Supplemental Material

Heavy metal tolerance of Fe(III)-reducing microbial communities in a contaminated creek bank soil

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Geochemistry of the study site.

In the pore water samples, Fe(II) was measured after HCl extraction with the phenanthroline method (13) as described previously (4, 10). Dissolved metals were measured in pore water samples acidified with nitric acid (65%, Roth, Karlsruhe, Germany) with ICP-MS (inductively coupled plasma – mass spectrometry; X-Series II, Quadrupol ICP-MS, Fa. Thermo Electron, Bremen). Each sample was measured 4 times. Fe(II) and metal concentrations are shown in Fig. S1.

DNA extraction and clone library construction from metal amended cultures. Culture samples were centrifuged (13,780 x g, 15 min) and DNA was extracted from the pellets using the PowerSoil DNA Isolation Kit (MOBIO Laboratories Inc., Carlsbad, CA, USA) with the alternative lysis method as given by the manufacturer. For PCR amplification the general domain *Bacteria* SSU rRNA gene primers fD1 (5'-AGA GTT TGA TCC TGG CTC AG-3') and rP2 (5'-ACG GCT ACC TTG TTA CGA CTT-3', 14) were used with the FailSafe PCR PreMix D (EPICENTRE Biotechnologies, Madison, WI, USA) and high yield Taq polymerase (Jena Bioscience, Jena, Germany). PCR products were gel-purified (Agarose Gel Extraction Kit, Jena Bioscience, Jena, Germany). PCR products of all three DNA extracts were combined during gel-purification. The cleaned PCR products were used to construct a clone library using the pGEM-T Vector System and JM109 High Efficiency Competent Cells (Promega, Madison, USA) following the manufacturer's instructions. The cloned inserts were amplified using the vector specific primers M13F and M13R. Restriction fragment length polymorphism (RFLP) analysis was used to group the clones into phylotypes based on pattern similarity after cutting with the restriction enzymes *Bsh*F1 and *Msp*I (Fermentas, St. Leon-Rot, Germany). Representative clones from each phylotype were sequenced bidirectionally (AGOWA GmbH, Berlin, Germany).

Sequences were assembled using BioEdit version 7.0.5.3 (7). Prior to comparative phylogenetic analysis, vector and primer sequences flanking the SSU rRNA gene inserts were

removed. Previously identified sequences with high sequence similarity to the clones obtained in this study were determined using the BLAST algorithm against the GenBank database available from National Center for Biotechnology Information (NCBI) (1). Clone sequences were checked for chimeras using the program Chimera Check from the Ribosomal Database Project II (5), the Bellerophon server (8), and Pintail (2). Clone sequences were grouped further into phylotypes based on a sequence similarity cut off of 97% using FastGroupII (15; Table S1). Statistical analyses were used to determine the sampling efficiencies and coverage of the clone library (6, 11). Sequences generated in this study were deposited in the GenBank database under the accession numbers HM992482 to HM992491.

Denaturing gradient gel electrophoresis (DGGE). DNA from the same extracts as used for clone library construction was amplified using the general domain *Bacteria* SSU rRNA gene primers mentioned above with the FailSafe PCR PreMix C (EPICENTRE Biotechnologies, Madison, WI, USA). Amplification was performed with the following temperature program: 5 min at 95°C, 30 cycles of 1 min at 95°C, 1 min at 56°C, 1 min at 72°C, and 10 min at 72°C. PCR products from this reaction were used for nested PCR in a similar temperature program as before with only 20 cycles and 54°C annealing temperature. For nested PCR the primers R1401 (5'-GCG TGT GTA CAA GAC CC-3') and F968GC (5'-GC clamp-AAC GCG AAG AAC CTT AC-3'; 9) and FailSafe PCR PreMix G (EPICENTRE Biotechnologies, Madison, WI, USA) were used. The nested PCR products were gel quantified using the software Gene Tools 4.0 (SynGene, Cambridge, UK) and 500 ng nested PCR product were loaded on the DGGE gel. Gradient gels contained 8% acrylamide and the gradient ranged from 50% to 65%. DGGE was run at 60°C and 100 V for 15 hours in 0.5 x TAE buffer (20 mM Tris, 0.5 mM EDTA, pH 8). Gels were stained with SybrGold (Invitrogen, Carlsbad, CA USA) for documentation.

Selected bands were cut from the gradient gels, incubated in 50µl TE-buffer for 4 h at room temperature, and stored at -20°C. Samples were reamplified with nested PCR as

described above, using the DNA-containing TE-buffer as template, with 30 and 25 amplification cycles. PCR products were purified (PCR Purification Kit, Analytic Jena, Jena, Germany), sequenced (AGOWA GmbH, Berlin, Germany) and sequence similarities were determined as described above.

