

THERMOLYSIN DIGESTION OF ESP

Aim

Digesting pro-esp using thermolysin in order to produce a mature, activated form of esp.

Procedure

1. Determine protein concentration using BCA or similar. Alternatively, a Nanodrop can be used to get a rough estimate.
2. Add thermolysin to a final concentration of 10 $\mu\text{g}/\mu\text{l}$.
3. Incubate at 37 °C, for a variety of times to achieve ideal digestion. Incubation times between 1h and 4h should achieve complete digestion.
4. To stop the reaction, add 0.5mmol/l EDTA
5. In order to evaluate digestion efficacy, samples can be run on a SDS-PAGE or Western Blot.
6. Mature Esp can be used for subsequent biofilm assays.

Sources

Sugimoto, S. et al. Cloning, expression and purification of extracellular serine protease Esp, a biofilm-degrading enzyme, from *Staphylococcus epidermidis*. J. Appl. Microbiol. 111, 1406–1415 (2011).