

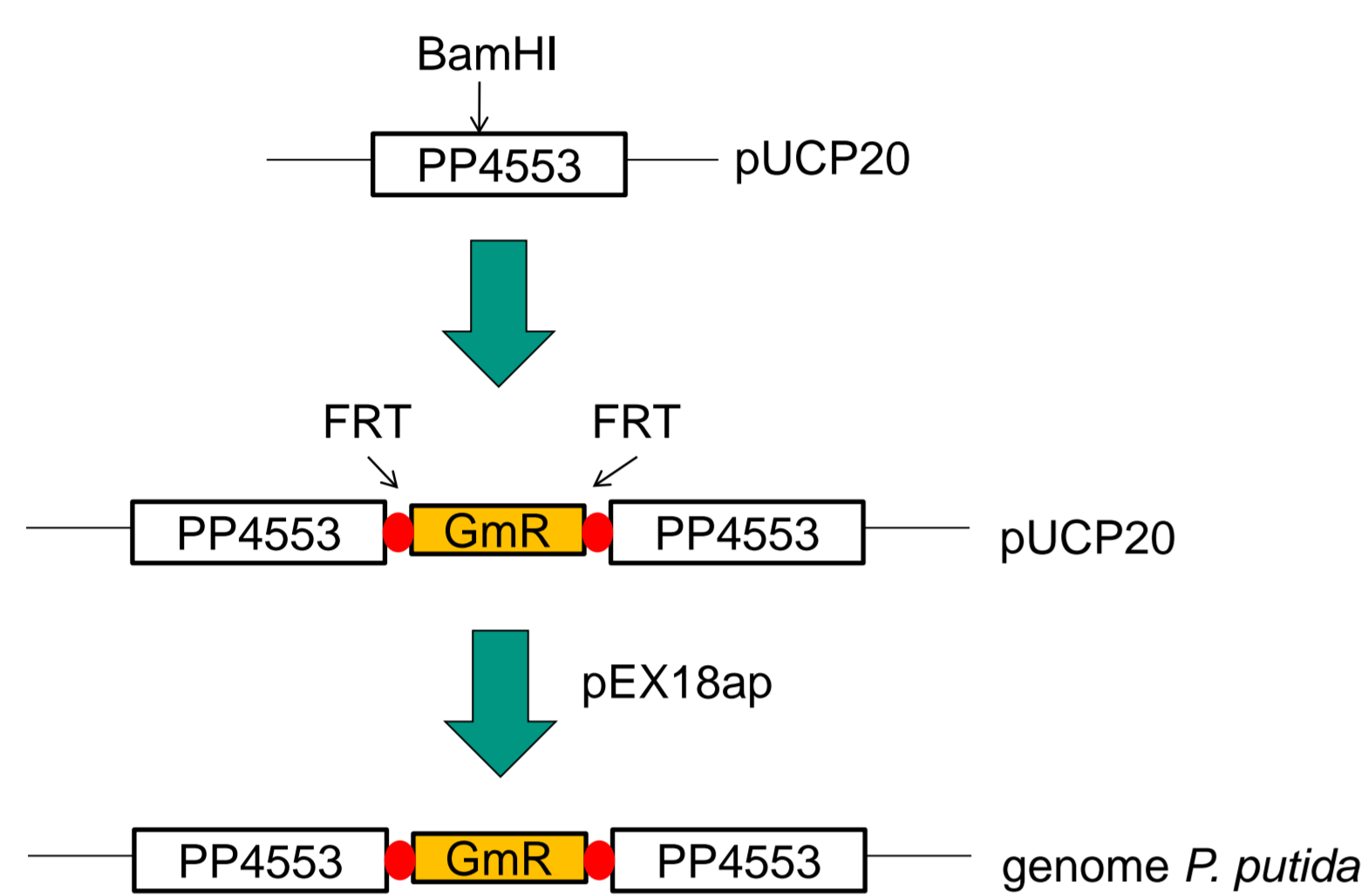
The role of the alternative sigma factor PP4553 in the stress response of *Pseudomonas putida*

Beatrix Bugert and Jörg Overhage

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 promoter 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].

