ITB, Stuttgart	EPP-SOP-ITB04
	Version 1.0

EPP - Standard Operating Procedure

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

Title: Selection of suitable *Pseudomonas putida* genetic backgrounds for small alcohols biotransformations

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experimenter 1	Wendy Escobedo		1st April 2019

Instruction

Selection of suitable Pseudomonas putida genetic backgrounds for small alcohols biotransformations

1 Introduction / Purpose

An efficient whole cell biocatalysis process relies on several factors, including the selected genetic background for heterologous protein expression. Thus, in order to avoid low product yields due to substrate and/or product consumption, or potential instability of the desired product owed to native enzymatic activities, it is important to assess stability of both substrate and expected product in the selected strain. The aim of this protocol is to identify the most suitable *P. putida* strains towards small alcohols stability, such as n-butanol, isobutanol and crotyl alcohol. We selected six different *P. putida* genetic backgrounds KT2440, GN346, EP1, EP2, EM42, and EM383, since those strains harbor different genetic features, regarding alcohol dehydrogenases or flagella assembly, in comparison with the parent strain KT2440 (figure 1).

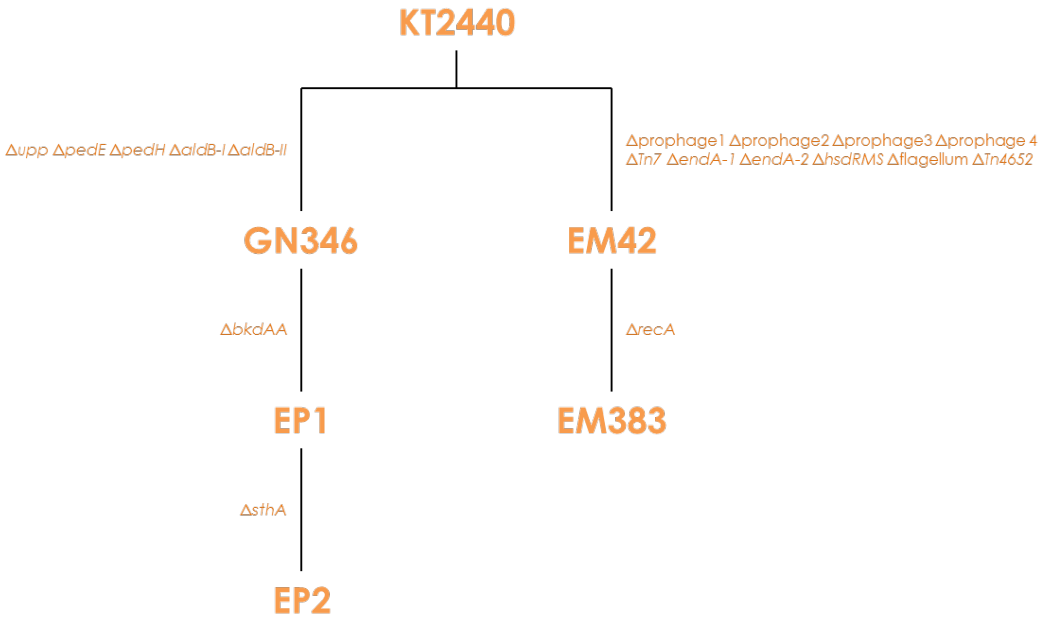

Figure 1. Genealogical tree of selected *P. putida* strains

2 Equipment and chemicals

2.1 Equipment

Spectrophotometer ultrospec 3100 pro
Clean bench Walner electronics
Fume hood Walner electronics

2.2 Chemicals

Isobutanol (2-methyl-1-propanol) (Fluka Chemika 99.5% GC, Cat. No. 58450)
1-butanol (Normapur 99.5%, Cat. No. 20810.323
Crotyl-alcohol (2-Buten-1-ol) (Alfa Aesar 96%, Cat. No. A10681)

2.3 Bacterial strains

P. putida KT2440
P. putida GN346
P. putida EP1
P. putida EP2
P. putida EM42
P. putida EM383
E. coli BL21(DE3)

3 Media and buffers

3.1 High-salt LB agar

10 g L^{-1} tryptone
5 g L^{-1} yeast extract
10 g L^{-1} sodium chloride
15 g L^{-1} agar agar

3.2 High-salt LB medium

10 g L^{-1} tryptone
5 g L^{-1} yeast extract
10 g L^{-1} sodium chloride

3.3 Buffer solution for resting cells (pH 7)

5.23 g L^{-1} KH_2PO_4
10.71 g L^{-1} K_2HPO_4
9 g L^{-1}Glucose

3.4 Substrate stocks (prepared in buffer solution)

Isobutanol (200 mM)
1-butanol (200 mM)
Crotyl alcohol (200 mM)

4 Procedures

This protocol is useful to identify the most suitable *P. putida* strains towards small alcohols stability, such as n-butanol, isobutanol and crotyl alcohol.

4.1 4.1 Resting cells production

Precultures: *E. coli* BL21(DE3) and the six selected *P. putida* genetic backgrounds KT2440, GN346, EP1, EP2, EM42, and EM383, were inoculated by triplicate from single isolated colonies in 5 mL high-salt LB media. Precultures were incubated o/n at 30°C and 180 rpm.

Cultures: biological triplicate cultures were inoculated with 1% (v/v) preculture in 4 mL high-salt LB media until stationary phase was reached. Optical density was measured over time, since all seven tested strains have different duplication times. Cultures were incubated o/n at 30°C and 180 rpm.

Resting cells production: For each strain, cultures were centrifuged (4,000 rpm, 4°C, 20 min). Cell pellets, were sequentially washed and centrifuged three times with cold buffer solution. Afterwards, cell pellets were suspended in sufficient cold buffer solution to reach an optical density of 0.5.

4.2 Assessment of stability/consumption of small alcohols in the different genetic backgrounds

For each strain, deep well plates containing 2 mL of resting cell solution (biological triplicates) were supplemented with 20 µL of each one of the different substrates to give a final concentration of 20 mM. Plates were tightly sealed with silicon mats and three layers of aluminum seals on top to avoid substrate decrease due to evaporation. Samples were incubated at 30°C and 180 rpm.

4.3 Analysis

In order to follow potential consumption of small alcohols in the different genetic backgrounds, samples were taken at different time points; 2, 4, 6, and 18 h. Further sample treatment regarding extraction and GC-MS analysis is described in the complimentary downstream protocol EPP-SOP-ITB06.

5 remarks/troubleshooting:

It is highly recommended to use always freshly prepared substrate stocks, since small alcohols are very volatile.

6 Biosafety:

No biosafety issues were associated with this protocol. The protocol was developed and performed at an S1 laboratory. The pipetting of small alcohols was performed in the fume hood due to the harmfulness of vapors (H226-315-318-335-336). For protection against the employed substrates, nitrile gloves are to be worn at all time when handling the samples.

7 Acknowledgements

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