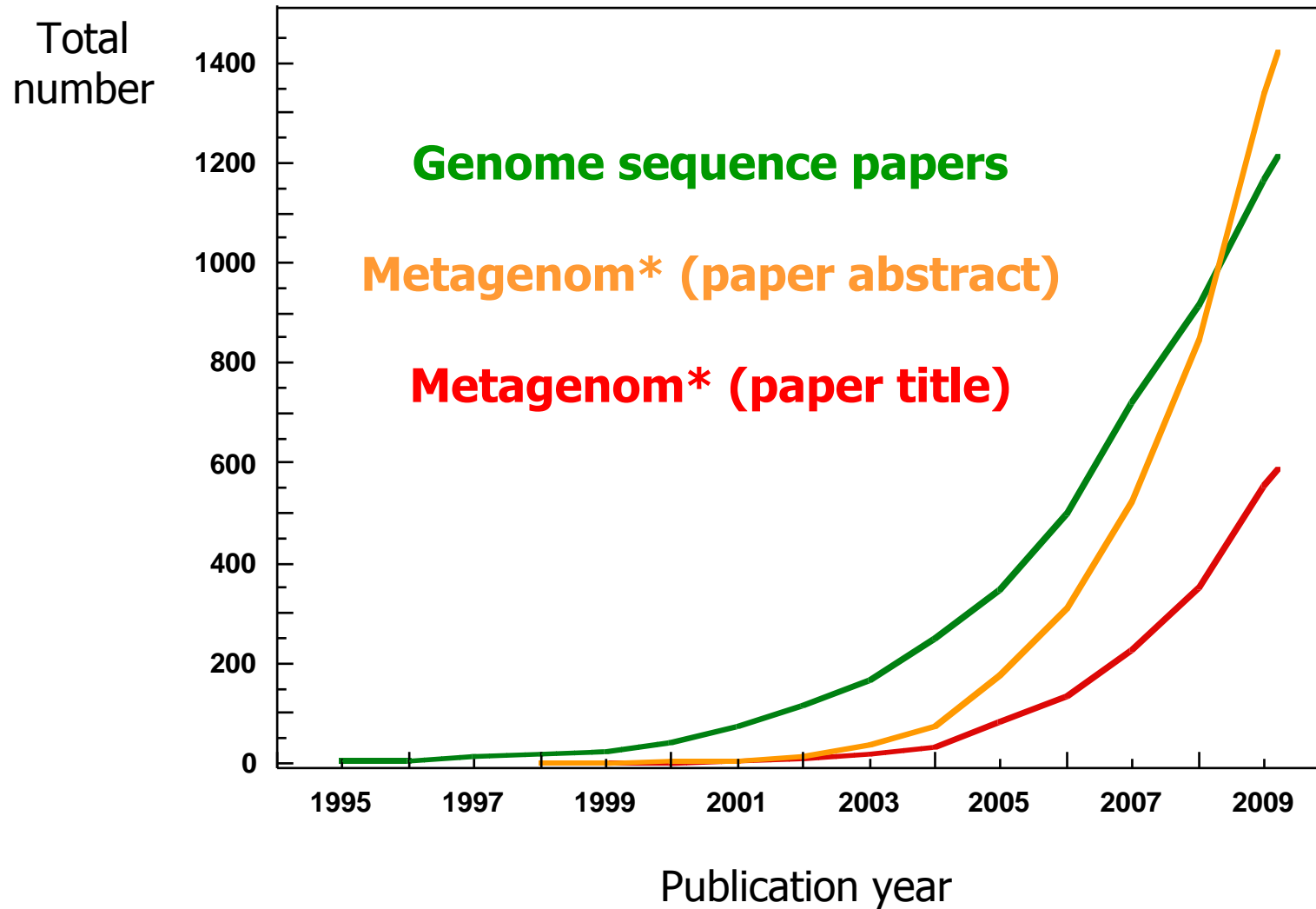


- Dichloromethane elimination by **inoculation of sequenced strains**
- **Sequence-based follow-up** > *metagenome?* > *metatranscriptome?*

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# metagenom\* vs complete genomes : publications



# The « soil » frontier: Complete sequencing of the dehalogenation function in soil?

- Huge, isolation-technique dependent diversity of soil...

Gans *et al.* (2005), revisiting DNA reassociation data from Torsvik *et al.*:

Non-polluted soil	$8.3 \cdot 10^6$ «phylotypes»
Heavily polluted soil	$7.9 \cdot 10^3$ «phylotypes»

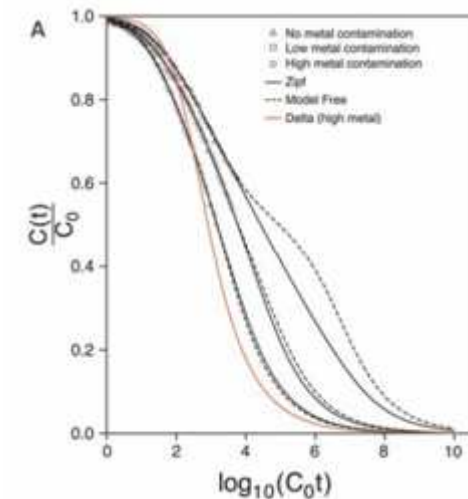
*Metagenome size estimate (assuming 5 Mb/genome):*

Non-polluted soil	$40 \cdot 10^4$ Gb ( $4 \cdot 10^{13}$ bases)
Heavily polluted soil	40 Gb ( $4 \cdot 10^{10}$ bases)

