# Developed biofilm assay suggests *Escherichia coli* Nissle 1917 may mediate biofilm inhibition in *Escherichia coli* K-12 in liquid co-culture

Alex Fung, Anderson Li, Helen Lin, Vivian Li

Department of Microbiology and Immunology, University of British Columbia, Vancouver, British Columbia, Canada

**SUPPLEMENTAL MATERIAL**

**TABLE. S1** Oligonucleotide primer pairs designed and used in this study, each flanking a gene of interest.

| Flanking Gene | | Sequence (5'-3') | Melting Temperature |
|---|---|---|---|
| *cpxA* | forward | AACGCCTGACGCCTTTCGAC | 60.6°C |
| | reverse | GGAAAATAACCCCCGGAGTGT | 57.2°C |
| *cpxR* | forward | CAAACATGCGTCAGGGGGTGT | 60.5°C |
| | reverse | GTCATCTGGCGTGAATCGAGC | 58.4°C |

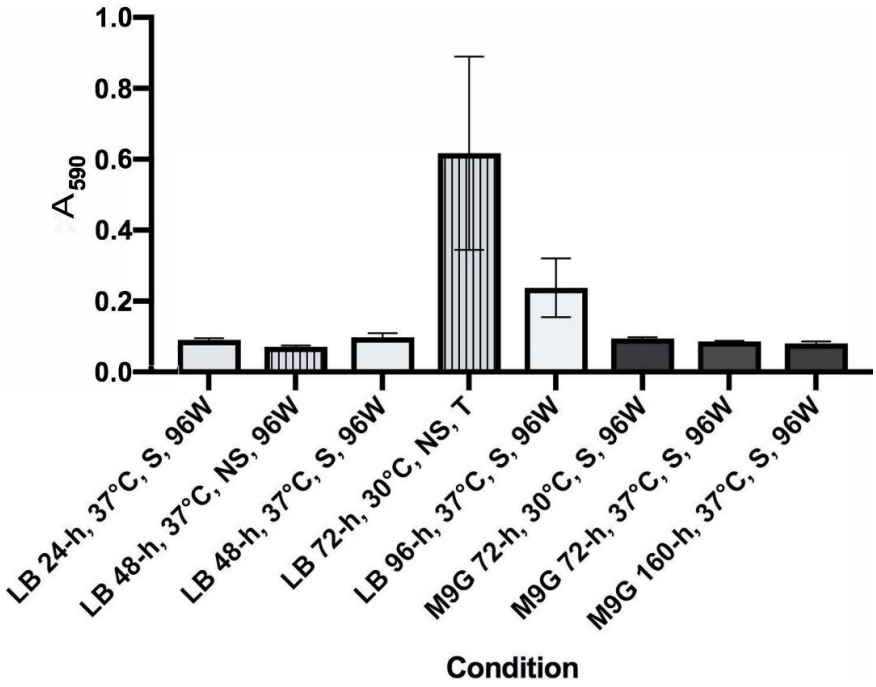


**FIG. S1** Optimization of growth conditions for expression of biofilm (A590) in K12. Conditions tested included incubation vessel (96-well plates, 96W; and glass test tubes, T), growth media (LB; and M9 minimal media + 0.4% dextrose, M9G), temperature (30°C and 37°C), aeration (shaking, S; and non-shaking, NS), and length of incubation (24, 48, 72, 96 and 160 h). Three technical replicates of each sample were averaged and the standard deviation is shown in error bars.

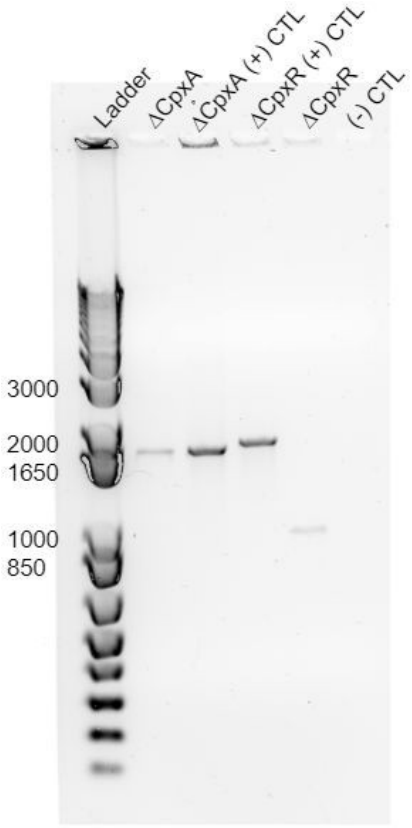


**FIG. S2** DNA agarose gel electrophoresis of the colony PCR results from the colonies of *E. coli ΔcpxA*, *E. coli ΔcpxR*, and *E. coli* K-12.

