Functional specificity of extracellular nucleases in *Shewanella oneidensis* MR-1

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-Supplemental Material-

Supplemental Table 1: Primers used in this study

Name	Sequence (5' → 3')
In-frame deletion of *endA* (SO_0833)	
MO11	CGACTGCAGGCTGTAACTCCATCGCTGG
MO12	CGCCAACAATTAGCCGAATAAGGTCGACAATG
MO13	TAATTGTTGGCGGTTCAACATTTATCCCAAAAATAATC
MO14	AGGATCCGCATTTGCAGCAAAATCGT
MO15	CGCGCGTATGGGATAAAT
MO16	CCAACCACTCAATTTACCCTTAG
In-frame deletion of *phoA* (SO_0831)	
PstI_KO_phoA_US_fwd	ACGCTGCAGAGGGCGCTAACGGCTTAGCC
BamHI_KO_phoA_DS_rev	ACGGGATCCGGCATGGGTGGCACCTCG
OL_KO_phoA_US_rev	GAGTATCACCCCACAGGGCTAGTTTGCTGC
OL_KO_phoA_DS_fwd	AGCCCTGTGGGGTGATACTCATATCAGATCCC
Check_KO_phoA_fwd_2	GTTTTCCCCCACCACCAGTAATTGACCATTAG
Check_KO_phoA_rev_2	GCTTGGTTTAGTGAATTTACCCCTGAGACC
Construction of pMal-P2X-EndA	
MO50	AGGATCCTCCCCTTCGCATCCAAGC
MO51	CGAGTCGACTCAATGATGATGATGATGATGTTCGGCTAATTGTTGGCGC
Overexpression of *endA*	
PstI_endA_fw	CACTGCAGAGGAGGAATTAACCATGTTGAACAACACCTCAATCG
XhoI_endA_rev	GTCACTCGAGTTATTCGGCTAATTGTTGGCGCAG
qRT-PCR	
MO25	GCAAGTATTGCTGTGCCTATTT
MO26	AGACAAGACCTTGGCTTGACTAA
recA-Q-PCR-651-for	TCACATCAACCGCACCAGAACG
recA-Q-PCR-781-rev	CGCTCTTGATCCTATCTACGCG
qPCR_phoA_fw	CTGGCAAGCTCTTCAAAGC
qPCR_phoA_rv	GTGTTTTTAGGTGGTGGAC
Operon mapping	
MO29	GCCTCATGATTTAGCACTAATACTTT
MO30	GTCATACGGGTATGGATGTGC
MO31	GCTTTGACTGGGTCAGTCTCA
MO32	CCAAAAAAGGCGAAACCC
MO33	CCATATAAAACAGTTGATAACCCCTT
MO34	GCACTAAAACTCAACTTGCACC
MO35	GCAAGATAATCATGACCATCGC
MO36	GGTCATTGATTTTAAGAATCGCC
MO37	CTTGACTAAAACTGCTTGGATGC
MO38	CGTGAGTGGCAATCGATTATG
MO39	GGATGTTCAGCTGTATTGGTATTG
MO40	CCAGTGGAAAGTCAATGATAACC

