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The *Vibrio campbellii* quorum sensing signals have a different impact on virulence of the bacterium towards different crustacean hosts

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ABSTRACT

Pathogenic bacteria communicate with small signal molecules in a process called quorum sensing, and they often use different signal molecules to regulate virulence gene expression. *Vibrio campbellii*, one of the major pathogens of aquatic organisms, regulates virulence gene expression by a three channel quorum sensing system. Here we show that although they use a common signal transduction cascade, the signal molecules have a different impact on the virulence of the bacterium towards different hosts, i.e. the brine shrimp *Artemia franciscana* and the commercially important giant freshwater prawn *Macrobrachium rosenbergii*. These results suggest that the use of multiple types of signal molecules to regulate virulence gene expression is one of the features that allow bacteria to infect different hosts. Our findings emphasize that it is highly important to study the efficacy of quorum sensing inhibitors as novel biocontrol agents under conditions that are as close as possible to the clinical situation.

1. Introduction

Pathogenic bacteria are often capable of infecting multiple host species (Faruque et al., 1998; Salanoubat et al., 2002; He et al., 2004; Seshadri et al., 2006; Defoirdt et al., 2007). This will require some level of flexibility in sensing of and responding to the environment since the bacteria will experience different environmental conditions in different hosts (especially when these are distantly related). One of the regulatory mechanisms involved in interacting with the environment is quorum sensing, a mechanism in which bacteria coordinate the expression of certain genes in response to the presence of small signal molecules (Hense et al., 2007). Quorum sensing systems have been reported to regulate the expression of virulence genes in many pathogenic bacteria, and many of them use different signal molecules to regulate virulence gene expression (Jayaraman and Wood, 2008; Ng and Bassler, 2009). However, it is often not clear why different signals are used, especially when they are controlling the same virulence factors and when they are using a common signal transduction cascade, as is the case in vibrios (Milton, 2006).

Vibrio campbellii BB120 (=ATCC BAA-1116; previously designated *Vibrio harveyi* (Lin et al., 2010)) and closely related bacteria are amongst the most important pathogens of aquatic organisms, causing significant losses in the aquaculture industry worldwide (Ruwandeepika et al., 2012). These pathogens can infect multiple hosts belonging

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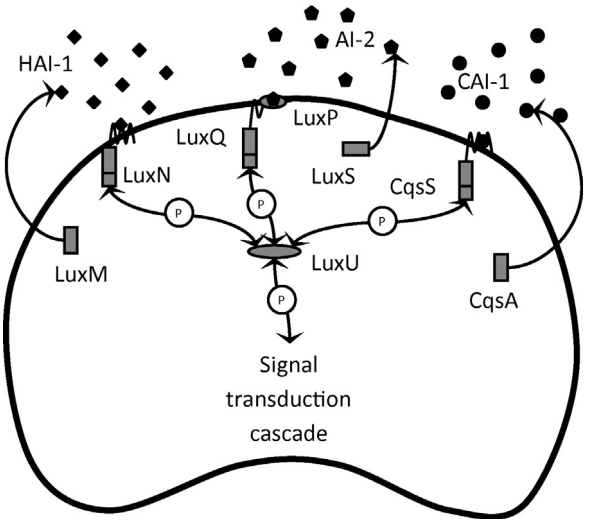

Fig. 1. Quorum sensing in *Vibrio campbellii*. The LuxM, LuxS and CqsA enzymes synthesize the autoinducers HAI-1, AI-2 and CAI-1, respectively. These autoinducers are detected at the cell surface by the LuxN, LuxQ and CqsS two-component receptor proteins, respectively. Detection of AI-2 by LuxQ requires the periplasmic protein LuxP. The receptors feed a common phosphorylation/dephosphorylation signal tranduscton cascade regulating the expression of target genes. "P" denotes b, c phosphotransfer.

