Supporting Information for:

Adaptive Evolution of *Escherichia coli* to Ciprofloxacin in Controlled Stress Environments: Contrasting Patterns of Resistance in Spatially Varying versus Uniformly Mixed Concentration Conditions

Jinzi Deng[1], Lang Zhou[2], Robert A. Sanford[3], Lauren A. Shechtman[4,5], Yiran Dong[1,6], Reinaldo E. Alcalde[2], Mayandi Sivaguru[1], Glenn Fried[1], Charles J. Werth[2], and Bruce W. Fouke[1,3,7]*

[1] Carl R. Woese Institute of Genomic Biology, University of Illinois Urbana-Champaign, Urbana, IL 61801
[2] Department of Civil, Architectural and Environmental Engineering, University of Texas at Austin, Austin, TX 78705
[3] Department of Geology, University of Illinois Urbana-Champaign, Urbana, IL 61801
[4]Department of Chemistry, University of Illinois Urbana-Champaign, Urbana, IL 61801
[5]Department of Integrative Biology, University of Illinois Urbana-Champaign, Urbana, IL 61801
[6]School of Environmental Studies, China University of Geosciences (Wuhan), Wuhan, 430074, China
[7]Department of Microbiology, University of Illinois Urbana-Champaign, Urbana, IL 61801

Summary Contents

MGC experiment with spatial gradient

Plasma binding of the PDMS cast and glass slides

The PDMS cast and a glass slide was washed with isopropyl alcohol and blow-dried using nitrogen. Then, the slide and PDMS were plasma treated in a plasma chamber (March Jupiter III RIE, March Instruments, Inc., Concord, CA) at 15W (reverse power 2W) for 45 s before gently pressed together. This enclosed the flow channels and well-array.

Hydrogel flow control elements fabrication

0.1 g poly (ethylene glycol) diacrylate (PEGDA) (26570-48-9, Sigma-Aldrich, St. Louis, MO) was dissolved in 0.5 ml of N_2 purged PBS in a 1 ml glass vial. Next, 3% 2-hydroxy-2-methylpropiophenone (HMPP) (405655-50ml, Sigma-Aldrich, St. Louis, MO) was added into the PEGDA solution as a photo initiator. The premixed PEGDA-HMPP solution was purged into and completely filled the well array, interconnecting throats, and flow channels. A mask was then placed over the MGC, so that only the first 3 rows of wells adjacent to each flow channel were visible. This was exposed to 1W/cm2 of UV for 10s (ABM Flood Exposure model 60, ABM-USA, Inc., San Jose, CA), and then non-crosslinked PEGDA solution was washed away from the flow channels and remaining well array with sterilized DI water. This resulted in solidified hydrogel along each boundary channel.

Pre-diffusion process

A 24 hours pre-diffusion process of the MGC was performed before the start of the experiment. LB media containing a ciprofloxacin concentration equivalent to $10 \times MIC_{original}$ was continuously infused into one of the boundary channels at 0.4 ml/h for 24 hours. Sterilized M9 media was continuously infused in the other boundary channel at the same flow rate. This process shortens the time of which ciprofloxacin and nutrient diffuse across the flow control elements into the well array.

Sterilization process

The MGC was sterilized before inoculation. 70% ethanol was infused into the well array and let sit for one hour. Then, sterilized DI water was infused into the well to displace the ethanol and wash the flow control elements that were in contact with ethanol.

Microscopy and image processing

A Carl Zeiss microscope observer Z1 equipped with a motorized stage was used to image bacterial dynamics in the MGC in real-time using epi-fluorescence microscopy. 27×26 mosaic tiled images of the entire well array were taken every 6 hours. The fluorescent area in each image, indicating *E. coli* 307 with GFP, was analyzed using Fiji software. The fluorescence images were converted into binary masks. Fluorescent area with grey values >50 was determined to be *E. coli* biomass, regardless of its shape due to elongation and aggregation of cells.

Extracting E. coli cells from the MGC

The top surface of the MGC was first wiped with 70% ethanol before sterilized LB media containing no ciprofloxacin was injected from one of the inoculation ports. The flow rate of the injection was kept at 100 µl/h for 20 min and then 1000 µl/h for 20 min followed with 10000 µl/h for 4 min. The fluid flushed through the well array was collected from the other inoculation port.

