Germination assay

DSM: see protocol „Media for *Bacillus subtilis*".

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 3 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^{-1}) and plate 100µl (=10^{-2})

Add 10µl from the 10^{-1} dilution to the second Eppi (=10^{-3}) and plate 100µl (10^{-4}).

Add 10µl from the 10^{-3} dilution to the third Eppi (=10^{-5}) and plate 100 µl (10^{-6}).

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 chould all give colonies.

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

Example: If the 10^{-6} plate has 150 Colonies: cells/ml=150/10^{-6}=150*10^{6}=150 000 000/ml=1.5*10^{8}/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^{-7}ml]=counted spores*2 500 000/ml

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

[*2 because of the Agarose-addition]

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