Poster

FleN and FlhF as new regulatory elements in the lifestyle switch in *Pseudomonas putida*

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ABSTRACT

Motivation: *Pseudomonas putida* is a soil bacterium that can be found in nature as individual motile cells or as part of sessile communities called biofilms (1). Biofilm formation could be considered an adaptive strategy as it provide higher resistance against adverse agents, antimicrobial treatments, ultraviolet radiation or dehydration, increasing bacteria survival (2). The switch from a planktonic lifestyle to biofilm formation in *P.putida* is regulated by FleQ and the intracellular levels of c-di-GMP (3). Isolation of insertion mutants in *flhF* gene, defective in biofilm formation, suggests the involvement of additional elements in the regulation of this process. In this work, we have characterized the transcriptional organization of *flhA, flhF, fleN* and *fliA* genes and the role of FlhF and FleN in biofilm development in *P.putida*.

Methods: Planktonic growth and biofilm formation curves, adhesion and *swimming*-motility assays. Gene expression analysis: β-galactosidase assays and RT-PCR. Electrophoretic mobility shift assays (EMSA).

Results and Conclusions: To test the role of FlhF and FleN in biofilm development, we carried out a phenotypic characterization of *ΔflhF* and *ΔfleN* mutants. Experiments shown that *ΔfleN* mutant is not able to form biofilm whereas *ΔflhF* mutant exhibits a wild-type phenotype. Adhesion assays indicate that *ΔfleN* mutant has a reduced adhesion, whereas *ΔflhF* can properly adhere to the surface. On the other hand, both mutant show reduced *swimming* motility. These resuts suggests that both, FleN and FlhF, are involved in *swimming* motility, but only FleN is necessary for biofilm formation, probably by altering adhesion capacity of the bacteria.

Bioinformatic tools predict that *flhF* and *fleN* form an operon with the upstream gene *flhA* and the downstream gene *fliA*. In order to corroborate this hypothesis, we have done RT-PCR using RNA from the mutants. Results shown that these genes are structured in a single operon: *flhAFfleNfliA*.

To determine a possible regulatory role of these elements in biofilm formation and motility, β-galactosidase assays were performed to analyse the expression of biofilm and flagellum related promoters. Results shown that FleN downregulates both types of promoters whereas FlhF is only involved in the regulation of one flagellar gene. The regulatory role of FleN has been further studied *in vitro* by EMSA with the regulator FleQ. Results suggest that FleN is required for FleQ to bind to its target promoters.

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