Supplementary Material - *Molecular Biology Methods*

DNA isolation protocols

The DNA from the aquatic community was isolated with the Aqua Pure genomic DNA Kit (BioRad) according to the manufacturer's protocol. The DNA of the soil sample was isolated as described previously [19]. Cosmid libraries were constructed in pWE15 (Stratagene, La Jolla, CA.) as previously published [19]. DNA fragments obtained after partial *Bsp134*I digestion were ligated into *Bam*HI restriction sites of the cosmid vector. Phage packaging mixtures obtained from Stratagene, and infection of *E. coli* VCS257 was performed using the manufacturer's protocol.

Identification of cellulase-positive clones, subcloning and DNA sequence analysis

Cellulase-positive clones were screened for by using a colorimetric assay on Congo red indicator plates [26] as previously described [1]. Cellulase positive cosmid clones were digested with the restriction enzymes *Eco*RI and *Sma*I respectively and subcloned in pTZ19R (Cm^R, Göttingen Genome Laboratory, G2L). These subclones were screened again for cellulase activity. Positive clones were sequenced.

Preparing of crude cell extracts of cellulase encoding cosmid clones.

For the preparation of the crude cell extracts 200 ml LB cultures with 0.2% (w/v) carboxymethylcellulose (CMC) containing ampicillin (100 µg/ml) were grown at 30°C to OD 1.0 -1.5. Cells were harvested and resuspended in 50 mM Tris-HCl pH8.0 prior to cell disruption through sonication (Sonicator UP 200S, Hielscher, Germany) at 50% amplitude and cycle 0.5 for 5 min. After centrifuging at 13000 rpm and 4°C for 30 min the crude cell extract was stored at -20°C till further use.

Overexpression of the celluase genes $celA_{10}$ $celA_{20}$ and $celA_{24}$

The cellulase encoding genes, $celA_{10}$ $celA_{20}$ and $celA_{24}$, were amplified from cosmid DNA using PCR in 35 cycles with primer pairs celA10-forward 5´-CTACATATGTTTGTTTT ACCCGTTCCG-3' and celA10-reverse 5`-GGATCCGGGGCTACAGAACTT-3', celA20-forward 5`-CGCGAGATATTTCATATGAAAAAATCCGTCCAG-3` and celA20-reverse 5`-GGATCCTTACAAACACTTCCGCTAAGG-3` and for celA24- forward 5`-GCTCATATGAAATTCCCGCATCACTGC-3' and celA24 reverse 5`-GGATCCTTTCTTATTTTCGCGCAGCAT-3' Primers were designed to introduce a 3'-*Bam*HI restriction site and a 5'-*Nde*I site into the cloned fragments. To increase cloning efficiency the PCR fragments were first ligated into pDrive (PCR Cloning Kit, Qiagen, Hilden, Germany), then excised with *Nde*I and *Bam*HI and ligated into pET19b (Novagen/Merck KGaA, Darmstadt, Germany). The recombinant clones were sequenced and the clones pJPpETCelA10, pJPpETCelA20 and pJPpETCelA24 were chosen for overproduction. *E. coli* BL21/pRIL, carrying the $celA_{10}$ gene, $celA_{20}$ gene and $celA_{24}$ gene respectively in pET19b, was grown to OD 0.6 in LB broth containing ampicillin (100 µg ml^{-1}) at 37°C and 150 rpm, and expression of the *celA* genes was induced by adding 0.05 M isopropyl thio-β-D-galactoside (IPTG) followed by an incubation at 30°C for 4 h. Cells were harvested and resuspended in 50 mM Tris-HCl pH8.0 prior to disruption through a French pressure cell at 20,000 psi cell pressure. The molecular mass of the CelA Proteins was determined by SDS-gel electrophoresis.

Directed evolution of $CelA_{10}$ employing Sequence saturation mutagenesis (SeSaM)

A SeSaM-Tv^{+} library for all four nucleotides in the coding strand of $celA_{10}$ was generated as described previously [27], using pET-19b vector specific primers SeSaM-fwd-F1 5'-CACACTACCGCACTCCGTCGCGACTCACTATAGGGGAATTGTGAGCGGA-3' and

SeSaM-rev-R4 5'-GTGTGATGGCGTGAGGCAGCGCAAAAAACCCCTCAAGACCCGTTTAGA-3' for SeSaM template generation. The purified PCR product of the *celA*$_{10}$ SeSaM-Tv^{+} random mutagenesis library was ligated in *Eco*RV-digested pBluescriptSK+ (Stratagene, La Jolla, CA) and transformed into *E.coli* DH5α. Clones were screened in deep well plates with 30% IL6 (v/v) using dinitro-salicylic acid reagent [24]. Clones with better activities than the wild type were tested with incubation over 17 h in buffer and up to 30% IL6 (v/v) and 20% IL7 (v/v) and IL8 (v/v) and sequenced.

