Flask Expression

Date: 2022-08-17
Tags: *1_Wetlab 3_Expression*
Created by: Stefanie Brands

(Written by Stefanie Brands_)

(_Last update: 2022.08.18_)

After cloning or receiving a new strain, flask expression is conducted with the aim to generate an active enzyme or folded protein.

{29.09.2017|Date of experiment}

Overnight precultures of {P. putida KT2440 pVLT33::pigC|expression strain} and the {empty vector strain (:as:)|:negative control:} are grown from a {single colony|inoculum} in {5|mL|:LB Kan:|growth media} at {30|°C|temperature} and {250|rpm|shaking} {overnight|time}.

{10.10.2017|Date of experiment}

Two {100|mL|:LB Kan:|expression media} cultures in {unbaffled Erlenmeyer|:flasks:} per strain were inoculated to an OD600 of {0.05|inoculation OD} with the overnight cultures.

Strain	OD600 preculture	V [mL] inoculation @OD600 = 0.05
P. putida KT2440 pVLT33::pigC	4.57	0.05 / 4.57 * 100 mL = 1.090 mL
P. putida KT2440 pVLT33 (EV)	7.92	0.05 / 7.92 * 100 mL = 0.631 mL

The cultures were incubated at {30|°C|temperature} and {250|rpm|shaking} to an OD600 of {0.4-0.6|induction OD} and induced by addition of {0.5|mM|:isopropyl-□-D-thiogalactopyranoside (IPTG):|inducer molecule}. {4|h|:after induction:|time of substrate supplementation}, the cultures were supplemented with {0.5|mM|:MAP and MBC:|substrates} in order to confirm the presence of active PigC enzyme.

During and after induction, samples of the corresponding volume of {1|mL|:OD = 1:|sample volume} were taken at {1|h|:intervals:} for SDS-PAGE analysis. Samples were pelleted for {1|min|centrifugation time} at {11,000|xg|centrifugation speed} and stored at {-20|°C|storage temperature}.

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Table 1 – Growth of expression cultures.

Time [h]			*P. putida* KT2440 pVLT33::*pigC*		*P. putida* KT2440 pVLT33 (EV)	
Date	Time	t [h]	OD_{600} (+ MAP/MBC)	OD_{600} (- MAP/MBC)	OD_{600} (+ MAP/MBC)	OD_{600} (- MAP/MBC)
10.10.2017	08:35	0	0.037	0.040	0.033	0.030
	10:35	2	0.082	0.077	0.123	0.117
	11:35	3	0.121	0.132	0.228	0.206
	12:35	4	0.203	0.184	0.447 (t0) +IPTG	0.407 (t0) +IPTG
	13:35	5	0.360	0.359	0.93	0.93
	14:35	6	0.635 (t0) +IPTG	0.612 (t0) +IPTG	1.64 (t2)	1.70 (t2)
	15:35	7	1.13	1.12	2.10	2.00
	16:35	8	1.59 (t2)	1.63 (t2)	2.05 (t4) +MAP/MBC	2.14 (t4) +MAP/MBC
	17:35	9	2.19	2.44	2.83	2.90
	18:35	10	2.69 (t4) +MAP/MBC	2.79 (t4) +MAP/MBC	3.07	3.31
11.10.2017	10:35	26	5.45 (t16)	5.48	5.60	7.00
	12:35	28	5.40	5.67	5.60 (t20)	7.05
	14:35	30	5.62 (t20)	5.71	5.87	6.96

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12.10.2017	10:35	50	5.32	5.35	5.23	6.16
	12:35	52	nd	nd	nd (t44)	nd
	14:35	54	nd (t44)	nd	nd	nd

blue - sample for SDS-PAGE (1 mL OD1)

orange - sample for prodiginine analysis (1 mL)

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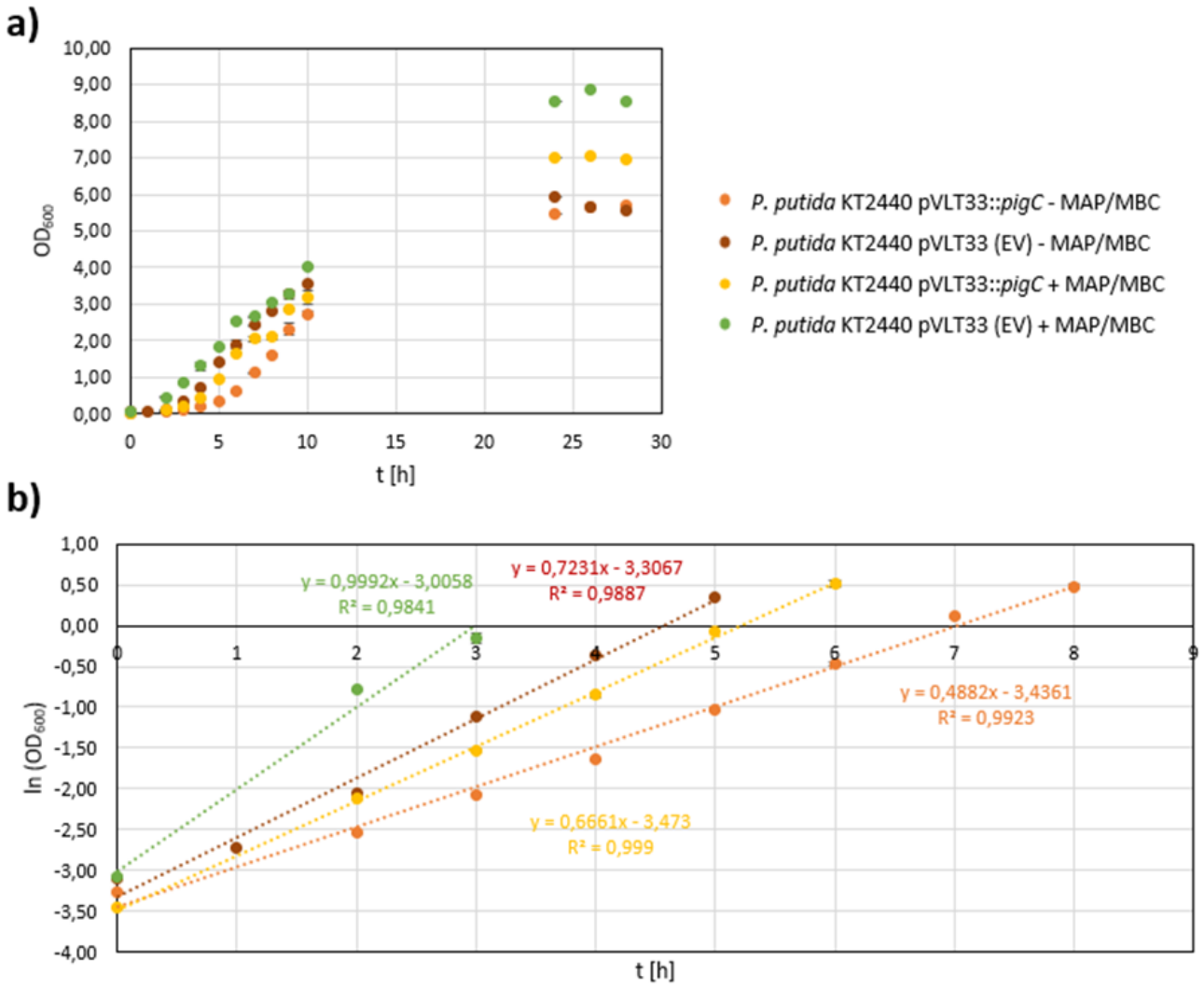