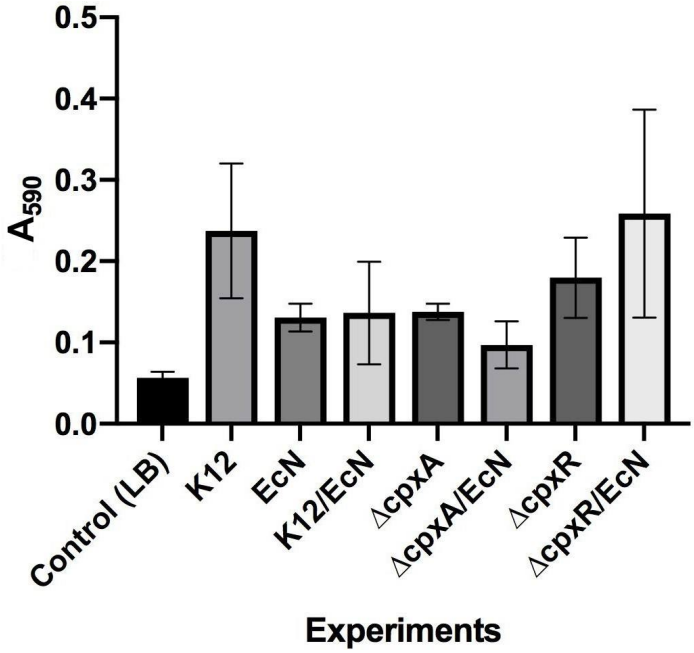


**FIG. S3** Co-culture of K12 with EcN demonstrates 2.3-fold decrease in K12 biofilm formation when compared to K12 monoculture. Bacterial cultures were incubated in LB for 96 h at 37°C in a polystyrene 96-well plate. Biofilm expression levels are shown as A590 values. Overnight cultures of K12, EcN, *E. coli*Δ*cpxA* and *E. coli*Δ*cpxR* were back-diluted in LB to a common OD600 value of 0.05.

Resulting cultures were used to inoculate a 96-well plate in the monoculture (200 μL of each) and

co-culture (100 μL of each bacterial culture) setups as indicated. Following incubation, the supernatant and planktonic cells were removed, and all wells and contents were washed, stained with 0.1% crystal violet, eluted with 30% acetic acid, and transferred to a new 96-well plate to obtain the A590 reading. The bars show means of three technical replicates of each bacterial culture with the error bars showing standard deviation. The negative control was LB medium.