« next generation » sequencing :  $\sim 2$  (+?) Gb / run

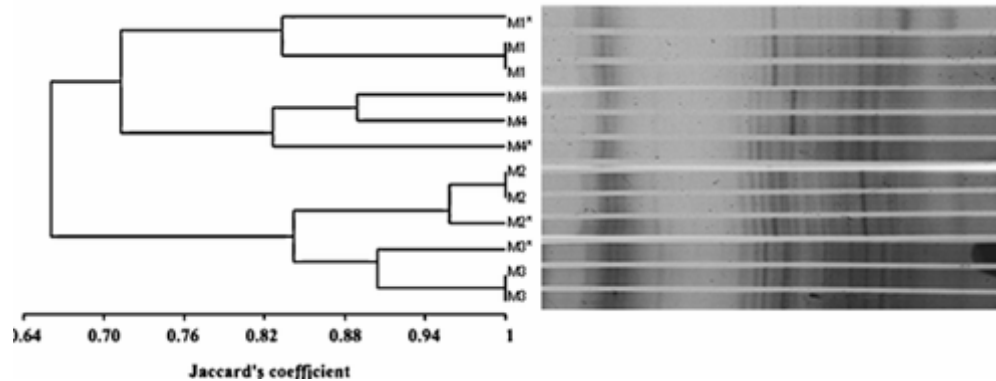
> In principle, today:

the complete metagenome of a heavily polluted soil may be sequenced



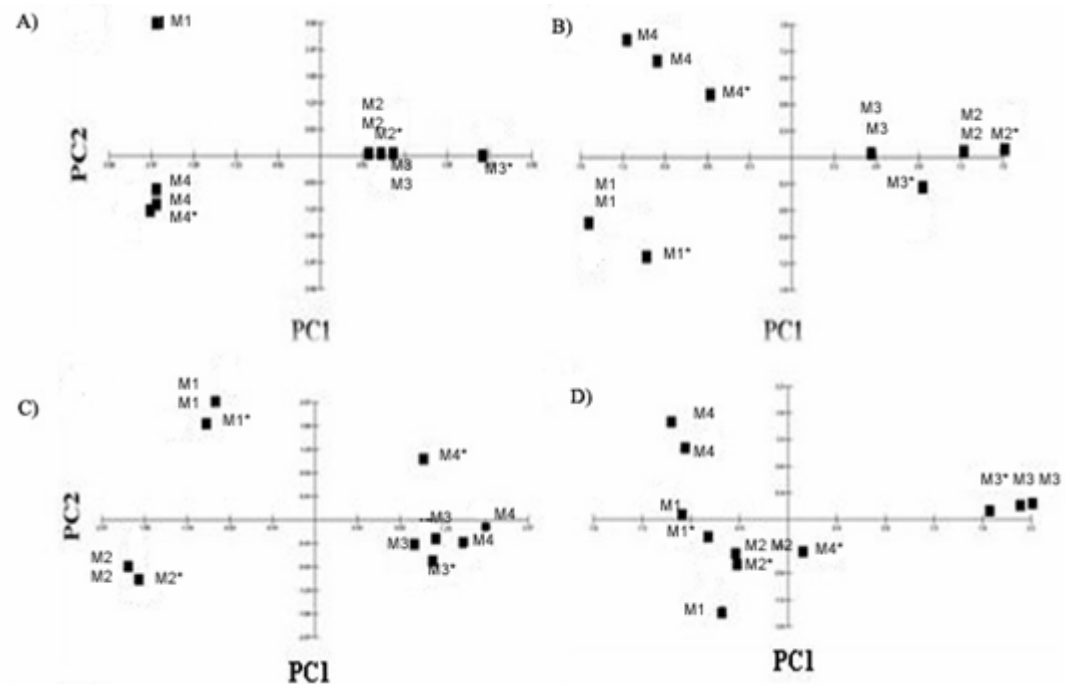


# Preparation of environmental DNA - still a major technical issue



DGGE gel and dendrogram constructed from similarity matching data produced from the DGGE profiles of one soil obtained with M1–M4. Two replicate DGGE analysis from a single DNA extract and one DGGE profile generated from a separate DNA extraction shown (asterisk)

**Principal Component Analysis:**  
**4 methods M1-M4,**  
**4 soils A-D**



Carrigg *et al.* (2007) DNA extraction method affects microbial community profiles from soils and sediment. *Appl. Microbiol. Biotechnol.* **77**, 955-964.

# Application of genomic studies in bioremediation

- Fundamental knowledge on microbial transformation of contaminants
- Development of new bioindicators for practical bioremediation :
  - Follow-up of relevant subpopulations
  - Fingerprinting (>forensics)
  - Estimation of biodegradation activity
  - Estimation of contaminant response
  - Assessment of horizontal gene transfer

***> For genomes AND metagenomes***

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*March 2010*

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[vuilleumier@unistra.fr](mailto:vuilleumier@unistra.fr)

Françoise Bringel, CNRS  
Thierry Nadalig, MC

**Technicians**

Christelle Gruffaz  
Yousra Louhichi

**PhD students**

Emilie Muller  
Muhammad Farhan

**Master students 2010**

Santiago Guerrero  
Louise Ott



**Université de Strasbourg**

Hubert Schaller (IBMP)  
Malek Alioua (IBMP)  
Dimitri Heintz (IBMP)  
Philippe Hammann (IBMC)  
Gwenaël Imfeld (LHyGES)  
Stéphane Le Calvé  
Michel Rohmer

**Metasoil (France)  
Terragenome (World)**

**Génoscope**



***Thank you***

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Julia Vorholt  
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**SIB Lausanne**

Marco Pagni