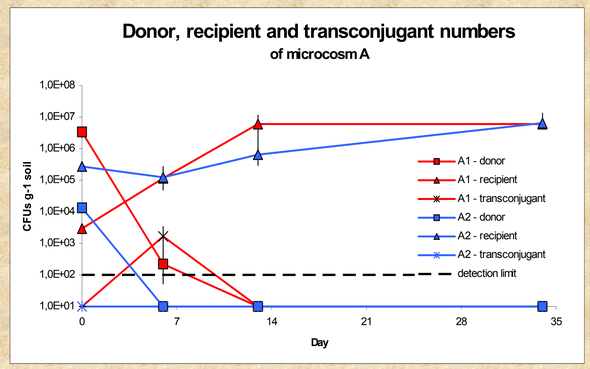
Introduction of mercury resistant bacterial strains to Hg(II) amended soil microcosms increases the resilience of the natural microbial community to mercury stress

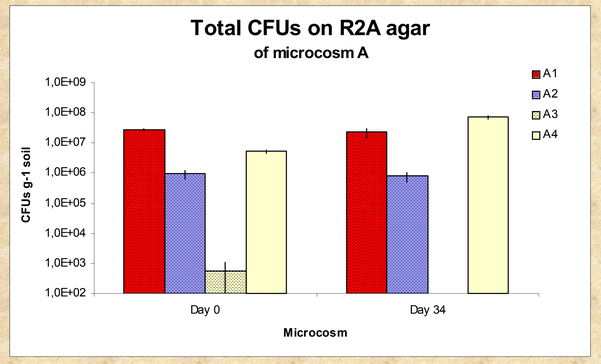
Julia R. de Lipthay, Lasse D. Rasmussen & Søren J. Sørensen
sjs@mermaid.molbio.ku.dk
Department of Microbiology, University of Copenhagen, Sølvgade 83 H, DK-1307 Copenhagen K , Denmark

Introduction

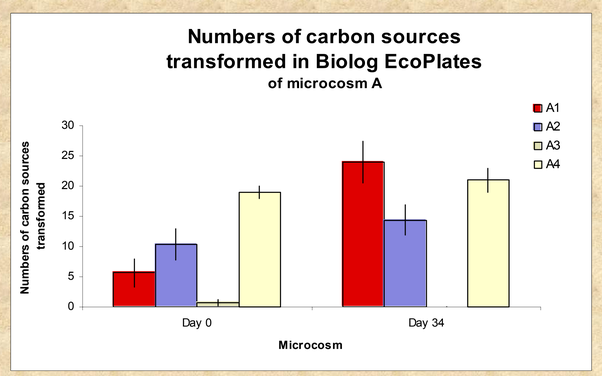
Heavy metals are among the most important groups of pollutant compounds, and they are highly persistent in the soil environment. Techniques that can be used for the remediation of heavy metal contaminated environments thus need to be evolved. In the present study we evaluated the effect of introducing a Hg resistance plasmid in subsurface soil communities. This was done in microcosms with DOE subsurface soils amended with 5-10 ppm of $HgCl_2$. Two microcosms were set up. In microcosm A we studied the effect of adding strain SØ3539 containing either the Hg resistance conjugative plasmid, pJORD 70, or the Hg resistance mobilizable plasmid, pPB117. In microcosm B we studied the effect of adding strain KT2442 with and without pJORD70. For both microcosms, the effect on the resilience of the indigenous bacterial community as well as the effect on the soil concentration of Hg was evaluated.



The conjugative Hg resistance plasmid, pJORD70, was transferred to the indigenous bacterial population. Transconjugants were detected at day 6, but at following sampling days they had disappeared. Transfer of the mobilizable plasmid, pPB117, to the indigenous subsurface microbial population was not detected.



Addition of Hg resistant *E. coli* strains to the Hg-amended subsurface soils significantly increased the survival of the culturable indigenous bacterial community. The effect was most pronounced with the addition of strain SØ3539/pJORD70 containing a conjugative Hg resistance plasmid.



Addition of Hg resistant *E. coli* strains to the Hg-amended subsurface soils significantly increased the number of carbon sources transformed in Biolog EcoPlates by the indigenous bacterial community. The effect was also here most pronounced with the addition of the strain containing a conjugative Hg resistance plasmid.

Acknowledgement

This study was supported by the Natural and Accelerated Bioremediation Research (NABIR) program, Biological and EnvironmentalResearch (BER), U.S. Department of Energy, with the project DE-FG02-99ER62864.