Agar shake technique. Agar shake media contained (per liter) 1.0 g NaCl, 0.4 g of $MgCl_2*6H_2O$, 0.1 g of $CaCl_2*H_2O$, 0.5 g KCl, 0.3 g of NH_4Cl and 0.6 g of KH_2PO_4, 20 mM $Fe(OH)_3$, 50 mM HEPES, 100 µM AQDS, 0.5 mM $FeCl_2$, 0.5% agar (AppliChem), 1 ml vitamin solution M141 (3), 1 ml trace element solution SL9 (3), and 1 mM each lactate and ethanol. Agar shake cultures were incubated at 22ºC in the dark for 3 weeks. Distinct black colonies were observed only for the cultures tolerant to 0.1 mM Cd or Cu. Colonies were picked using sterile, anaerobic technique and transferred to fresh agar shakes. After 5 subsequent transfers in agar shake media, colonies were transferred to the liquid media described above without agar and with 0.1% yeast extract and 30 mM bicarbonate as a buffer. DNA was extracted from cell pellets using the PowerSoil DNA Isolation kit (Mo Bio Laboratories, Solana Beach, California) according to manufacturer's instructions and then PCR amplified with 16S rRNA gene primers fD1 and rP2 (see above). Strains were sequenced at Macrogen Inc. (Macrogen Europe, Amsterdam, Netherlands) and assembled using Geneious Pro version 4.6.4 (Biomatters, http://www.geneious.com). Fe(II) formation was determined as described above and carbon utilization was determined with high-performance liquid chromatography (4).

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Figure S1: Fe(II) and average metal concentrations of the pore water in the soil profile at the bank of the contaminated creek Gessenbach as measured monthly in 2007. The corresponding soil horizons are given at the right side of the graphs. This figure includes Fe(II) concentrations from August which have been published previously (4).

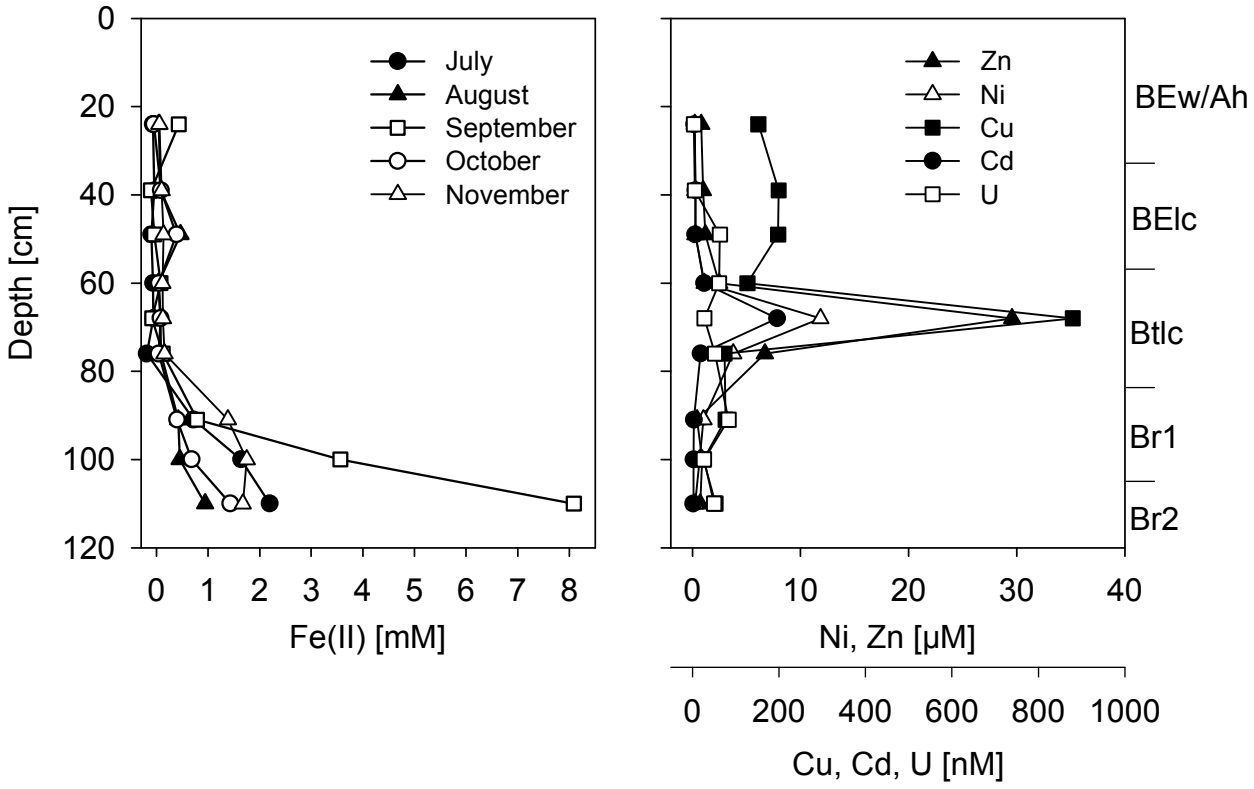

Table S1: Summary of phylogenetic affiliation and distribution of SSU rRNA gene clones from clone libraries of metal amended enrichment cultures.

Phylogenetic group	Clone designation	Nearest relative (accession number)	% Identity	Number of clones per phylotype
Betaproteobacteria	SB60	*Dechloromonas* sp. MissR (AF170357)	99	1
Deltaproteobacteria	SB3	Clone IRD18B06 (AY947909)	97	8
	SB8	*Geobacter psychrophilus* (AY653549)	98	21
	SB54	*Geobacter* sp. Ply1 (EF527233)	98	2
Firmicutes	SB4	*Anaerobranca* sp. Clone SRB2 (DQ069229)	96	3
	SB5	*Sedimentibacter* sp. Clone JN18_A14_H (DQ168650)	99	2
	SB18	*Sedimentibacter* sp. Clone BRS2 (AY221992)	98	4
	SB25	Clone BSV34 (AJ229194)	97	2
	SB52	*Clostridiaceae* clone dgC-140 (AB218342)	92	1
	SB15	Clone ZZ14C3 (AY214191)	98	1