WCSB, Wageningen University	IBISBA-SOP-WU16
	Version 1.0

EPP - Standard Operating Procedure

(only for selected experiments intended to transfer results from one lab to the other)

Title: Cultivation of *P. putida* (and *E. coli*) in rich-, minimal-, and selective medium

distribution list			
changes to prior version:			
	name	signature	date
Experimenter 1	Rita Volkers		26/3/2019

Instruction

Cultivation of P. putida (and E. coli) in rich-, minimal-, and selective medium

1. Introduction / Purpose

Described here are the recipes of rich (LB)-, minimal (M9 and deBont)-, and selective media used for cultuvation of *P. putida*. *E. coli* can also be cultivated in the rich- and selective media.

Keywords: Cultivation – medium – *E. coli* – *P. putida* – rich – selective – minimal

2. Equipment and chemicals

2.1. Equipment

2.2. Chemicals

3. Media and Buffers

Rich medium

- **LB (1 L):** 10 g Tryptone + 5 g Yeast Extract + 10 g NaCl (Sterilization by autoclaving)
- **LB-agar (1 L):** 10 g Tryptone + 5 g Yeast Extract + 10 g NaCl + 7.5 g Bacteriological agar (Sterilization by autoclaving) * Store at > 55°C for less than 2 days to keep it liquid before pouring plates

Minimal media
deBont minimal medium (DB) components:

- **100x deBont buffer (1L):** 388 g K_2HPO_4 + 163 g NaH_2PO_4 (or 212 g $NaH_2PO_4 \cdot 2H_2O$) (Sterilization by autoclaving; check if pH+7.0; concentration 3.6 M)
- **100x $(NH_4)_2SO_4$:** 200 g $(NH_4)_2SO_4$ (Sterilization by autoclaving)
- **100x deBont trace metals:**

Component	**Stock (L^{-1})**
EDTA	1000 mg
$MgCl_2 \cdot 6H_2O$	10 g
$ZnSO_4 \cdot 7H_2O$	200 mg

$CaCl_2 \cdot 2H_2O$	100 mg
$FeSO_4 \cdot 7H_2O$	500 mg
$Na_2MoO_4 \cdot 2H_2O$	20 mg
$CuSO_4 \cdot 5H_2O$	20 mg
$CoCl_2 \cdot 6H_2O$	40 mg
$MnCl_2 \cdot 2H_2O$	100 mg

- EDTA must be added first and dissolved completely before adding the rest. Add 100 mg of EDTA to 25 ml of water and add drops of 10M NaOH until the EDTA is completely dissolved. Add this solution to the rest of the water and bring the pH back to 4 with concentrated HCl.
- The solution should be clear, initially green, then yellow and finally pink upon longer storage. Store in a cool, dark place.
- (Sterilization by autoclaving)
- **Glucose stock 2M (1 L):** 360.3 g Glucose (Sterilization by autoclaving or filtering to prevent possible caramelization)

DB medium composition:

- **DB (1 L):** 100 ml Glucose stock 2M + 10 ml 100x deBont buffer + 10 ml 100x $(NO_4)_2SO_4$ + 10 ml 100x Trace elements + 870 ml autoclaved dH_2O
- **2.2 % (w/v) agar (1 L):** 22 g Agar
- **DB-agar (1 L):** 100 ml Glucose stock 2M + 10 ml 100x deBont buffer + 10 ml 100x $(NO_4)_2SO_4$ + 10 ml 100x Trace elements + 233 ml autoclaved dH_2O + 637 ml 2.2% (w/v) agar

M9 medium components:

- **5x M9 medium salts (1 L):** 56.4 g Sigma Aldrich 5xM9 salts mix (Sterilization by autoclaving)
- **20% (w/v) glucose (1 L):** 200g glucose monohydrate (Sterilization by autoclaving or filtering to prevent possible caramelization)
- **$MgSO_4$ 1 M (1 L):** 24.65 g $MgSO_4 \cdot 7\ H_2O$ (Sterilization by autoclaving)
- **$CaCl_2$ 1M (1 L):** 14.07 g $CaCl_2 \cdot 2\ H_2O$ (Sterilization by autoclaving)
- **Thiamin (50 mL):** 50 mg thiamin-HCl (Filter sterilization 0.22 µm filter)

M9 medium composition:

- **M9 (1 L):** 200 ml M9 salts + 150 ml 20% (w/v) Glucose + 1 ml 1M $MgSO_4$ + 300 µl 1M $CaCl_2$ + 1 ml Thiamin + 10 ml 100x deBont trace metals + 637 ml autoclaved dH_2O
- **M9-agar (1 L):** 200 ml M9 salts + 150 ml 20% (w/v) Glucose + 1 ml 1M $MgSO_4$ + 300 µl 1M $CaCl_2$ + 1 ml Thiamin + 10 ml 100x deBont trace metals + 637 ml 2.2% (w/v) agar

Selective medium

- Selective medium is a medium of choice plus antibiotics.

- Antibiotics are prepared in 1000x stocks in such way that the volume of antibiotic in microliters that had to be added to a culture is the total volume of the culture in milliliters (e.g. Liquid culture of 20 ml of LB medium would require 20 µl of antibiotic). All the antibiotics are dissolved in water and sterilized by filtering unless otherwise specified:

- **Ampicillin 150 mg/ml (10 ml):** 1.5 g Ampicillin
- **Kanamycin 50 mg/ml (10 ml):** 0.5 g Kanamycin
- **Streptomycin 50 mg/ml (10 ml):** 0.5 g Streptomycin
- **Gentamicin 10 mg/ml (10 ml):** 0.1 g Gentamicin
- **Carbonicillin 500 mg/ml (10 ml):** 5 g Carbonicillin
- **Chloramphenicol 30 mg/ml (10 ml):** 0.3 g Chloramphenicol in 100% ethanol

- Add the desired antibiotic to the medium before use. The medium should not be hot (not above a hand-warm temperature), otherwise the antibiotics will break down.

4. Procedures

Cultivate *P. putida* at 30°C with shaking at 250 rpm in liquid medium or on solid medium without shaking. Cultivate *E. coli* at 37°C with shaking at 250 rpm in liquid medium or on solid medium without shaking.

5. Remarks / troubleshooting

6. Biosafety

No biosafety issues are associated with this protocol.

7. Acknowledgements

IBISBA (grant agreement No 730976) and EmPowerPutida (grant agreement No 635536) projects have received funding from the European Union's Horizon 2020 research and innovation programme.