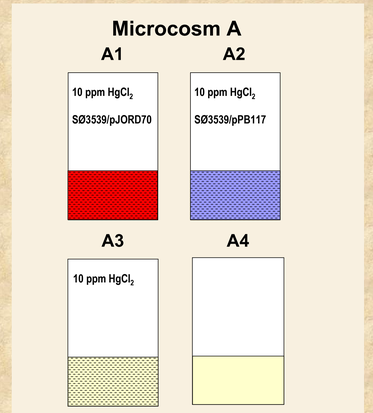
Donor - Recipient - Transconjugant

$LB_{50ppm\,Hg\,Kan}$ MM_{glu} $MM_{glu\,50ppm\,Hg}$

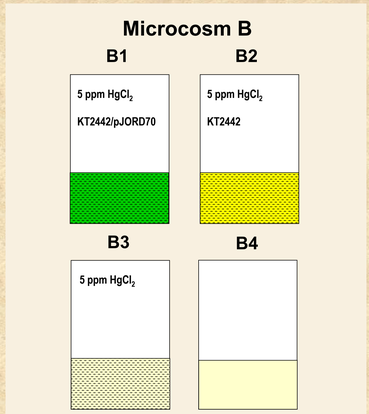
Total CFU

R2A

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



Microcosms A were set up with 100 g of DOE subsurface soil DABNV-01-02 (location D-Phyto) and amended with: A1, 10 ppm $HgCl_2$ and 10^8 cells g^{-1} soil of PBS-washed *E. coli* SØ3539/pJORD70; A2, 10 ppm $HgCl_2$ and 10^8 cells g^{-1} soil of PBS-washed *E. coli* SØ3539/pPB117; A3, 10 ppm $HgCl_2$ and PBS (same volume as in A1 and A2); A4, PBS. Microcosms were incubated at 24°C, and samples were taken at days 0, 6, 13, and 34.



Microcosms B were set up with 100 g of DOE subsurface soil DABNV-01-02 (location D-Phyto) and amended with: B1, 5 ppm $HgCl_2$ and 10^8 cells g^{-1} soil of PBS-washed *P. putida* KT2442/pJORD70; B2, 5 ppm $HgCl_2$ and 10^8 cells g^{-1} soil of PBS-washed *P. putida* KT2442; B3, 5 ppm $HgCl_2$ and PBS (same volume as in A1 and A2); B4, PBS. Microcosms were incubated at 24°C, and samples were taken at days 0, 2, 7, and 14.

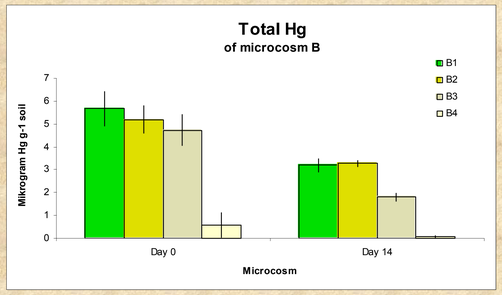
Total Hg Quantified using a Jerome 431-x mercury vapor analyzer

Whole cell biosensors for detection of bioavailable Hg

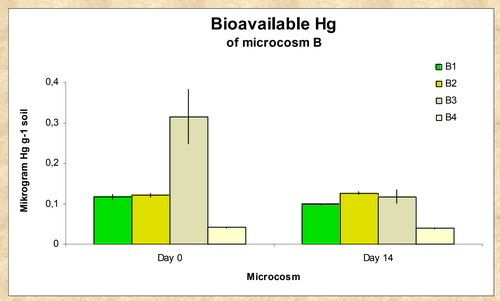
Total CFU

R2A

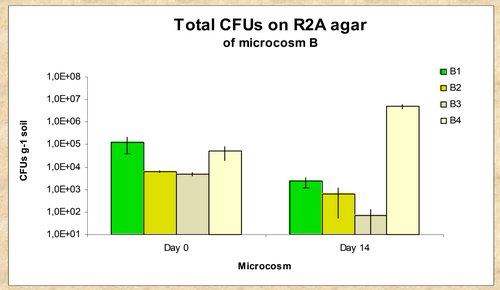
Genetic Diversity Using DGGE with Eubacterial primers



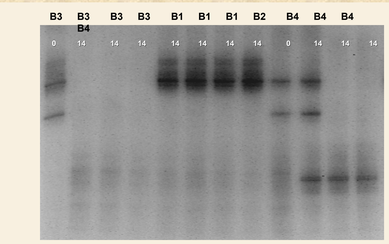
The concentration of Hg was significantly reduced during the 14-day incubation in microcosms B. The higher Hg concentration observed in B1 and B2 indicate that Hg was adsorbed to the biomass of the *P. putida* strains.



The bioavailable fraction of Hg was significantly reduced by the addition of the *P. putida* strains. This indicates that Hg was adsorbed to the bacterial biomass.



Amendment of 5 ppm Hg to the DOE subsurface soil significantly reduced the biomass of the culturable indigenous bacterial community. However, addition of *P. putida* KT2442/pJORD70 had a significant effect on the survival of the indigenous bacterial community.



Addition of *P. putida* strains to Hg amended subsurface soil had a significant effect on the diversity of the indigenous bacterial community. Thus, additional five bacterial phylotypes were observed in microcosms B1 and B2 compared to B3.

Conclusions

- Amendment of 5-10 ppm $HgCl_2$ to DOE subsurface soils significantly reduced the biomass of the culturable indigenous bacterial community.
- The addition of a high bacterial biomass to the Hg-amended subsurface soils had a significant effect on the survival of the culturable indigenous bacterial community - and thus on the resilience to Hg stress.
- In situ transfer of a conjugative Hg resistance plasmid to indigenous subsurface soil bacteria was demonstrated.
- Data indicate that strains harboring a Hg resistance, conjugative plasmid exert the highest effect on the resilience of the indigenous bacterial community to Hg stress.
- Addition of bacterial strains (with or without Hg resistance plasmids) did not reduce the total Hg concentration in subsurface soil. However, there was a transient effect on the bioavailable fraction of Hg in the soil, indicate that Hg was adsorbed to the bacterial biomass.