

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

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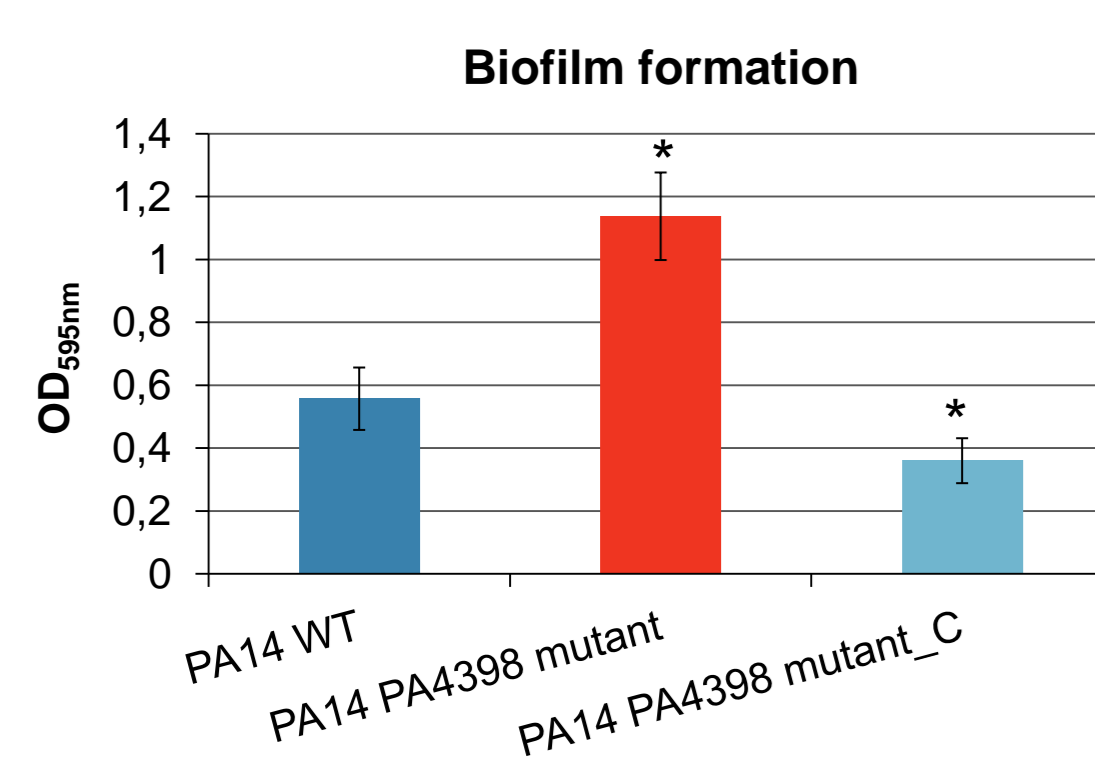
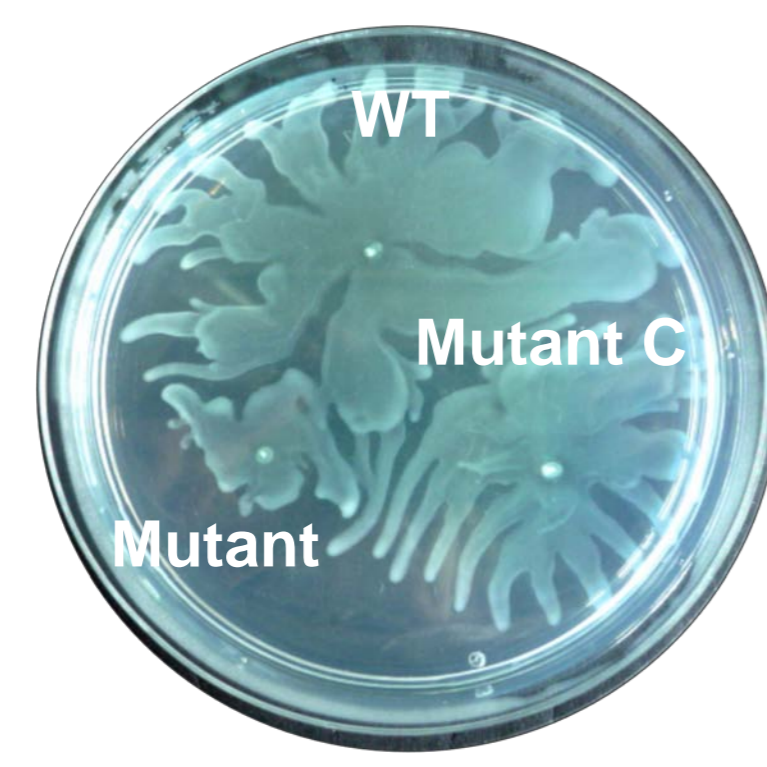
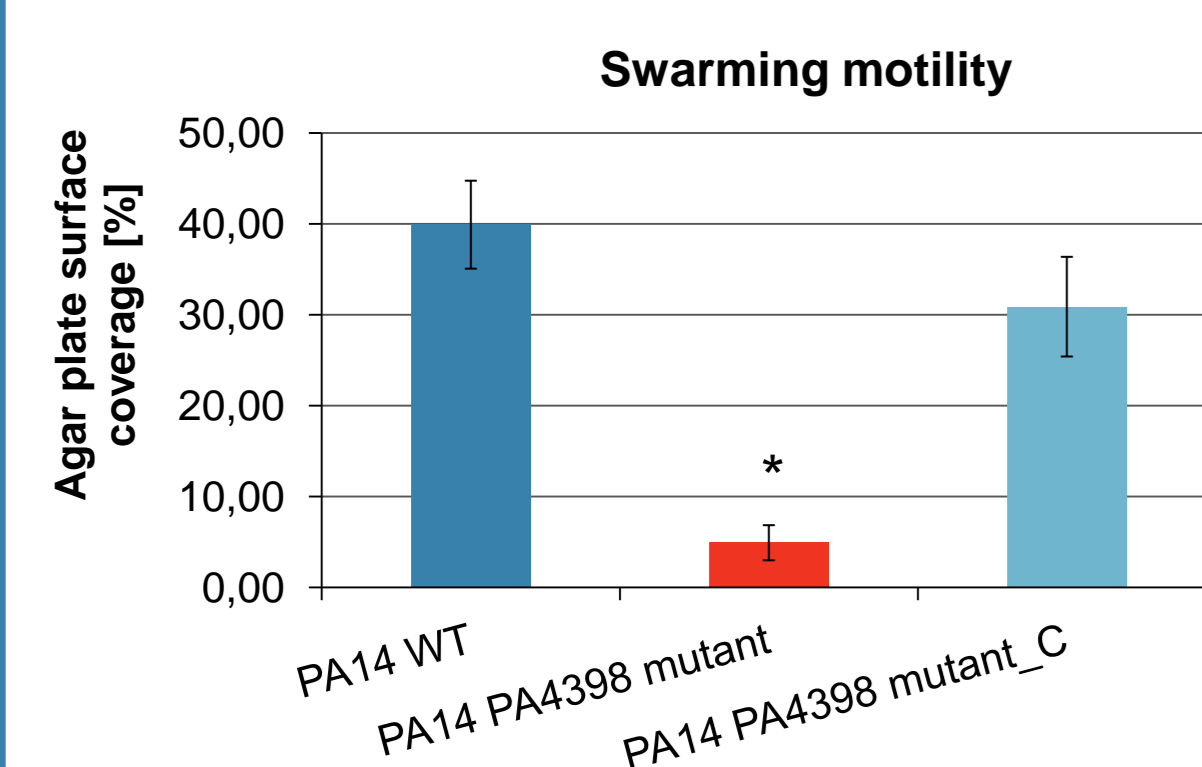
## Introduction

