
Development of genetic tools to study the phenotypic heterogeneity of a marine pathogen

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Abstract

Marine organisms are facing a dual and cumulative threat, *i.e.* pathogenic microorganisms reinforced by global warming. Bacteria belonging to the genus *Vibrio* are one of the most common pathogens in oceans and are responsible for massive death of molluscs. However, despite these ecological and economical concerns, virtually nothing is known about the behaviour of bacterial individuals during infection. Does every single cell of an isogenic pathogen behave the same, or is the bacterial population composed of different sub-populations, behaving differently to divide the labour and conquer its new ecological niche. This "divide and conquer" strategy is the focus of a new field of research in microbiology, but this field remains typically restricted to mammal pathogens.

Here, the aim was to start unravelling the behaviour of *Vibrio harveyi* ORM4, a pathogen infecting the abalone, working at the single cell level to account for the phenotypic heterogeneity. The major limiting step was to genetically modify this strain, as the genetic tools typically available for model organisms are not necessarily valid for environmental and marine bacteria. An electroporation protocol was developed to introduce exogenous DNA in various *Vibrio* strains. Moreover, we constructed bioreporters to follow the expression of genes at the single cell level, targeting relevant virulence genes. Importantly, we could demonstrate for the first time that an isogenic population of *V. harveyi* is composed of 2 populations with varying expression states of a flagellar promoter.

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This work is a fundamental step to understand the behaviour of marine pathogens, and will deepen our understanding on the extent of microbial phenotypic heterogeneity during microbial infections.