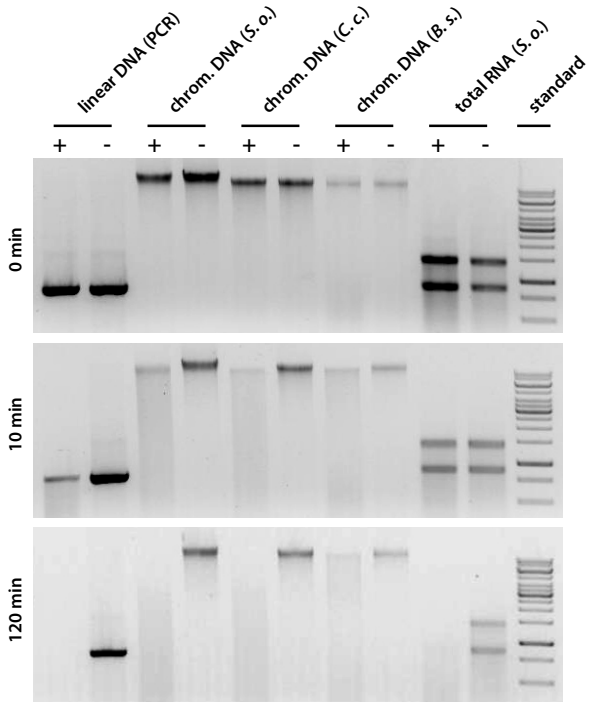

Figure S1: Degradation of different nucleic acids by MBP-EndA. 0.09 U of MBP-EndA (+) or the equimolar amount of MBP (-) that was purified in parallel were added to the indicated type of nucleic acid and incubated at 30 °C for the indicated amount of time prior to separation on a 1.0 % agarose gel. The chromosomal DNA was prepared from *Shewanella oneidensis* MR-1 (*S.o.*), *Caulobacter crescentus* (*C.c.*), and *Bacillus subtitilis* (*B.s.*), the total RNA was prepared from *Shewanella oneidensis* MR-1.

