Karlsruhe Institute of Technology

Institute of Functional Interfaces (IFG)
RG Bacterial stress response and process engineering

Role of alternative sigma factor PP4553 in stress response and biofilm formation of *Pseudomonas putida* KT2440

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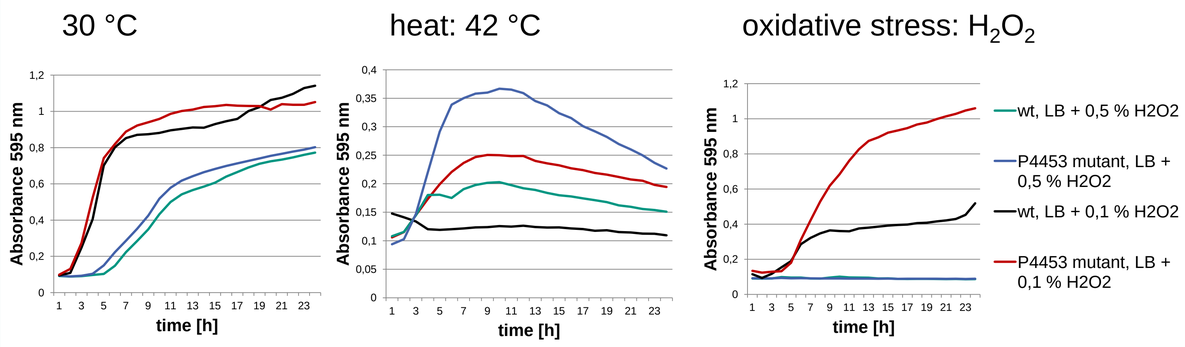
Introduction

Pseudomonas putida is a Gram-negative and non-pathogenic soil bacterium, which is well known for its extremely metabolic versatility. Because of this, *P. putida* offers a considerable potential for biotechnological applications. The remarkable versatility is at least in parts driven by sophisticated and coordinated regulation of gene expression mediated by a repertoire of transcriptional regulators, in particular the so called sigma factors. Sigma factors are essential for prokaryotic transcription initiation and enable specific binding of the RNA polymerase to the respective promotor recognition sites. Bacteria generally contain one housekeeping sigma factor and a pool of alternative sigma factors, which are activated in response to different and often stressful conditions. *P. putida* exhibits with 24 a striking number of alternative sigma factors [1], one of which is open reading frame PP4553. To analyze this putative sigma factor in more detail, we constructed a gene knock-out deletion mutant of PP4553 in *P. putida* KT2440.

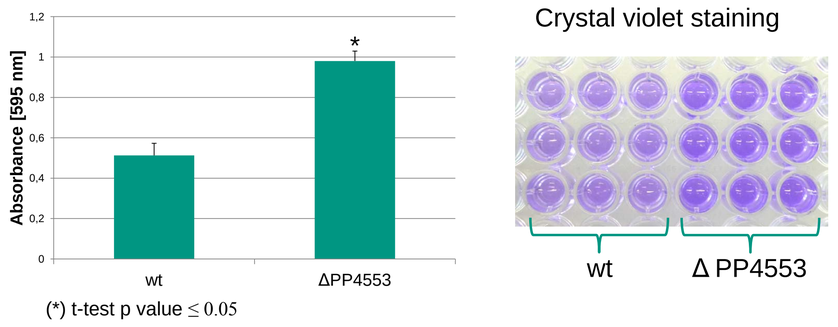
METHODS & RESULTS

Growth curves under different stress conditions



P. putida cultures were grown for 24h in a 96-well microtiter plate under shaking in a tecan microplate reader.

PP4553 knock-out mutant exhibits more biofilm formation



(*) t-test p value ≤ 0.05

P. putida wt and knock-out mutant were grown in 96-well microtiter plates in M9 medium for 24 h at 30 °C. Biofilm formation was determined by crystal violet staining.

