Antimicrobial assay

Standard curve for antimicrobial assay:

1. To obtain a standard curve for CFU/ml with respect to different OD_{600} values (0.1, 0.2, 0.3, 0.4, 0.5), grow the cells to the desired OD_{600} values and plate at 10^{6} dilution.
2. Incubate the plates at +37°C for over 12 hours, and calculate the CFUs corresponding to each OD_{600} value from the plates. Thus, the bacterial concentrations during the antimicrobial assay can be estimated photometrically at 600nm.

Activity testing:

1. Grow bacterial cultures at +37°C until the OD_{600} value reaches 0.05.
2. Incubate bacterial samples with the antimicrobial peptides of interest and appropriate controls at +37°C with shaking for 45 minutes. The samples were incubated at 37°C, shaking for 45 mins with the respective peptides Nisin, DCD1L, LL37 and antibiotic chloramphenicol including negative control.
3. After incubation, measure the OD values of the cells.
4. The bactericidal activity of the tested reagents can be expressed as: [1 – (cells after peptide incubation)/(cells before peptide incubation)] × 100, which represents the percentage of cells that were killed.

Cellulose nanofiber (CNF) binding assay

1. Prepare a 1% CNF mixture (=1g / 100mL). For each CNF+protein mixture use 150µg of CNF and an equal volume of protein solution. Vary the protein concentration (from 0.5µM to 50µM). Alternatively, 200µg of CNF can be mixed with 200µg of protein, and the amount of CNF can be varied (2x, 10x, 100x etc.)
2. Mix the CNF+protein mixtures well by pipetting up and down in an Eppendorf tube. Incubate for 10 minutes - 1 hour at RT.
3. Centrifuge the mixtures at ~4,000 x g for 10 minutes. Transfer the supernatant (containing all the proteins not bound to CNF) to a new Eppendorf tube. The pellet contains CNF and all the proteins that bound to it.
4. Prepare samples for SDS-PAGE: take part of the supernatant and mix it with SDS sample buffer. Heat at +95°C for 5 minutes. As controls prepare samples with the same protein solutions without binding to CNF.
5. Run the samples on 12% polyacrylamide gel. Fix the gel with fixing solution (10% acetic acid, 50% methanol and water mixture) and stain with Coomassie Blue.
6. Image the gel. Any bands that do not appear in the respective protein sample with CNF indicate proteins that have bound to CNF.