Pseudomonas aeruginosa requires the DNA-specific endonuclease EndA to degrade eDNA to disperse from the biofilm

Kathryn E. Cherny and Karin Sauer#

SUPPLEMENTARY INFORMATION

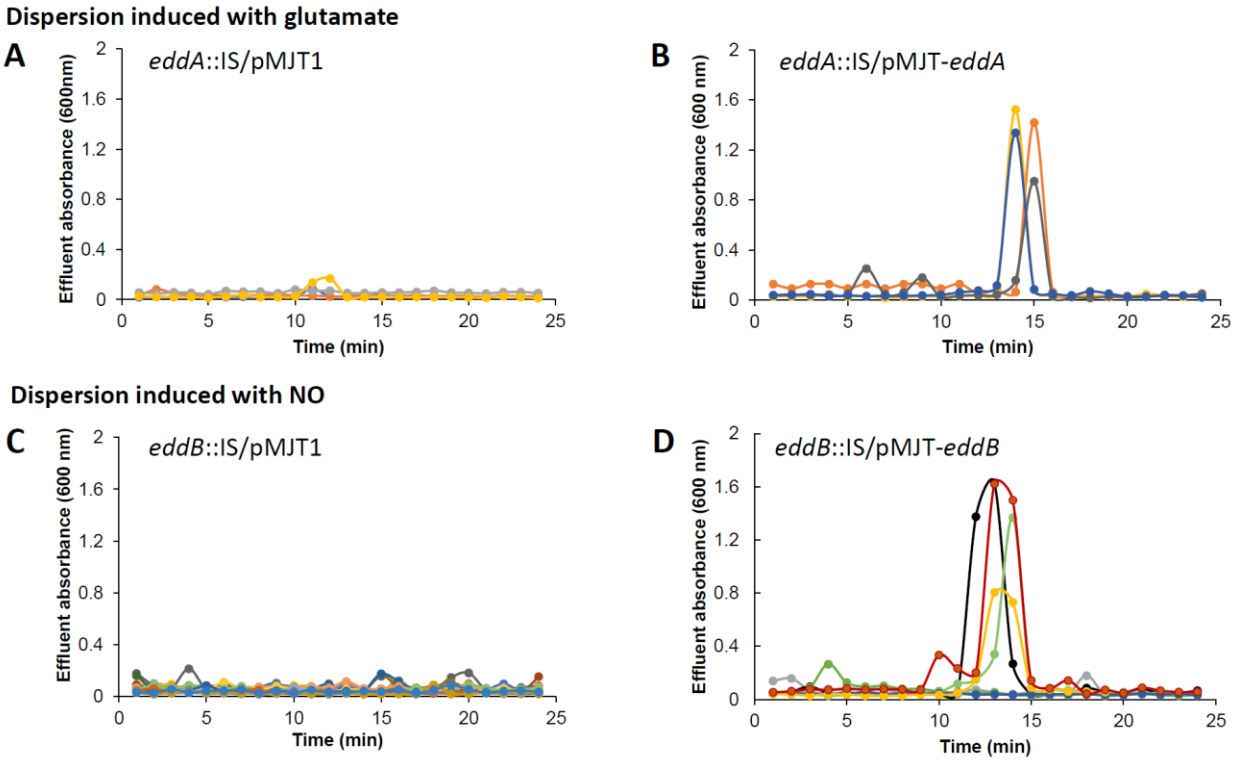

Figure S1. Multi-copy expression of *eddA* and e*ddB* in eddA::IS and eddB::IS mutant strains, respectively, restore the dispersion response to wild-type levels. Biofilms were grown for 5 days in 5-fold diluted VBMM in tube reactors supplemented with carbenicillin for plasmid maintenance and 0.1% arabinose to ensure expression of *eddA* and *eddB*, respectively. Dispersion was induced the addition of glutamate or nitric oxide (NO) to the growth medium. Representative dispersion profiles in response to glutamate are shown for (A) *eddA*::IS/pMJT1, (B) *eddA*::IS/pMJT-*eddA*, and in response to NO for (C) *eddB*::IS/pMJT1, and (D) *eddB*::IS/pMJT-*eddB*. Dispersion assays were performed in triplicate consisting of at least 4 technical replicates.