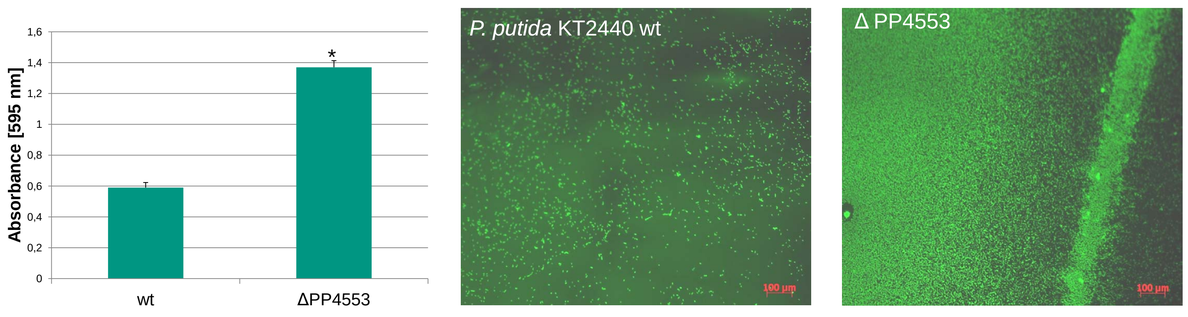
MIC – Minimal Inhibitory Concentration

	Colistin [µg/ml]	Ciprofloxacin [µg/ml]	Aztreonam [µg/ml]	Tobramycin [µg/ml]	H_2O_2 [% v/v]
wt	0,25	0,0039	0,5	0,125	0,001
ΔPP4553	1	0,015	4	0,125	0,005

Antibiotics of different classes were used to determine the minimal inhibitory concentration of *P. putida* wildtyp and knock-out deletion mutant. Cultures were grown in MH or LB medium at 30 °C. MIC was observed in 96-well microtiter plates with the unaided eye [2].

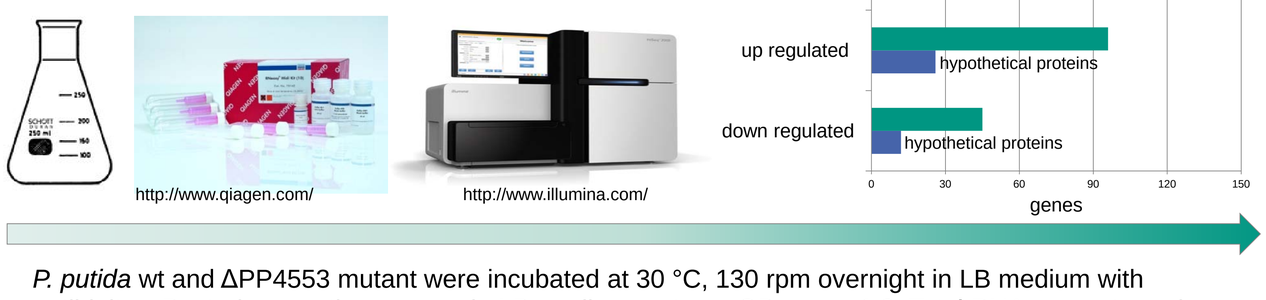
Mutation in the sigma factor PP4553 leads to increased attachment

Attachment on 96-well plates and glass slides



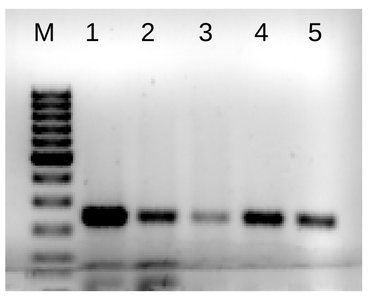
Cultures were grown in LB medium for 1 h at 30 °C. Attachment was determined in 96-well microtiter plates by crystal violet staining or on glass object slides by fluorescence microscopy.
(*) t-test p value ≤ 0.05

RNASeq



P. putida wt and ΔPP4553 mutant were incubated at 30 °C, 130 rpm overnight in LB medium with antibioitcs. Bacteria were then grown in LB medium up to an OD_{600nm} ~ 1,3. Total RNA was extraced according to the protocol provied by Quagen (Rneasy midi kit) and rRNA was removed (MicrobExpress Kit Ambion) , RNA Seq was performed with an Illumina HiSeq1000.

PP4553 is involved in stress response



[M] marker, GeneRuler 100 bp
[1] without stress, exp. growth
[2] heat, exp. growth
[3] heat, stat. phase
[4] red. NH_4Cl concentration, stat. phase
[5] control, genomic DNA

P. putida cultures were grown in LB medium under different stress conditions at 30 °C. RNA was isolated for cDNA synthesis. cDNA was used for qRT-PCR. Sigma factor PP4553 is expressed under different cultur conditions

Summary

The mutation in the sigma factor PP4553 reveals an growth advantage of the mutant compared to the wildtyp under different stress conditions. The knock-out deletion mutant exhibits increased attachment and biofilm formation. Furthermore raised resistance to antibiotics and oxidative stress was confirmed. qRT-PCR shows the expression of sigma factor PP4553 under different conditions. Gene expression analysis using RNASeq reveals 141 differenital expressed genes of 5446 total genes with 96 up and 45 down regulated genes. Further data evaluation is in progress.

References:
[1] Enviromental Microbiology (2002); 4 (12): 842-855
[2] Nature Protocols (2008); 3 (2): 163-175

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