
Studying host pathogen interactions by a mutagenesis approach : the case of *Vibrio tapetis* type IV secretion system (T4SS) in the brown ring disease (BRD) affecting the Manila clam *Ruditapes philippinarum*

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Abstract

BRD is a disease affecting the Manila clam *Ruditapes philippinarum* due to the Gram-negative bacterium *Vibrio tapetis*. Recently, Dias et al. (2018) demonstrated the high level of conservation of the gene cluster coding for the T4SS in strains of *V. tapetis* pathogenic to clams. This system is well known to be responsible of pathogenicity in multiple diseases and can deliver proteins directly into host cells to cause infection. Our approach is to delete genes potentially linked to pathogenicity in order to improve knowledge on the mechanisms associated to the infection by *V. tapetis* in the context of BRD.

For our first study, we choose to target the gene *VirB4*, encoding an ATPase subunit of the T4SS, in order to prove its role in BRD. Our approach is based on a mutagenesis technique by two homologous recombinations enabling to delete only the targeted gene. To test pathogenicity of our *Delta-VirB4* strain, we performed an *in vitro* standardized virulence test based on the ratio of non-adherent hemocytes in exposed samples (to the WT strain or the *Delta-VirB4* strain) / in control samples.

We succeeded to construct a *V. tapetis* strain lacking the gene *VirB4* by optimizing the conjugation protocol in order to improve the efficiency of our technique. Our virulence test shows a loss of adherence properties of clam hemocytes in the presence of the WT strain but not in the presence of the *Delta-VirB4* strain, revealing then a loss of *V. tapetis* cytotoxic activity in absence of the gene *VirB4*.

These results strongly suggest that the T4SS plays an essential role in the pathogenic capacity of the bacterium. With this technique we have developed, we will be able to better understand the genes and thus the mechanisms related to the pathogenicity of *V. tapetis* in the context of BRD.

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