

Single cell growth rate and morphological dynamics revealing an “opportunistic” persistence

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Supplementary Information:

Figure S1. Cell width variation without antibiotic treatment.

Figure S2 and video 1. Concentration gradient profiles of fluorescein during the first 8 h.

Figure S3. An example of resurrection of an inhibited *E. coli* cell at $C_{AM} = 2$ mg/L.

Protocols for inhibition experiments with and without cell lysate extract.

Figure S4: The growth curves of *E. coli* under different inhibition conditions in 96-well plates (n = 3).

Figure S5. Concentration - inhibition rate curves for *Comamonas Denitrifican*.

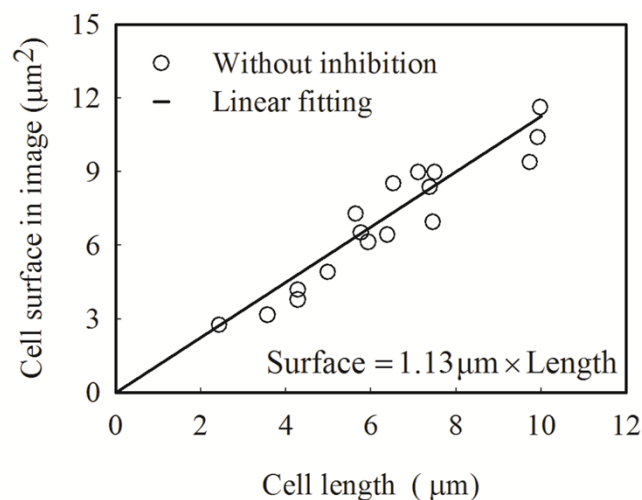


Figure S1. Cell width variation without antibiotic treatment. Cell length versus cell surface area shows an excellent linear relationship, suggesting a constant cell width. The derived cell width from the fitting is 1.13 μm .

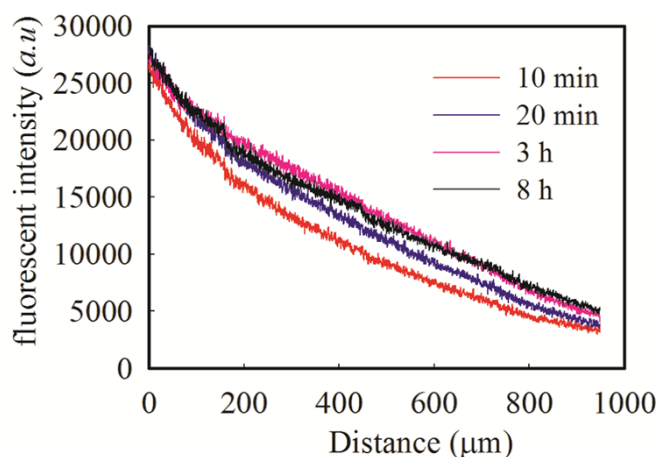


Figure S2 and video 1. Concentration gradient profiles of fluorescein during the first 8 h of microfluidic flow at 5 $\mu\text{l}/\text{min}$. The fluorescence intensity of fluorescein solution is directly proportional to its concentration. Fluorescein and amoxicillin have similar molecular weights, and consequently similar diffusion characteristics.

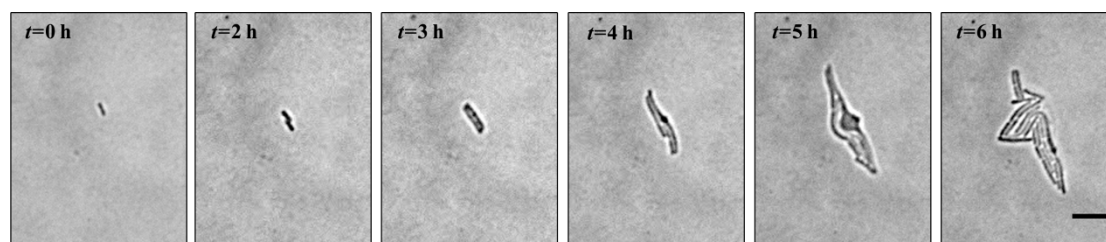


Figure S3. An example of resurrection of an inhibited *E. coli* cell at $C_{AM} = 2 \text{ mg/L}$. Scale bar 10 μm .

PROTOCOLS

Inhibitory Tests in 96 well plates. To investigate the role of cell cytoplasm on bacterial persistence to amoxicillin, inhibition tests with and without cell lysate extract were carried out in 96-well plates using *E. coli*. First, cell cytoplasm was extracted from *E. coli* as follow. *E. coli* were cultured overnight to reach stationary phase (OD₆₀₀ is about 1.0 - 1.2). They were then collected, washed with PBS, and lysed with an ultrasonic cell disruption system (JY92-II D, SCIENTZ) at 70% of 350W. The treatment lasted for 10 minutes with repeated cycles of 4 seconds ultrasonication followed by 2 seconds cooling. The crude lysate was then filtered through a 0.22 µm membrane filter to remove cell wall debris, and gave rise to a cytoplasm rich filtrate in PBS. The filtrate, denoted as cell extract, has an OD₆₀₀ value of 0.08, close to that of LB broth (OD₆₀₀ = 0.07).

For the inhibitory tests, each well contained 5 µl of bacterial suspension and 295 µl culture medium. Three different culture mediums were tested, including LB broth, cell extract, and a mixture of LB broth and cell extract at a ratio of 1:1. A series of amoxicillin concentrations covering the range of concentrations gradient formed on chip, namely at 1, 2, 3, 4, and 5 mg/L, were prepared in the respective culture medium. Each concentration was added to three wells. The plates were placed on an orbital shaker at a speed of 150 rpm and maintained at 22°C. The OD₆₀₀ of each well was recorded every 10 minutes for 10 hours with a plate reader (Synergy HT, Biotek Company).

