Adaptation of subsurface microbial communities to mercury

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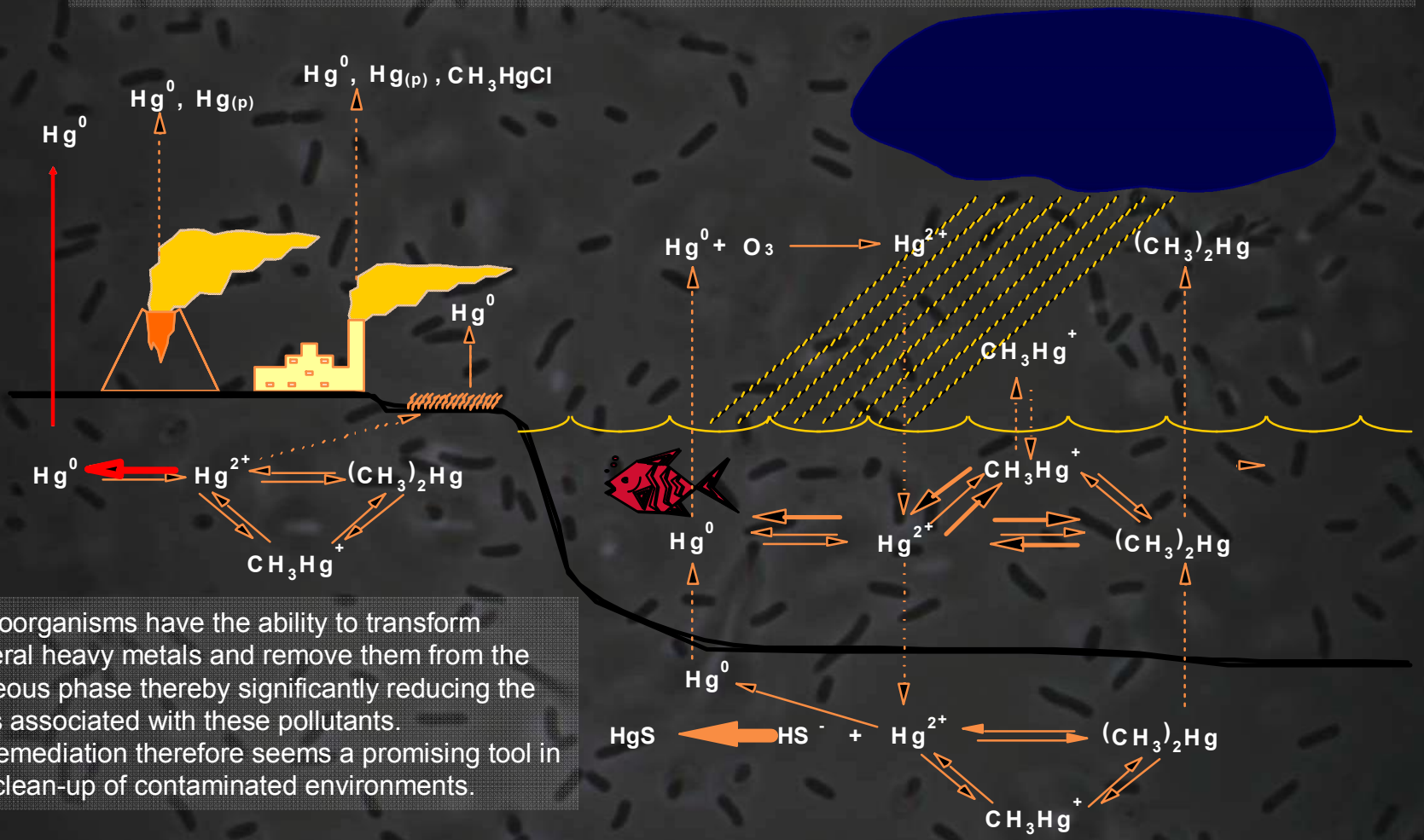
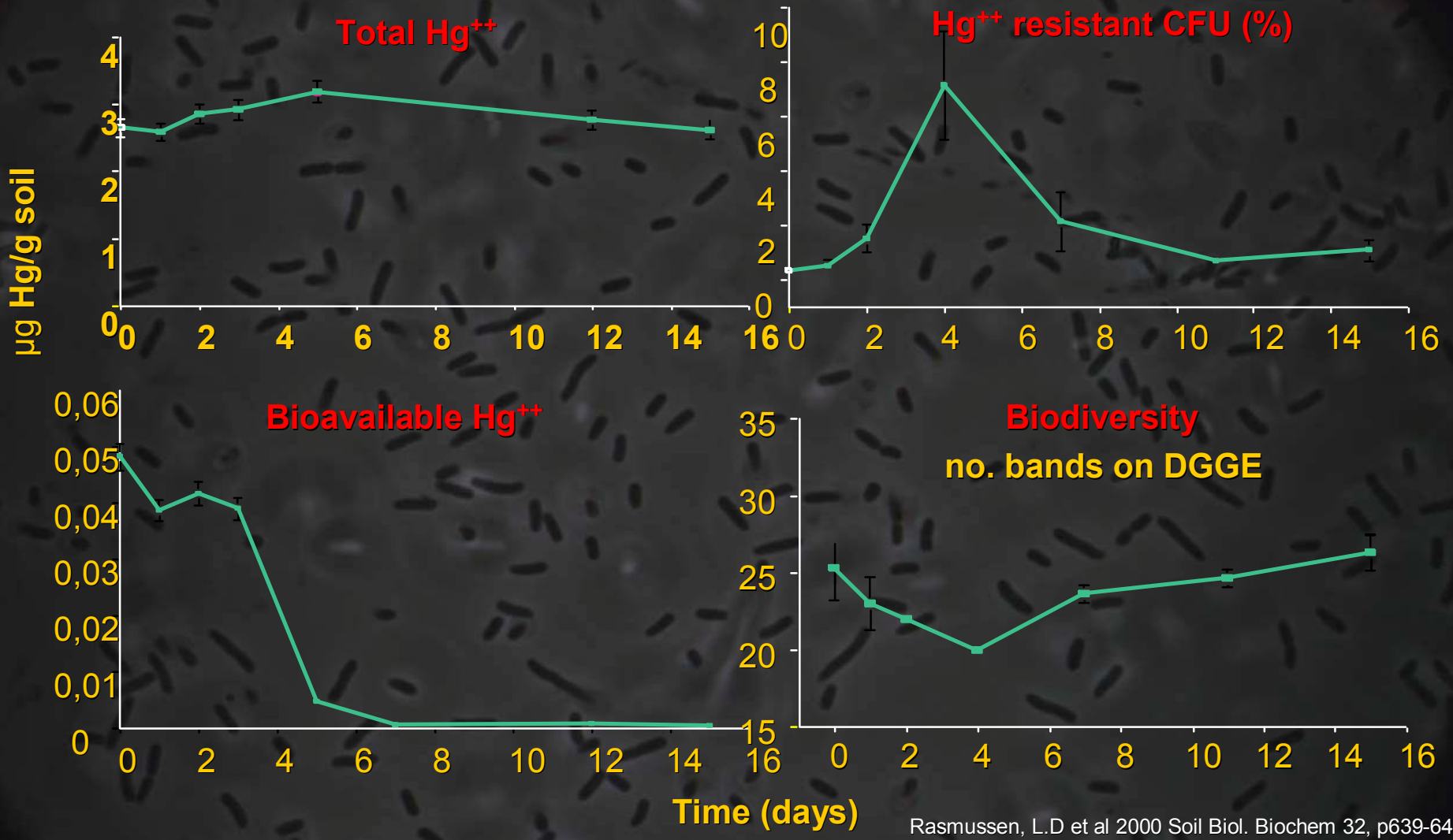
Mercury Cycle in the Biosphere

Knowledge on the response and adaptation of soil bacterial communities to heavy metals is of high relevance as these contaminants are widely distributed in terrestrial environments. Heavy metals as mercury (Hg) arise from e.g. spread of fertilisers, and burning of fossil fuels - and naturally by weathering of rocks.



Microorganisms have the ability to transform several heavy metals and remove them from the aqueous phase thereby significantly reducing the risks associated with these pollutants. Bioremediation therefore seems a promising tool in the clean-up of contaminated environments.

