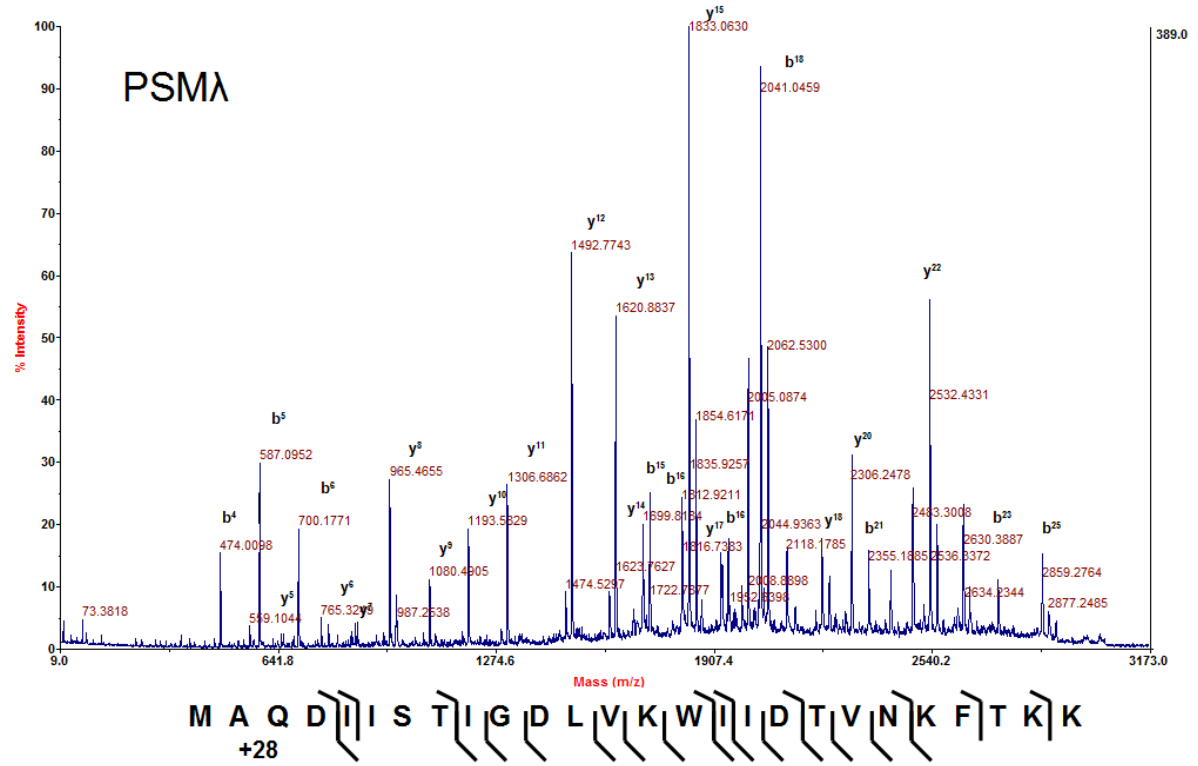
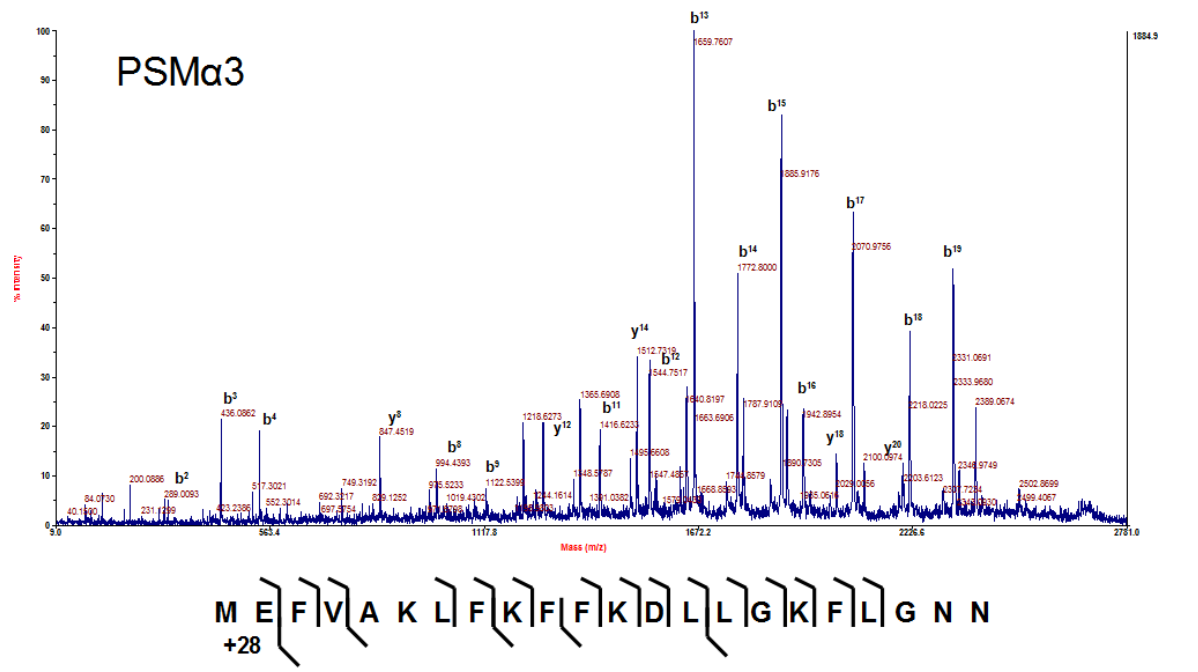
