

Potential uses of genomic investigations for natural remediation of chlorinated pollutants

Equipe Adaptations et Interactions Microbiennes dans l'Environnement
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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?

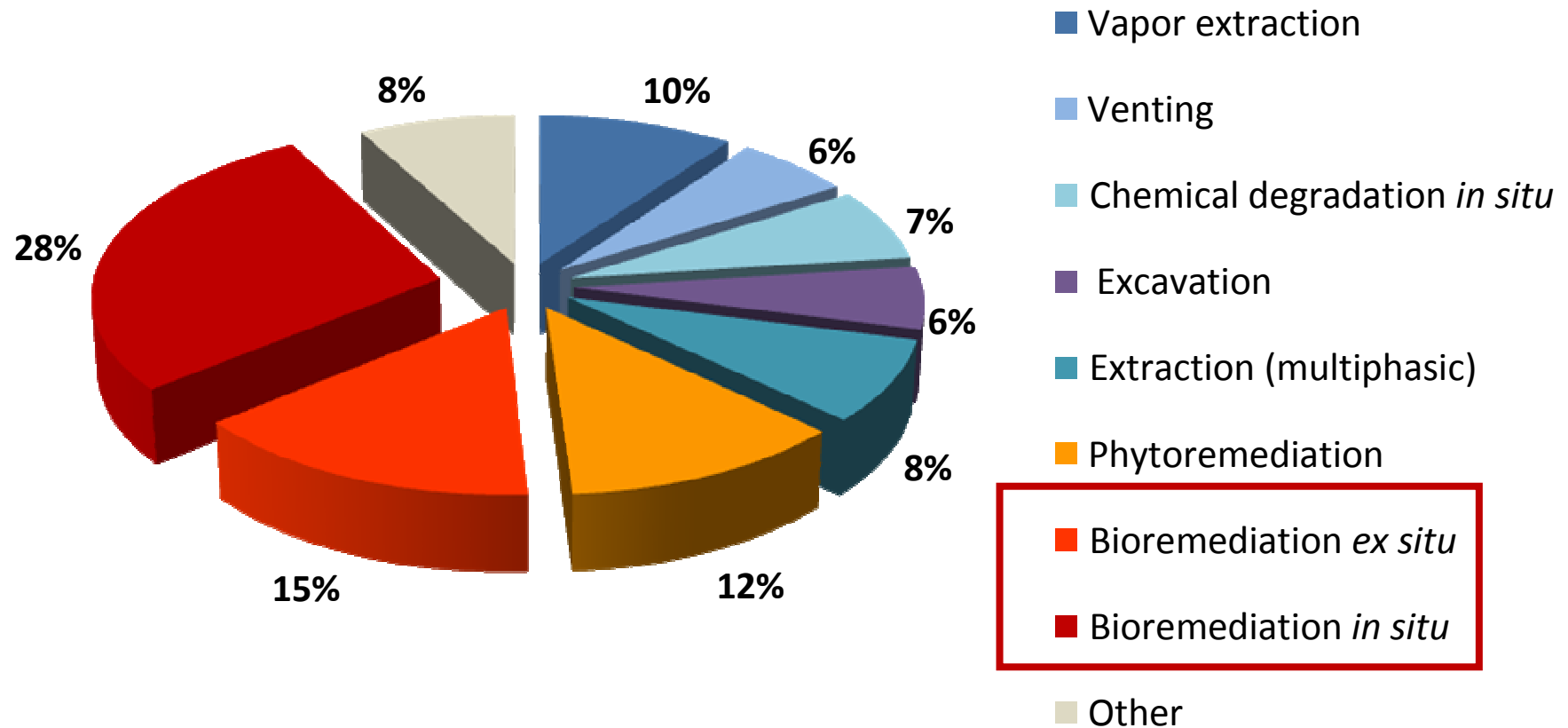
Why bother about environmental pollution?

>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

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Pathway Prediction System | PredictBT Workshops | Biochemical Periodic Tables

Microbial biocatalytic reactions and biodegradation pathways.

