Bacillus subtilis – Germination Protocol

Materials

- Tryptone
- Yeast extract
- NaCl
- H$_2$O$_4$
- Agar
- Becker
- Measuring cylinder
- DSM medium

Apparatus

- Neubauer Chamber
- Autoclave
- Digital analytical balance
- Incubator
- Drigalski spatula

Methods

Part 1 – preparation of spore solution

1. First step, follow our Sporulation protocol. As result, now you have a solution with both bacteria and spores.

2. Take 10 μL of the resulting solution to follow the Cell counting with Neubauer Chamber protocol. It is used to distinguish between spores and bacteria, so that sporulation efficiency can be measured. The spore number is also important to know the germination rate. After counting, safely discard this sample.

3. Heat a sample until reach 120°C in an autoclave. When this temperature is reached, turn off the autoclave immediately. Now you have only spores alive in your solution.
Part 2 – Spore germination using LB Medium (10X)²

1. Add 100 g of tryptone, 50 g of yeast extract, 100 g of NaCl and 15 g of agar in a becker;
2. Dissolve components in 1000 mL of H₂Od;
3. Sterilize the medium by autoclaving for 20 minutes.
4. Seed in Petri plate.
5. To check spores viability, prepare dilutions using the protocol described in part 1.
6. Use 3 microtubes (1.5 mL) for each dilution. The first contains 900 μl of DSM, the second and third 990 μl each.
7. Add 100 μl of cells (=10⁻¹) and plate 100 μl (=10⁻²) to the first microtube.
8. Add 10 μl from the 10⁻¹ dilution to the second microtube (=10⁻³) and plate 100 μl (10⁴).
9. Add 10 μl from the 10⁻³ dilution to the third microtube (=10⁻⁵) and plate 100 μl (10⁶).
10. Incubate all plates at 37°C. After 12h check for colony formation periodically.
11. Calculate the spore's viability (concentration) following this formula:

   a. Viable spores/ml = amount of colonies/dilution.

12. Comparing the result with spore concentration counted at Neubauer chamber, the germination efficiency is achieved.

References: