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ORIGINAL ARTICLE

Luminescence, virulence and quorum sensing signal production by pathogenic *Vibrio campbellii* and *Vibrio harveyi* isolates

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Keywords
quorum quenching, quorum sensing, *Vibrio harveyi*.

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Abbreviations
AHL, Acylated homoserine lactone; AI-2, Autoinducer 2; CAI-1, Cholerae autoinducer 1; HAI-1, Harveyi autoinducer 1.

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Abstract

Aims: To study the relationship between luminescence, autoinducer production and virulence of pathogenic vibrios.
Methods and Results: Luminescence, quorum sensing signal production and virulence towards brine shrimp nauplii of 13 *Vibrio campbellii* and *Vibrio harveyi* strains were studied. Although only two of the tested strains were brightly luminescent, all of them were shown to produce the three different types of quorum sensing signals known to be produced by *Vibrio harveyi*. Cell-free culture fluids of all strains significantly induced bioluminescence in the cholerae autoinducer 1, autoinducer 2 and harveyi autoinducer 1 reporter strains JAF375, JMH597 and JMH612, respectively. There was no relation between luminescence and signal production and virulence towards brine shrimp.
Conclusions: There is a large difference between different strains of *Vibrio campbellii* and *Vibrio harveyi* with respect to bioluminescence. However, this is not reflected in signal production and virulence towards gnotobiotic brine shrimp. Moreover, there seems to be no relation between quorum sensing signal production and virulence towards brine shrimp.
Significance and Impact of the Study: The results presented here indicate that strains that are most brightly luminescent are not necessarily the most virulent ones and that the lower virulence of some of the strains is not due to a lack of autoinducer production.

Introduction

Aquaculture comprises all forms of culture of aquatic animals and plants in fresh, brackish and marine environments (Pillay and Kutty 2005). According to FAO statistics, it is one of the fastest growing food-producing industries worldwide (FAO, 2004). However, disease outbreaks are considered as a significant constraint to the development of the sector, with a global estimate (made by the World Bank in 1997) of disease losses in the range of US$ 3 billion per year (Subasinghe *et al.* 2001). Bacteria belonging to the species *Vibrio harveyi* and closely related species such as *Vibrio campbellii* are amongst the most important bacterial pathogens in aquaculture. *V. harveyi* and *V. campbellii* are genetically related species with a DNA–DNA similarity value of 69% and a 16S rRNA similarity higher than 97% (Gomez-Gil *et al.* 2004). Disease caused by these bacteria is often referred to as luminescent vibriosis as many of these bacteria are luminescent (Aguirre-Guzmán *et al.* 2004). Although almost all types of cultured animals can be affected by these bacteria, the most serious problems have been reported in penaeid shrimp culturing, and luminescent vibriosis has become a major constraint on shrimp production in South America and Asia (Austin and Zhang 2006).

Virulence of several pathogenic bacteria has been shown to be linked to quorum sensing, bacterial cell-to-cell communication by means of small signal molecules (De Kievit and Iglewski 2000). *Vibrio harveyi* has been

reported to use a multi-channel quorum sensing system. The first channel of this system is mediated by the harveyi autoinducer 1 (HAI-1), an acylated homoserine lactone (AHL) (Cao and Meighen 1989). The second channel is mediated by the so-called autoinducer 2 (AI-2), which is a furanosyl borate diester (Chen *et al.* 2002). The chemical structure of the third autoinducer, called cholerae autoinducer 1 (CAI-1) is still unknown (Henke and Bassler 2004b). All three autoinducers are detected at the cell surface and activate or inactivate target gene expression by a phosphorylation/dephosphorylation signal transduction cascade. Phenotypes that were found to be controlled by this quorum sensing system include bioluminescence (Bassler *et al.* 1993) and the production of several virulence factors such as a type III secretion system (Henke and Bassler 2004a), extracellular toxin (Manefield *et al.* 2000) and a siderophore (Lilley and Bassler 2000). Recent research at our laboratories showed that quorum sensing regulates virulence of the bacterium towards brine shrimp (*Artemia franciscana*) and rotifers (*Brachionus plicatilis*) (Defoirdt *et al.* 2005; Tinh *et al.* 2007). Moreover, quorum sensing-disrupting halogenated furanones were shown to neutralize the negative effects of pathogenic isolates belonging to the species *V. harveyi*, *V. campbellii* and *Vibrio parahaemolyticus* (Defoirdt *et al.* 2006; Tinh *et al.* 2007).

This study aimed at detecting quorum sensing signal production by several different pathogenic isolates belonging to the species *V. harveyi* and the closely related *V. campbellii* and linking signal production and luminescence of the strains to virulence towards gnotobiotic brine shrimp nauplii.

Materials and methods

Bacterial strains and growth conditions

The bacterial strains that were used in this study are described in Table 1. All strains were stored in 40% glycerol at −80°C. Of these stored cultures, 10 μl was inocu-