Mercury contaminated soils

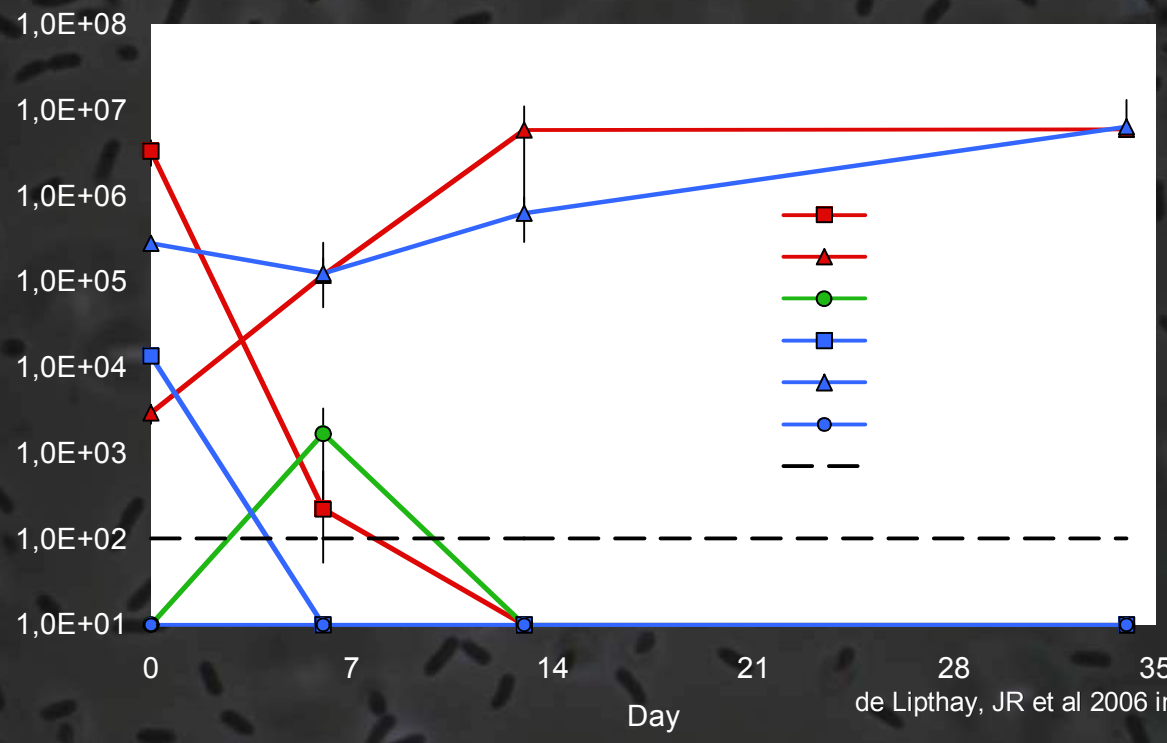


Rasmussen, L.D et al 2000 Soil Biol. Biochem 32, p639-646

Contaminated subsurface soil

Can we introduce Hg-resistance plasmids in natural bacterial populations, and thereby speed up adaptation and stimulate microbial activities in contaminated subsurface soils?

Donor, recipient and transconjugant numbers of microcosm A



de Lipthay, JR et al 2006 in prep

Project tasks

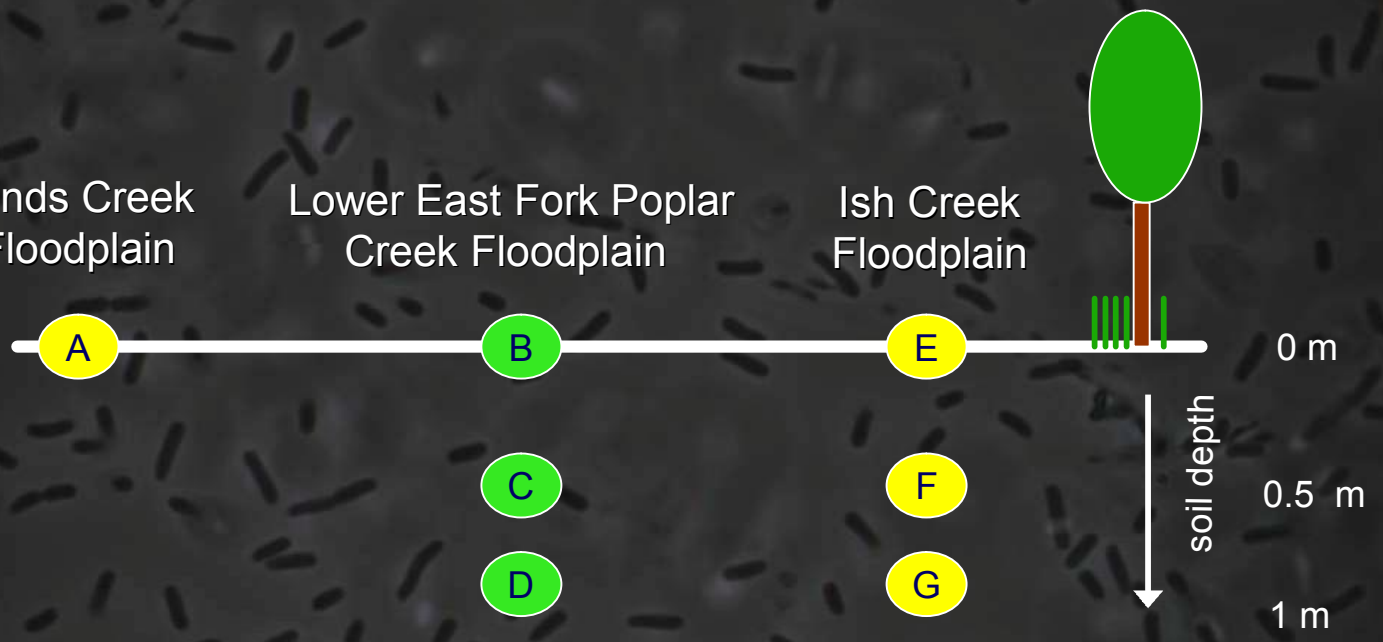
1) Isolation and characterization of hitherto uncultured bacteria of relevance for biotransformation of metals *(University of Copenhagen, NERI)*

2) Horizontal gene transfer to “non-culturable” subsurface bacteria *(University of Copenhagen)*

3) Significance of mobile genetic elements for microbial community adaptation to pollutant stress *(Rutgers University, University of Copenhagen)*

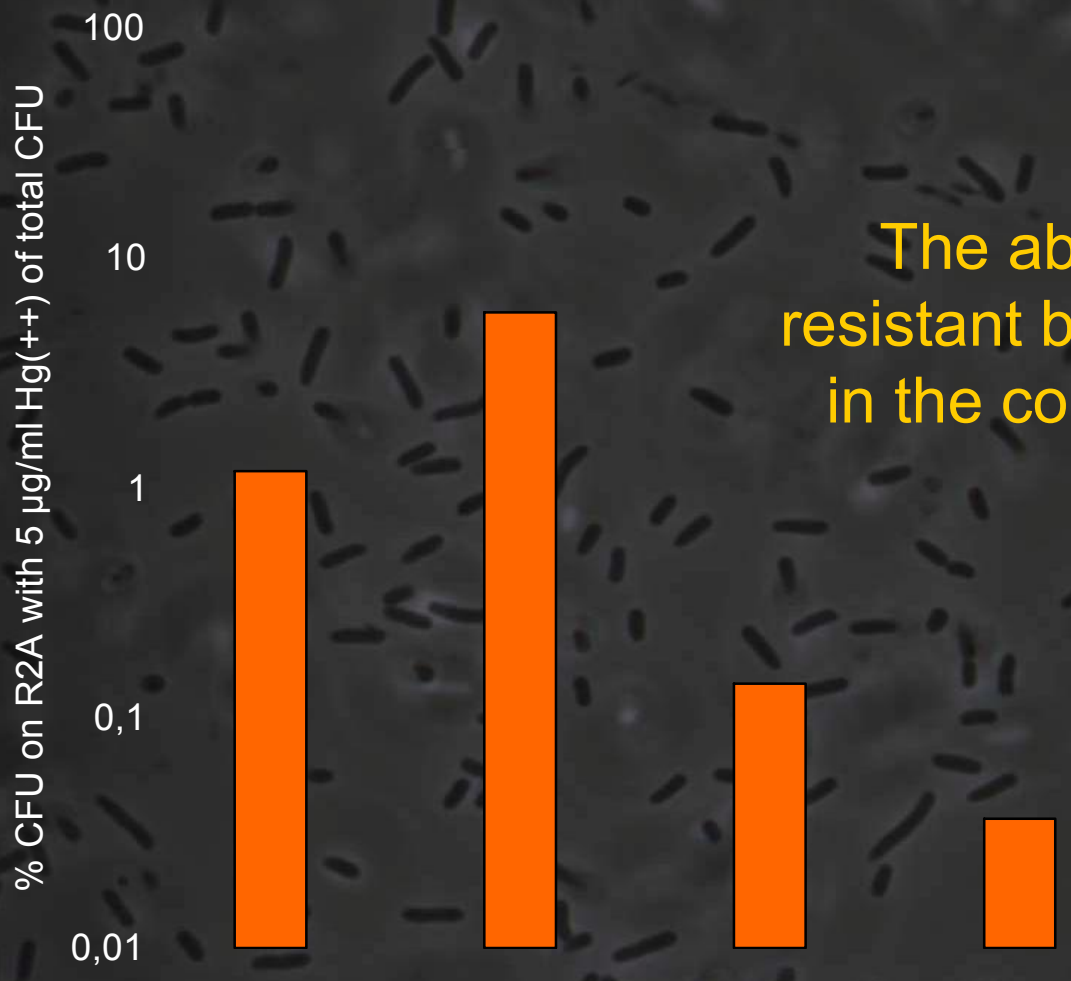
4) Biostimulation of transformation rates in ground water and sediment *(University of Connecticut)*

