

OD₆₀₀ test

Aim: The aim is to test the activity of our *Bacillus subtilis* strains (particularly the lysostaphin effect) on *Staphylococcus epidermidis* or *aureus* cultures.

Protocole

Day 1:

- Make liquid cultures of the *Bacillus* strains you want to test in 5 mL of TSB. Incubate overnight at 30 °C.
- Make a liquid culture of *S.epidermidis* in 5ml of LB. Incubate overnight at 37°C.

Day 2:

- Before starting, switch the spectrophotometer on.
- Measure the OD₆₀₀ of each culture. Continue the incubation of *Bacillus* cultures until all have the same OD₆₀₀ (OD₆₀₀ ≈ 4).
- Centrifuge each culture 4 minutes at 5000 rpm.
- For *S.epidermidis* culture: remove the supernatant and add M63 or another non nutritive medium to obtain a culture with an OD₆₀₀ ≈ 0,8.
For *Bacillus* cultures, filter the supernatants on 0,22 µm filters.
- Put 0,5mL of *S.epidermidis* culture and 0,5mL of *Bacillus* cultures you want to test in each spectrophotometer tank.
- Make the blank with the appropriate medium mix. Measure OD₆₀₀ during 6 or 12 hours. Don't forget to shake the culture 1 minute before each measure to homogenize the solution.