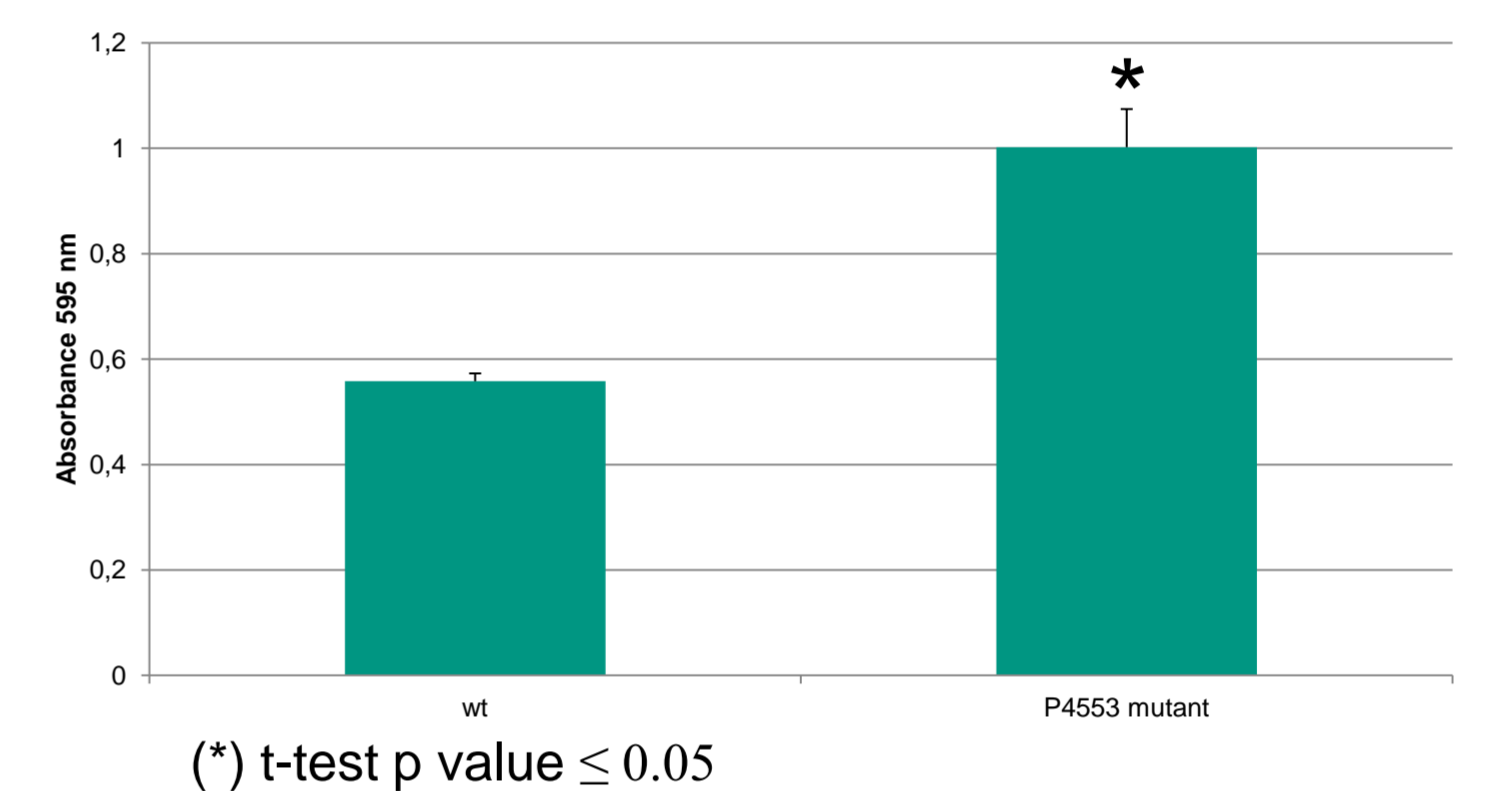
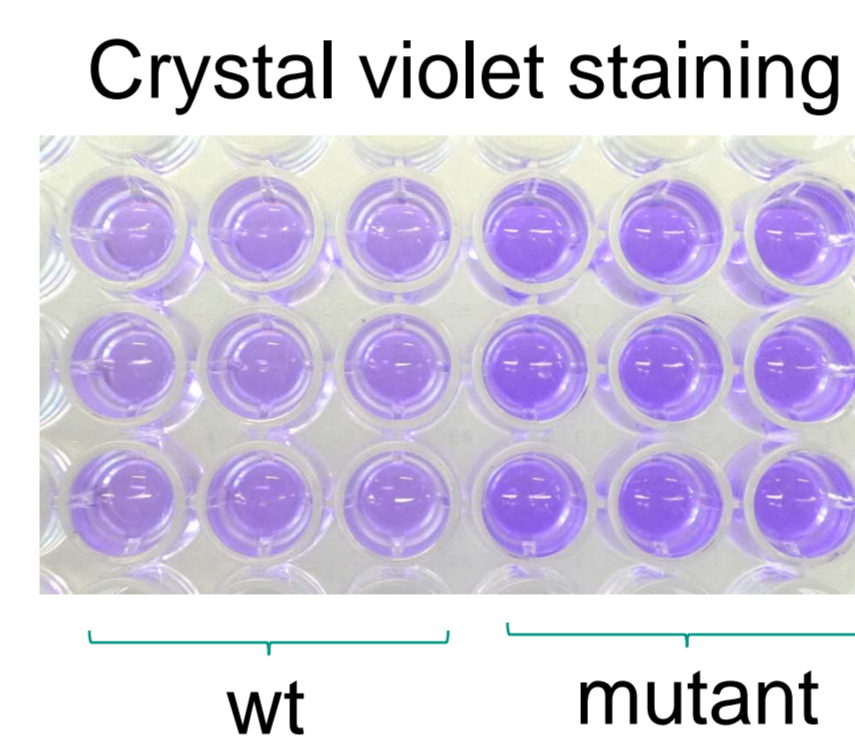
Construction of the *P. putida* PP4553 knock-out mutant