Fluorescein dye diffusion experiment

0.5 mM fluorescein dye was infused into one of the boundary channels. Fluorescent images were taken every 10 minutes for the first hour and then every 30 minutes until the end of the experiment. An image at 7 h 20 min is shown in Figure S4a.

Fluorescein dye at concentrations of 0.5mM, 0.25mM, 0.1mM, 0.05mM and 0.025mM was loaded into the MGC consecutively to calibrate the relationship between fluorescence intensity and concentration (Figure S4c).

Concentration profiles of the fluorescein dye experiment and ciprofloxacin diffusion modeling across the well array at 7 h 20 min was normalized using the boundary concentration of the dye and ciprofloxacin respectively and were plotted in Figure S4b.

Experiments in Uniformly Mixed Concentration Conditions

Optical density measurement

Thermo Spectronic GENESYS 20 (Model number 4001/4, Thermo Fisher Scientific, Waltham, MA) was used to measure the OD_{600} of the control and the samples exposed ciprofloxacin conditions similar to the lower region of the MGC.

MIC measurement

Fresh LB media containing a range of ciprofloxacin concentrations were inoculated with 5×10^5 CFU/ml of each *E. coli* sample[1] and followed with incubation at 37 ˚C. The OD_{600} of each culture was measured after 16 h of incubation. There was a step change between each successively higher ciprofloxacin concentration, so an MIC range was determined from the maximum ciprofloxacin concentration showing growth (i.e., $OD_{600}>0.1$) and the next highest ciprofloxacin concentration with no growth (i.e., $OD_{600}\sim0$).

