Construction of *gfp*-tagged *Shewanella* strains

Since a previously used Tn*7* delivery system for *gfp* tagging (Lambertsen *et al.*, 2004) is based on vectors that are stably maintained in *Shewanella oneidensis* MR-1, we constructed a modified delivery system. To that end, pBK-miniTn7-*gfp3* (Lambertsen *et al.*, 2004) was digested with NotI, releasing a fragment harboring constitutively expressed *egfp* and a chloramphenicol resistance cassette. This fragment was subsequently ligated into the NotI site of the Tn*7* delivery vector pUC18-R6KT-miniTn7T (Choi *et al.*, 2005) that, in contrast to the previously used delivery vectors, does not replicate in *S. oneidensis* MR-1. The resulting plasmid pUC18-R6KT-miniTn7T-egfp was used for tagging *S. oneidensis* strains by three-parental mating from the DAP-auxotroph *E. coli* WM3064 together with *E. coli* WM3064 harboring the helper plasmid pTNS2. Selection for tagged *S. oneidensis* strains was carried out using LB plates containing chloramphenicol but lacking DAP. Fluorescence of the strains was determined by fluorescence microscopy using an upright Zeiss Image MI (Oberkochen, Germany) equipped with a Cascade 1K camera (Visitron Systems, Puchheim, Germany) and a Zeiss Plan Apochromat 100×/1.4 differential interference contrast objective. Comparative growth and biofilm experiments ensured that no detrimental effects based on fluorescent protein expression occurred. The Gfp-tagged strain also served as host strain for introducing targeted gene disruptions.

Choi KH, Gaynor JB, White KG, Lopez C, Bosio CM, Karkhoff-Schweizer RR, and Schweizer HP. (2005). A Tn*7*-based broad-range bacterial cloning and expression system. *Nature Methods* **2**: 443-448.

Lambertsen L, Sternberg C, and Molin S. (2004). Mini-Tn7 transposons for site-specific tagging of bacteria with fluorescent proteins. *Environ. Microbiol.* **6**: 726-732.

Heterologous production of SO_2685 (predicted major head subunit of MuSo2) and SO_2963 (predicted major capsid protein of LambdaSo).

Genes to be overexpressed were amplified from template genomic DNA using primers listed in Supplemental Table 2. The resulting PCR products were ligated in-frame into pASK-IBA3plus (IBA GmbH, Göttingen, Germany).

Overproduction of SO_2685 and SO_2963 was conducted in *E. coli* DH5α λpir harbouring the corresponding expression vector. Gene expression was induced by adding anhydrotetracycline (Sigma-Aldrich, Taufkirchen, Germany) at a final concentration of 200 ng• ml^{-1} to exponentially growing cultures followed by incubation for 4 h at 37°C.

For purification of the recombinant proteins, cell sediments were resuspended in lysis buffer (10 mM HEPES, 150 mM NaCl, 0.5 mg• ml^{-1} lysozyme, pH 7) and lysed by a French Press (SLM-AMINCO/Spectronic) three times at 18,000 p.s.i. These steps were repeated twice. Inclusion bodies were finally harvested by centrifugation at 1,000 x g for 30 min and stored at -20°C. The total protein concentration was determined via Bio-Rad Protein assay (Bio-Rad Labratories GmbH, Munich, Germany) following the manufacturer's instructions.

SO_2685 and SO_2963 inclusion bodies were resuspended in sample buffer {Laemmli, 1970 #5437}, heated at 99°C for 5 min and resolved by SDS-PAGE on a 11 % polyacrylamide gel. The relevant band was excised from gels stained with 0.1% Coomassie dye in water.

Table S1: Sequence of primers used in this study

Primer name	Sequence

in-frame deletions

ΔMuSo1
dMu-1-up-EcoRI-fw	CGAATTCTCGGTGCTAAGGTGATTGC
dMu-1-up-overlap-rev	AGGTTATAGCTTAGTTAGCTCACAATGTTAATACGA
dMu-1-dwn-overlap-fw	CTAACTAAGCTATAACCTTGCTCCAGCACC
dMu-1-dwn-PstI-rev	CGACTGCAGCAATGACGGGCTGATATTTG
dMu1-check-fw	GCGAAACCGTTTTGATACAC
dMu1-check-rev	CGAAGACTCTATCTACAGGCCAA

ΔMuSo2
dMu-2-up-PstI-fw	ACCCTGCAGTGTTCGGAC
dMu-2-up-overlap-rev	AAGCTTGCCCGATCCCGTTTACATCCTACA
dMu-2-dwn-overlap-fw	ACGGGATCGGGCAAGCTTATTTCTTCGCTT
dMu-2-dwn-BamHI-rev	CGGATCCTCGAATGTGACCGTTGTGG
dMu2-check-fw	GCCTGATAACGACAAACAAATTT
dMu2-check-rev	CCCAGTTTGTGAAGGTTATGAG