Soil samples



Soil	Site	Depth (inch)	Hg status	Total Hg (ug pr g soil)	Bioavailable Hg (ng pr g soil)
Soil B	Poplar Creek	0-2"	Contaminated	12.5 +/- 1.4	< d.l.
Soil C	Poplar Creek	18-22"	Contaminated	7.6 +/- 1.0	0.83 +/- 0.43
Soil E	Ish Creek	0-2"	Reference	0.2 +/- 0.05	< d.l.
Soil F	Ish Creek	18-22"	Reference	0.06 +/- 0.01	< d.l.

CFUs on mercury amended R2A plates



The abundance of Hg resistant bacteria was higher in the contaminated soils.

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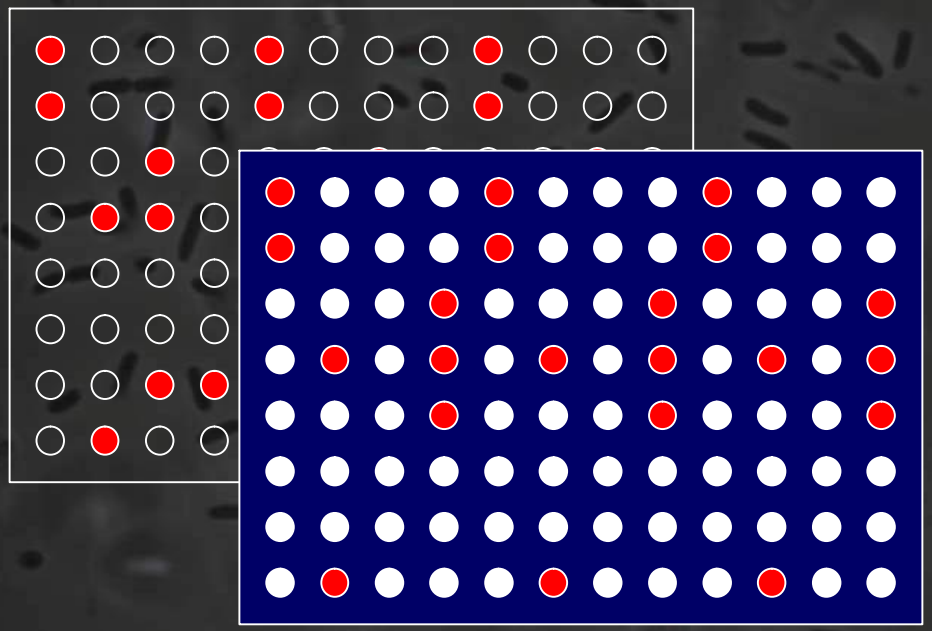
Adaptation Test

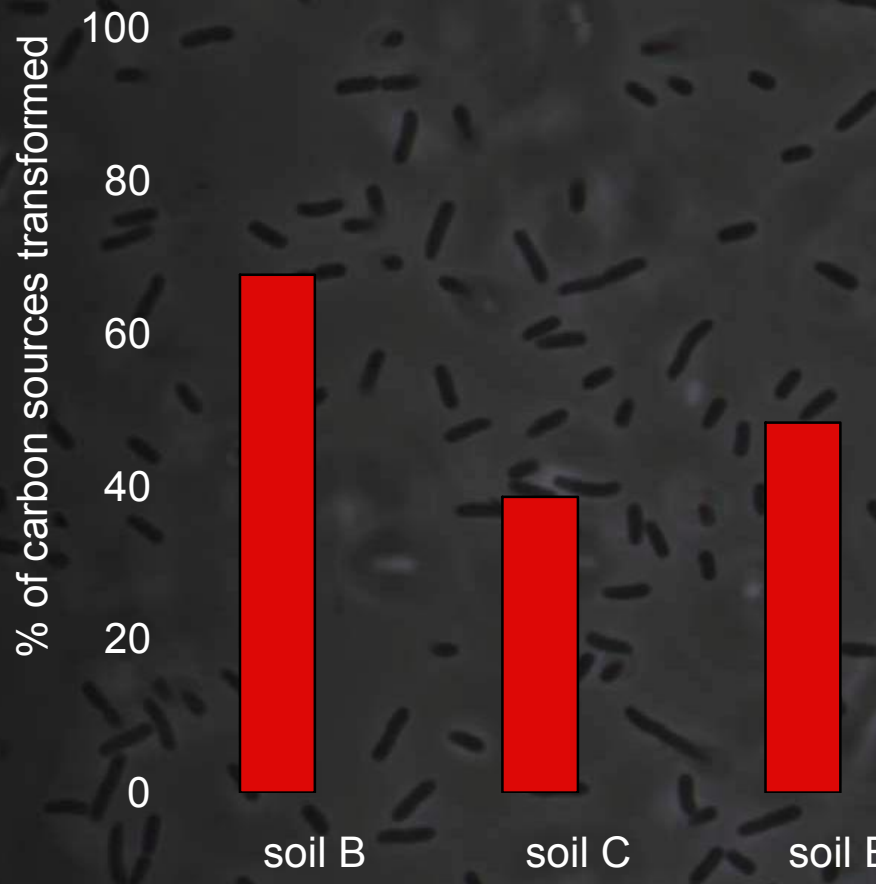
0µg Hg^{++}/ml



1µg Hg^{++}/ml

EcoPlates containing 3 x 31 different sole carbon sources and a tetrazolium redox dye

Adaptation to Hg^{++}



The surface soils were better adapted to Hg than the subsurface soil

The contaminated soils were better adapted to Hg than noncontaminated

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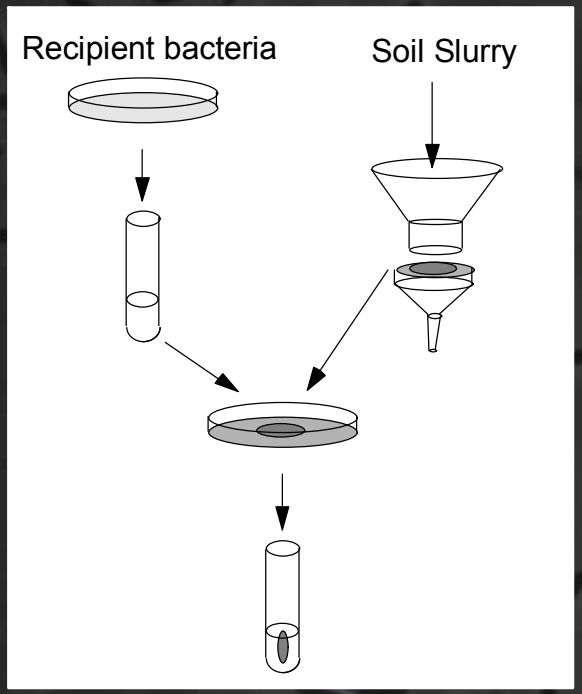
Conclusions

- The **mercury tolerance** of the bacterial communities was **higher in the contaminated soils** than in the control soils despite very low bioavailable mercury concentrations in both types of soils. This indicates that an exposed soil will maintain its ability to tolerate mercury - even when the exposure is low.

- The **high adaptive potential** of subsurface microbial communities **suggest** - similar to surface soils – that **transfer of the *mer* operon by horizontal gene exchange** may play a role for community adaptation to the applied mercury stress.

