ITB, Stuttgart	EPP-SOP-ITB01
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EPP - Standard Operating Procedure

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

Title: Applying two-phase based whole cell system for bioconversions

distribution list

changes to prior version:

	name	signature	date
experimenter 1	Max-Philipp Fischer		10th April 2018

Instruction

Applying two-phase based whole cell system for bioconversions

1 Introduction / Purpose

For LinD catalyzed bioconversions resulting in highly volatile or gaseous molecules, a two-phase based whole cell system was established. Here, a whole cell reaction mixture is used for the bioconversion, while a second organic phase consisting of cyclohexane is added thereto in order to trap the products. Analysis can then be performed by injection into GC/MS directly out of the second phase.

2 Equipment and chemicals

2.1 Equipment

Shimadzu GC/MS-QP2010 equiped with PAL AOC-5000 Auto Injector
column: HP-5msi (30 m, 0.25 mm, 0.25 µm; Agilent technologies)

2.2 Chemicals

Cyclohexane (99.5 %, Roth)

2.3 Bacterial strains

E. coli Jm83(DE3)

3 Media and buffers

3.1 LB agar

10 g/L tryptone

5 g/L yeast extract

5 g/L sodium chloride

15 g/L agar agar

3.2 LB *medium*

10 g/L tryptone

5 g/L yeast extract

5 g/L sodium chloride

3.3 TB medium

12 g/L tryptone

24 g/L yeast extract

5 g/L glycerol

10 % (v/v) KPi buffer (170 mM KH_2PO_4, 720 mM K_2HPO_4)

3.4 Reaction buffer

100 mM NaPi containing 2 % (w/v) sorbitol, pH 6.0

3.5 DTT solution

20 mM DTT in reaction buffer

3.6 Substrate stock

200 mM substrate (2-methyl-3-buten-2-ol or 3-buten-2-ol) in DMSO

4 Procedures

4.1 Expression

E. coli Jm83(DE3) containing the LinD plasmid precultures were grown o/n at 37 °C and 180 rpm in 4 mL LB media. Main cultures were inoculated with 1 % (v/v) main culture in 4 mL TB media. Induction was performed after incubation for 4 h at 37 °C and 180 rpm using 1 mM final concetration of IPTG. Expression was performed o/n at 16 °C and 180 rpm.

4.2 Bioconversions

Cells were harvested and resuspended in 3 mL reaction buffer. 850 µL of the cell suspension was transferred into a 2 mL GC vial and 100 µL DTT solution was added. After addition of 900 µL cyclohexane, the reaction was started by addition of 50 µL substrate solution and the vial sealed airtight. The reactions were incubated for 18 h at 30 °C and 180 rpm. As controls, samples containing buffer instead of cell suspension and 10 mM of substrate as well as ones containing 10 mM of the product isoprene were treated analogously.

4.3 Analysis

The GC vials containing the reaction mixtures were measured directly without any further extraction steps. Therefore, 1 µL of the organic cyclohexane phase was injected into GC/MS (split ratio: 50 (2-methyl-3-buten-2-ol) or 20 (3-buten-2-ol); injection temperature: 250 °C). Helium served as carrier gas with a constant pressure of 26.7 kPa and the detection temperature was 280 °C. Measuring was performed isothermally at 70 °C for 3 min in case of 2-methyl-3-buten-2-ol and at 40 °C for 2 min in case of 3-buten-2-ol, respectively. Substrate 2-methyl-3-buten-2-ol and product isoprene were quantified in SIM mode with selected m/z ions of 71.0 and 67.0, respectively, with retention times of 1.65 min and 1.56 min, respectively, using calibration curves. Product butadiene was quantified in SIM mode with selected m/z ion of 54.0 with retention time of 1.47 min.

5 remarks/troubleshooting:

- GC vials need to be closed thoroughly in order to prevent evaporating of the products
- Water miscible substrates cannot be detected due to lack of real extraction step
- *E. coli* Jm83(DE3) is well tolerable against cyclohexane

6 Biosafety:

No biosafety issues were associated with this protocol when applied to *E. coli*. The protocol was developed and performed at an S1 laboratory. The pipetting of cyclohexane as well as isoprene was performed in the fume hood due to the harmfulness of cyclohexane vapors (H225-304-315-336-410) and isoprene vapors (H: 224-350-341-412). For protection against these compounds, nitrile gloves are to be worn at all time when handling the samples.