Karlsruhe Institute of Technology

Institute of Functional Interfaces (IFG)
RG Bacterial Stress Response and Process Engineering

The sensor kinase PA4398 regulates swarming motility and biofilm formation in *Pseudomonas aeruginosa* PA14

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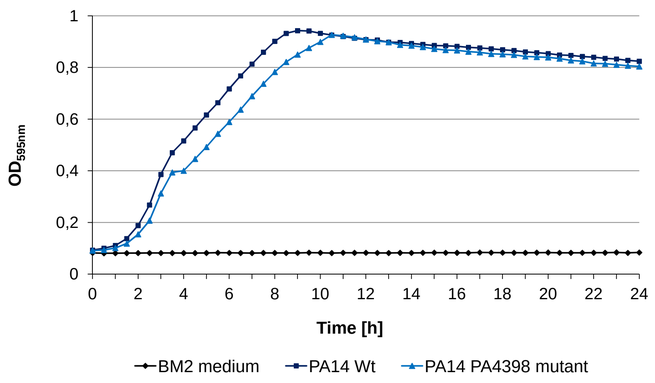
Introduction

Multicellular behavior is an important process central to the pathogenesis of *P. aeruginosa*. Biofilms are surface-associated communities which are formed in a sequential process on a variety of biotic and abiotic surfaces. Bacteria living in biofilms are more resistant against antimicrobial agents and are protected from the host immune response. In addition to biofilm formation, swarming motility represents a second surface-associated community behavior of the human pathogen *P. aeruginosa*. Recently, we have shown that swarming can be considered as a distinct physiological state with a tailored metabolic lifestyle or a complex adaptation of *P. aeruginosa* in response to viscous environments [1]. Biofilm formation and swarming motility seem to be inversely regulated. However, the regulatory networks involved in the regulation of these two social behaviors are not yet fully understood. During a screening for swarming deficient mutants [2] we identified a two-component sensor kinase transposon mutant (PA4398) in *P. aeruginosa* PA14 with defects in the ability to swarm on semisolid surfaces. In this study, we constructed a knock-out mutant of PA4398 in *P. aeruginosa* PA14 and phenotypically characterized this sensor kinase mutant in more detail.

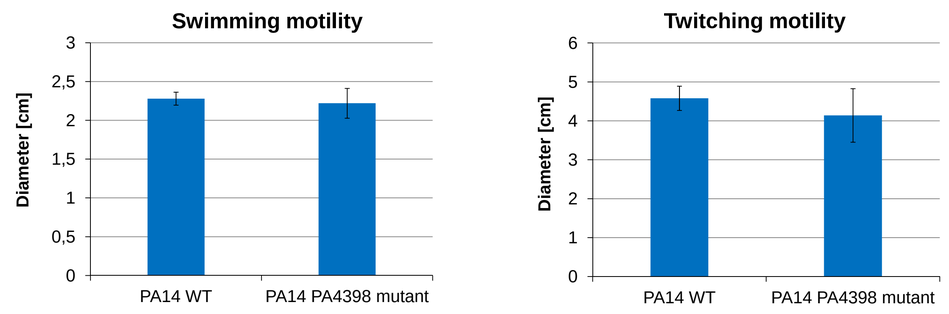
Methods and Results

Growth of *P. aeruginosa* PA14 in BM2 medium



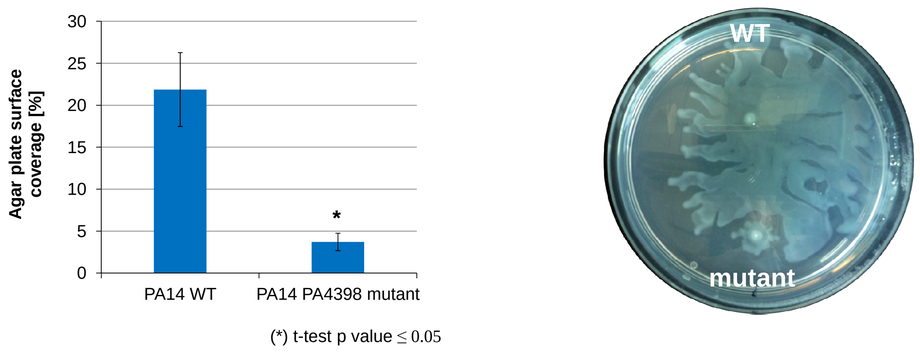
P. aeruginosa PA14 cultures were grown in a 96-well microtiter plate under shaking conditions at 37 ℃ in BM2 minimal medium.

