Sporulation

1. Overnight culture

- Inoculate your culture of *Bacillus subtilis* in 4 ml LB-medium
- Let them grow overnight at 37°C, 200 rpm

2. Exponential growth

- Measure the OD_{600} of your overnight culture and dilute it in LB-medium to an OD_{600} off around 0.1-0.2/ml in 10 ml LB
- Let the cells grow to an OD_{600} of 0,8/ml (37°C, 200 rpm)

3. Sporulation

- Centrifuge 10 ml of the cells at 13,000 x g for 1 minute
- Wash the pellet with 1 x PBS
- Re-suspend the pellet in 5 ml DSM (Difco Sporulation Medium)
- Let the cells grow for 24 hours at 37°C (200 rpm)

4. Lysozyme treatment (additional for spore purification)

- Treat the samples with lysozyme (15 mg/ml) at a dilution of 1:6
- Incubate for 1 h at room temperature
- Wash 6 times with 1 x PBS

5. Additional:

→ count spores using a Neubauer improved counting chamber and make aliquots with a defined number of spores per aliquot (e.g. 100 Million spores per 500 µl)

(Note: always use fresh DSM since the $FeSO_4$ is rusting when it is in dilution)

Data from the purification:

In forward and side scatter you can easily distinct the difference between the purified and unpurified samples (figure 5). Purification leads to a better and solid read out in

further experiments and applications.

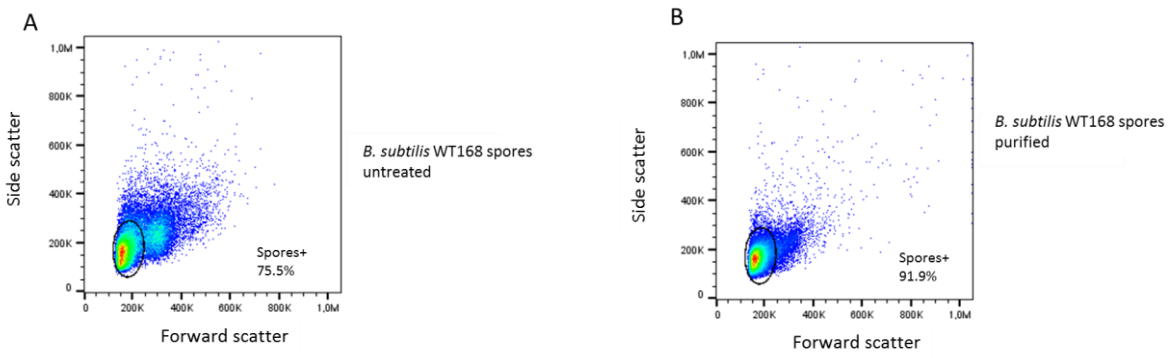

Figure 7. *Bacillus subtilis* spore purification.
(A) The spores of *Bacillus subtilis* WT168 were analyzed using flow cytometry. The set gate for spores shows that 75.5% of the sample consisted of spores. (B) The spores of *Bacillus subtilis* WT168 were treated with lysozyme for 1 h. The flow cytometry analysis shows that after purification the amount of spores is considerably higher with 91.9%.

DSM – Sporulation Medium

8 g	Nutrient Broth
1 g	KCl
1 ml	$MgSO_4$ (1 M)
1 ml	$MnCl_2$ (10 mM)
1000 ml	Aqua bidest

Autoclave and add:

0.5 ml $CaCl_2$ (1 M)
1 ml $FeSO_4$ (1 M)