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

- [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

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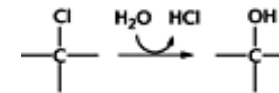
<http://umbdd.msi.umn.edu>

Bioremediation of chlorinated pollutants: Dehalogenases

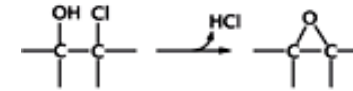


- hydrolytic
- thiolytic
- dehydrodehalogenation
- Methyl group transfer
- oxydative
- reductive
- respiratory

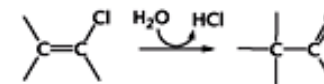
Hydrolytic dehalogenation



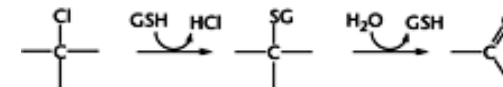
Lyase-catalysed dehalogenation



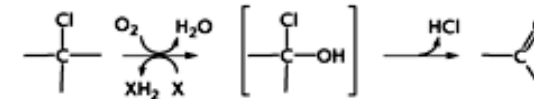
Hydratase-catalysed dehalogenation



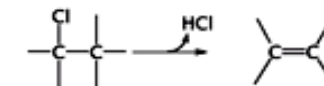
Glutathione S-transferase-dependent dehalogenation