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 complex adaptation of *P. aeruginosa* in response to a viscous environment leading to increased antibiotic resistance and virulence gene expression [1]. During an extensive screening of the PA14 transposon mutant library [2] for swarming deficient mutants, we identified a two-component sensor kinase mutant (PA4398) with defects in the ability to swarm on semisolid surfaces [3].



To analyze the function of this sensor kinase in more detail, we constructed a PA14 knock-out mutant of PA4398, phenotypically characterized it and performed transcription analysis using microarrays.

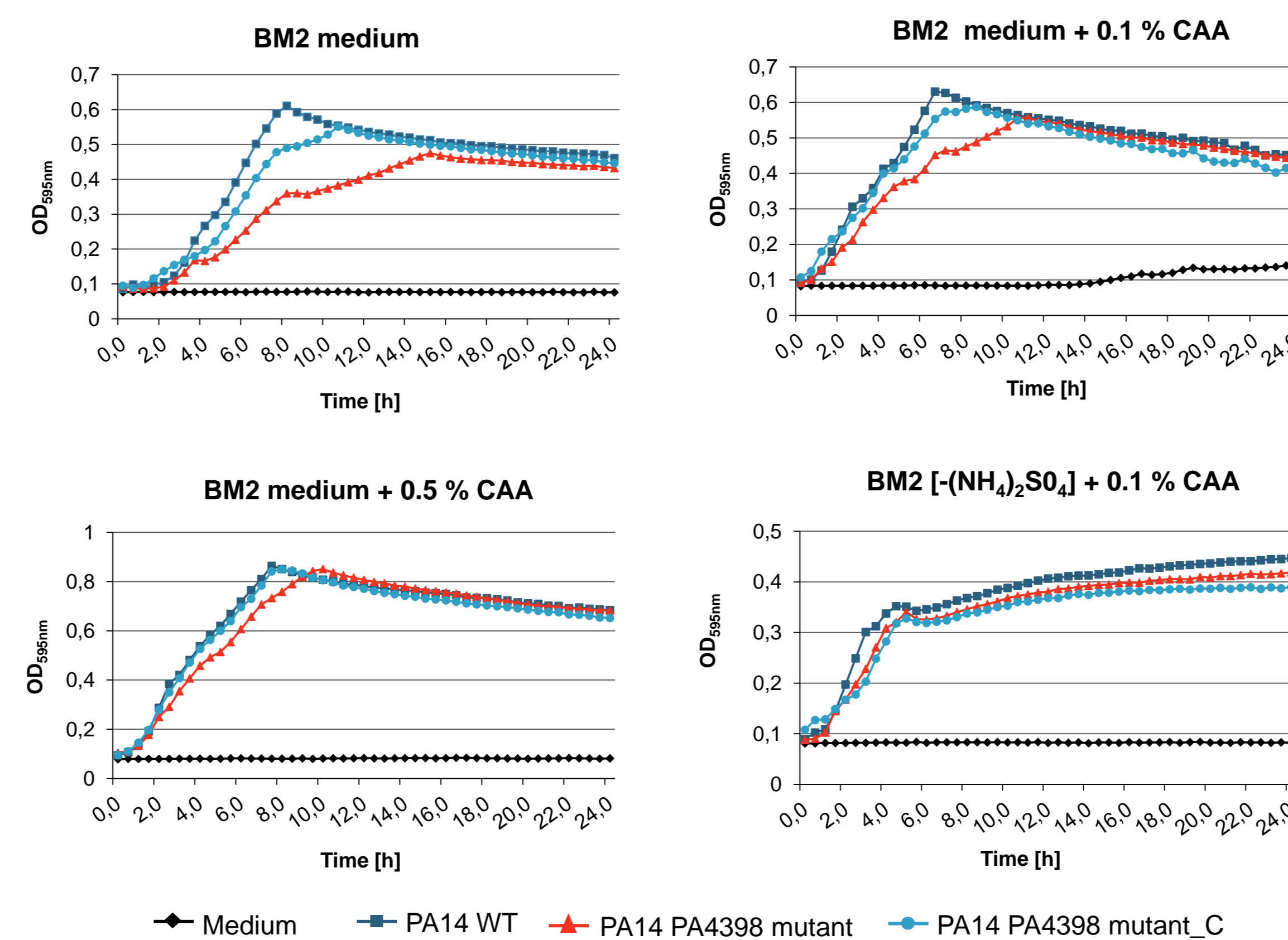
## PA14 PA4398 mutant exhibits a defect in swarming motility and an increase in biofilm formation



**Swarming motility** of *P. aeruginosa* PA14 WT and PA4398 mutant was performed on BM2 [-(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>] + 0.1 % CAA for 20 h at 37 °C. **Biofilm formation** was analysed after incubation in BM2 + 0.5 % CAA for 24 h at 37 °C in 96-well microtiter plates.

