Nitrogen Limitation and Evolution of Swimming Motility in Aflagellate Mutant Strains of *Pseudomonas fluorescens* SBW25

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Introduction

Swimming motility, an important trait for successful root colonization, by *Pseudomonas fluorescens* SBW25 requires flagella, expression of which is activated in a hierarchical manner by the master regulator FleQ. A non-motile, aflagellate strain, AR2 Δ*fleQviscB* had been shown to reacquire flagella driven motility through 2 step mutation of a related two-component regulator, the NtrBC nitrogen sensor:regulator. Overexpressed NtrC-P is assumed to activate flagella expression. NtrBC responds to nitrogen limitation by upregulating expression of operons involved in nitrogen assimilation, including *glnAntrBC* [encoding: glutamine synthetase (GS), NtrBC] and *glnKamtB* (encoding PII and AmtB ammonium channel).

Aims and Research Hypothesis

Aims: To investigate the influence of different nitrogen sources and different motility phenotypes on the evolution pathway and probability/frequency of re-establishment of swimming motility in aflagellate P. *fluorescens* SBW25 Δ*fleQ* strains.

Research hypothesis: The physiological status of NtrBC under nitrogen limitation will increase the probability of identification of evolved swimming isolates of *P. fluorescens* SBW25 Δ*fleQ* strains carrying mutations in *ntrBC*. Also, as mutation of ntrBC has global effects and impact on cell fitness, it was hypothesized that other enhancer binding proteins (EBP) might rescue loss of *fleQ*, particularly in nitrogen replete conditions (Figure 1).

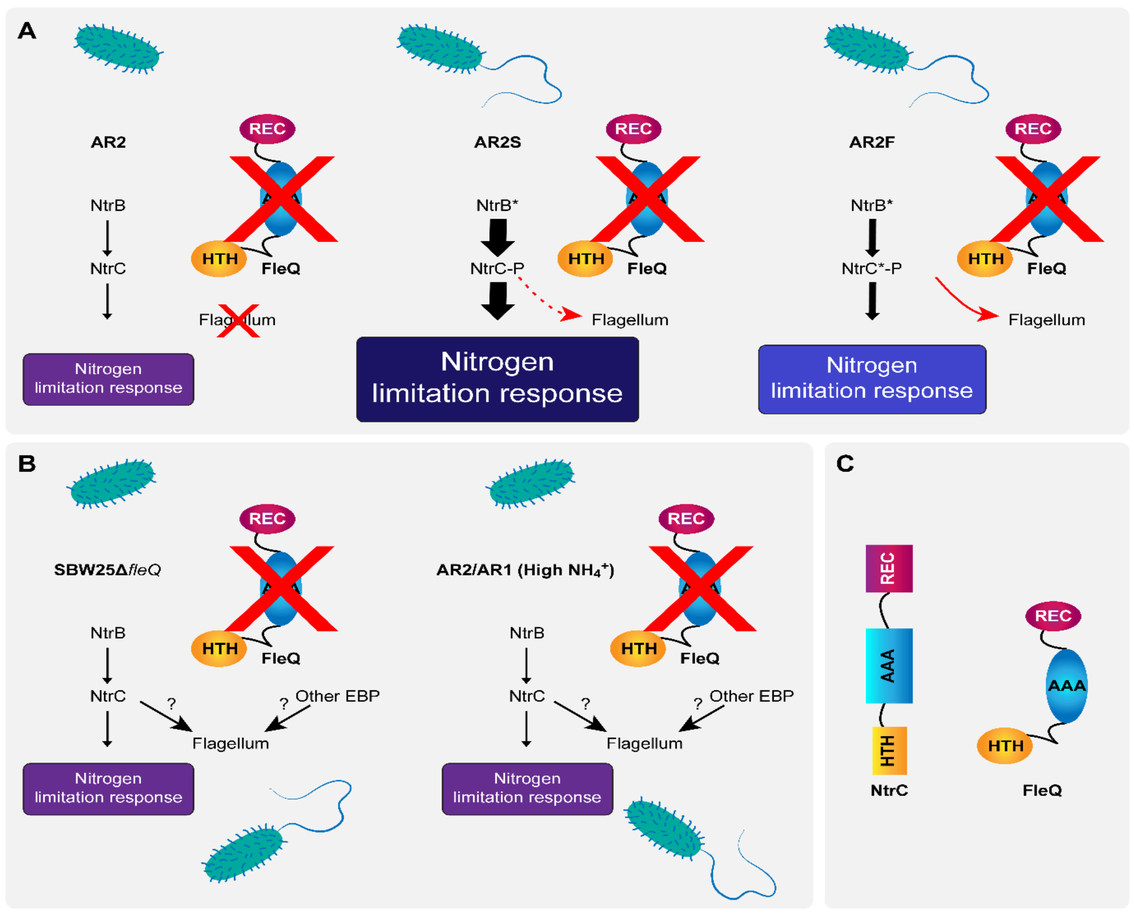

Figure 1. (A) Route of evolution of swimming motility in the non-motile parent strain, AR2 (Science 347: 6225 pp. 1014-107). (B) Hypothesis that other Enhancer Binding Proteins (EBPs) might complement loss of FleQ. (C) Similarity of NtrC and FleQ domain structure. REC, receiver domain; AAA, ATP binding and hydrolysis domain; HTH, helix-turn-helix, DNA binding domain.