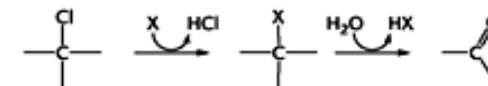
Dehalogenation by oxygenase



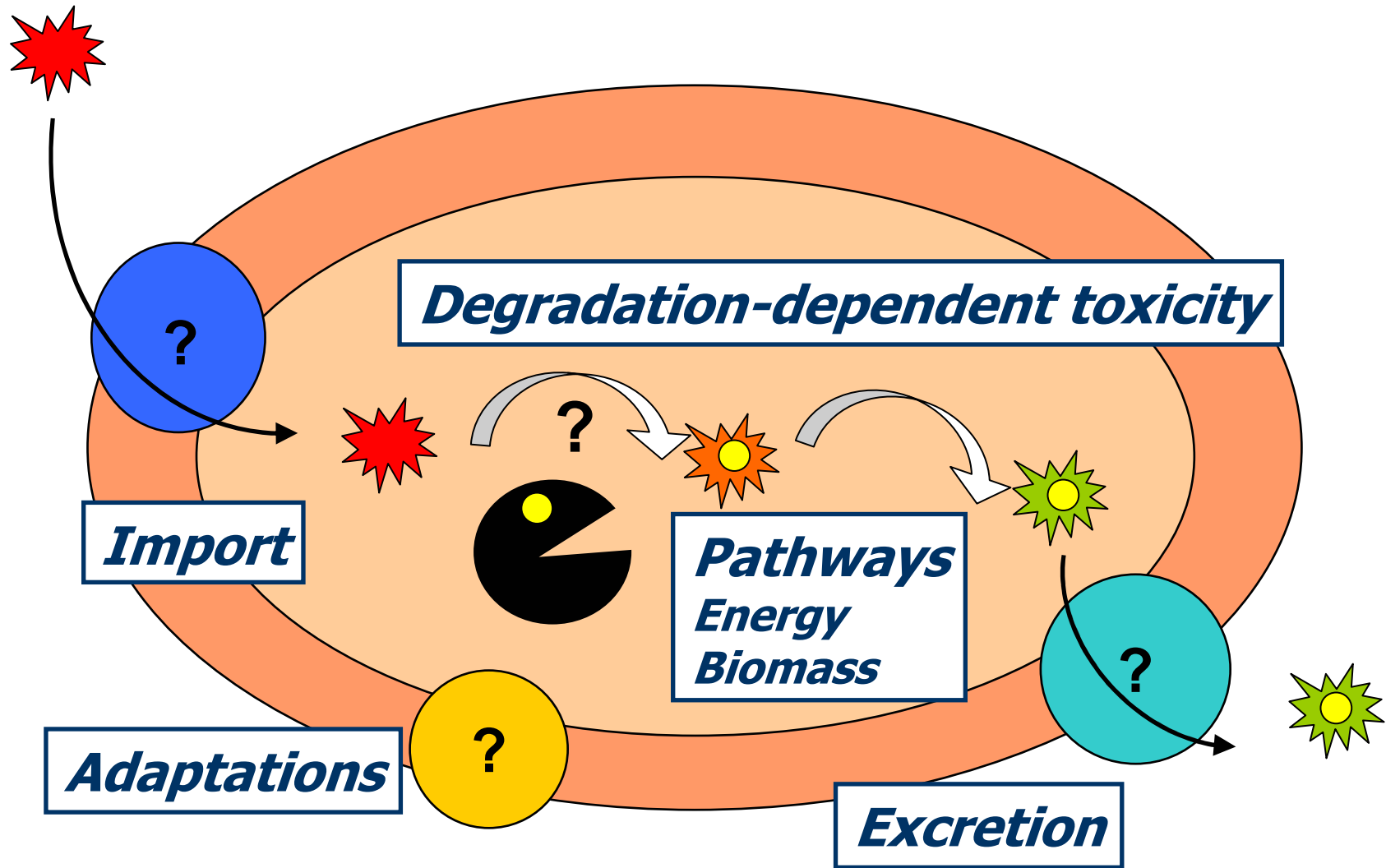
Dehydrohalogenation



Dehalogenation by methyltransferase/dehydrogenase



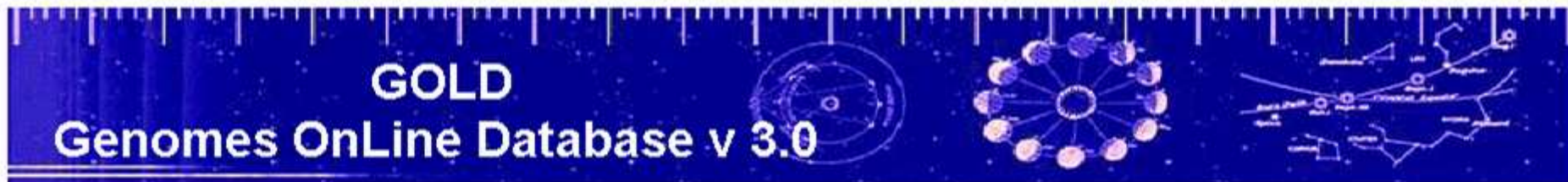
Microbial degradation of halogenated pollutants: It's not just the enzymes!




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Genomic information databases



<p>Contact: Genomesonline</p>	<p>Last Update: 2010-03-12</p>	<p>Location www.genomesonline.org</p>
<p>1217 Published Complete Genomes</p>	<p><u>Search GOLD:</u> 6926 genome projects</p>	<p>208 Metagenomes</p>
<p>202 Archaeal Ongoing Genomes</p>	<p>3976 Bacterial Ongoing Genomes</p>	<p>1323 Eukaryotic Ongoing Genomes</p>
<p> GOLD RSS Feeds</p>	<p>Click to save all data: DOWNLOAD</p>	
<p>PROJECT TYPE DISTRIBUTION</p>	<p>SEQUENCING STATUS DISTRIBUTION</p>	<p>PHYLOGENETIC DISTRIBUTION</p>

