Study of expression heterogeneity of flagellar genes in an insect pathogenic bacterium Xenorhabdus using single cell technologies

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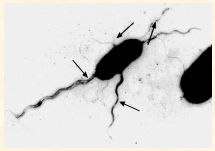
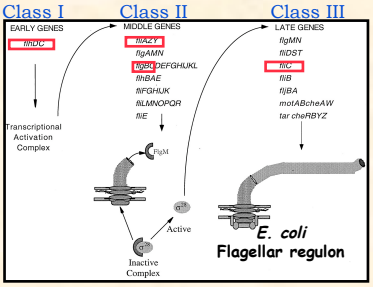
Introduction. *Xenorhabdus nematophila* is a motile Gram-negative bacterium that forms a mutualistic association with a nematode and their association is highly pathogenic for a wide range of insect larvae. Upon insect entry, *Xenorhabdus* is released by its nematode partner, escapes insect defence responses, invades tissues and hemolymph (the insect equivalent of blood) and secretes virulence factors involved in the death of insect. Among the regulatory networks involved in the virulence of *Xenorhabdus*, the flagellar cascade (cf figure) controls the production of flagellum, an extracellular appendage required for bacterial motility, and exoenzymes such as protease, lipase and hemolysins[1].

In this study, we investigated flagellar gene expression dynamics at single-cell resolution using flow cytometry. Promoter sequence of *X. nematophila flgB* gene (class II gene) was first inserted into pPROBE-*gfp*[AAV], a highly stable vector to create a transcriptional fusion with the reporter gene *gfp*[AAV][2]. This *gfp* gene version encodes an unstable GFP with a half-life of ~60 min in *E. coli*. Destablized GFP was used as an expression monitoring system since it allows studies of fine-tuned transcriptional process. This construction was then transferred into *X. nematophila* and *flgB* expression was analysed.



X. nematophila : a motile and flagelled bacterium



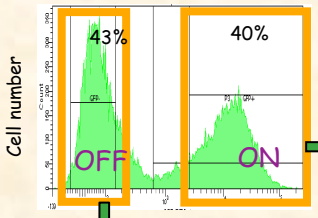
Références: (1) Lanois A, Jubelin G, Givaudan A (2008) Mol Microbiol 68: 516-533.
(2) G. Jubelin, S. Pagès, A. Lanois, M. H. Boyer, S. Gaudriault , J.-B. Ferdy and A. Gi.vaudan (2011). Environmental Microbiology. May;13(5):1271-84.

Bimodal expression of P*flgB-gfp*[AAV] fusion in *X. nematophila*

Cell sorting

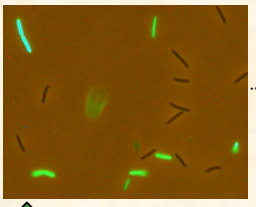
was performed with exponential grown culture using a FACSAria cell sorter during 2 hours in PBS buffer.

Flow cytometry analysis



After subculturing …

Fluorescence microscopy



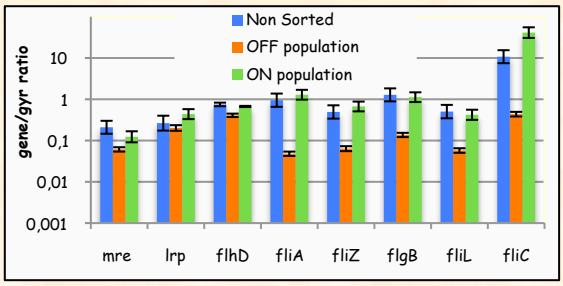
…each subpopulation is able to produce both states.

Expression heterogeneity remains temporal.

Sorted cells analysis

A/ qRT-PCR :

RNA purification from 10 ^{7} cells using Qiagen microprep RNeasy kit

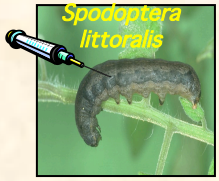


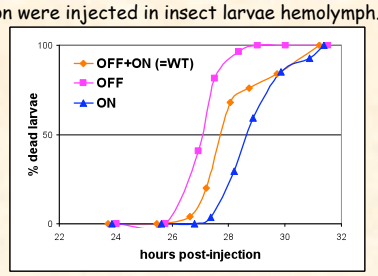
(i) This result confirms the differential level of *flgB* gene expression observed with the *gfp* reporter gene.

(ii) The "OFF" population displays lower levels of class II and class III mRNA, but not class I.

B/ Pathology test :

200 bacteria of each population were injected in insect larvae hemolymph.





flgB- sorted cells displayed an hyper-virulent phenotype in our insect model *Spodoptera*. In contrast, flgB^{+} sorted cells have a delayed virulence suggesting that the production of flagella is deleterious to the bacterial virulence.

Conclusion and perspectives

- This work reveals **a bimodal expression of *flgB* flagellar gene in *X. nematophila*.**

- Previous experiment using a class III gene (*fliC*) gave the same results. These data mean a mixed population of **flagellated and non-flagellated bacteria occurs at the mid-exponential growth phase.**

- Because heterogeneity (or phenotypic noise) is often associated with **positive feedback loop in gene networks**, we are currently examining the involvement of flagellar regulators in this phenomenon.