(\*) t-test p value ≤ 0.05

## Growth analysis of *P. aeruginosa* PA14 PA4398 mutant under different nitrogen conditions



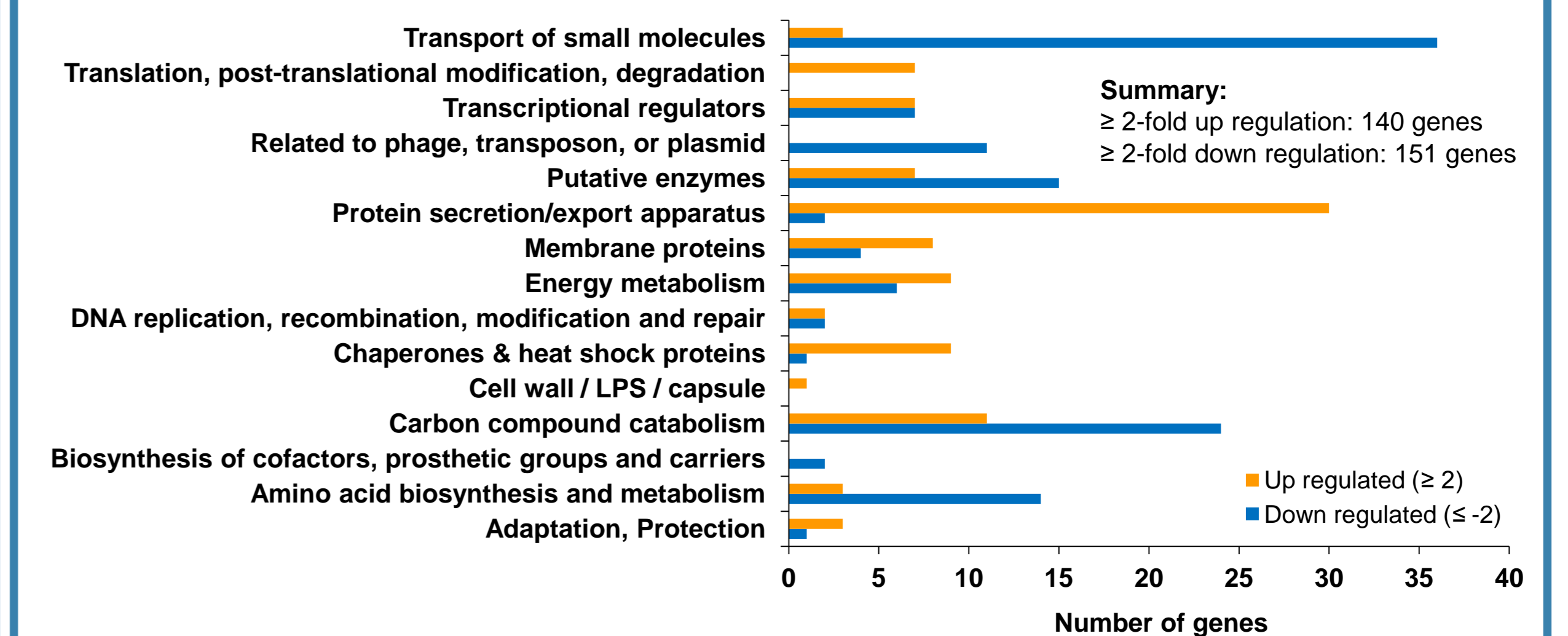
Growth analyses of PA14 PA4398 mutant in BM2 medium containing different N sources suggest a role of PA4398 in nitrogen metabolism.

## 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
Polymyxin 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 °C in MH medium.

## Transcription analysis of PA14 PA4398 mutant



### Selected up and down regulated genes:

	Public ID	Gene designation	Gene function
Up regulated genes (FC ≥ 2)	PA1691 - PA1697	<i>pstTSRQPON</i>	Type III secretion system
	PA1699 - PA1713	<i>popN/pcr123DGVH/popBD</i>	
	PA1715 - PA1724	<i>pstBCDEFGHIJK</i>	
	PA4385 - PA4386	<i>groEL, groES</i>	
PA4760 - PA4762	<i>dnaJ, dnaK, grpE</i>		
Down regulated genes (FC ≥ 2)	PA1070 - PA1074	<i>braGFEDC</i>	Branched-chain amino acid transporter
	PA2247 - PA2250	<i>bdkA1, bdkA2, bdkB, lpdV</i>	Amino acid degradation
	PA0633/ PA0754/ PA0755/ PA0887/ PA1070/ PA4505/ PA5545	- / - <i>opdH/ acsA</i> <i>braG/ dppD</i> -	Mutation known for swarming defect [3]; [4]

## Summary

- PA4398 mutant exhibits reduced swarming motility in comparison to wildtype cells. No significant differences regarding twitching and swimming motility were observed (data not shown).
- In contrast, a mutation in the sensor kinase leads to a 2-fold increase in biofilm formation after 24 hours of growth.
- Differences in growth of PA14 PA4398 mutant compared to PA14 WT in BM2 media suggest a role of PA4398 in nitrogen metabolism.
- The PA4398 mutant is less susceptible towards colistin and polymyxin B than PA14 WT.

### References:

- [1] *J. Bacteriol.* (2008), 190(8):2671-9.  
[2] *Proc Natl Acad Sci U S A* (2006), 103(8):2833-8.  
[3] *J. Bacteriol.* (2009), 191(18):5592-602.  
[4] *J. Bacteriol.* (2007), 189(5):2164–2169.

### Acknowledgements:

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