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

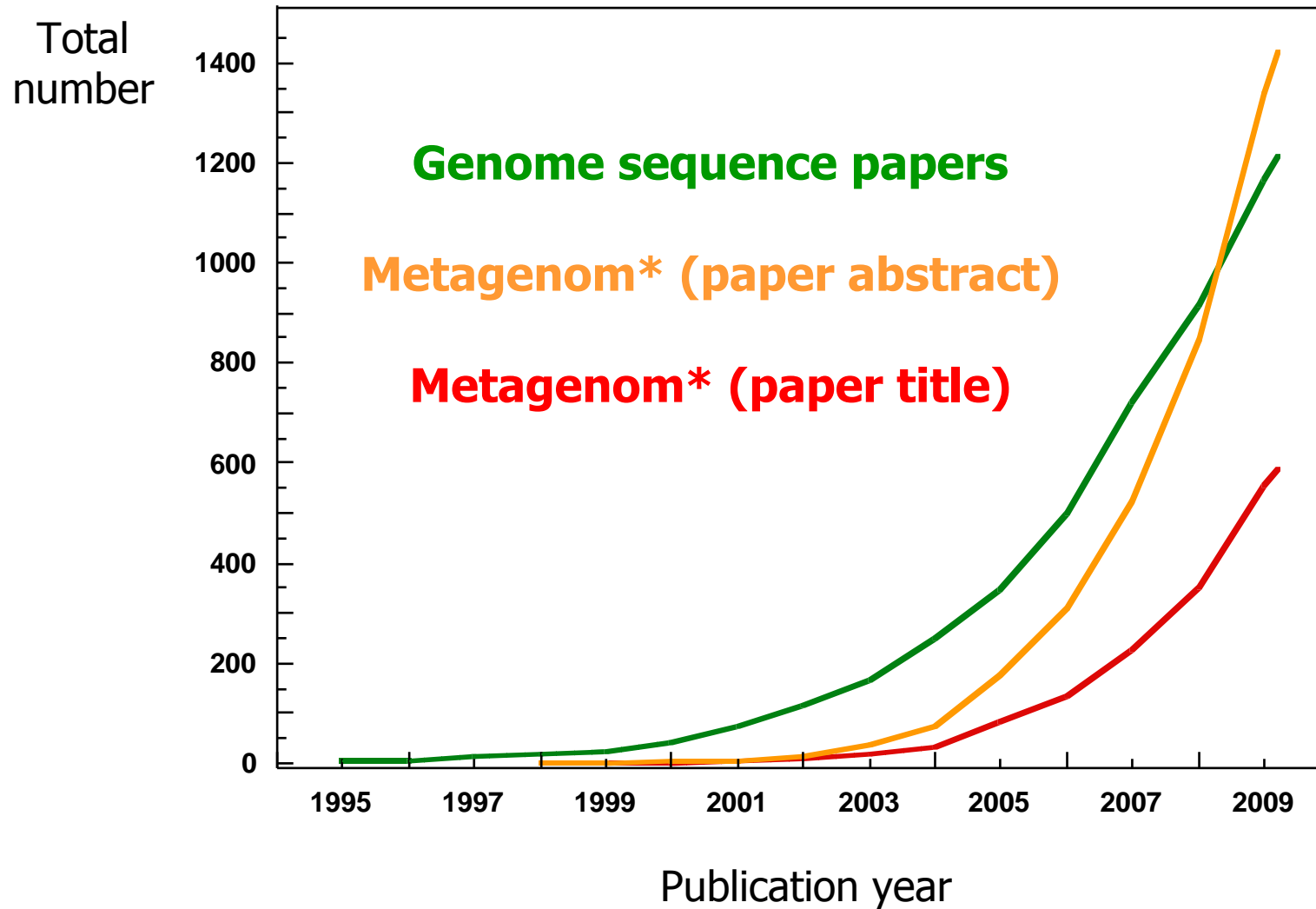


- Dichloromethane elimination by **inoculation of sequenced strains**
- **Sequence-based follow-up** > *metagenome?* > *metatranscriptome?*

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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»

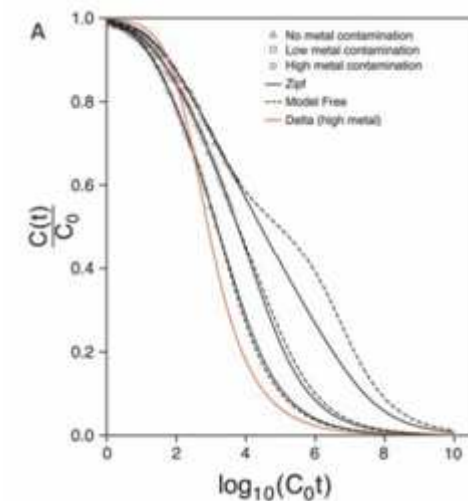
Metagenome size estimate (assuming 5 Mb/genome):