Fig. 1: a. Growth curves of P. putida KT2440 pVLT33::pigC and P. putida KT2440 pVLT33 (EV) with and without substrates (MAP/MBC). **b.** Growth rates of the cultures.

{25.10.2017|Date of experiment}

Samples from the expression cultures were thawn on ice and resuspended in {30|µL|:PBS buffer:|resuspension volume}. After {1|min|:sonification:} in an ultrasonic bath, resuspended samples were mixed with {10|µL|:4x SDS loading dye:}, incubated for {5 min at 95°C|incubation} and {vortex(:ed:) thoroughly|process} in order to shear genomic DNA. The boiled samples were spinned down for {1 min at maximal speed|centrifugation} and {5|µL|(:of:) :supernatant:} was loaded on a {10|%|:SDS-PAGE separating gel:|acrylamide concentration}.

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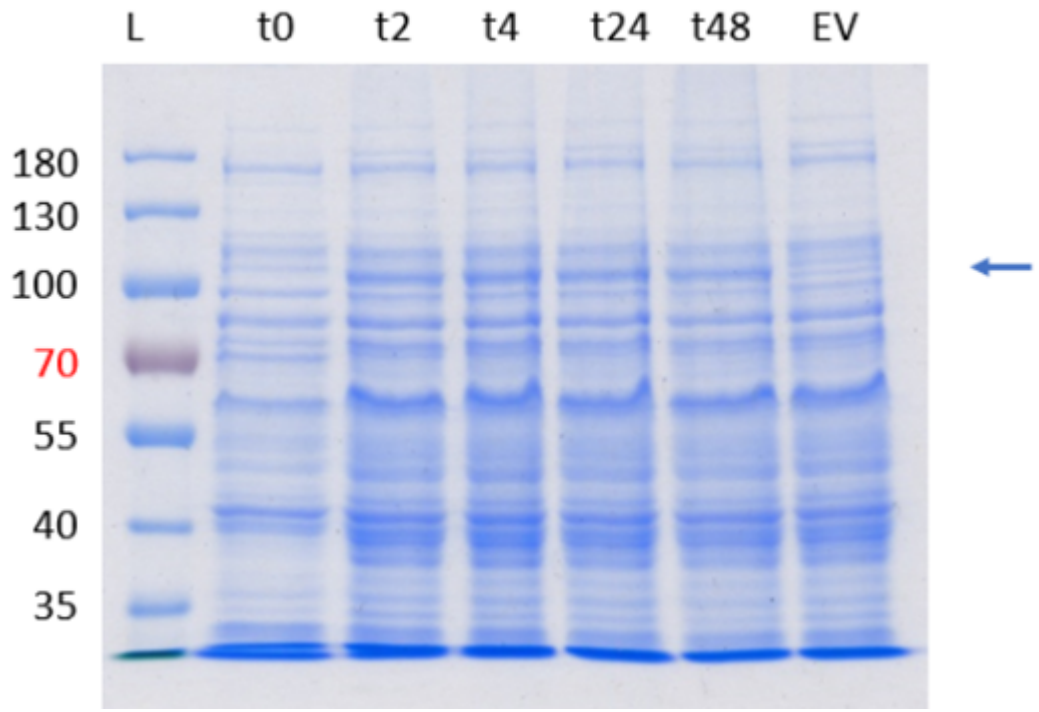

Fig 2: SDS-PAGE of pigC expression with P. putida KT2440 pVLT33::pigC. EV = empty vector control after 48 h. The arrow marks the PigC size of ~ 100 kDa.

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Attached files

fig1.png
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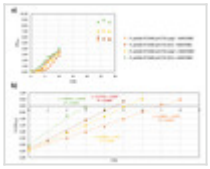

fig2.png
sha256: aaba59bc60e0a24c052fa549279e3c6c717cfea7d65af79fcb0ecfcdb1bfd4a4