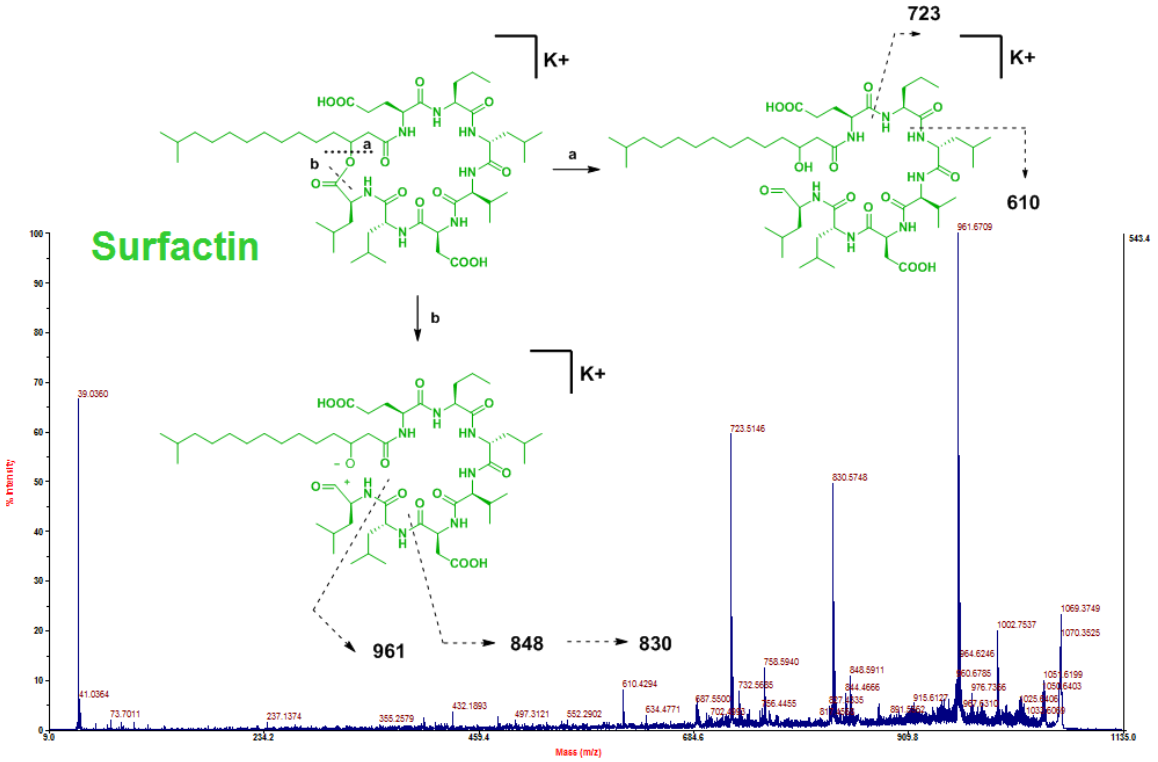