to distantly related taxa, ranging from mollusks over crustaceans to fish (Defoirdt et al., 2007). The species is also one of the model organisms in studies on quorum sensing in bacteria (Ng and Bassler, 2009). *V. campbellii* BB120 contains a three-channel quorum sensing system, with three different types of signal molecules (HAI-1, AI-2 and CAI-1, respectively) feeding a common signal transduction cascade (Fig. 1). HAI-1, harveyi autoinducer-1, is 3-hydroxybutanoyl-L-homoserine lactone; AI-2, autoinducer-2, is the furanosyl borate diester 3A-methyl-5,6-dihydrofuro(2,3-D)(1,3,2)diox-aborole-2,2,6,6A-tetraol; and CAI-1, cholerae autoinducer-1, is (Z)-3-aminoundec-2-en-4-one (Ng and Bassler, 2009; Ng et al., 2011). *V. campbellii* quorum sensing has been documented to control the expression of different virulence genes, including *vhp* metalloprotease (Mok et al., 2003; Ruwandeepika et al., 2011), a siderophore (Lilley and Bassler, 2000), type III secretion system components (Henke and Bassler, 2004a), chitinase A (Defoirdt et al., 2010) and three phospholipase genes (Natrah et al., 2011). We previously reported that AI-2 and CAI-1, but not HAI-1, are needed for full virulence of this bacterium towards brine shrimp larvae (Defoirdt et al., 2005; Defoirdt and Sorgeloos, 2012). Here, we report that HAI-1 and AI-2, but not CAI-1, are needed for full virulence towards the commercially important giant freshwater prawn *Macrobrachium rosenbergii*. These data suggest that the use of different types of signal molecules to regulate virulence gene expression is one of the features that allow bacteria to infect different hosts.

2. Materials and methods

2.1. Bacterial strains and growth conditions

V. campbellii strains used in this study are described in Table 1. All strains were stored at −80 °C in 40% glycerol and the stocks were streaked onto Luria-Bertani agar containing 12 g l^{-1} Instant Ocean synthetic sea salt (Aquarium Systems Inc., Sarrebourg, France) (LB_{12}) or 35 g l^{-1} Instant Ocean (LB_{35}) for use in giant freshwater prawn and brine shrimp, respectively. After 24 h of incubation at 28 °C, a single colony was inoculated into 5 ml fresh LB broth with appropriate salinity and incubated overnight at 28 °C under constant agitation (100 min^{-1}).

Table 1
Vibrio campbellii strains used in this study.

Strain	Relevant information	Reference
BB120	Wild type from which all other strains are derived	Bassler et al. (1997)
BB120RifR	Spontaneous rifampicin resistant mutant of BB120	This study
BB152	Mutation in *luxM* (HAI-1 synthase)	Bassler et al. (1994)
MM30	Mutation in *luxS* (AI-2 synthase)	Surette et al. (1999)
MM77	Mutation in *luxM* and *luxS*	Surette et al. (1999)
MM77RifR	Spontaneous rifampicin resistant mutant of MM77	This study
JMH603	Mutation in *cqsA* (CAI-1 synthase)	Henke and Bassler (2004b)

2.2. Selection of rifampicin resistant mutants of V. campbellii BB120 and MM77

100 μl of densely grown cultures of BB120 and MM77 (OD_{600} of 1) were inoculated into tubes containing 5 ml of fresh LB_{12} broth supplemented with 50 mg l^{-1} rifampicin (Sigma) and incubated for 5 days at 28 °C under constant agitation (100 min^{-1}). The grown cultures were inoculated into fresh LB_{12} broth with 50 mg l^{-1} rifampicin and incubated overnight. The grown cultures were stored at −80 °C in 40% glycerol until use.

2.3. Signal molecules

HAI-1, N-3-hydroxybutanoyl-L-homoserine lactone (Sigma) was dissolved in distilled water at 1000 mg l^{-1}. AI-2 precursor (S)-4,5-dihydroxy-2,3-pentadione (DPD) was obtained from OMM Scientific Inc. (Dallas, Texas, USA).

2.4. Axenic hatching of brine shrimp

Decapsulation and hatching of axenic brine shrimp was performed as described previously (Defoirdt et al., 2005), with some modifications. Briefly, 200 mg cysts (Ocean Nutrition Europe, Essen, Belgium) were hydrated in a 50 ml tube containing 18 ml distilled water for 1 h. Sterile cysts were obtained via decapsulation using 660 μl NaOH (32%) and 10 ml NaOCl (50%). The suspension was gently shaken under a laminar flow hood. The reaction was stopped after 2 min by adding 14 ml $Na_2S_2O_3$ (10 g l^{-1}). The decapsulated cysts were washed with fresh autoclaved synthetic sea water (35 g l^{-1} Instant Ocean) over a 100 μm sieve and transferred to two sterile 50 ml tubes, each containing