Exogenic plasmid isolation

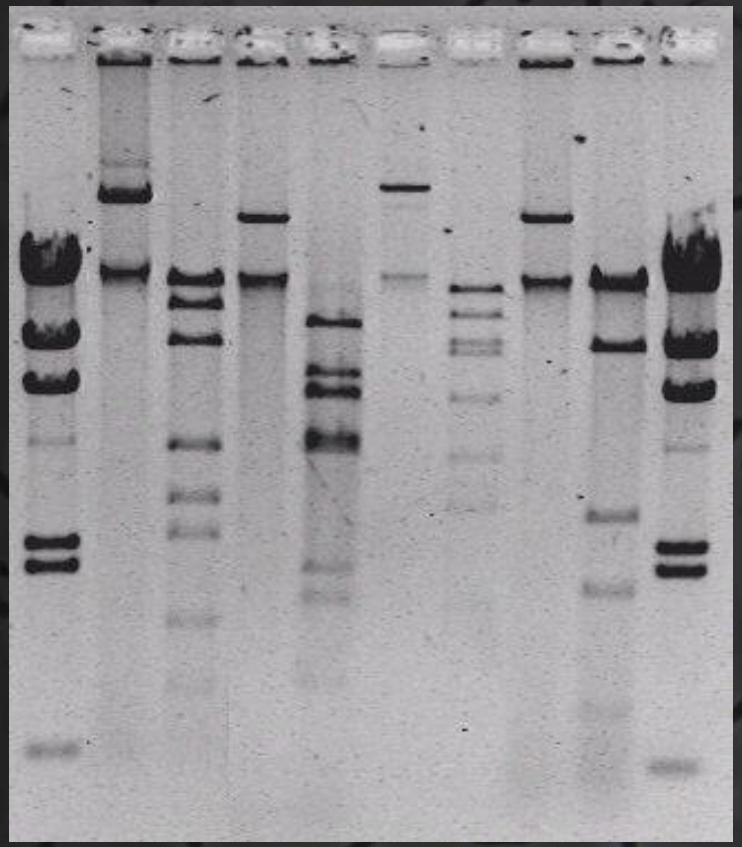


12 replicate isolations experiments from each soil with *E.coli* and *P.putida* as recipient bacteria

Plasmid	Soil B	Soil C	Soil D	Soil E	Soil A
p1	+	+	+	-	-
p2	+	-	-	-	-
p2	+	-	-	-	-
p4	+	-	-	-	-

Exogenous plasmid isolation

The plasmids and EcoRI Restriction cutting



The four different plasmids were all beloning to the same Inc P1-beta group

Plasmid	Recipient	Size	Transfer efficiency
p1	*E.coli*	57.3 Kb	7.58×10^{-6}
p2	*P.putida*	33.6 Kb	1.12×10^{-7}
p3	*E.coli*	68.1 Kb	4.69×10^{-4}
p4	*E.coli*	36.0 Kb	5.59×10^{-6}

