# Isolating LiaS From *Enterococcus faecalis* and *Enterococcus faecium*

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In many hospital settings the increased use of antibiotics have allowed for the emergence of deadly multidrug resistant pathogens. Many of these strains of bacteria have developed a Cell Envelope Stress Response (CESR). In *Enterococci* strains specifically, it consists primarily of the LiaFSR signaling pathway, where mutations occur in the nucleotide sequence- leading to early stages of antibiotic resistance. LiaS of this pathway has two transmembrane regions, so the structure and mechanism of this protein is unknown due to its insolubility. In this experiment two strains of *Enterococcus* bacteria were used- *E. faecalis* and *E. faecium*. Primers for each species were designed specifically to clone the LiaS protein without the transmembrane region for increased solubility. Despite this, the amount of soluble protein was not very concentrated, though did slightly increase after being grown in BioSilta media at the optimum temperature. This led to the conclusion that LiaS interacts closely with the cell membrane despite being cloned without its transmembrane region. With the LiaS protein that was purified, *in vitro* phosphorylation of LiaR and crystallization of the His-kinase region of LiaS will be attempted.

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