ΔlambdaSo
dlambda-up-EcoRI-fw	CGAATTCAATTGCTTTAGCCGAGGCA
dlambda-up-overlap-rev	AATGAGTGAGGTAAGCTTTCGTTCGTTTGG
dlambda-dwn-overlap-fw	AAGCTTACCTCACTCATTAGTATCTTGCTCTTCTGA
dlambda-dwn-PstI-rev	CGACTGCAGGCGGATTTCAGTGTTTCCAG
dlambda-check-fw	TGAAAGCGCACATTTCCA
dlambda -check-rev	GAAACCCTTGTCGGTGAAAA

lacZ-reporter fusion
LacZ-fw-Pst/Hind	GTCGACCTGCAGCCAAGCTTGCTCCCGTCGT
LacZ-rv-XhoI	CATACTCGAGTTCCTTACGCGAAATACGGGCAGAC
Pmot-fw-Hind	GCTTGGATCCGAGGAGCTGTATCTTCGAGTGTAC
Pmot-rv-Bam	TAAAGGATCCTGGTTACCAGCAACGTTAATGGAG

Protein overproduction
SO2963-EcoRI-fw	CGAATTCCCAAACCCAAATTTT
SO2963-PstI-rev	CGACTGCAGGGCAAAAGTAAAGTTAC
SO2685-EcoRI-fw	CGAATTCGCTACTGAAGCACAAGT
SO2685-NcoI-rev	GACCATGGGCAGTAACTAAAGGC

q-PT-PCR

dSO2653- qPCR -fw	GATCACCTCTCACGCGCAT
dSO2653- qPCR -rev	GCGTATTGAAATGTCACCTATTGA
SO2658-qPCR-fw	GTAAAGCCATTGAGGTTGACCT
SO2658-qPCR -rev	CTAAACAAAAGCCGCCGC
SO2985-qPCR-fw	CCACCCGACTCTCACGAT
SO2985-qPCR -rev	GGTGAAAGCAGATTTGGAAGAT
SO2999-qPCR-fw	GGCCATATTGTGGCAGATAAA
SO2999-qPCR-rev	CTTATCTTTTAACCAAGAAACGGTG
recA-Q-PCR-651-for	TCACATCAACCGCACCAGAACG
recA-Q-PCR-781-rev	CGCTCTTGATCCTATCTACGCG

PCR with eDNA

SO0577-Seq-fw	CGCAACGGTTACTATATCCACTCG
SO0577-Seq-rev	CGATAATGGCGTTCATCGGCG
dSO0641-up-EcoRI-fw	CGAATTCGGATGGTCGTATGGTGTCG
dSO0641-up-overlap-rev	AAGAAAGTGCGGTAAGGACGTGAAGTAATTCTCA
SO1066-XmaI-fw	GACCCGGGGAAAATGTTAATAAGTTAACAGCTGTT
SO1066-NcoI-rev	GACCATGGATAACGGCGACGACGTTG
SO1773-up-EcoRI-fw	CGAATTCTGCAATTCCTTTTTATGCACTG
SO1773-up-NdeI-rev	CGTACATATGGTTTGTATCTAGGGCAACTGCTTG
SO2685-EcoRI-fw	CGAATTCGCTACTGAAGCACAAGT
SO2685-NcoI-rev	GACCATGGGCAGTAACTAAAGGC
dSO2882-up-EcoRI-fw	CGAATTCGGTAGCGCCTGGACTTGG
dSO2882-up-overlap-rev	TAAATACCAGCGCTTTTGGTAATGCTCG
SO2993-BamHI-fw	CGGATCCCCCGTCACCTCCTTCTGTT
SO2993-SphI-rev	GAGCATGCCTTATTTTGTATTGTCACAATTACCGA
dSO3000-EcoRI-fw	CGAATTCGGTTGGATGTTGCCAGGT
dSO3000-KpnI-rev	GAGGTACCCGTTATCAAACAACCAATTGCTAC
SO4405-up-EcoRI-fw	CGAATTCGGGATATTCCTTTACTTATGGCTAAC
SO4405-up-NdeI-rev	CGTACATATGGCTTAACTCTGAAATGTTGTCATGTT

PCR using ΔMuSo1 DNA

dSO0644-EcoRI-fw	CGAATTCGCGTGAAAAGTTATGGCTAAAA
dSO0644-KpnI-rev	CGATAATGGCGTTCATCGGCG