Supplementary Fig. S1. TOFTOF MS spectrum and annotation of delta-toxin (PSMλ), *m/z* 3035, obtained from an *S. aureus* colony co-incubated with *B. subtilis*.

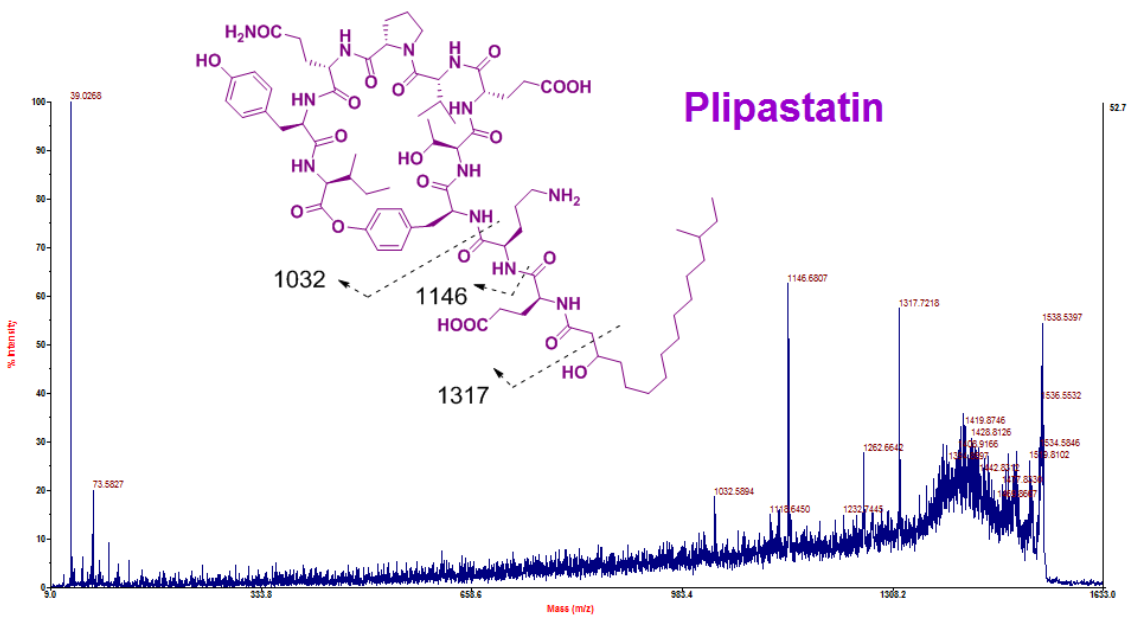


Supplementary Fig. S2. TOFTOF MS2 spectrum and annotation of PSMα3, *m/z* 2635, obtained from an *S. aureus* colony co-incubated with *B. subtilis*.

Gonzalez, D. J., Haste, N. M., Hollands, A., Fleming, T., Hamby, M., Pogliano, K., Nizet, V. & Dorrestein, P. C. (2011). Microbial competition between *Bacillus subtilis* and *Staphylococcus aureus* monitored by imaging mass spectrometry. *Microbiology* **157**, 2485–2492.

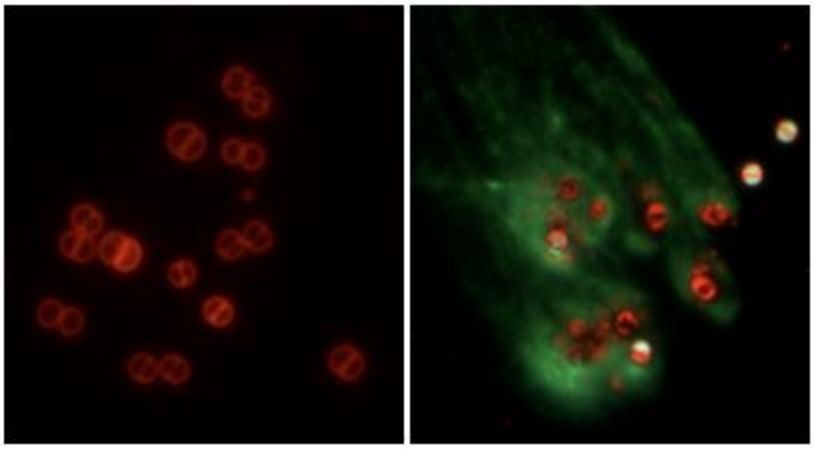


Supplementary Fig. S3. TOFTOF spectrum and fragmentation annotation of surfactin, *m/z* 1075. The surfactin ion was visualized from a *B. subtilis* sample obtained from a colony grown adjacent to *S. aureus*.



Supplementary Fig. S4. TOFTOF spectrum and fragmentation annotation of plipastatin, *m/z* 1544. The plipastatin was visualized from a *B. subtilis* sample obtained from a colony grown adjacent to *S. aureus*.

Gonzalez, D. J., Haste, N. M., Hollands, A., Fleming, T., Hamby, M., Pogliano, K., Nizet, V. & Dorrestein, P. C. (2011). Microbial competition between *Bacillus subtilis* and *Staphylococcus aureus* monitored by imaging mass spectrometry. *Microbiology* **157**, 2485–2492.



Supplementary Fig. S5. Fluorescence microscopy of *S. aureus* incubated with surfactin at 10 and 100 μg ml^{-1}. The dye FM4-64 was used to visualize cell-membrane integrity and the dye SYTOX Green was used to stain nucleic acid, indicating membrane damage. (a) *S. aureus* incubated with surfactin at 10 μg ml^{-1}, a concentration that does not lyse the bacterial wall. (b) *S. aureus* incubated with surfactin at 100 μg ml^{-1}, a concentration that lyses the bacterial cell wall and exposes intracellular nucleic acid.

Gonzalez, D. J., Haste, N. M., Hollands, A., Fleming, T., Hamby, M., Pogliano, K., Nizet, V. & Dorrestein, P. C. (2011). Microbial competition between *Bacillus subtilis* and *Staphylococcus aureus* monitored by imaging mass spectrometry. *Microbiology* **157**, 2485–2492.