Supplementary Material – Additional FIGURE legends

FIG. 1. Effects of various additives on cellulase activities; of **A)** solvents, detergents **B)** bivalent metals and EDTA on the activities of the Cellulases $CelA_{10}$, $CelA_{20}$ and $CelA_{24}$. Activity was measured using the standard assay and control activity was taken as 100%.

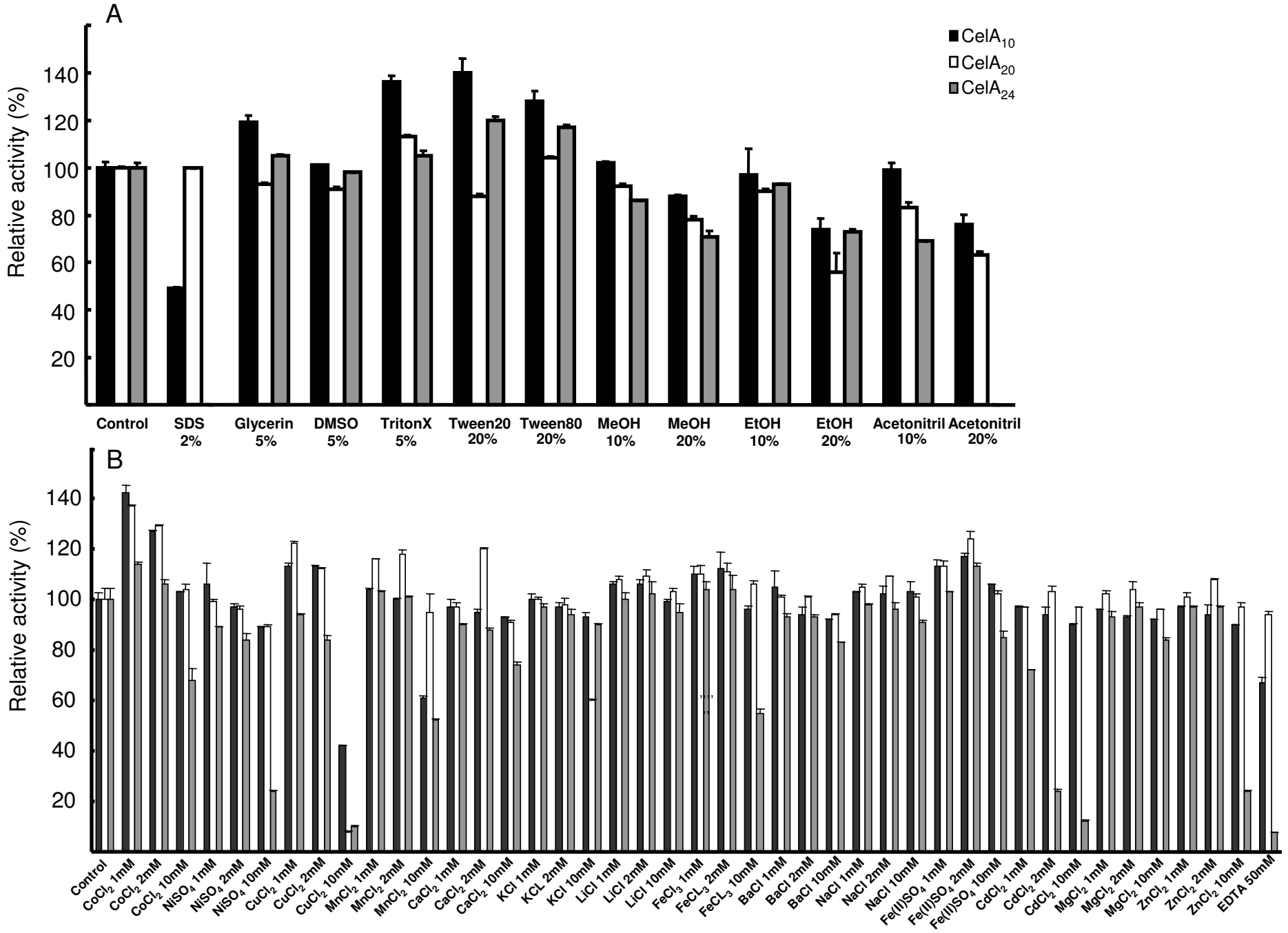

Figure 1; supplementary Material, Pottkämper et al. 2009