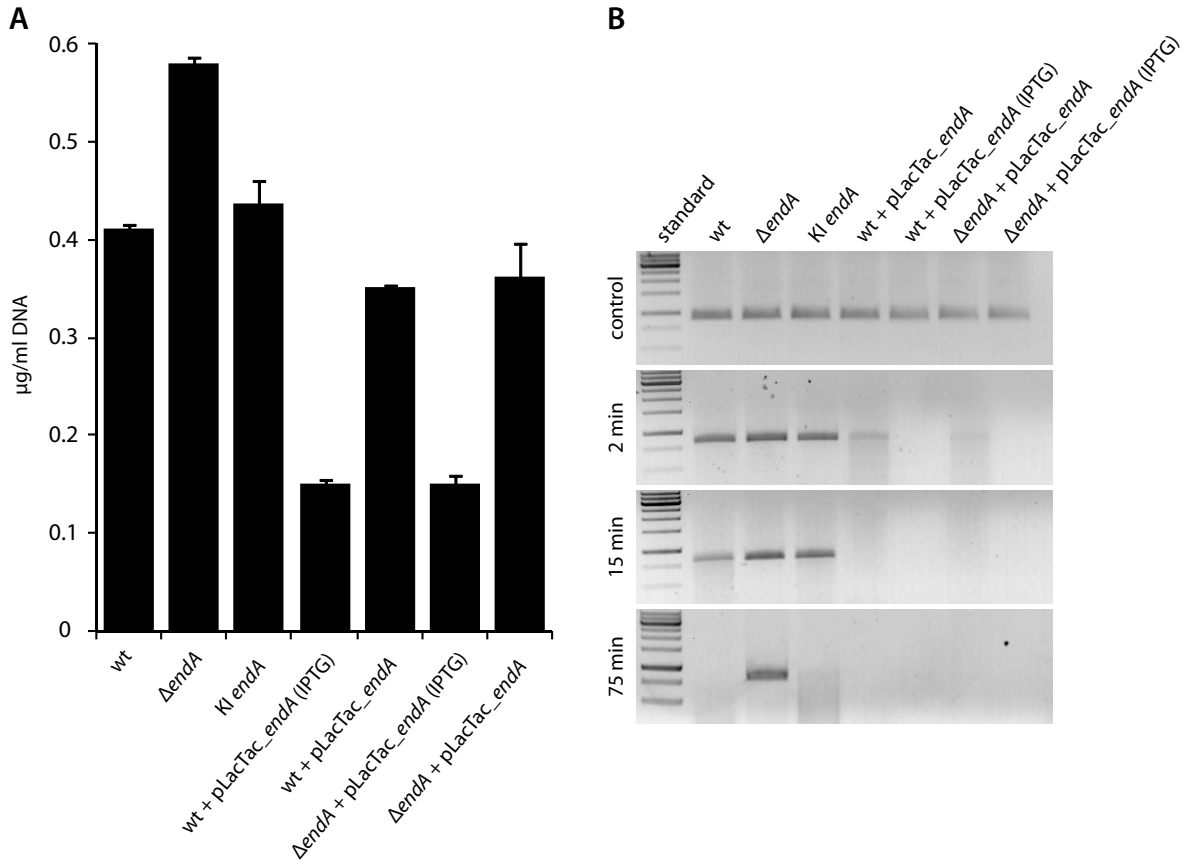

Figure S2: Nuclease activity in supernatants of the complemented *endA* mutant and a strain overexpressing *endA*. A) Amount of eDNA in cultures of the indicated strains grown in 4M medium for 24 hours. To induce overexpression of *endA* from pLacTac-EndA, IPTG was added prior to inoculation (IPTG). **B)** Vizualization of the degradation of a 1.2 kbp PCR fragment by supernatants of the indicated strains. Samples were taken prior addition of the supernatant (control) and at the indicated time points.

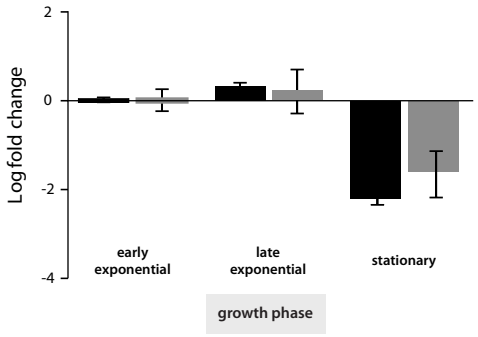

Figure S3: Growth-dependent regulation of *phoA*. The transcript levels of *phoA* compared to those of *endA* were determined by qRT-PCR. Displayed is the regulation of cells in the late exponential growth phase (OD_{600} 2.0) and stationary growth phase (OD_{600} 6.0) compared to the early exponential growth phase in LB medium (OD_{600} 0.6).

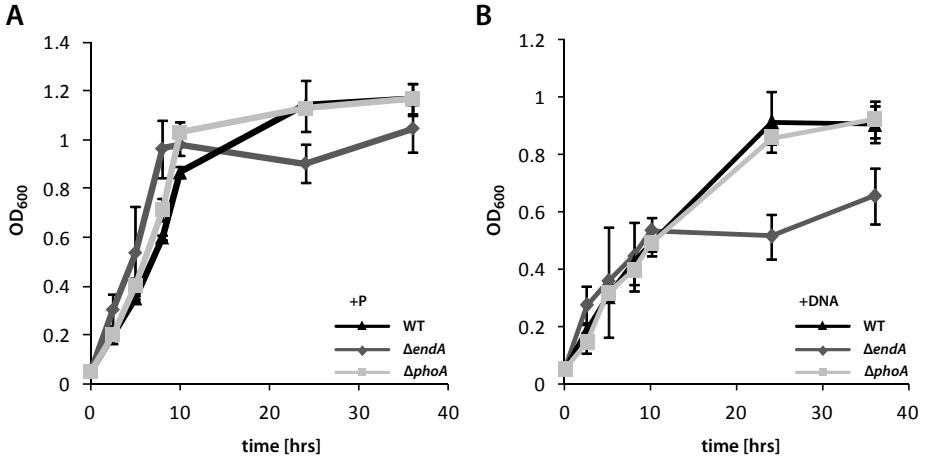

Figure S4: Contribution of EndA and PhoA to aerobic growth during exponential growth phase. Growth of the wild type (black triangles), the *ΔendA* mutant (dark grey diamonds), and the *ΔphoA* mutant (light grey squares) was followed for 36 hours in M1 mineral medium supplemented with either 0.86 mM NaH_2PO_4 **(A)**, or salmon sperm DNA (0.5 g • l^{-1}; **B**). The error bars represent the standard deviation.

