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Identification and Imaging of Peptides and Proteins on *Enterococcus faecalis* Biofilms by Matrix Assisted Laser Desorption Ionization Mass Spectrometry

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S1. *In situ* MALDI-MS imaging of identified proteins. It was not possible to obtain MALDI-MS imaging for either the EF1734 or EF1885 proteins due to low S/N for the proteins identified by top down proteomics. However, the bottom up proteomic approach was able to image several proteins on biofilms grown on MALDI-MS plates. Figure S1 shows the spatial localization of trypsin digested peptides in intact *E. faecalis* V583 biofilms with *m/z* 730.1 corresponding to the BioY family protein (a biotin transporter), *m/z* 712.1 corresponding to the GTP binding protein (multifunctional protein family), *m/z* 1389.4 and m/z 1487.4 corresponding to tyrosyl-tRNA synthase (a protein synthesis associated protein), *m/z* 819.3 corresponding to glyceraldehyde-3-phosphate dehydrogenase (a glycolysis enzyme and possible cell surface virulence factor) and *m/z* 659.3 corresponding to an endogenous peak not associated with any protein. The MS images show that different proteins were expressed differentially within the biofilm. For example, the MS images show the GTP binding protein (Figure S1b) was localized more at the periphery of the scanned region while the other proteins were localized more at the center. Furthermore, the peptide peaks at *m/z* 1389.4 and 1487.4 (Figure S1c) displayed very similar spatial distributions, confirming the fact that both these peptides were associated with the same protein, tyrosyl-tRNA synthase. Spatial localization of the endogenous peak at *m/z* 659.3 (not associated with any protein, see Figure S1e) indicates that all proteins identified are localized only in the region where the bacterial cells were more concentrated.

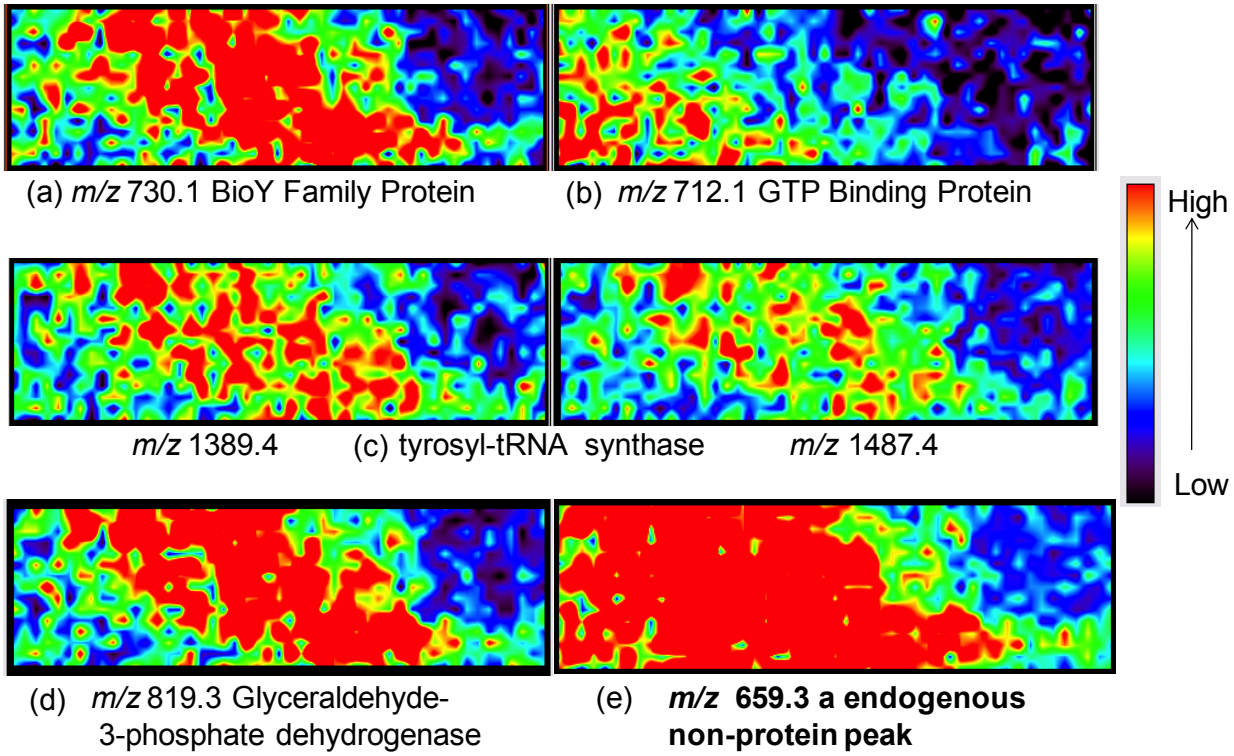

Figure S1: 2 × 7 mm MALDI-MS images of trypsin digested peptides (a to d) with the corresponding protein identification and (e) an endogenous peak indicating the localization of bacterial cells in the biofilm.

S2. Scanning electron microscopy (SEM).

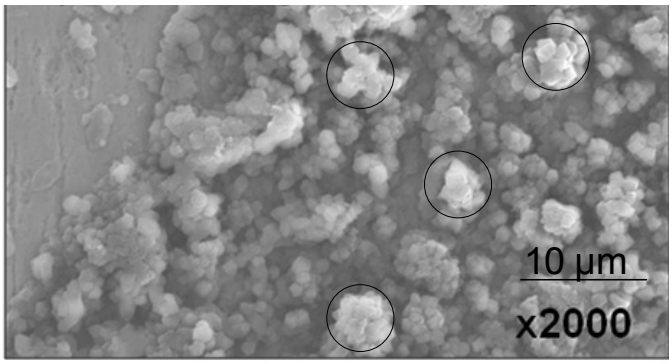

Figure S2: SEM image of *E. faecalis* V583 biofilm sprayed with CHCA matrix showing the cells enclosed in CHCA matrix crystals. The largest matrix crystals formed (circled) are less than 10 µm wide.