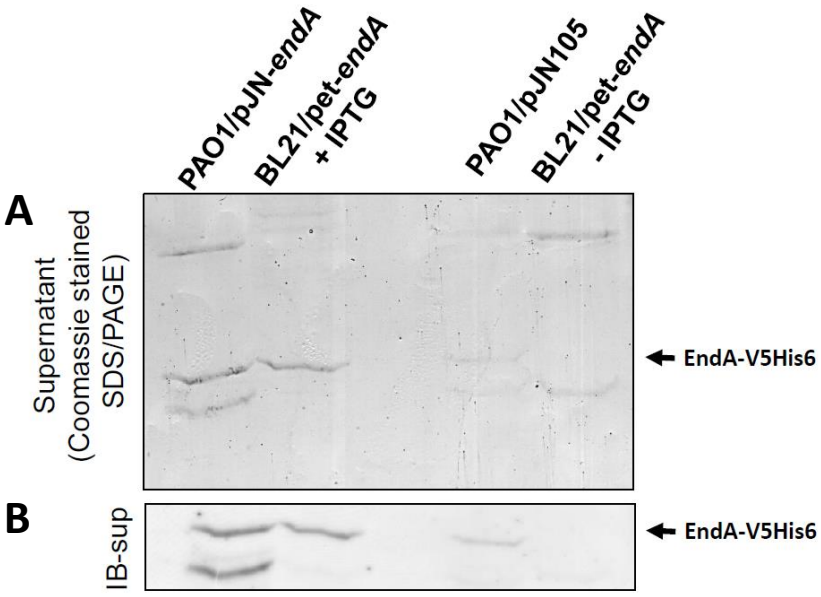

Figure S2. Confirmation of EndA being secreted by *P. aeruginosa* and *E. coli*. Culture supernatants were obtained from *P. aeruginosa* PAO1/pJN105 and PAO1/pJN-*endA*-V5/6xHis cells grown planktonic to exponential phase and post arabinose induction, and from *E. coli* BL21/pet-*endA*-V5/6xHis grown in the absence and presence of IPTG. (A) Image of Coomassie stained SDS-gel. A total of 1.5 µg of concentrated supernatant was loaded. (B) Image of immunoblot probed for the presence of V5/6xHis-tagged EndA using anti-V5 antibodies. A total of 1.5 µg of concentrated supernatant protein was transferred for immunoblot analysis.

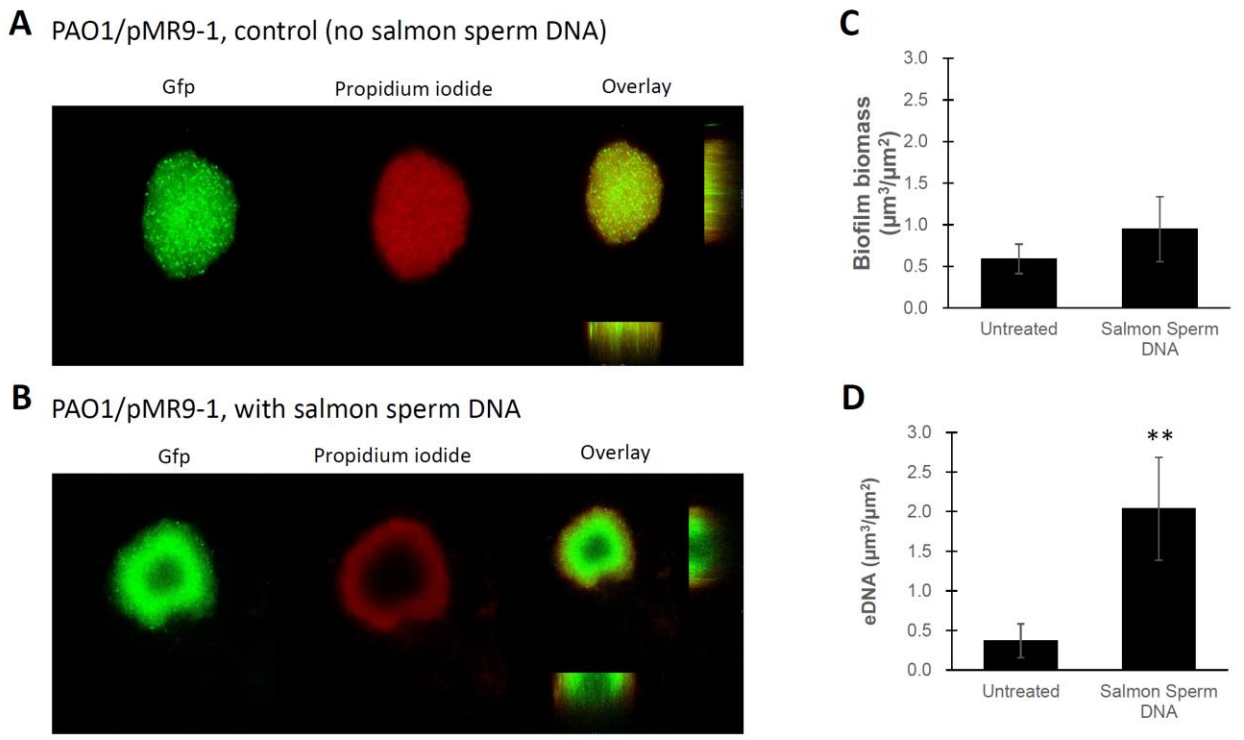

Figure S3. Visualization and quantitation of eDNA present in biofilms. (A) Representative images of flow-cell grown *P. aeruginosa* PAO1/pMRP9-1 biofilms constitutively expressing gfp following 2 days of growth in 24-well plates under static conditions with media changes every 12 h. The biofilm biomass was visualized by Gfp. eDNA was visualized following staining with propidium iodide. White bar, 100 µm. (B) Representative images of biofilms by PAO1/pMRP9-1 following 2 days of growth in 24-well plates under static conditions with media changes every 12 h following exposure to exogenous salmon sperm DNA (5 mg/ml). The biofilm biomass was visualized by Gfp, while eDNA was visualized following staining with propidium iodide. White bar, 100 µm. (C) Quantitative analysis of the biofilm biomass in the absence and presence of exogenous salmon sperm DNA using COMSTAT. (D) Quantitative analysis of the biofilm-associated eDNA in the absence and presence of exogenous salmon sperm DNA using COMSTAT. Experiments were performed in triplicates, with each repeat comprising 6 technical replicates. Error bars represent standard deviation. **, significantly different compared to PAO1/pJN105 (<0.005) as determined using ANOVA.