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

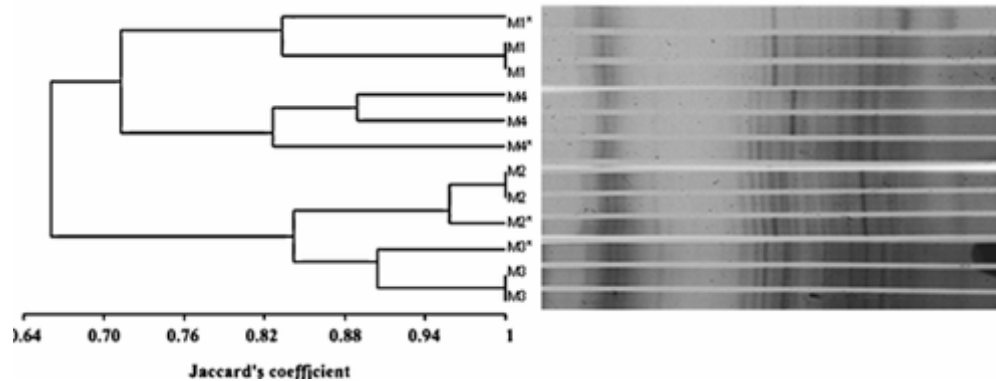
« next generation » sequencing : ~ 2 (+?) Gb / run

> In principle, today:

the complete metagenome of a heavily polluted soil may be sequenced

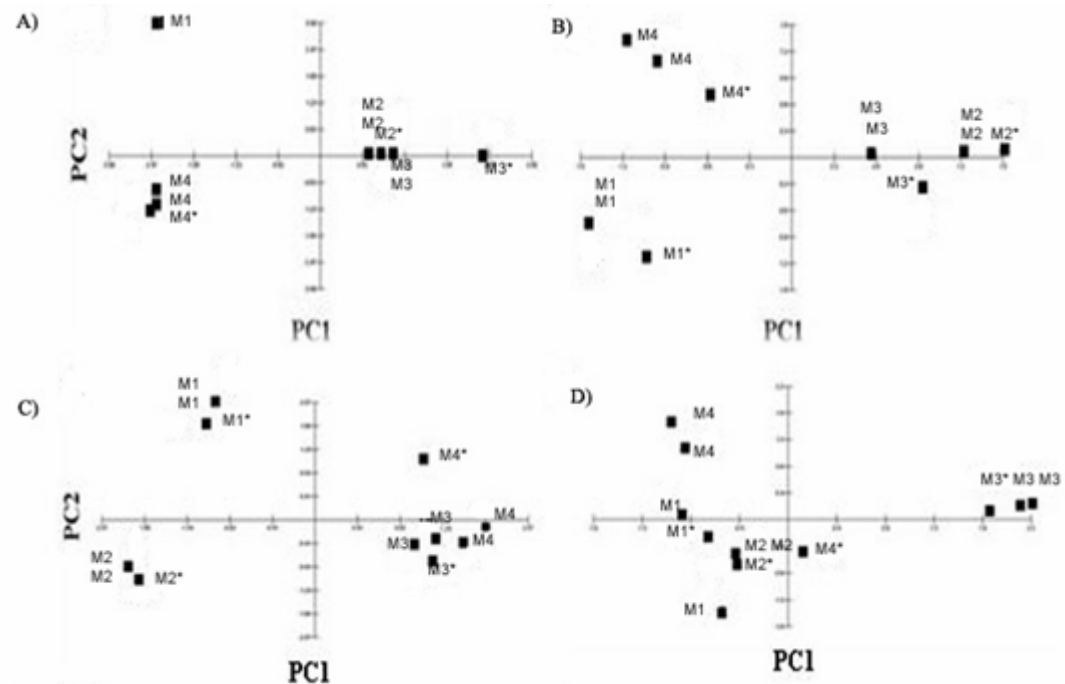


Preparation of environmental DNA - still a major technical issue



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

Adaptations et interactions microbiennes dans l'environnement (UMR 7156 Uds-CNRS)

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Gwenaël Imfeld (LHyGES)
Stéphane Le Calvé
Michel Rohmer

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

Génoscope



Thank you

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