Capture efficiency of *Escherichia coli* in fimbriae-mediated immunoimmobilization

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Experimental

Materials

PBS buffer salt and APTES were purchased from Sigma-Aldrich (St. Louis, MO). N-[ß-maleimidopropyloxy]-succinimide ester (BMPS) was purchased from Pierce Biotechnology (Rockford, IL). The wafer was purchased from Silicon Inc. (Boise, ID, Cat. #: 415400-01977).

Immunoimmobilization of living **E. coli** *cells on antibody-modified substrates*

The antibody-modified substrates (silicon chips) were incubated with bacterial cultures, with a cell concentration of ~5×10^8 cfu/ml unless otherwise specified, under ambient conditions for ~45 min. The substrates with immobilized cells were then washed gently with PBS buffer to remove excess and loosely attached cells. The immobilized cells are then imaged using an optical microscope to evaluate the immobilization efficiency. A polished Si surface makes the imaging of bacteria extremely efficient in that a single cell immunoimmobilized within a 200×200 μm^2 field of view of the microscope can easily be spotted. At least three samples were investigated to evaluate the immobilization efficacy of each antibody.

Preparation of antibody microarray

The antibody microarray on glass slides was prepared using a microplotter (Bio-Rad, VersArray Chipwriter Compact System) at room temperature with the humidity inside the plotter chamber adjusted to 90%. The glass slides were cleaned and activated by following the procedure for silicon substrates described above except for the deposition of the antibodies by the microplotter. A microarray consisting of a 6×45 matrix was prepared using three antibodies: anti-CFA/I, anti-K88ac and anti-987P, with each antibody plotted as two separated rows of 45 spots (Figure 7B). The antibody microarray was kept inside the chamber with 98% humidity for 1 hr to allow the covalent linking of antibody molecules to the substrate, then washed with LB medium and PBS buffer to remove excess antibody.

Optical and fluorescence imaging

All the optical (bright field) and fluorescence imaging was done using an Olympus BX61 microscope in reflectance mode (for non-fluorescent strains) or epifluorescence mode (for fluorescent strains). Three filter cubes were used to image the blue, green and red emissions of the DAPI, FITC and TRITC fluorescent stains, respectively. Reflection mode images were taken near the edges of antibody-modified circular areas on silicon chips so that the sharp boundaries of the immobilized cells could be imaged clearly, as in Figures 2-4.

Atomic force microscopy imaging

Atomic force microscopy (AFM) imaging of *E. coli* strains was conducted using a Veeco AFM (MultiMode V, Santa Barbara, CA) to reveal their fimbriae [1, 2]. The cells were immobilized on freshly cleaved mica disks by incubating ~100 µl of bacterial culture on the mica for 30 min followed by rinsing with PBS buffer and Nanopure water and slow drying in air. The cells on the mica disks were imaged in air using tapping mode. The fimbriae on the cell surfaces can be seen clearly in the amplitude images, as shown in Figure 1.

Supplementary Results

Figure S1 Immunoimmobilization of 9.1360 on a substrate modified with mAb against K99 (A) and with pAb against K99 (B).

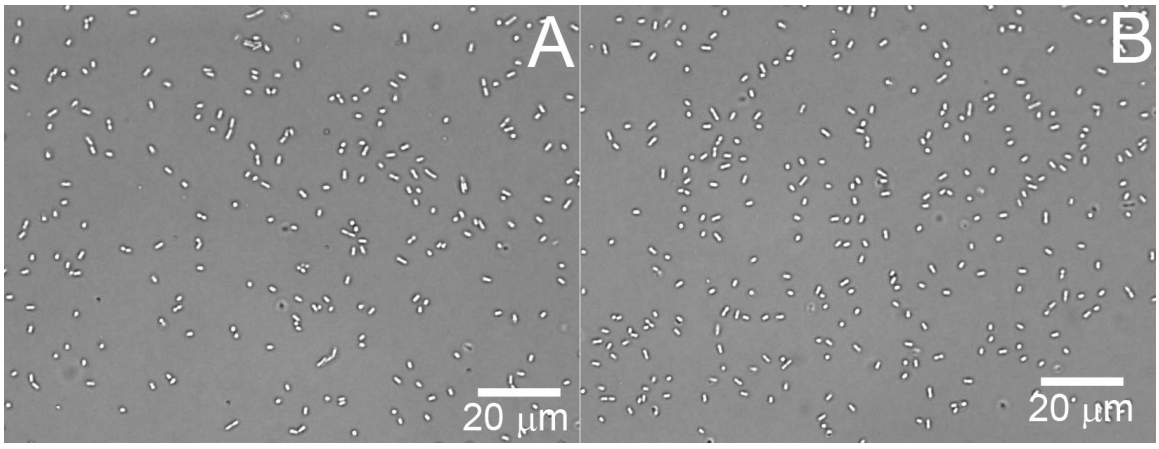


Figure S2 Immobilization of H72-pBAD-cfa on silicon substrates modified with anti-CFA/I: (A) Bacteria were grown in a medium without arabinose (0 µg/ml) and only minimum amount of CFA/I fimbriae were expressed; (B) Bacteria were grown in a medium containing arabinose at a concentration of 3 µg/ml so the CFA/I expression level was enhanced. All other experimental conditions were kept identical. The dark area in the images was due to a small air bubble in the liquid light guide cable coupled to the light source.