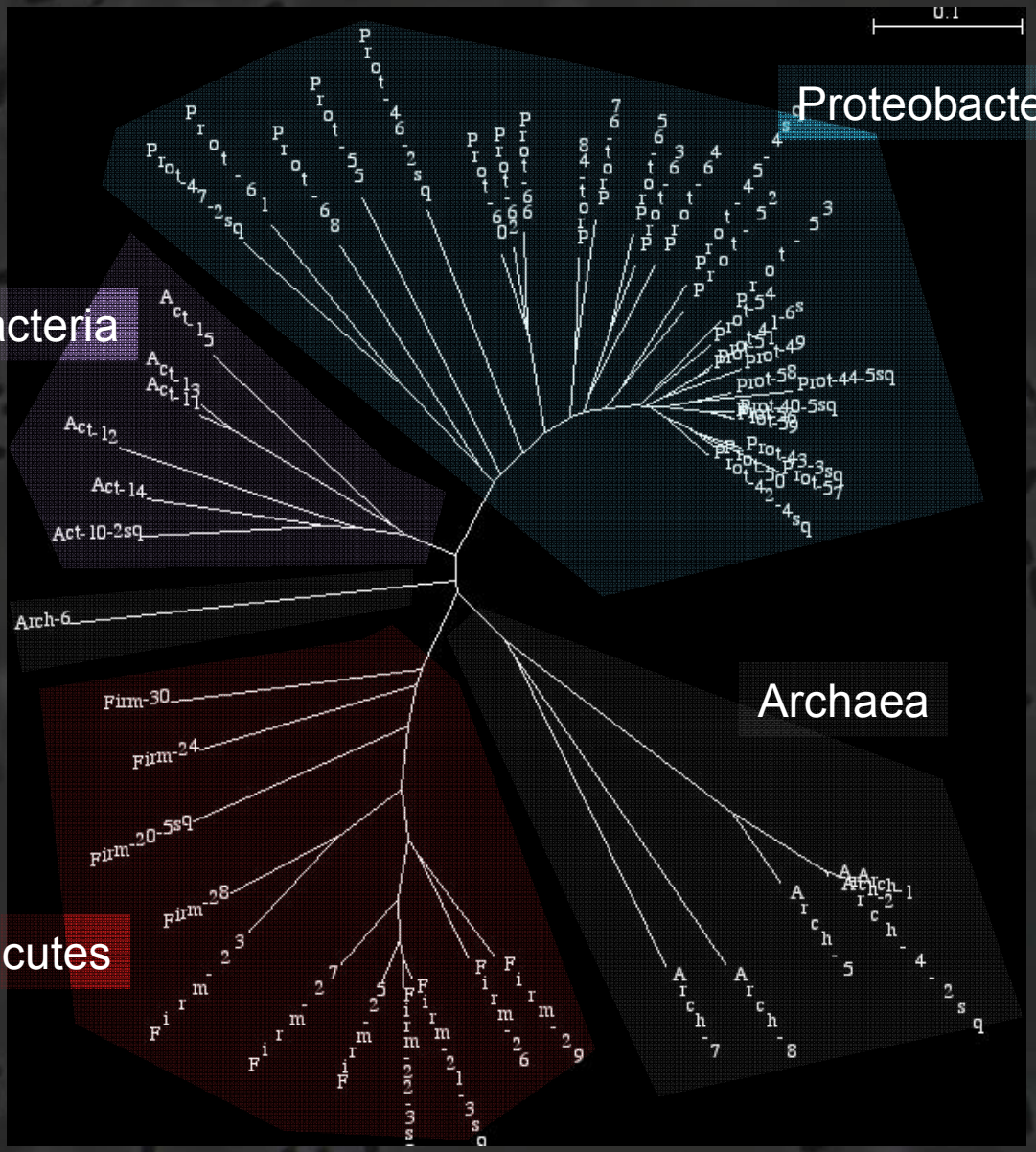
Gram-negative mercury resistance

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

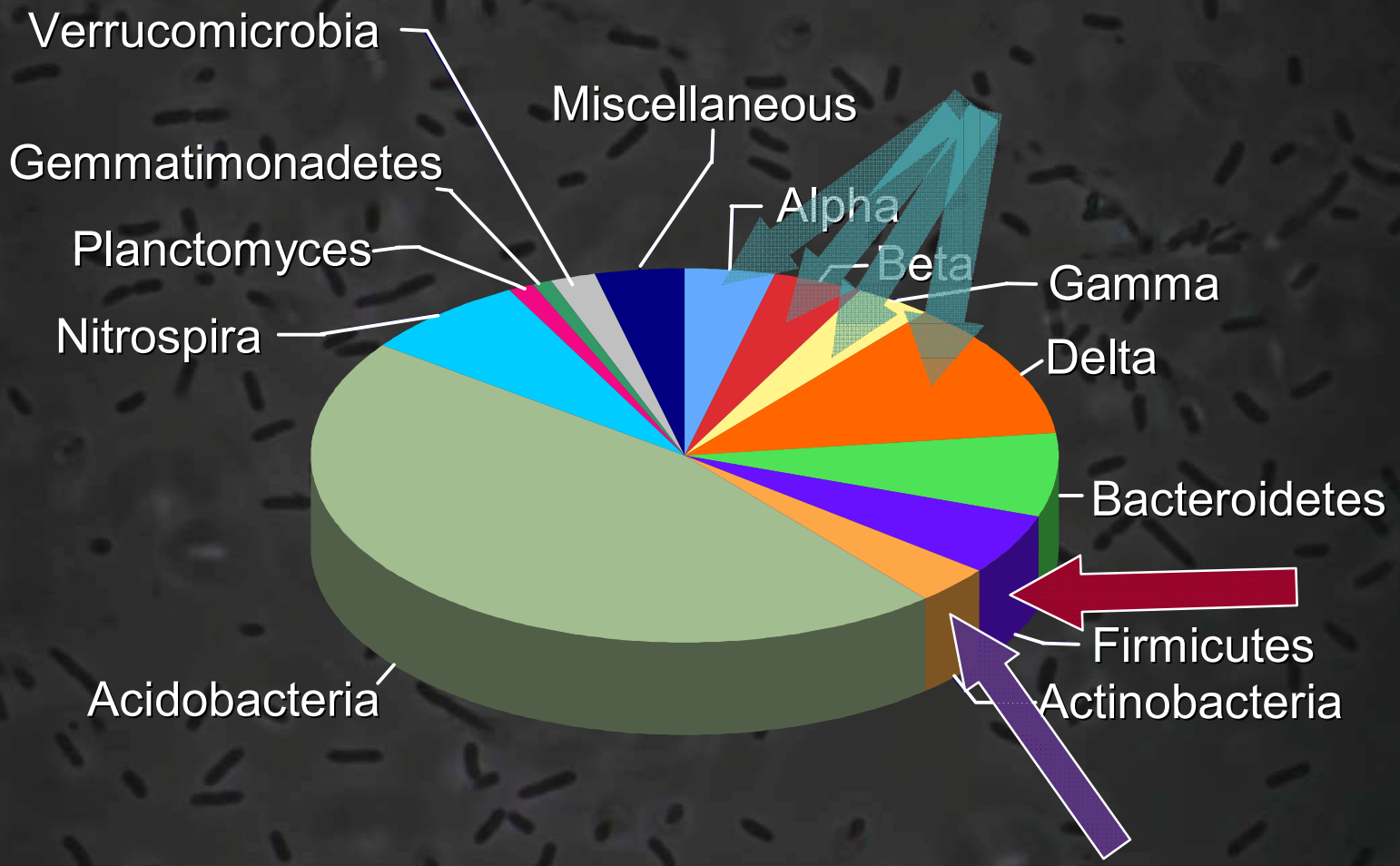
- The *merA* gene encodes a mercuric reductase that reduces Hg^{++} to volatile Hg(0).
- MerP and MerT are involved in the transportation of Hg^{++} to MerA in the cytosol.
- The *merR* gene encodes a Hg responsive regulator.

Barkay et al. FEMS microbiology Reviews 2003 27:355-384

Prokaryotic mercuric reductase protein diversity



16S rDNA clone library



merA PCR

	Soil B	Soil C	Soil D	Soil E	Soil F
Control	+ + +	+ + -	+ + +	- - -	- - -
Hg amended	+ + +	+ + -	+ + +	+ + -	+ + +

+: *merA* present; -: *merA* abscent
The table indicates in how many of three replicate soil samples*merA* could be detected.

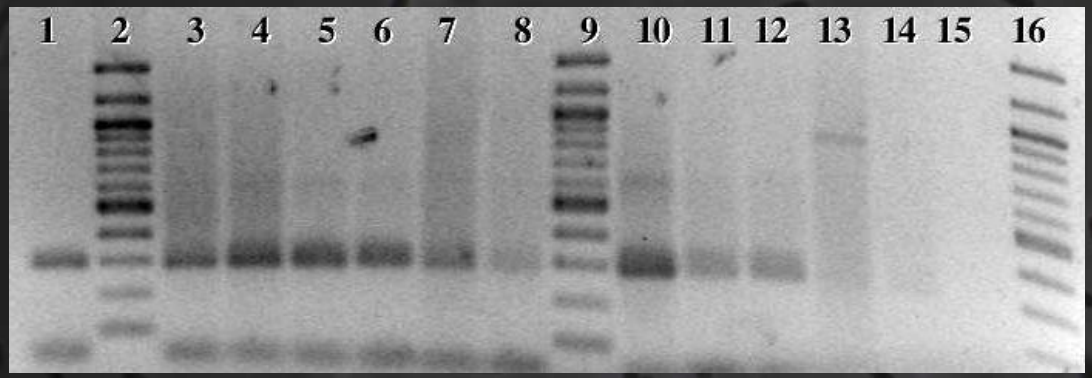
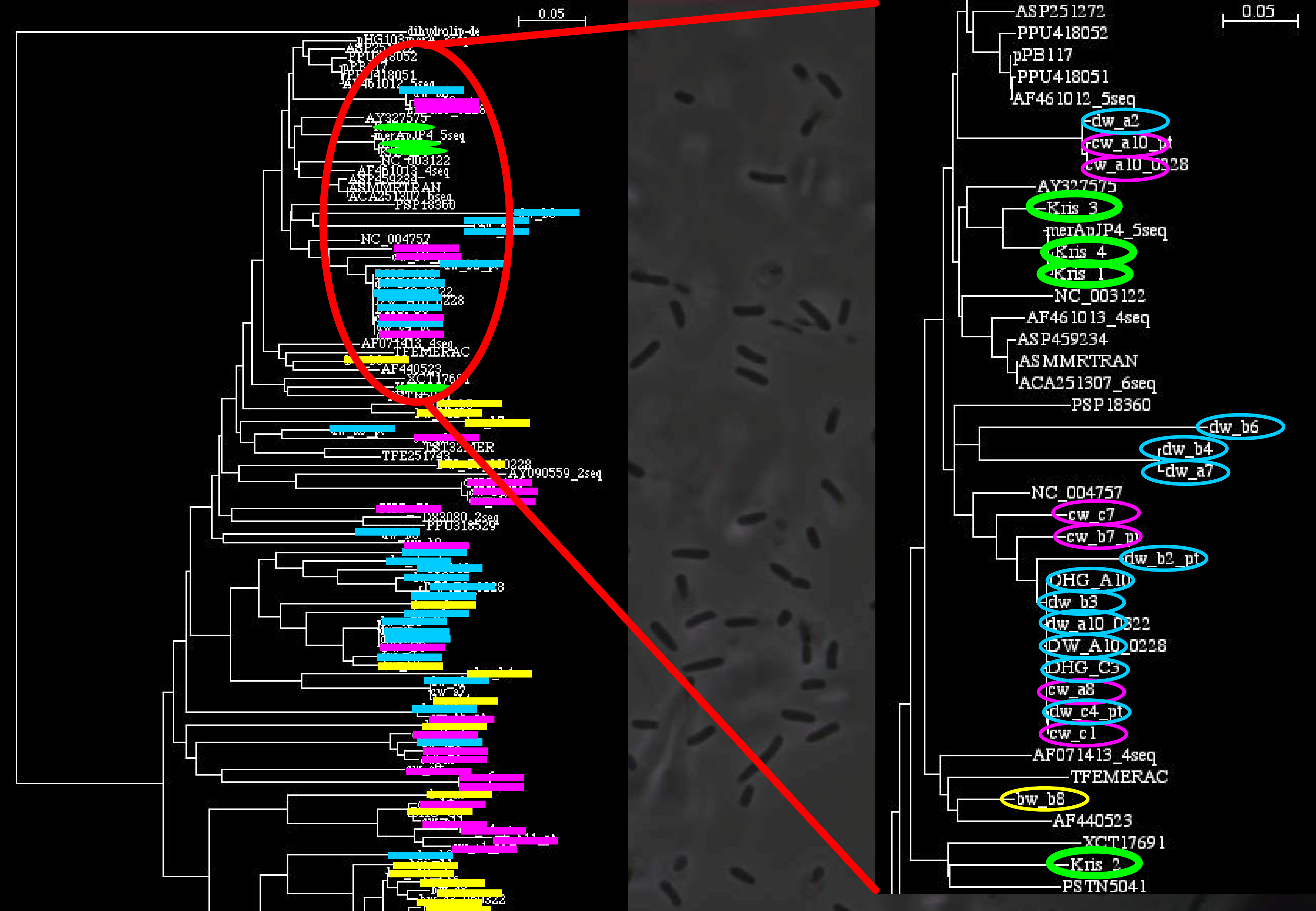