Materials and Methods

Evolution of swimming motility in the aflagellate strains, SBW25 Δ*fleQ* (*Fla*$^-$, *Visc*$^+$), AR1 (*Fla*$^-$, *Visc*$^-$), and AR2 (*Fla*$^-$, *Visc*$^-$) was performed in 0.25 % M9 –glucose agar medium with glutamine, glutamate (nitrogen limiting) or ammonium (nitrogen replete) as nitrogen source. Colony spreading phenotypes of evolved isolates on minimal and rich media were monitored and quantitated by time lapse photography. Mutations were identified by targeted sequencing of segments of *ntrB*, *ntrC* and the entire *glnK* gene or by whole genome sequencing (WGS).

Results and Conclusions

Results: As predicted, swimming motility of the sessile strains, AR1 and AR2, evolved later with ammonium as N-source (mean = 5.53 days, *SD* = 0.61 days, *n* = 19) compared to glutamine (mean =2.88 days, *SD* = 0.89 days, *n* = 17) or glutamate (mean = 2.94 days, *SD* = 0.80 days, *n* = 17), Table 1 and Figure 2.

Conclusions: Mutation in NtrB is the primary evolutionary pathway for re-establishment of swimming motility in *P. fluorescens* Δ*fleQ* strains irrespective of nitrogen status.

Table 1 Summary of results

Strain	M9 Culture Medium[B]			LB (Mean OD_{600})	Swimming Motility Phenotype	Swarming	Mean Rate of Colony Expansion in 8 h (mm^2/h)	*ntrB* nt Change	NtrB AA Change	[a]Area (mm^2) Mean (*SD*)
	NH_4^+ (Mean OD_{600})	Glutamine (Mean OD_{600})	Glutamate (Mean OD_{600})							
[c]SBW25	0.5328	0.8171	0.6886	1.5010	WT	WT	26.3	*WT*	*WT*	12328.7 (6204.0)
[c]SBW25Δ*fleQ*	0.5777	0.8701	0.6486	1.2748	None	None	1.3	*WT*	*WT*	11104.5 (6969.0)
FleQS5	0.1068	0.8192	0.2262	1.2634	Medium	Spidery	5.1	***WT**	***WT**	751.9 (559.0)
FleQS7	0.0347	0.8400	0.3204	1.2002	Slow	Spidery	4.4	*WT*	*WT*	652.8 (52.0)

Note: [a]Mean area (mm^2) covered by different strains in 24 h when grown on swarming medium. [b]Glucose as the carbon source. [c]Data for strains SBW25 and SBW25Δ*fleQ* (15 cm diameter plates).
WT wild type; AA amino acid; nt nucleotide.
The strains shaded red grew more or the area covered by them was greater that the strains shaded yellow.
All the evolved strains did not show any mutation in the target sequence for *ntrC*, and there were no mutations present in *glnK*.
*The results from WGS recognised that this strain was a mix population, which carried two non-identical single point mutations in *ntrB*: T97P/D228G. The mutation D228G was not situated in the region covered by the primers, hence initially it was regarded as *WT*.

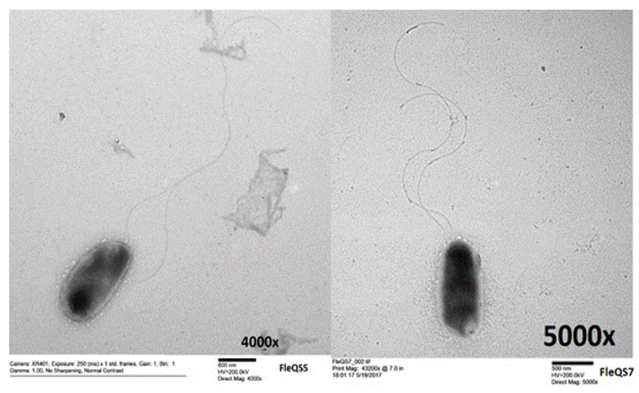

Figure 2 TEM of evolved strains FleQS7 and FleQS5
TEM of strain FleQS7 with flagella in the lophotricus position and strain FleQS5 with a polar flagellum and a lateral flagellum. The cells were grown prior to EM in 3 mL LB (poured into 50 mL conical centrifuge tube) and incubated ON at 26 °C on a rotary shaker at 95 rpm.

References

1. Altamirano Junqueira, A. E. (2019) *Evolution of swimming motility in aflagellate strains of Pseudomonas fluorescens SBW25*. PhD thesis, University of Reading. (http://centaur.reading.ac.uk/85599/)
2. Science 347: 6225 pp. 1014-107

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