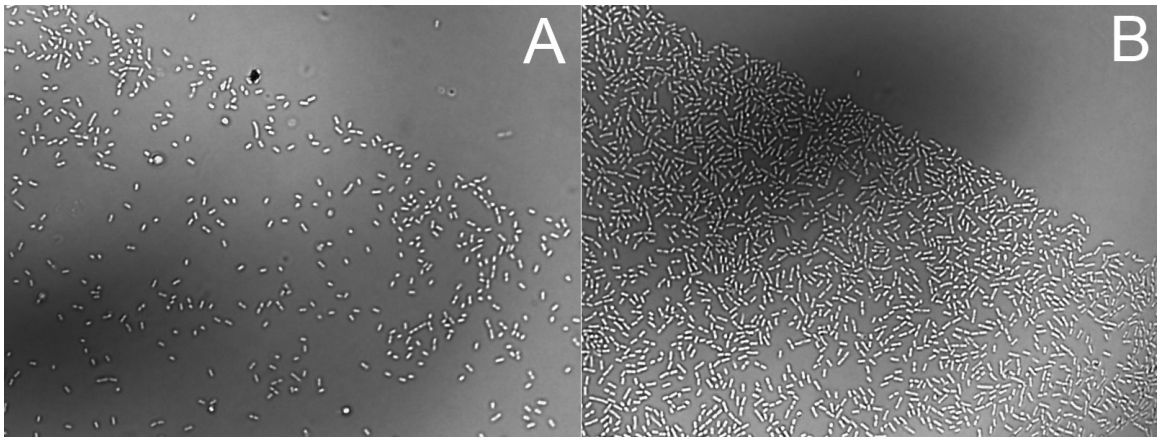
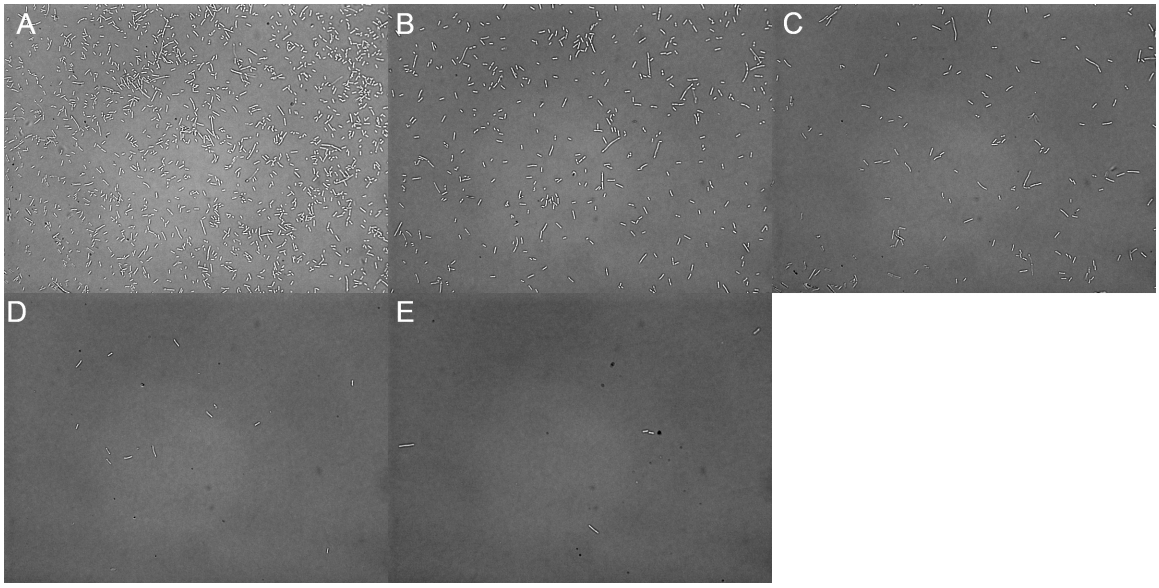


Figure S3 Detection limit of *Salmonella* Typhimurium H72-pBBScfa. The antibody modified substrates (silicon) were incubated in serial diluted bacterial cultures for 45min and observed under optical microscope after rinsing with PBS buffer. The cell concentration is 1.0×10^8 cfu/ml (A), 1.0×10^7 cfu/ml (B), 1.0×10^6 cfu/ml (C), 1.0×10^5 cfu/ml (D), 1.0×10^4 cfu/ml (E). Immobilized cells can be clearly identified at the cell concentration of 1.0×10^4 cfu/ml (panel E).



(1) Suo, Z.; Yang, X. H.; Avci, R.; Deliorman, M.; Rugheimer, P.; Pascual, D. W.; Idzerda, Y. *Anal. Chem.* **2009**, *81*, 7571-7578.

(2) Suo, Z. Y.; Yang, X. H.; Avci, R.; Kellerman, L.; Pascual, D. W.; Fries, M.; Steele, A. *Langmuir* **2007**, *23*, 1365-1374.