

## Germination assay

(Jennifer Emenegger + Jara Radeck, 2012)

DSM

### Day 1:

- Inoculate your strains in DSM with antibiotics and let grow overnight. Let grow for 2 days if strain is growing slowly. Use W168 and spo0A mutant as a control

### Day2:

- Prepare 6 plates for each strain (LB+AB)
- Make 3 aliquots à 500µl into 1.5 ml Eppis. One of them gets diluted and plated right away. The two others get heat shocked (at least 60°C for at least 40 minutes).
- Dilute and plate the heat shocked cells
- Pour 500µl 1%Agarose (From the gel room) into the second tube of hat shocked cells (still hot). Mix and take 3µl into the Neubauer-Zählkammer.
- Count spores and cells in 2 group-squares (contains 4x4 of the smallest squares) and calculate the average

### How to make the dilutions:

Prepare 3 Eppis for each dilution. The first contains 900µl of DSM, the second and third 990µl each. Add 100µl of cells (=10<sup>-1</sup>) and plate 100µl (=10<sup>-2</sup>)

Add 10µl from the 10<sup>-1</sup> dilution to the second Eppi (=10<sup>-3</sup>) and plate 100µl (10<sup>-4</sup>).

Add 10µl from the 10<sup>-3</sup> dilution to the third Eppi (=10<sup>-5</sup>) and plate 100 µl (10<sup>-6</sup>).

Incubate plate at 37°C and check for colonies regularly.

### Day 3 or 4:

Note the amount of colonies and the dilution. In non-heat shocked samples, spores and cells should all give colonies.

Cells+spores/ml= amount of colonies/dilution.

Example: If the 10<sup>-6</sup> plate has 150 Colonies: cells/ml=150/10<sup>-6</sup>=150\*10<sup>6</sup>=150 000 000/ml=1.5\*10<sup>8</sup>/ml

For the heat-shocked samples, not the cells, but only the germinable spores give colonies.

To calculate the cell density from the cell counting:

Take the average amount of spores per group-square (e.g. 35).

Spores/ml=counted spores\*2/Volume=counted spores\*2/[8\*10<sup>-7</sup>ml]=counted spores\*2 500 000/ml

Example: 35\*2 500 000/ml=87 200 000/ml

[\*2 because of the Agarose-addition]