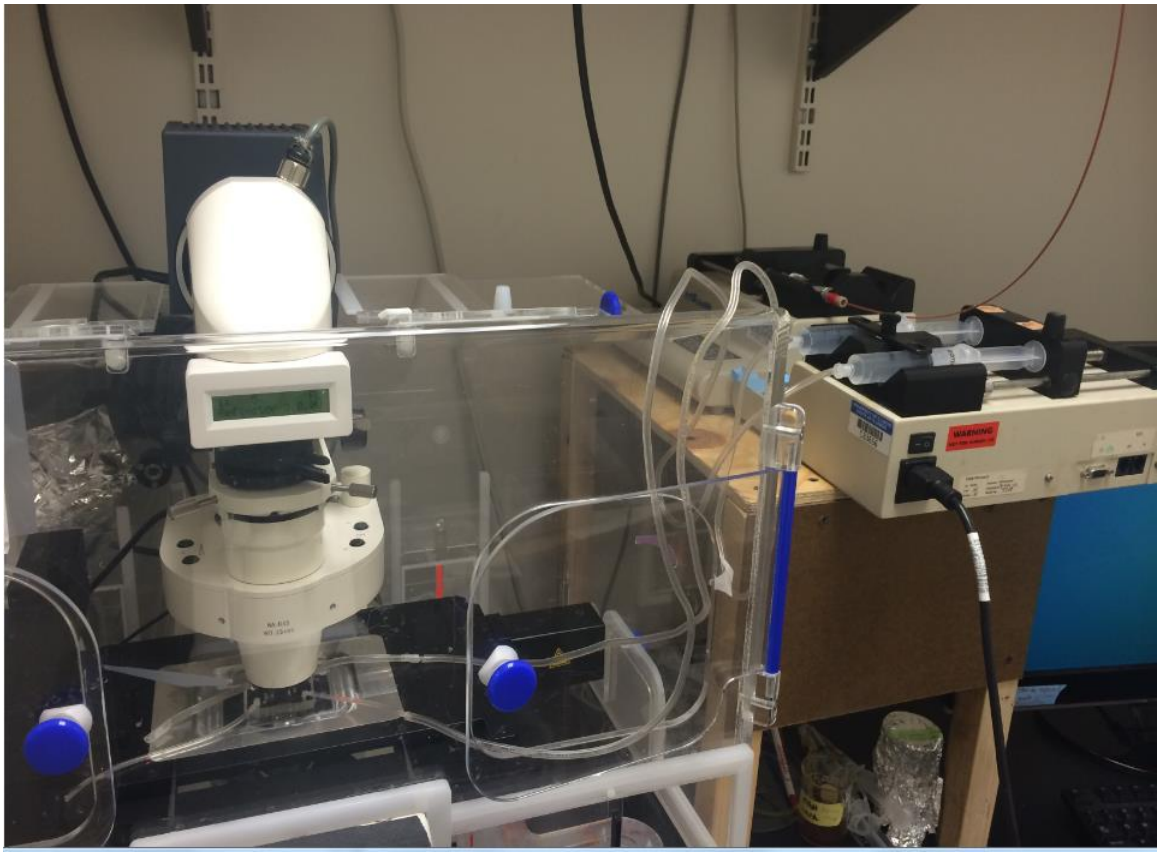

Figure S1. A photo of the MGC connected to a syringe pump by fittings and tubing. A temperature control chamber was mounted outside the microscope stage to keep the MGC at the desired temperature. 1/16” ID tubing (McMaster-Carr, 51135K11) and fittings (McMaster-Carr, 5463K39) were used to connect the MGC with the syringe pump (Cole Parmer Instrument Company, 74900 series).

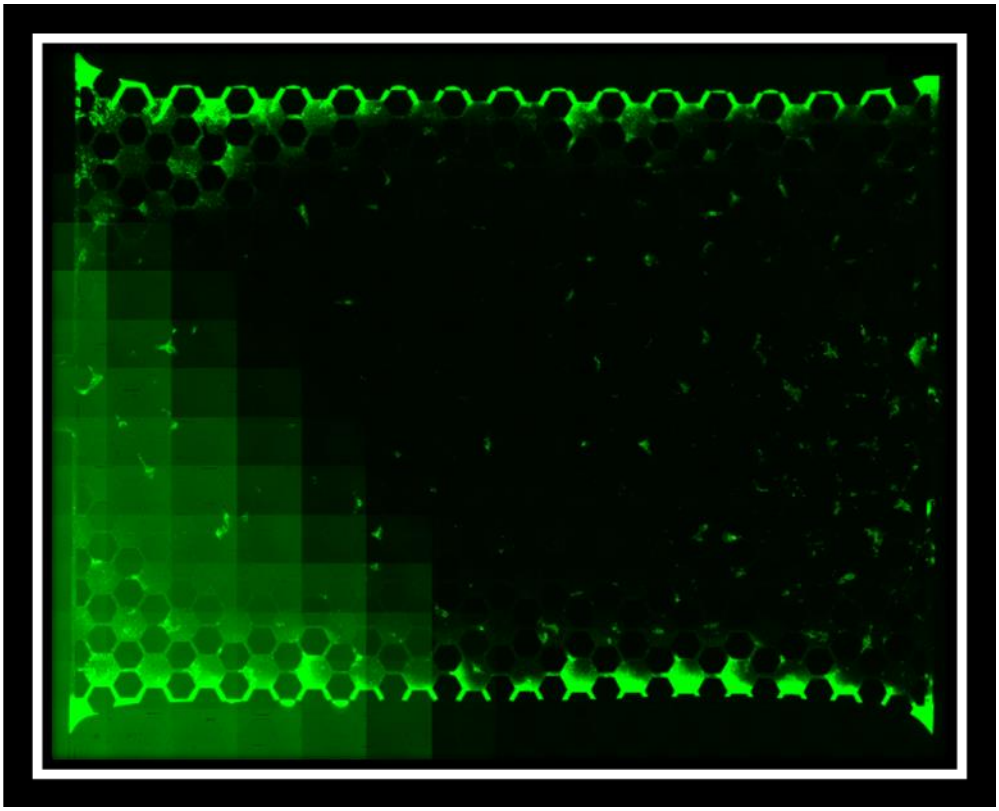

Figure S2. Tiled fluorescent images of fluorescent *E. coli* 307 in the well array of a control MGC experiment with no ciprofloxacin exposure. Both boundary channels of the MGC were infused with nutrients only. No ciprofloxacin was administrated into the system. The highly fluorescent areas are closest to the hydrogel and boundary channels (containing nutrients), where fluorescent *E. coli* cells were denser.