Figure show representative gel of one replicate

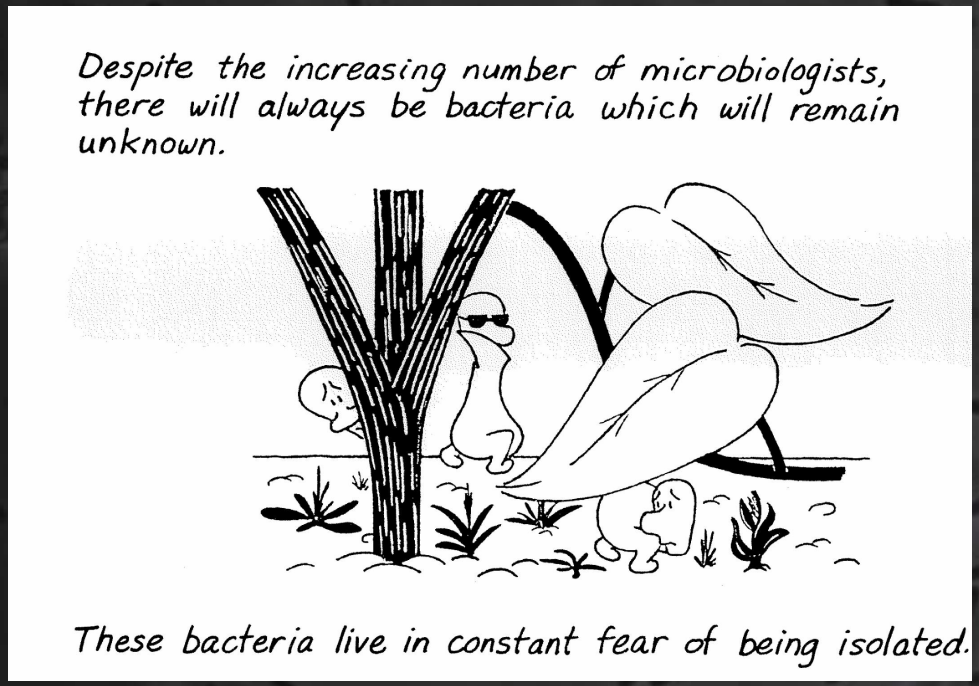
Lane 1: positive control (plasmid pHG103). Lane 15: negative control (H_2O).
Lanes 2, 9 & 16: 100 bp ladder.
Lanes 4+10: soil B; lanes 5+11: soil C; lanes 6+12: soil D; lanes 7+13: soil E; lanes 8+14: soil F.
Lanes 4-8 show data from the Hg amended soils, while lanes 10-14 show data from the control soils.

- Initially, *merA* genes were only found in control soils from the contaminated site.
- Following soil Hg amendment, *merA* genes were found in all soils - indicating the adaptive potential of the subsurface soils.

de Lipthay, JR et al 2006 in prep

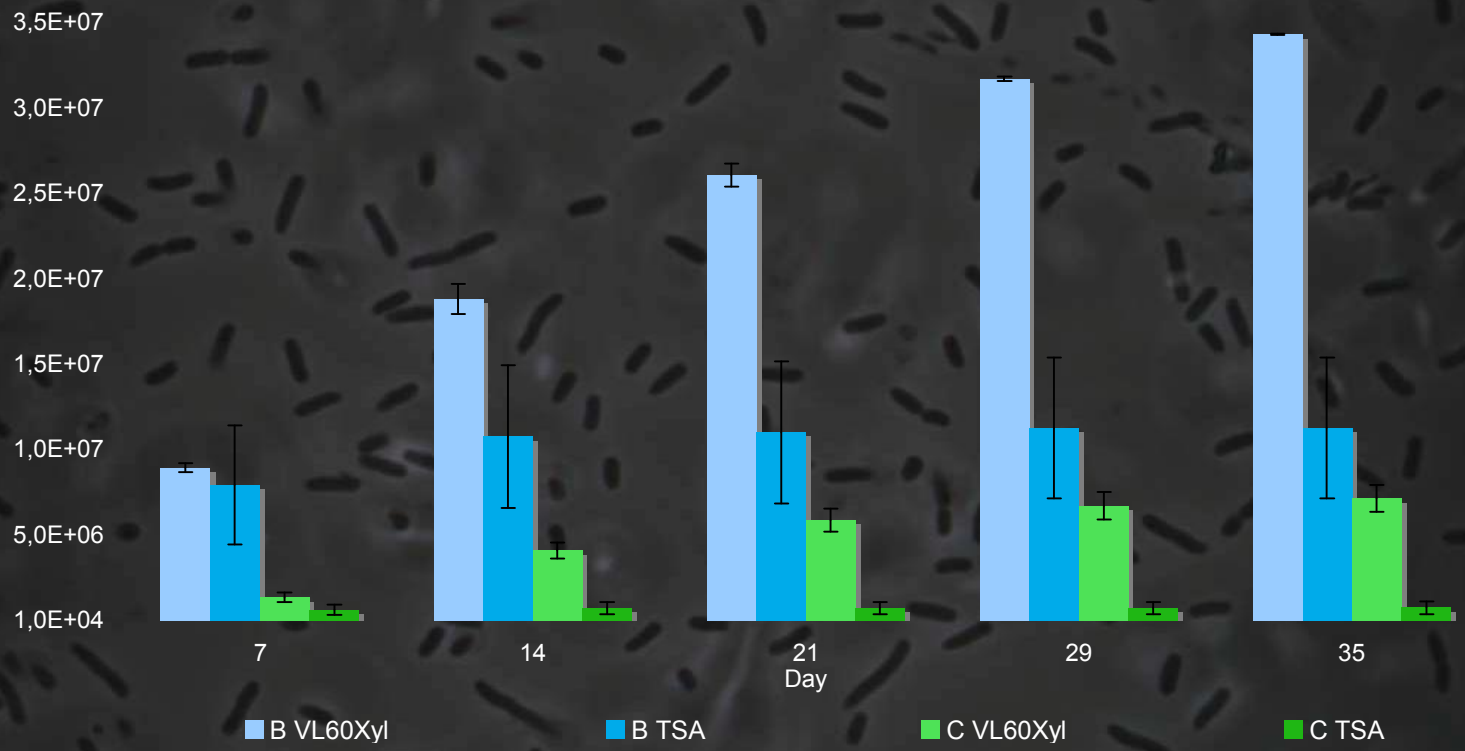

0.05
ASP251272
PPU418052
pPB117
PPU418051
AF461012_5seq
dw_a2
cw_a10_pt
cw_a10_0228
AY327575
Kris_3
merApJP4_5seq
Kris_4
Kris_1
NC_003122
AF461013_4seq
ASP459234
ASMMRTRAN
ACA251307_6seq
PSP18360
dw_b6
dw_b4
dw_a7
NC_004757
cw_c7
cw_b7_pt
dw_b2_pt
DHG_A10
dw_b3
dw_a10_0322
DW_A10_0228
DHG_C3
cw_a8
dw_c4_pt
cw_c1
AF071413_4seq
TFEMERAC
bw_b8
AF440523
XCT17691
Kris_2
PSTN5041
NC_006512

