GROWTH CONDITION, STORAGE, MEDIA & ANTIBIOTICS FOR *BACILLUS SUBTILIS*

Growth conditions:

B. subtilis growth best at 37°C and has a doubling time of 30 min. Always use flasks that comprise at least 5x times the volume of media used, and always use lids that are able to allow air passage. *B. subtilis growths strictly aerobe!*

Storage:

We use three types of storage conditions for *B. subtilis* cells.

1. **Glycerol-stocks** for long-term storage and back-up: Freeze two independent clones of your *B. subtilis* strain at -80°C with glycerol (1.3 ml fresh overnight culture + 50% glycerol).

2. **DSM-Plate:** Long-term storage for frequent used strains. Strike out your cells on a DSM plate. *B. subtilis is able to produce spores, that are very resistant to all kind of environmental conditions. So storage of spores is a reliable way of storage.*

3. LB-plates: For everyday use you can leave your *B. subtilis* strains on LB-plates **at room temperature.** ***Note: B. subtilis is cold sensitive, never store B. subtilis cells at 4°C.***

Media

Luria-Bertani (LB) broth:

Tryptone	10 g
Yeast extract	5 g
NaCl	10 g
H_2O (dest)	ad 1.000 ml

- for LB plates: add 15 g/l of agar
    - important: cool down the agar solution to 50°C before adding antibiotics

Difco Sporulation Medium (DSM):

Nutrient Broth	8 g

KCl	1 g
$MgSO_4$ (1 M)	1 ml
$MnCl_2$ (10 mM)	1 ml
H_2O (bidest)	ad 1.000 ml
Add after autoclave:	
$CaCl_2$ (1 M)	0,5 ml
$FeSO_4$ (1 mM)	1 ml

- for DSM plates: add 15 g/l of agar
    - important: cool down the agar solution to 50°C before adding antibiotics

Starch plates:

Nutrient Broth (Difco)	7,5 g
Starch	5 g
Agar	15 g
H_2O (dest)	ad 1.000 ml

Chemical defined medium (CSE):

1×C-Salts	100 ml
Tryptophan (5 mg/ml)	1 ml
Ammoniumeisencitrat (2,2 mg/ml)	1 ml
III'-Salts	1 ml
Potassium glutamate (40%)	2 ml
Sodium succinate (30%)	2 ml

5×C-Salts (1 l)

KH_2PO_4	20 g
$K_2HPO_4 \times 3\ H_2O$	80 g

$(NH_4)_2\ SO_4$	16,5 g

III'-Salts (1 l)

$MnSO_4 \times 4\ H_2O$	0,232 g
$MgSO_4 \times 7\ H_2O$	12,3 g

- autoclave (or filtrate) each component separately and put them together freshly before starting your experiment
- Optionally: addition of media additives, for example pyruvate (0.5% final concentration) or glucose (1% final concentration)

Antibiotics

- Indicated are 1.000-times stock solutions
- Dissolve in the specific solvent and filtrate by using 0.2 µm filters
- Store at -20°C

Strain	Antibiotic	Concentration	Dissolve in	Color code
B. subtilis	Kanamycin	10 mg/ml	H_2O	Black (one bar)
	Chloramphenicol	5 mg/ml	70% ethanol	Blue
	MLS selection:			Red
	Erythromycin	1 mg/ml	70% ethanol	
	Lincomycin	25 mg/ml	H_2O	
	Spectinomycin	100 mg/ml	H_2O	Purple
	Tetracyclin	10 mg/ml	H_2O	Orange
	Bacitracin	50 mg/ml	H_2O	-
E. coli	Ampicillin	100 mg/ml	H_2O	Green
	Kanamycin	50 mg/ml	H_2O	Black (two bars)
	Streptomycin	20 mg/ml		
Rhodobacter	Tetracyclin	1 mg/ml	H_2O	Orange
	Spectinomycin	25 mg/ml	H_2O	Purple
	Kanamycin	25 mg/ml	H_2O	Black

Further substances

- IPTG: 1 M (dissolve in H_2O; store at -20°C)
- X-Gal: 100 mg/ml (dissolve in DMF; color code: blue X; store at -20°C)
- BCIP (5-Brom-4-chlor-3-indoxylphosphat-p-toluidinsalz): 50 mg/ml (dissolve in DMF; store at -20°C)