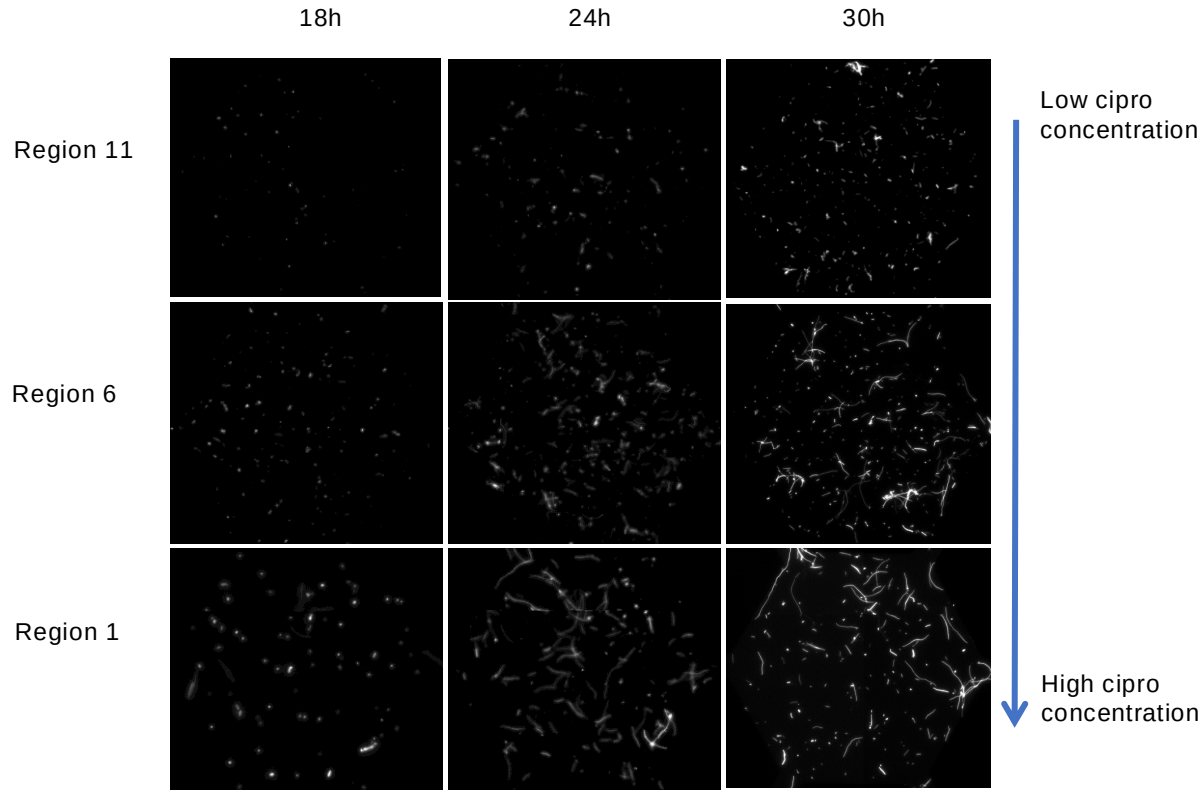

Figure S3. Progression of elongation of *E. coli* cells when exposed to the spatial gradient of ciprofloxacin in the MGC.

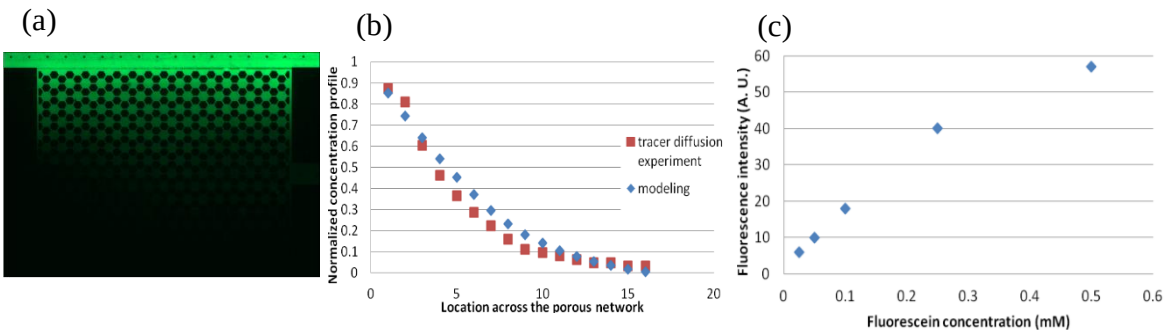

Figure S4. Fluorescein dye diffusion experiment. (a) An image of fluorescein diffusion in the MGC. (b) Normalized concentration profile of the fluorescein dye and the simulated ciprofloxacin concentration across the well array, with 1 being the highest concentration in the boundary channel. (c) Fluorescence intensity calibration using different concentrations of the fluorescein dye.