First, the gene coding for the *P. putida* sigma factor PP4553 was cloned into pUCP20. The gentamicin resistance cassette from the plasmid pPS856 was inserted into the gene. By the use of the suicide vector pEX18ap the construct was integrated into the *P. putida* KT2440 genome.

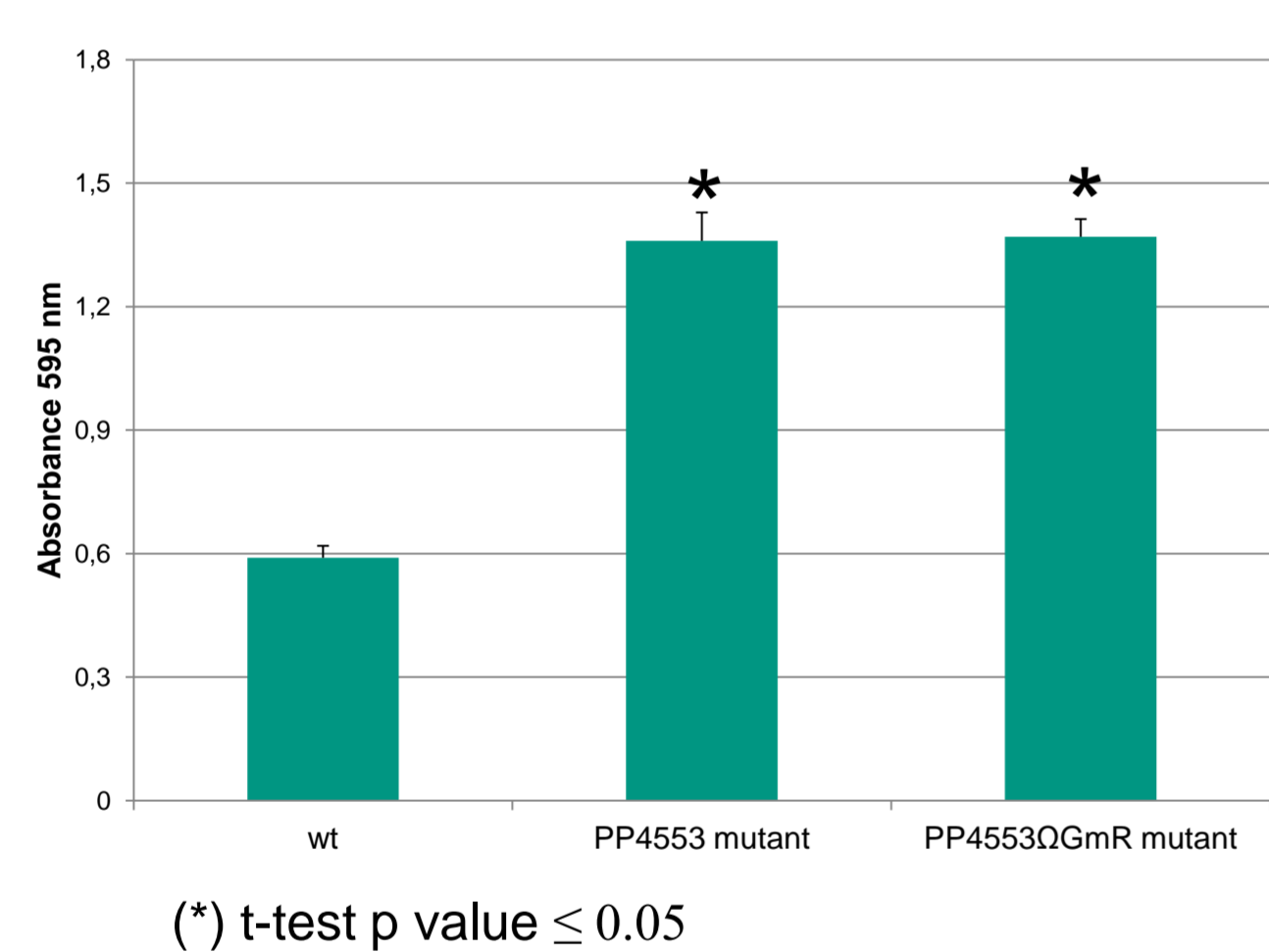
The PP4553 knock-out mutant exhibits more biofilm formation