Cultivation of Hg^{++} resistant subsurface bacteria



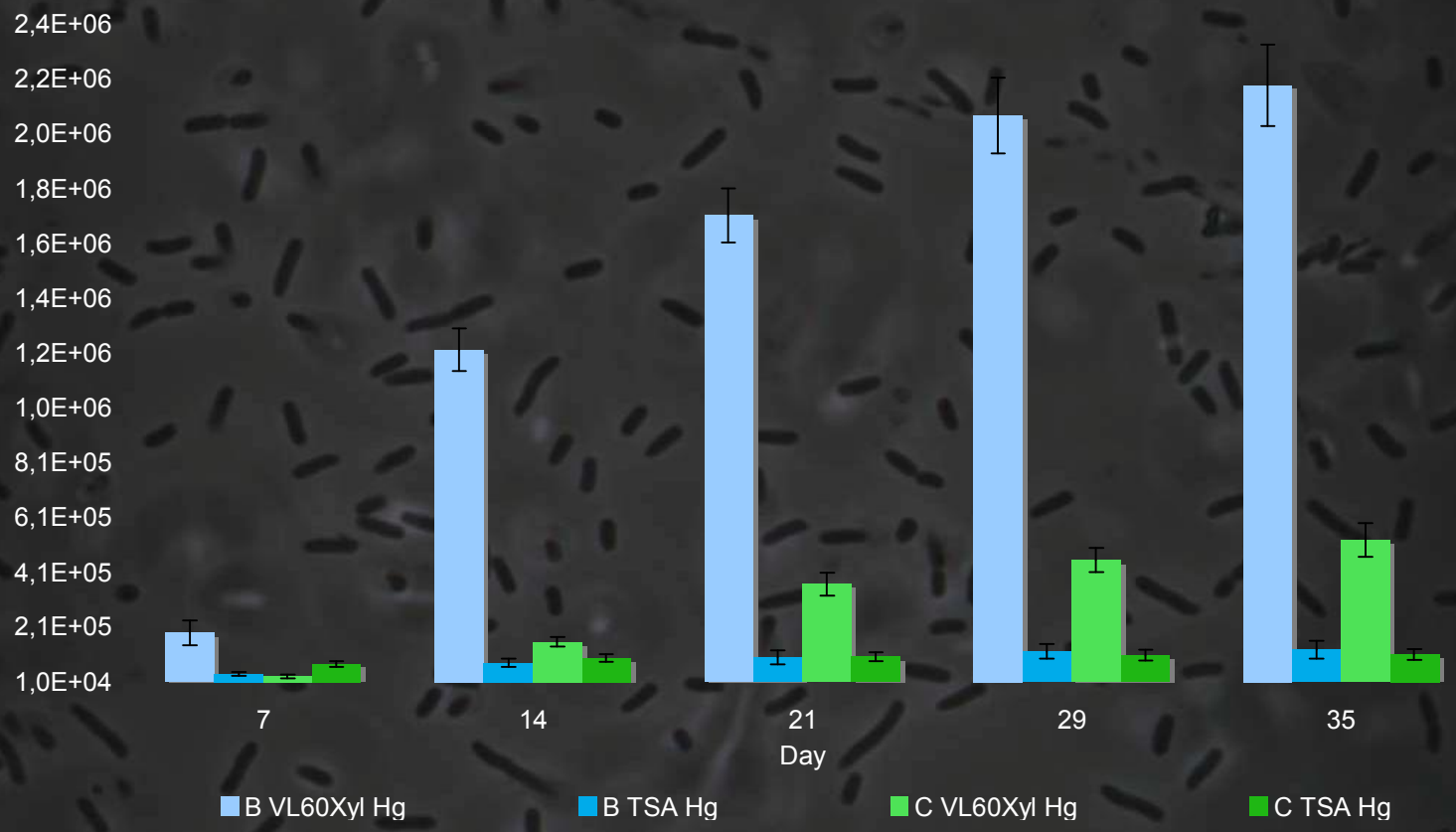
- Microcultivation approach that simulates the natural growth conditions
  - See our poster tonight!
- Use dilute media as proposed by Janssen et al. (AEM 2002) and long-term incubation (6-12 weeks)

