Karlsruhe Institute of Technology

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

Alternative Sigma factor PP4553 is a negative regulator of antibiotic resistance and biofilm formation in *Pseudomonas putida* KT2440

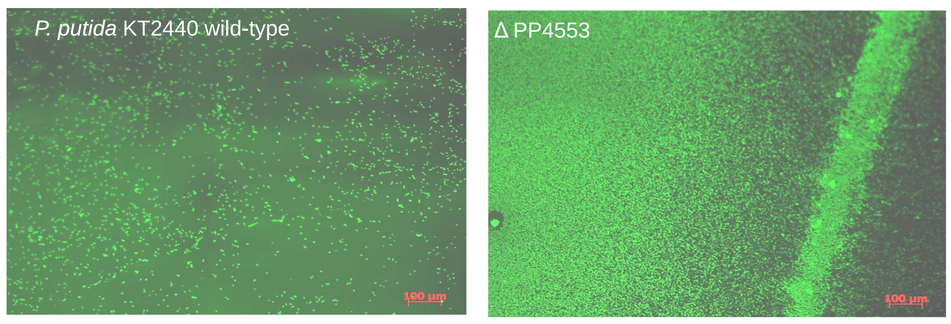
Beatrix Bugert[1], Andreas Doetsch[2], Olivier Armant[3] and Joerg Overhage[1]

[1]Institute of Functional Interfaces, KIT Karlsruhe; [2]Helmholtz Centre for Infection Research, Braunschweig; [3]Institute of Toxicology and Genetics, KIT Karlsruhe

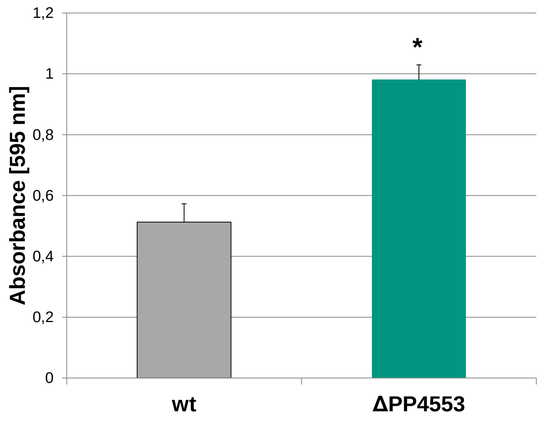
Introduction

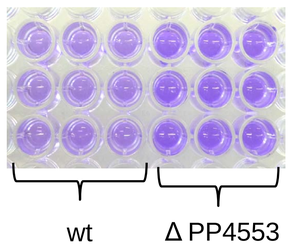
Pseudomonas putida is a Gram-negative soil bacterium, which offers a considerable potential for biotechnological applications [1]. This bacterium exhibits a remarkable metabolic versatility, which is at least in parts driven by sophisticated and coordinated regulation of gene expression mediated by a repertoire of transcriptional regulators, in particular the so called sigma factors [2]. Sigma factors are key regulators activated in response to different environmental and often stressful conditions, and are involved in controlling bacterial growth, survival and lifestyle (e.g. biofilm growth).
In order to identify new key regulators governing biofilm formation in *P. putida* KT2440, we analyzed selected sigma factors for altered biofilm formation and recognized PP4553 to be involved in adhesion and biofilm development. We constructed the respective knock-out deletion mutant *P. putida* ΔPP4553, phenotypically characterized this mutant in more detail and performed transcriptome analysis using RNA-Sequencing .

P. putida ΔPP4553 mutant exhibits increased adhesion and biofilm formation



Adhesion (1 h, 30 °C, LB broth) was determinated on glass object slides by fluorescence microscopy.





(*) t-test p value ≤ 0.05

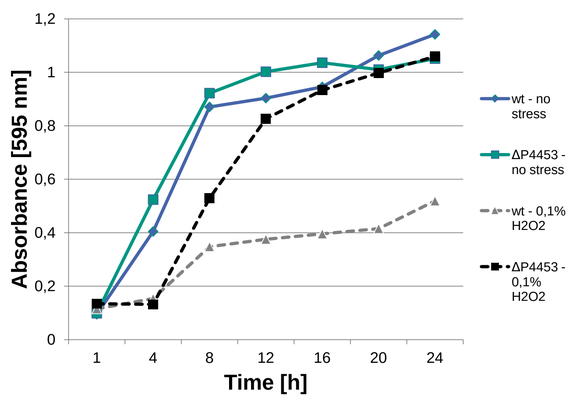
Biofilm formation (24 h, 30 °C, M9 broth) was determinated in 96-well microtiter plates by crystal violet staining.

