

# Tailocins expression and purification

## **solutions:**

- Mitomycin C 500 µg/mL
- Tn50 buffer:
  - NaCl 50 mM
  - MgSO<sub>4</sub>·7H<sub>2</sub>O 8 mM
  - sterile water

## **Method:**

- Grow your Pseudomonas on a NYB (or KB) petri dish (it might take 48 hours to get colonies)
- Early in the morning, start a liquid culture with one colony from your plate in 100 mL of NYB
- Incubate at 30 °C until O.D. reaches approximately 0.4-0.6
- Add 0.5 µL/mL of mitomycin C and incubate overnight, the culture should clear up due to the lysis of the cells
- Centrifuge to eliminate the remains of cells
- Filter the supernatant with 0.22 µm filters, from here all the steps should be done in sterile conditions
- Add 10% (w/v) of PEG6000 or PEG8000, shake until all is dissolved
- Incubate overnight at 4°C under gentle agitation (the tubes should lay flat)
- The next morning, centrifuge 1h at 10'000 rpm in the centrifuge (mark the side of the tube on which the pellet should form, it might not be visible)
- Resuspend the pellet in 5 mL of Tn50 buffer (pipette up and down several times, scratching the side of the tube where the pellet should have formed)
- Ideally, incubate one more night under gentle agitation at 4 °C to completely dissolve all PEG particles (we obtained good results without doing it though).
- Keep the purified tailocins in the fridge at 4°C, they might lose some activity with time but can be conserved for several weeks at least.

## Soft agar overlay assay

- overnight liquid culture of the target strain in NYB
- melt LB soft agar medium in the microwave
- pour 5-6 mL of molten LB soft agar in a falcon tube, wait until the medium is warm but not hot (do not wait too long or the medium will solidify)
- inoculate the medium with 60  $\mu$ L of liquid culture of the target strain
- Pour the inoculated LB soft agar on a NA medium plate, tilt in all directions in order to make sure the soft agar covers the whole plate (be fast, it solidifies quickly)
- Spot drops of 2-5  $\mu$ L of your purified tailocins on the plate, let them dry before .
- Incubate overnight at room temperature (leaving the plates on the bench is fine) and observe the formation of lysis plaques.
- To differentiate tailocins from phage killing, spot a 1:2 serial dilution of your extract. Phages will produce small individual lysis plaques when diluted whereas tailocins only produce a fainter big plaque.

# Electron microscopy (EM) protocol

## Materials

- A sufficient amount of your sample: we used 100  $\mu\text{L}$  for each tailocins extraction (0.5  $\mu\text{g}/\text{mL}$  and 3  $\mu\text{g}/\text{mL}$  of mitomycinC, control)
- Electron microscope
- Uranyl acetate 1%
- 400mesh carbon coated copper EM grids.

Disclaimer: Our EM pictures were taken by a professional at the EM facility at University of Lausanne, Switzerland

## Instructions

3 $\mu\text{L}$  of phage suspension was adsorbed on a glow-discharged copper 400 mesh grid coated with carbon (EMS, Hatfield, PA, US) during 1minute at RT. Then they were washed with three drops of distilled water followed by staining with uranyl acetate (Sigma, St Louis, MO, US) 1% in  $\text{H}_2\text{O}$  for 1 minute. Excess of uranyl acetate was drained on blotting paper and the grid was dried for 10 minutes before image acquisition.

Micrographs were taken with a transmission electron microscope Philips CM100 (Thermo Fisher Scientific, Hillsboro, USA) at an acceleration voltage of 80kV with a TVIPS TemCam-F416 digital camera (TVIPS GmbH, Gauting, Germany).