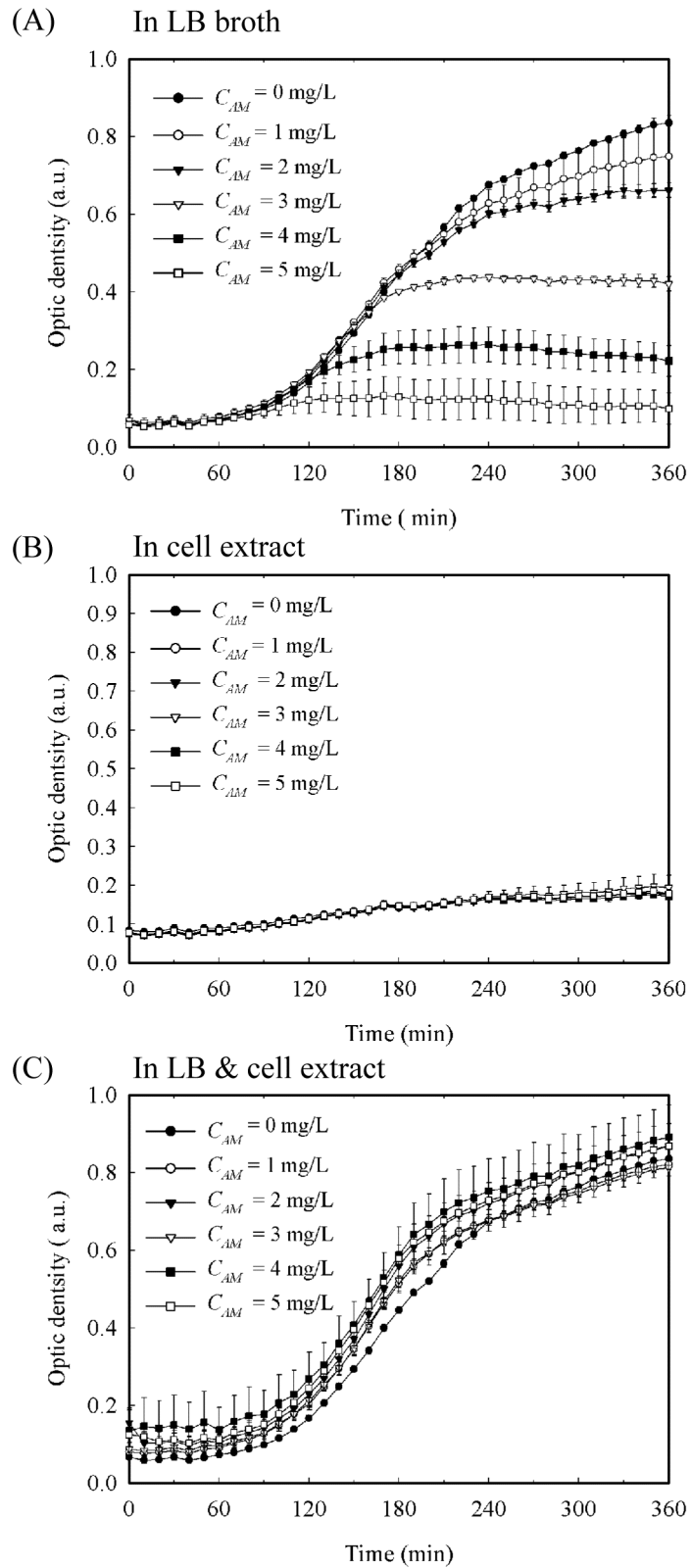


Figure S4: The growth curves of *E. coli* under different inhibition conditions in 96-well plates ($n=3$). (A) In LB broth; (B) In cell extract and (C) In the mixture of LB and cell extract at a ratio of 1:1. All data points have error bars of one standard deviation.

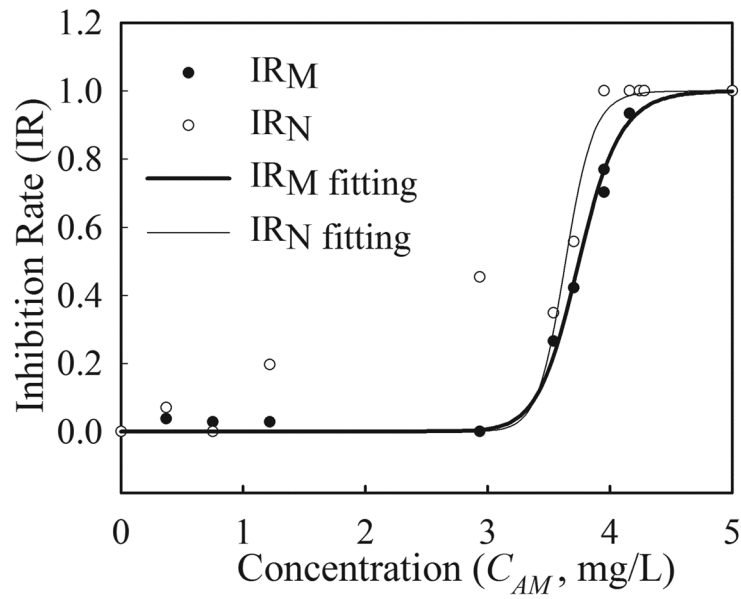


Figure S5: Concentration - inhibition rate curves for *Comamonas Denitrifican*. The logistic function described in Equation 4 was used for curve fitting. The estimated logistic function is as follows: $IR_M = 1/[1+(C/3.75)^{-22.0}]$ and $IR_N = 1/[1+(C/3.64)^{-31.5}]$