Cultivation a' la Janssen



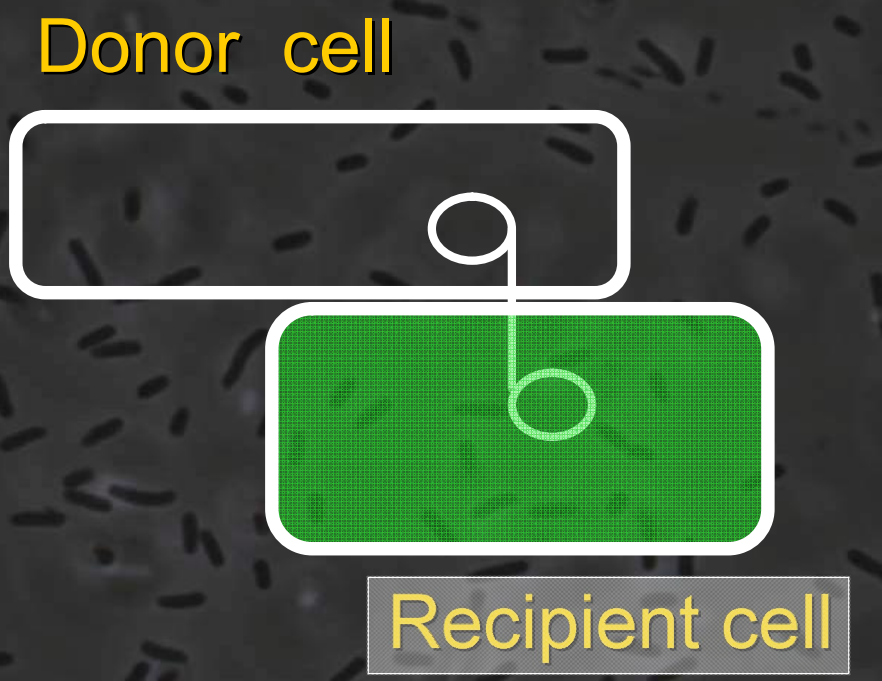
Oregaard, G et al 2006 in prep

Cultivation of Hg tolerant bacteria

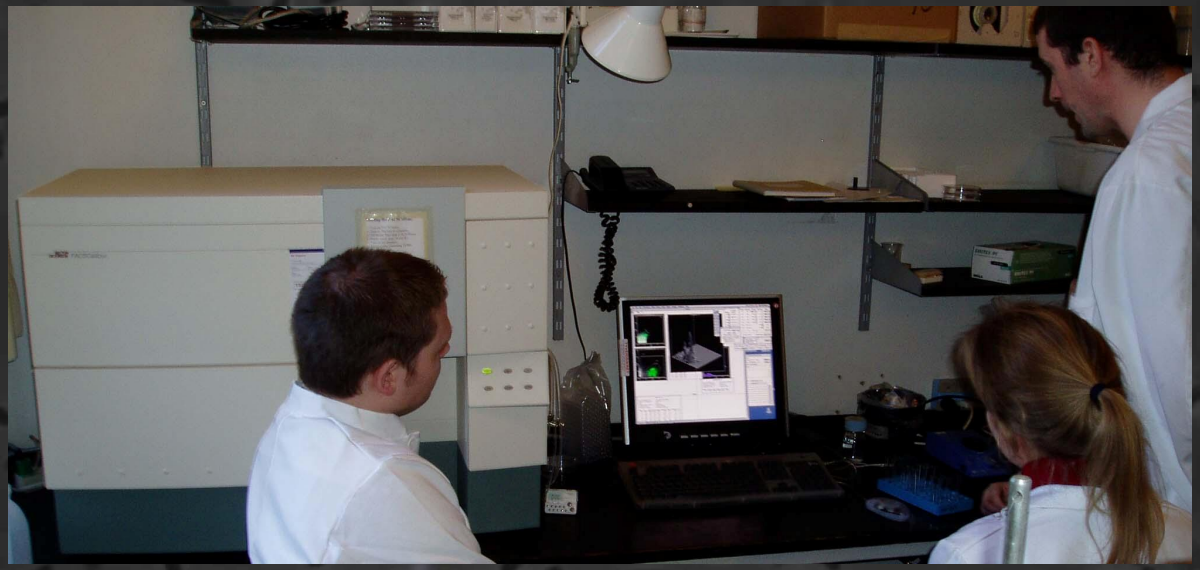


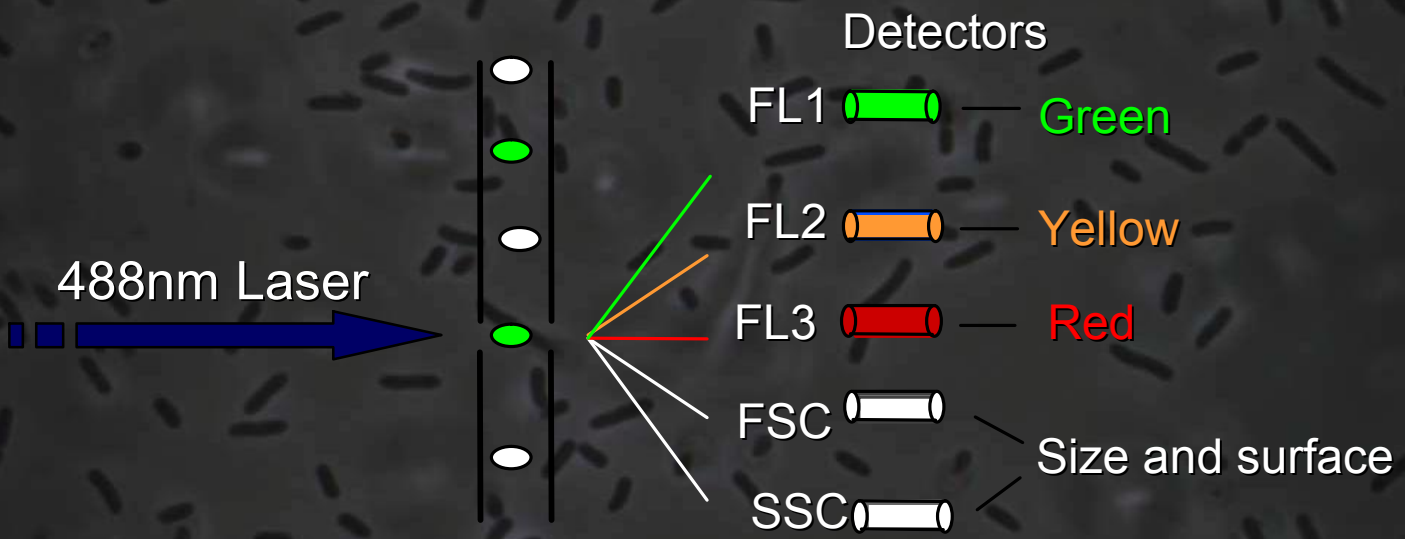
Oregaard, G et al 2006 in prep

Direct detection of HGT



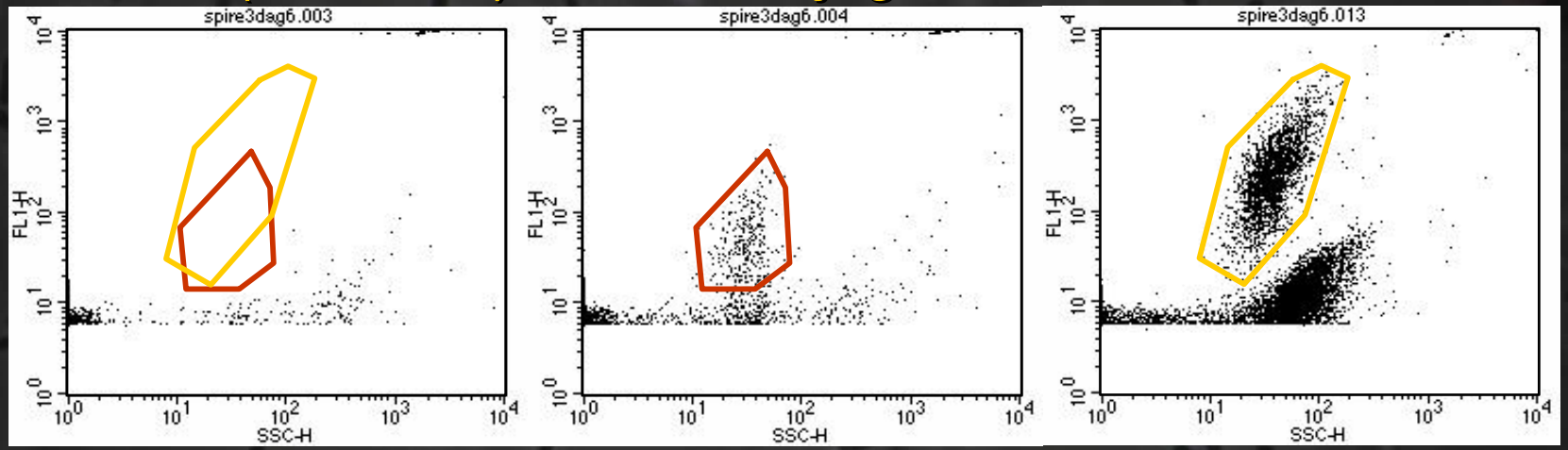
Flow cytometry





Direct detection of HGT

- Control (no donor) Transconjugants Donors



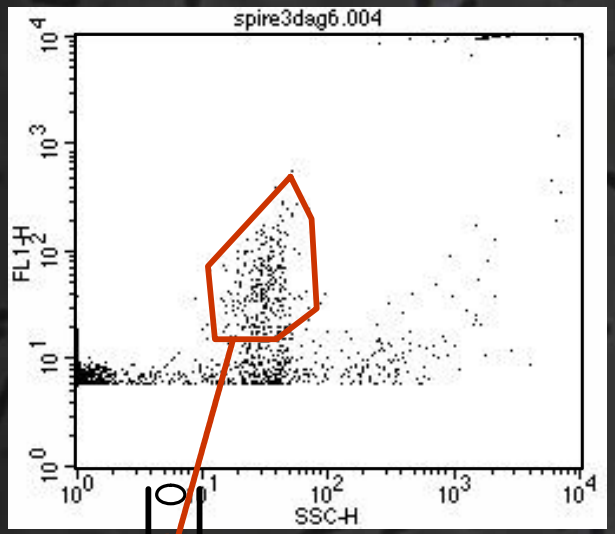
T/D

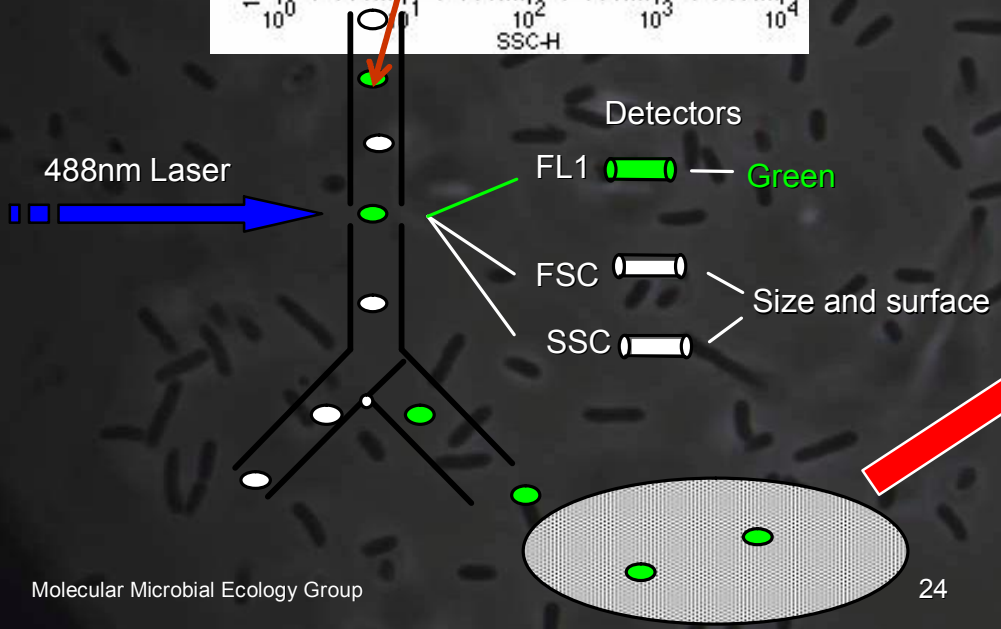
- Rhizosphere 12-38% 100-1000

Cultivation based detection underestimate the frequency of HGT

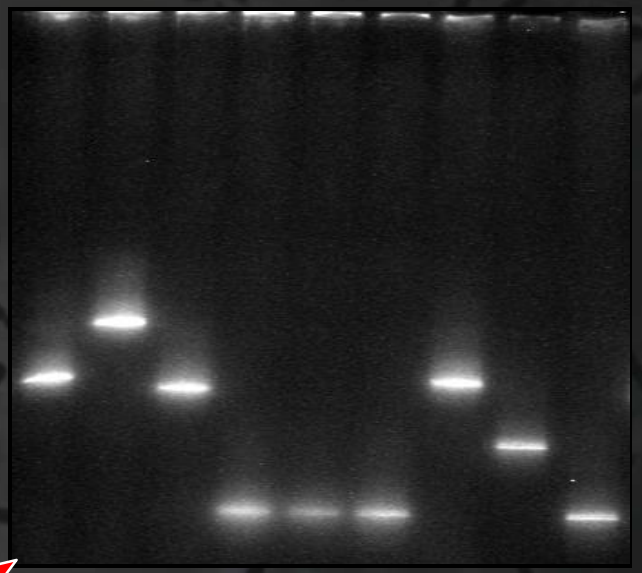
Sørensen, SJ., et al 2005 Nature Reviews Microbiology 3 (9): p700-710

Sorting transconjugant bacteria for molecular analysis





DGGE analysis of 16S rDNA clones from sorted transconjugant cells



Sørensen, SJ., et al 2005 Nature Reviews Microbiology 3 (9): p700-710

Conclusions

- We have isolated several Hg^{++} resistance plasmid from subsurface bacteria.
- We can culture hitherto unculturable Hg^{++} tolerant subsurface bacteria.
- We have a new method for detecting plasmid transfer between subsurface bacteria without cultivation.
- We have developed DNA-microarray for characterization of plasmids.

In the last year we will characterize and tag the isolated plasmids. Then we will study the transfer at *in situ* conditions and evaluate the possibility to use HGT as a mean to stimulate the heavy metal transformation.