The PA4398 mutant exhibits normal swimming and twitching motility



Swimming motility of *P. aeruginosa* PA14 (WT and PA4398 mutant) was analysed after an incubation for 18 h at 30 ℃ on LB plates, containing 0.3 % (w/v) agar. **Twitching motility** was performed for 48 h at 37 ℃ with 1 % (w/v) LB agar plates.

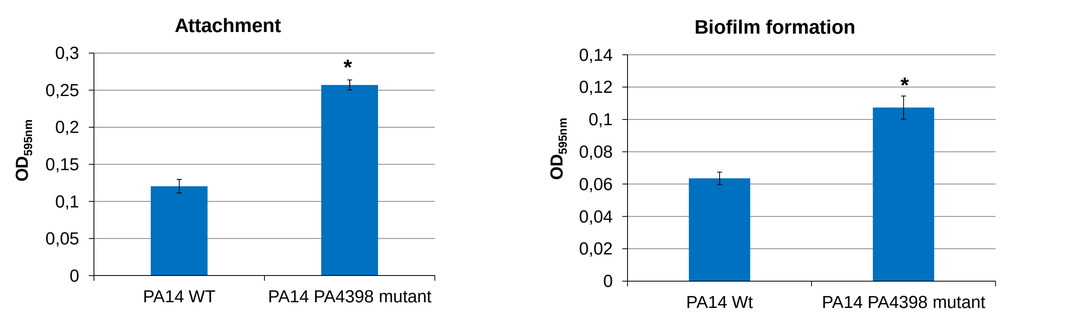
The sensor kinase mutant (PA4398) exhibits a defect in swarming motility



(*) t-test p value ≤ 0.05

P. aeruginosa (PA14 Wt; PA4398 mutant) cultures were grown in LB medium to an optical density (OD_{595nm}) of 1. 1 µl of these cultures were incubated on BM2 [-$(NH_4)_2SO_4$] + 0.1 % CAA agar plates. Shown is a representative swarm plate after 18 h of incubation at 37 ℃.

Biofilm formation of PA14 PA4398 mutant is increased



(*) t-test p value ≤ 0.05

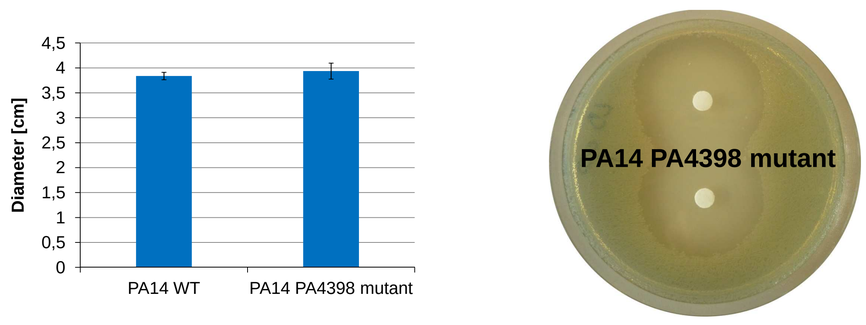
The Attachment of *P. aeruginosa* PA14 was analysed after incubation in BM2 + 0,1 % CAA for 1 h at 37 ℃ while biofilm formation was measured after 24 h at 37 ℃ in BM 2 + 0,5 % CAA in 96-well microtiter plates.

Antibiotic resistance of PA14 PA4398 mutant

Antibiotics	PA14 WT (µg/ml)	PA14 PA4398 mutant (µg/ml)
Tobramycin	0,25	0,25
Meropenem	1	1
Tetracyclin	8	8
Ciprofloxacin	0,03	0,03
Colistin	0,5	1
Polymixin B	0,25	0,5

The minimal inhibitory concentration (MIC) was determined after incubation of PA14 WT and PA4398 mutant together with selected antibiotics at defined concentrations for 17 h at 37℃ in MH medium.

Oxidative stress response of the sensor kinase mutant



Overnight cultures of PA14 WT and PA4398 mutant were adjusted to an OD_{595} = 0.1 and plated on LB agar. A circular „Whatman“ paper (diameter 0,5 mm), soaked in 30 % H_2O_2 was placed on top. After 20 h of incubation at 37 °C the formed zone of inhibition was measured. Shown is a representative plate of the PA14 PA4398 mutant.

Summary

- PA4398 mutant exhibited reduced swarming motility in comparison to wildtype cells whereas no significant differences regarding growth rate, twitching and swimming motility were observed.
- In contrast, a mutation in the sensor kinase leads to an increase in biofilm formation after 24 hours of growth.
- In addition there were no significant differences between PA14 WT and the PA14 sensor kinase mutant regarding MIC of selected antibiotics and during oxidative stress by H_2O_2.

References:
[1] *J. Bacteriol.* (2008), 190(8):2671-9.
[2] *J. Bacteriol. (*2009), 191(18):5592-602.

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