Phenyl-lactic acid is an active ingredient

in bactericidal supernatants of Lactobacillus crispatus

Omar Abdul-Rahim, Qihao Wu, Travis K. Price, Giuseppe Pistone, Katherine Diebel,

Tim S. Bugni, Alan J. Wolfe

Supplemental Information

Supplemental Figure 1. Concentrations of known inhibitory molecules in LC40 CFS. Hydrogen peroxide concentrations were determined by KMNO₄ titration. Organic acid concentrations were determined by UV-Vis spectrophotometry. H₂O₂ 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.

10¹⁰

10⁹

10⁸ 10⁷

10⁶

10⁵

10⁴

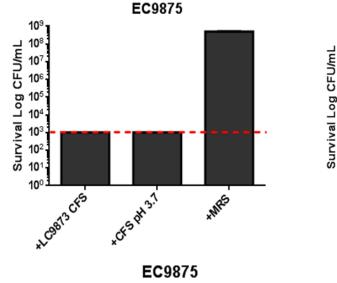
10³

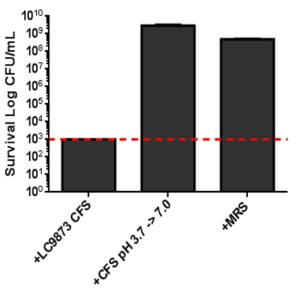
10²

10¹

10⁰

*109873015



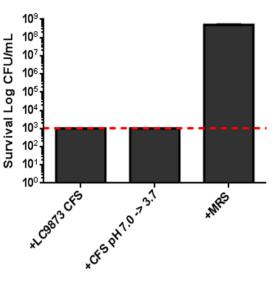


EC9875

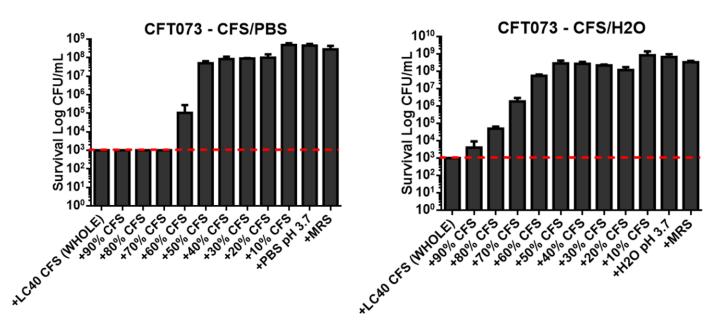
*NRS

*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.