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.



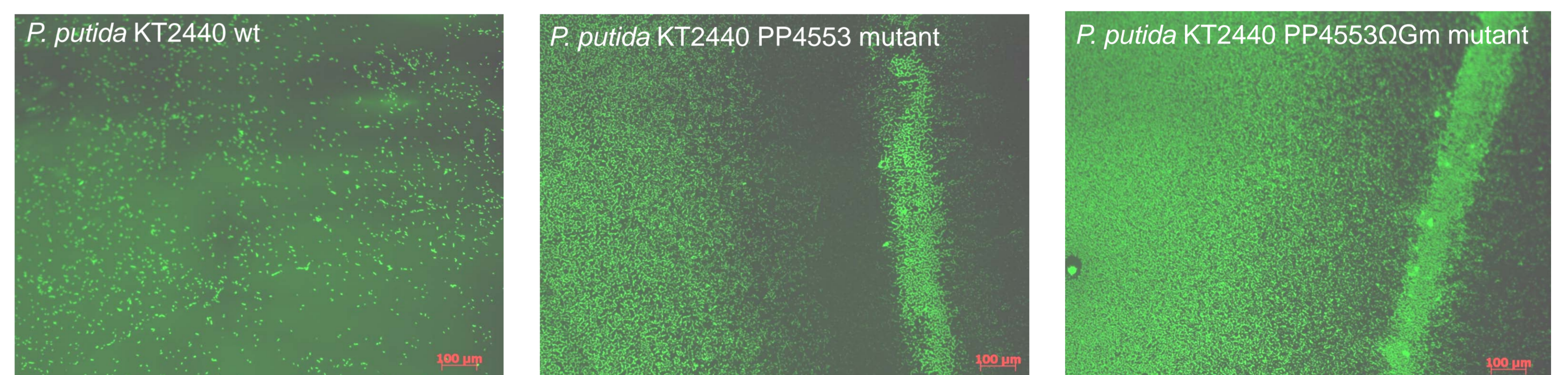
Mutation in the sigma factor PP4553 leads to increased attachment