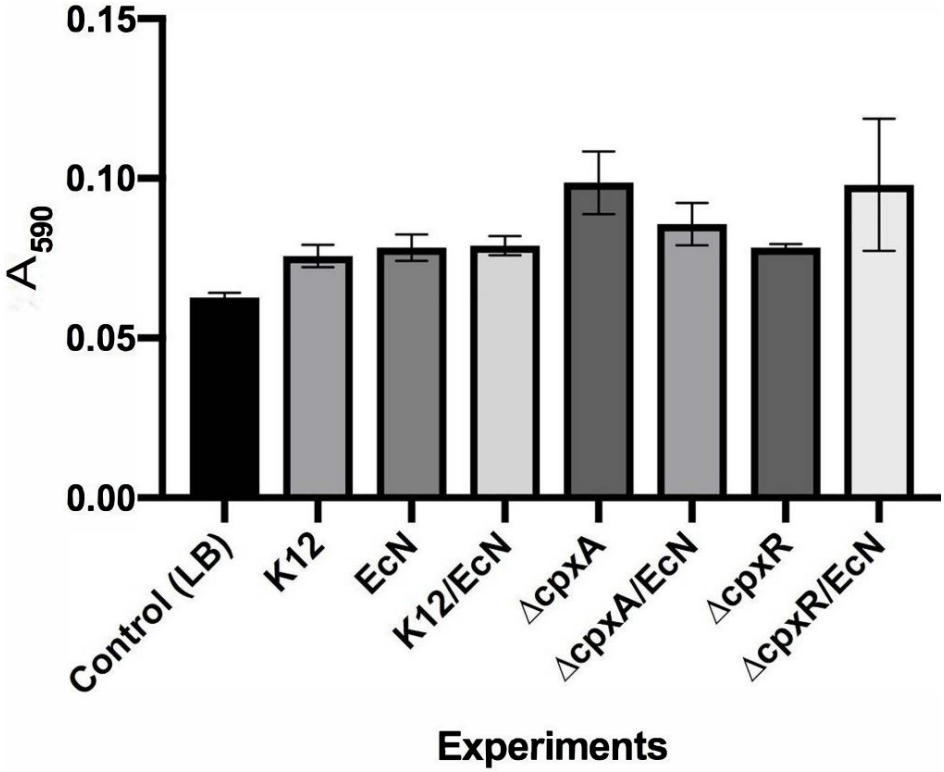


**FIG. S4** Repeat of the experimental setup and biofilm assay as described in Fig. S3, controlled for evaporation bias**.** Biofilm expression levels are shown as A590 values. The bars show means of three technical replicates of each bacterial culture with error bars showing standard deviation. The negative control was LB medium. Experimental wells were surrounded by wells with 200 µL LB to prevent evaporation. Findings from this repeat experiment, controlled for evaporation, are not consistent with previous experiment.

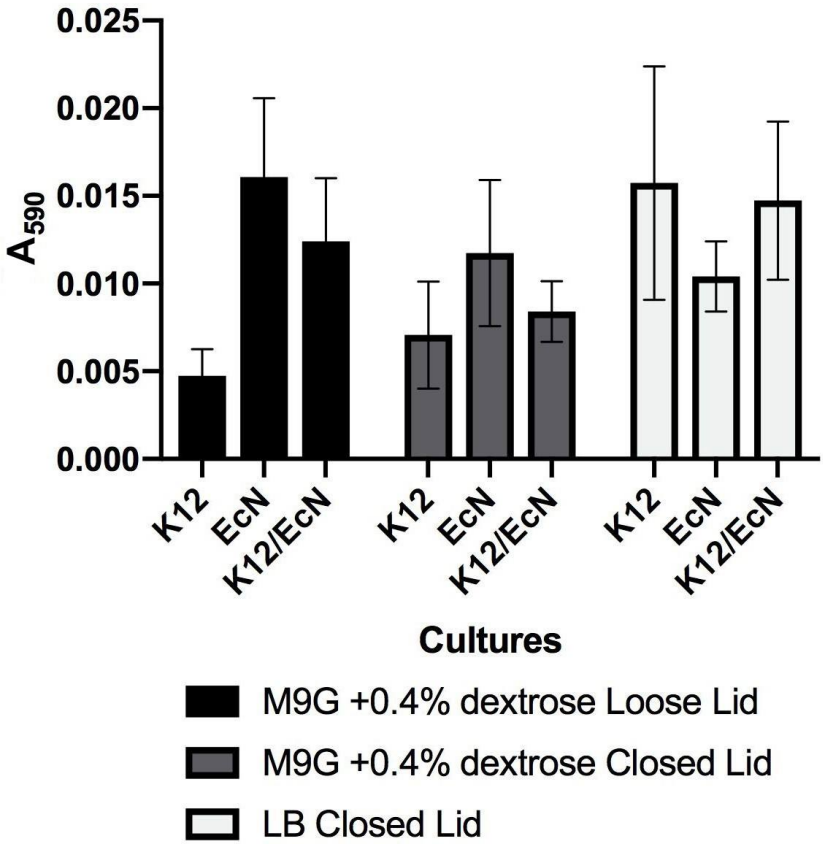


**FIG. S5** Biofilm quantification in planktonic cells of K12, EcN and co-culture K12/EcN**.** The expression of biofilm was measured by A590 values after a crystal violet assay was performed. The planktonic cells of overnight bacterial cultures grown in polypropylene test tubes were removed and seeded in a polypropylene 96-well plate and incubated at 37°C, without shaking, for 96 h. The bars show the mean value of three technical replicates for each bacterial culture, and the error bars indicate the standard deviation.