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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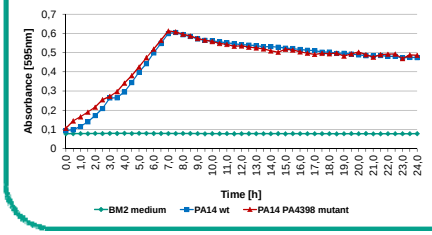
INTRODUCTION

Multicellular behavior is an important process central to the pathogenesis of *P. aeruginosa*. In addition to biofilm formation, swarming motility represents a second surface-associated community behavior of this human pathogen. 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 a viscous environment leading to increased antibiotic resistance and virulence gene expression [1]. 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.

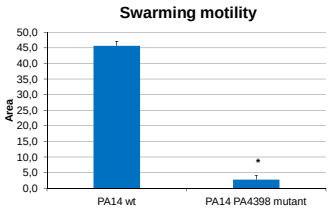
METHODS & RESULTS

Growth of *P. aeruginosa* PA14 and PA4398 mutant

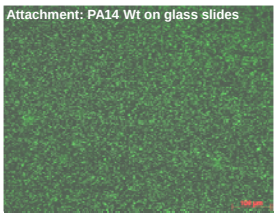


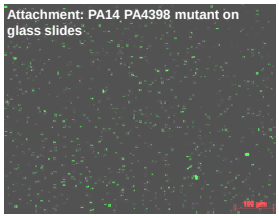
P. aeruginosa PA14 cultures were grown in a 96-well microtiter plate under shaking conditions at 37 °C in BM2 minimal medium.

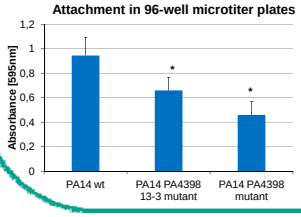
Mutation in the sensor kinase PA4398 leads to decreased attachment and swarming motility



P. aeruginosa (PA14 wt; PA4398 mutant) cultures were grown in LB medium to an optical density (OD_{595nm}) of 1. One µl of these cultures were incubated on BM2swarm [-$(NH_4)_2SO_4$ + 0.1 % CAA] agar plates [1]. Shown is a representative swarm plate after 18 h of incubation at 37 °C.



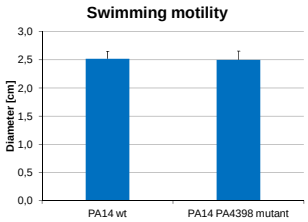


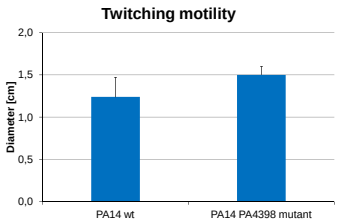


P. aeruginosa PA14 cultures were grown in BM2 medium containing 0.1 % CAA for 1h at 37 °C. Attachment was determined in 96-well microtiter plates by crystal violet staining or on glass object slides by fluorescence microscopy [2].

(*) t-test p value ≤ 0.05

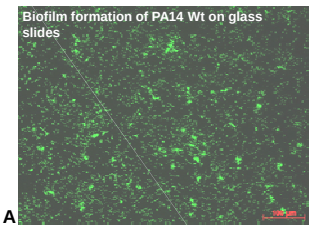
The PA4398 mutant exhibits normal swimming and twitching motility



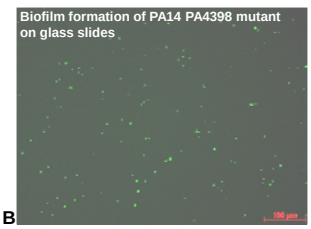


Swimming: One µl of overnight cultures were incubated on 0.3 % (w/v) LB agar plates for 18 h at 30 °C [2].
Twitching motility was performed on 1.5 % (w/v) LB agar plates using 2 µl of overnight cultures and an incubation for 48 h at 37 °C [2].

PA4398 mutant is impaired in biofilm formation

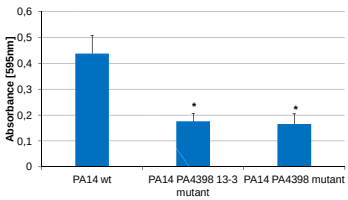


A



B

C



(*) t-test p value ≤ 0.05

PA14 overnight cultures were diluted 1:100 in BM2. Biofilm formation was examined under static conditions on glass object slides or in 96-well microtiter plates after 16 h of incubation at 37 °C. Visualization of biofilm formation was implemented after immobilization (3 % formaldehyde) and SYTO9 staining by fluorescence microscopy [A; B] or crystal violet staining [C].

SUMMARY

- The PA4398 mutant exhibited reduced swarming mobility, attachment and biofilm formation in comparison to wildtype cells whereas no differences regarding growth rate, twitching and swimming motility were observed.
- Preliminary microarray analysis of mutant strain PA14 13_3 suggests a role of sensor kinase PA4398 in nitrogen and iron metabolism in *P. aeruginosa* PA14 (data not shown).

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

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