PP4553 plays a role in antibiotic resistance and oxidative stress response

	Meropenem [µg/ml]	Ciprofloxacin [µg/ml]	Aztreonam [µg/ml]	Tobramycin [µg/ml]	Polymyxin B [µg/ml]	Tetrazyklin [µg/ml]
wild-type	0,062	0,0039	0,5	0,125	0,125	4
ΔPP4553	**0,125**	**0,015**	**4**	0,125	**0,25**	**8**

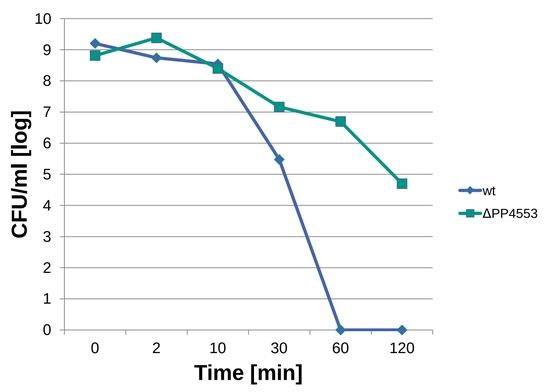
Minimal inhibitory concentration (24 h, 30 °C, MH broth) was analyzed in 96-well microtiter plates.

Growth curves



Cultures were grown in a Tecan microplate reader (24 h, 30 °C, LB broth).

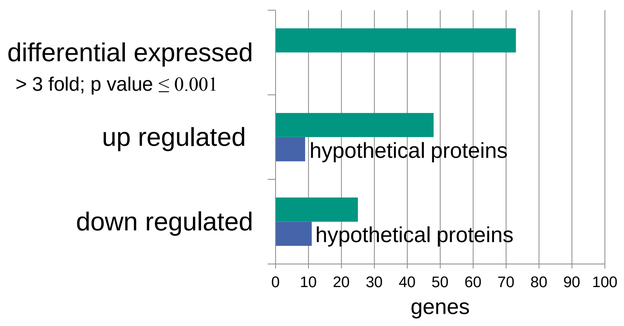
Time - Kill curve



Kill curve was determinated for Ciprofloxacin [0,3 µg/ml] (30 °C, MH broth).

Gene expression analysis using RNA-Sequencing

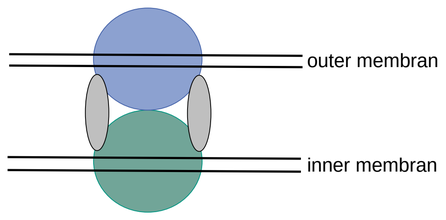
Deep Sequencing facility, KIT



Cultures of *P. putida* wild-type and mutant strain ΔPP4553 were incubated to an OD_{600nm} of ~ 1,3. Total RNA was extracted, rRNA was removed and RNA-Seq was performed with an Illumina HiSeq1000.
Data analysis is still in progress.

RNA-Sequencing revealed up regulation of an efflux pump

locus tag	gene name	fold change	product name
PP_1384	ttgC	3,0	RND efflux system, outer membrane lipoprotein
PP_1385	ttgB	4,5	transporter hydrophobe/amphiphile efflux
PP_1386	ttgA	7,0	efflux transporter RND family, MFP subunit
PP_1387	ttgR	5,5	TetR family transcriptional regulator



Summary

- Alternative sigma factor PP4553 is a negative regulator of **adhesion** and **biofilm formation**.
- *P. putida* ΔPP4553 shows enhanced **resistance** to antibiotics and oxidative stress.
- Gene expression analysis indicated up-regulation of genes coding for **TtgABC efflux pump**.

adhesion ↑ **resistance ↑** **biofilm formation ↑**

References:
[1] Enviromental Microbiology (2002); 4: 842-855
[2] FEMS Microbial Rev (2008); 32:38-55

Acknowledgements:
The Research Group *Bacterial Stress Response and Process Engineering* is financed by the concept for the future of the Karlsriuher Institute of Technology (KIT) within the German Excellence Initiative (DFG) and the BIF program within the Helmholtz Association

Contact:
KIT – Campus North
Institute of Functional Interfaces (IFG)
beatrix.bugert@kit.edu