Attachment on 96-well microtiter plates



P. putida KT2440 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.

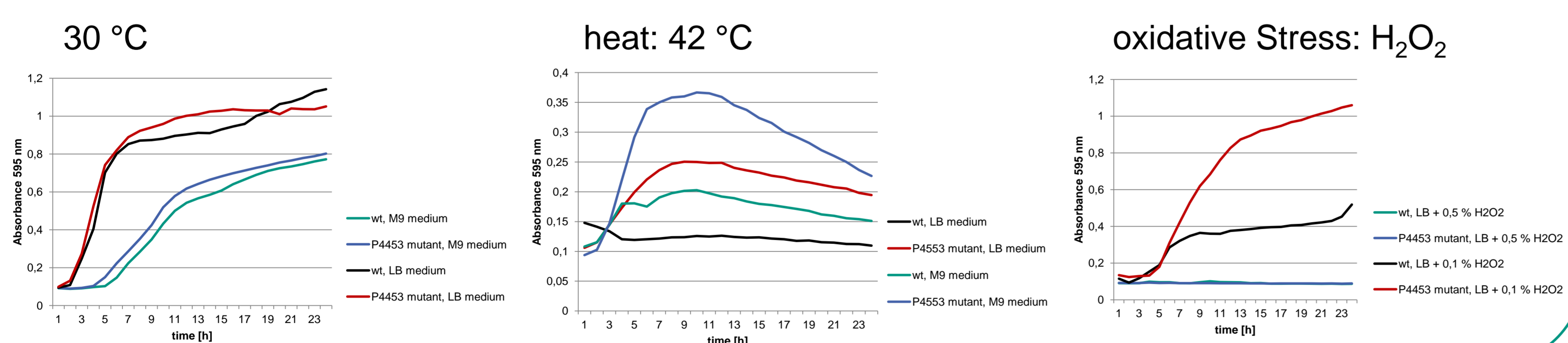
Attachment on glass slides



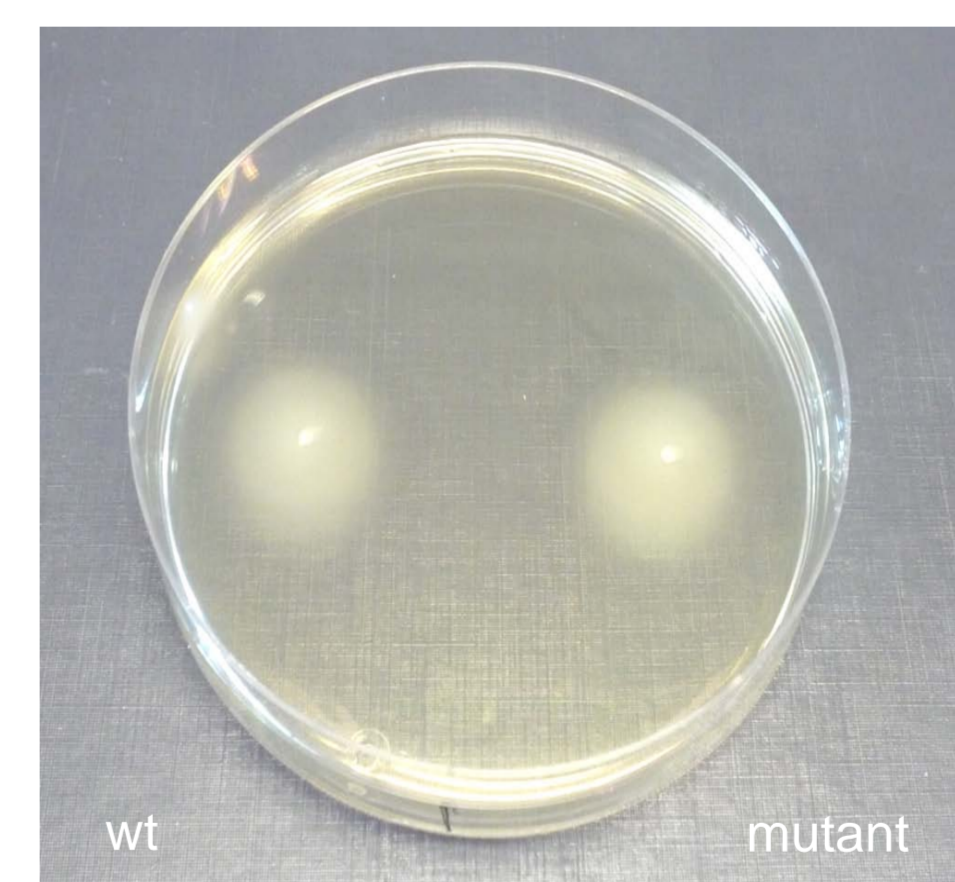
Visualization of attached cells was implemented by fluorescence microscopy after immobilization (3 % formaldehyde v/v) and SYTO9 staining.

Growth under different stress conditions

P. putida cultures were grown in a 96-well microtiter plate under shaking. Stress conditions were varied:



The PP4553 mutant exhibits normal swimming motility



Swimming: 1 μ l of overnight cultures were incubated on 0.3 % (w/v) LB agar plates for 18 h at 30 °C.

MIC – Minimal Inhibitory Concentration

	Gentamicin [μ g/ml]	Kanamycin [μ g/ml]	Tetracyclin [μ g/ml]	H ₂ O ₂ [% v/v]	NaClO [μ g/ml free chlorine]
wt	0.125	8	0.125	0.0005	256
PP4553 mutant	16	8	1	0.001	256

Cultures were grown in MH or LB medium at 30 °C. MIC was determined in 96-well microtiter plates with the naked eye.

Summary

- Increased attachment and biofilm formation of the knock-out mutant was observed.
- Growth under different stress conditions reveal an advantage of the mutant compared to the *P. putida* wt.
- Raised resistance of the mutant to antibiotics and oxidative stress was confirmed. Tolerance to disinfectants was not affected.
- The *P. putida* PP4553 knock-out mutant shows normal swimming motility.

References:

[1] Environmental Microbiology (2002) ; 4 (12): 842-855

Contact:

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

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