## Phenyl-lactic acid is an active ingredient

## in bactericidal supernatants of Lactobacillus crispatus

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## **Supplemental Information**

Supplemental Figure 1. Concentrations of known inhibitory molecules in LC40 CFS. Hydrogen peroxide concentrations were determined by KMNO<sub>4</sub> titration. Organic acid concentrations were determined by UV-Vis spectrophotometry. H<sub>2</sub>O<sub>2</sub> and organic acid concentrations in CFS were lower than concentrations determined to exhibit inhibitory activity against common uropathogens.

CFS Molecule	Inhibitory Concentration	Average Lactobacilli Concentration	LC40 Produced Concentration
Hydrogen Peroxide	2.5mM	0.03 - 2.6mM	11.51uM
Lactic Acid	70mM	62mM	9.52mM
Acetic Acid	8.33mM	13.8mM	7.26mM

Supplemental Figure 2. The killing activity of *L. crispatus* CFS can be restored upon reversion to low pH. CFS samples of *L. crispatus* isolate LC9873 (starting pH 3.9) were modified to pH 3.7 or pH 7.0, incubated on benchtop for 1 hour, and then modified further (3.7 modified to 7.0, 7.0 modified to 3.7) before being tested for killing activity against *E. coli* strain EC9875.

10<sup>10</sup>

10<sup>9</sup>

10<sup>8</sup> 10<sup>7</sup>

10<sup>6</sup>

10<sup>5</sup>

**10**<sup>4</sup>

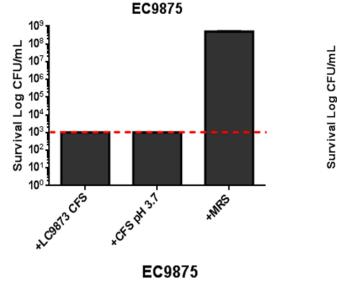
10<sup>3</sup>

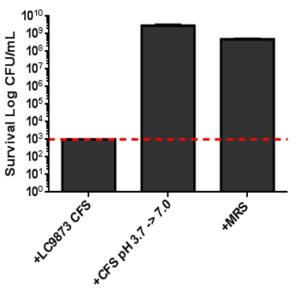
10<sup>2</sup>

10<sup>1</sup>

10<sup>0</sup>

\*109873015



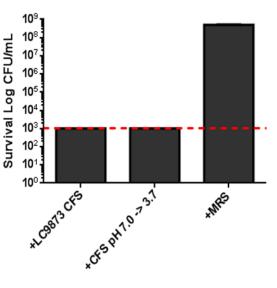


EC9875

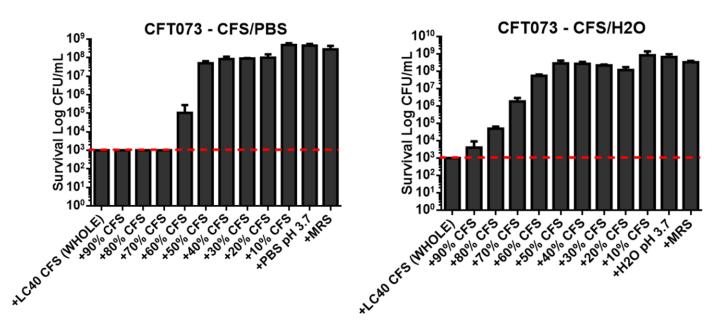
\*NRS

\*ctsph1.9

EC9875



**Supplemental Figure 3. Dilution of CFS abrogates killing activity.** CFS samples from *L. crispatus* isolate LC40 were serially diluted in either pH 3.7 1X PBS (left) or pH 3.7 1X H2O (right) before being tested for killing activity against *E. coli* UPEC strain CFT073.



<u>Supplemental Figure 4 PLA and Methyl-PLA Chemical Structures</u> The chemical structures of both PLA (left) and methyl-PLA (right). Compared to methyl-PLA, PLA exhibited increased pH sensitivity.

