

Bacillus subtilis – Germination Protocol

Materials

- Tryptone
- Yeast extract
- NaCl
- H₂O_d
- Agar
- Becker
- Measuring cylinder
- DSM medium

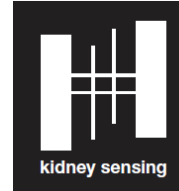
Apparatus

- 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 immediately¹. 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 H₂O;
3. Sterilize the medium by autoclaving for 20 minutes.
4. Shed 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 (10⁻⁴).
9. Add 10 µl from the 10⁻³ dilution to the third microtube (=10⁻⁵) and plate 100 µl (10⁻⁶).
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:

1. Edwards JL Jr, Busta FF, Speck ML. Thermal inactivation characteristics of *Bacillus subtilis* spores at ultrahigh temperature. *J. Appl Microbiol.* 1965 Nov; **13(6)**:851-7.
2. Ghosh S, Setlow P. The preparation, germination properties and stability of superdormant spores of *Bacillus cereus*. *J Appl Microbiol.* 2010 Feb; **108(2)**:582-90.