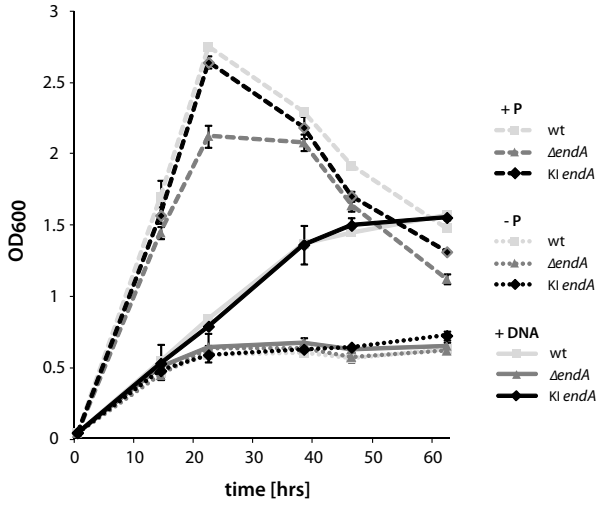

Figure S5: Reintegration of endA into the corresponding deletion strain restores growth on eDNA as sole source of carbon. Growth of the wild type (black squares), the *ΔendA* mutant (grey triangles), and the complemented mutant (KI *endA*, black diamonds) was followed for 62 hours in M1 mineral medium supplemented with either 0.86 mM NaH_2PO_4 (dashed lines), salmon sperm DNA (0.5 g • l^{-1}; solid lines), or no source of phosphorus (dotted lines). The error bars represent the standard deviation.

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-Supplemental Data-

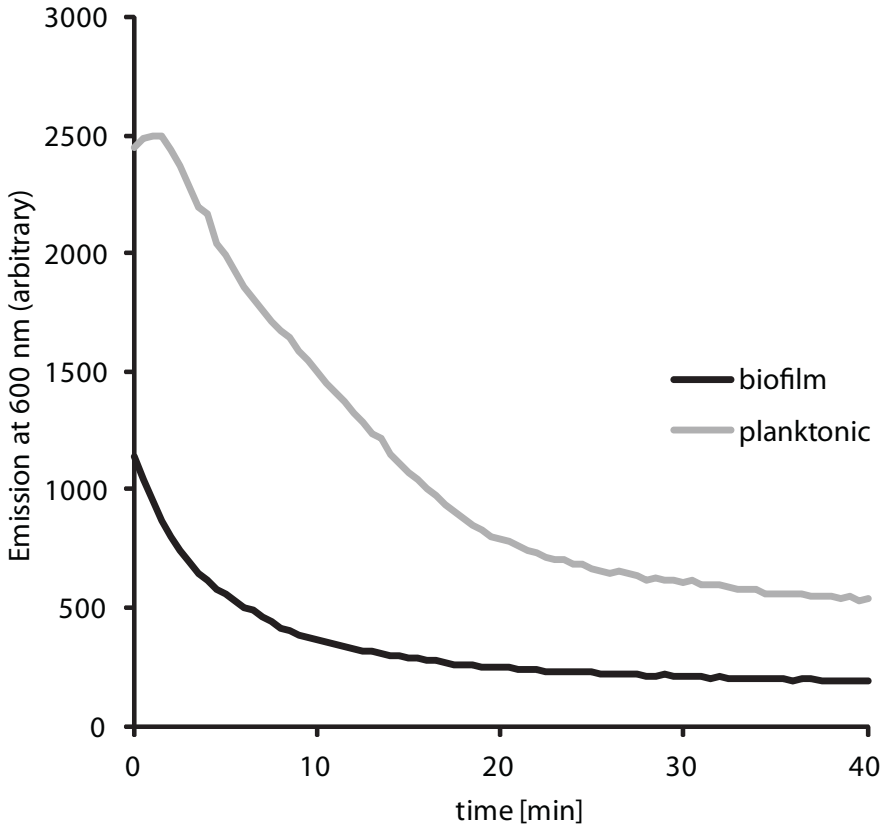

Figure S6: Activity of MPB-EndA. Activity of highly enriched MBP-EndA on eDNA in the supernatant of planktonic cultures (light grey) and on eDNA prepared from the matrix of statically grown biofilms. The activity was determined by the loss of fluorescence of DNA-bound GelRed™ nucleic acid stain due to DNA degradation.