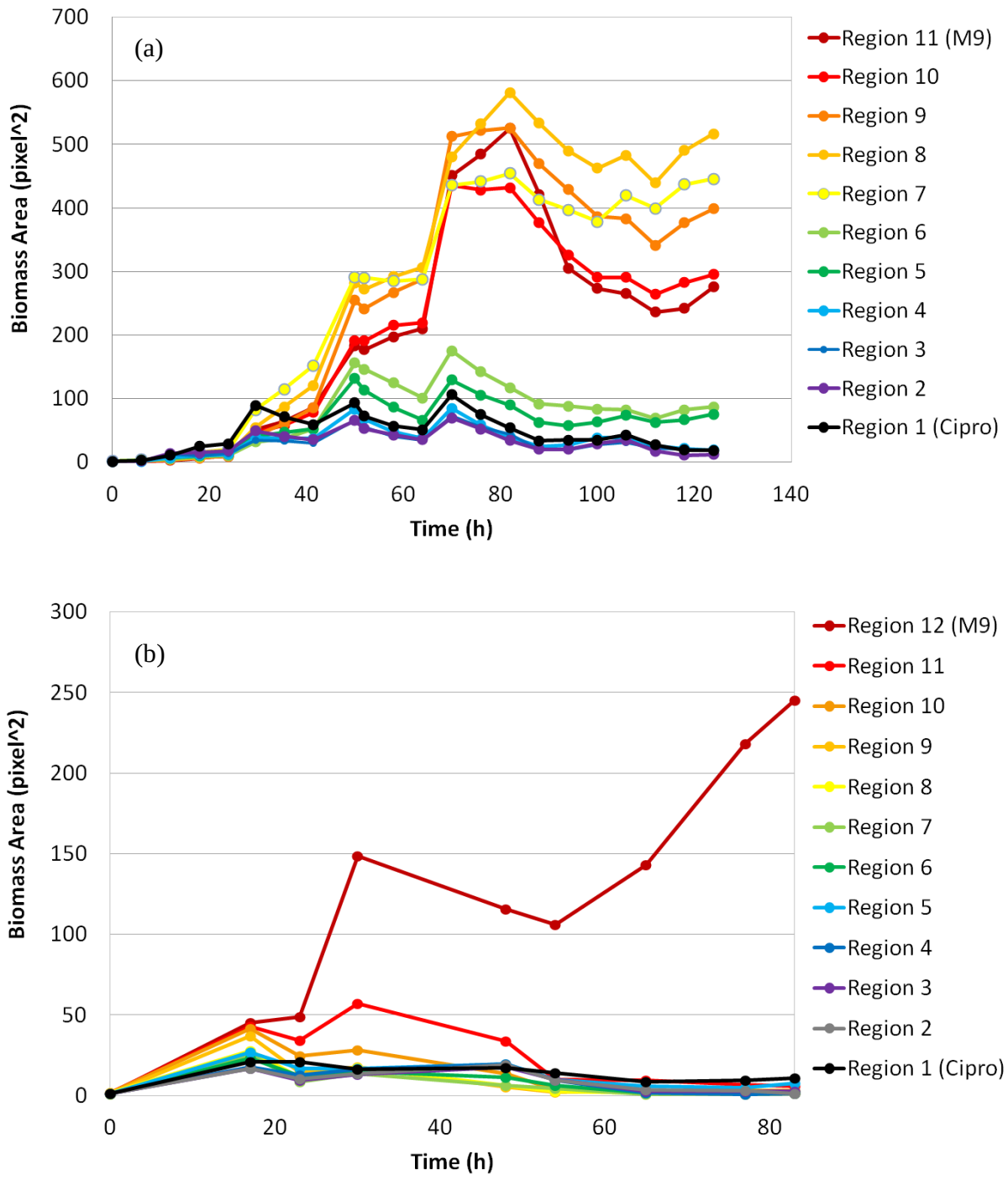

Figure S5. Dynamic spatial distributions of biomass area across the well array of replicate MGC experiments.

Reference:

1. Wiegand, I.; Hilpert, K.; Hancock, R. E. W., Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nat Protoc* **2008,** *3*, (2), 163-175.