Table 1 Bacterial strains used in this study

Strain	Relevant features and/or synonyms	Reference
LVS3	Isolate that enhances growth and survival of *Artemia*	Verschuere *et al.* (1999)
Vibrio campbellii strains		
LMG 21361	= CAIM415 = Z1; isolated from sea water from shrimp (*Litopenaeus* spp.) broodstock tank, Mexico	Soto-Rodriguez *et al.* (2003); Gomez-Gil *et al.* (2004)
LMG 21362	= CAIM333 = M1; isolated from sea water from shrimp (*Litopenaeus* spp.) broodstock tank, Mexico	Soto-Rodriguez *et al.* (2003); Gomez-Gil *et al.* (2004)
LMG 21363	= CAIM372 = PN9801; isolated from the lymphoid organ of diseased shrimp (*Penaeus* spp.) juveniles, Philippines	Soto-Rodriguez *et al.* (2003); Gomez-Gil *et al.* (2004)
LMG 22888	= CAIM416 = Z2; isolated from sea water from shrimp (*Litopenaeus* spp.) broodstock tank, Mexico	Soto-Rodriguez *et al.* (2003); Gomez-Gil *et al.* (2004)
LMG 22889	= CAIM417 = Z3; isolated from sea water from shrimp (*Litopenaeus* spp.) broodstock tank, Mexico	Soto-Rodriguez *et al.* (2003); Gomez-Gil *et al.* (2004)
LMG 22890	= CAIM395 = STD3-131; isolated from diseased shrimp (*Litopenaeus* spp.) postlarvae, Ecuador	Soto-Rodriguez *et al.* (2003); Gomez-Gil *et al.* (2004)
LMG 22895	= CAIM223; isolated from the hepathopancreas of diseased shrimp (*Litopenaeus* spp.), Mexico	Bruno Gomez-Gil
Vibrio harveyi strains		
LMG 22891	= CAIM88; isolated from the hemolymph of shrimp (*Litopenaeus* spp.), Mexico	Bruno Gomez-Gil
LMG 22893	= CAIM148; isolated from the hemolymph of diseased shrimp (*Penaeus* spp.), Mexico	Bruno Gomez-Gil
LMG 22894	= CAIM151; isolated from the hemolymph of diseased shrimp (*Penaeus* spp.), Mexico	Bruno Gomez-Gil
VIB 571	Isolates from sea bass (*Dicentrarchus labrax*), Spain	Zhang *et al.* (2001)
VIB 645	Isolated from sea bass (*Dicentrarchus labrax*), Tunisia	Zhang *et al.* (2001)
Vibrio harveyi BB120 and mutants		
BB120	Wildtype from which strains BNL258, JAF375, JAF483, JAF553, JMH597 and JMH612 are derived	Bassler *et al.* (1997)
JAF375	*luxN*::Cm^R *luxQ*::Kan^R	Freeman and Bassler (1999)
JMH597	*luxN*::Tn5 *cqsS*:: Cm^R	Henke and Bassler (2004b)
JMH612	*luxPQ*::Tn5 *cqsS*:: Cm^R	Henke and Bassler (2004b)

LMG, Laboratory of Microbiology collection (Ghent University, Belgium); CAIM, Collection of Aquacultural Important Micro-organisms (CIAD/Mazatlán Unit for Aquaculture, Mazatlán, Mexico).

lated into Marine Broth (Difco Laboratories, Detroit, MI, USA) and incubated at 28°C under constant agitation (100 rev min^{-1}). Spectrophotometry at OD_{600} was used to measure growth. Luminescence was measured with a Lumac Biocounter M2500 luminometer (Lumac b.v., Landgraaf, the Netherlands).

Preparation of cell-free culture fluids

Cell-free culture fluids were prepared by removing the cells from the growth medium by centrifugation at 4000 ***g*** for 5 min in a microcentrifuge. The cleared fluids were passed through 0·22 μm Millipore filters (Millipore, Bedford, USA) and stored at −20°C.

Detection of autoinducers

Cell-free culture fluids of the strains were tested for the presence of *V. harveyi* autoinducers by an assay slightly modified from Surette and Bassler (1998) using the double mutants JAF375 (sensor HAI-1^-, sensor AI-2^-, sensor CAI-1^+), JMH597 (sensor HAI-1^-, sensor AI-2^+, sensor CAI-1^-) and JMH612 (sensor HAI-1^+, sensor AI-2^-, sensor CAI-1^-) as reporters for CAI-1, AI-2 and HAI-1, respectively. The reporter strains were grown at 28°C with shaking (100 rev min^{-1}) in Marine Broth to an OD_{600} of approximately 1 and diluted 1/5000 in fresh medium. Of the diluted reporter cultures, 50 μl was mixed with 50 μl of cell-free culture fluids in 3-ml test tubes. Cell-free culture fluids of *V. harveyi* BB120 were used as a positive control; fresh medium was used as a negative control. The test tubes were incubated at 28°C and luminescence was measured every hour with a Lumac Biocounter M2500 luminometer (Lumac b.v) until the luminescence with fresh medium was minimal (after 3–4 h).

Axenic hatching of Artemia

All experiments were performed with high quality hatching cysts of *Artemia franciscana* (EG® Type, batch 6940; INVE Aquaculture, Baasrode, Belgium). Of cysts, 200 mg was hydrated in 18 ml of tap water for 1 h. Sterile cysts and nauplii were obtained via decapsulation, adapted from Marques *et al.* (2004). Briefly, 660 μl of NaOH (32%) and 10 ml of NaOCl (50%) were added to the hydrated cyst suspension. The decapsulation was stopped after 2 min by adding 14 ml of $Na_2S_2O_3$ (10 g l^{-1}). During the reaction, 0·22 μm filtered aeration was provided. The decapsulated cysts were washed with filtered (0·22 μm) and autoclaved artificial seawater containing 35 g l^{-1} of Instant Ocean synthetic sea salt (Aquarium Systems Inc., Sarrebourg, France). The cysts were resuspended in a 50-ml tube containing 30 ml of filtered and autoclaved artificial seawater and hatched for 24 h on a rotor (4 rev min^{-1}) at 28°C with constant illumination (approximately 2000 lux).

Challenge tests

Challenge tests were performed as described in Defoirdt *et al.* (2006). Briefly, after hatching, groups of 20 nauplii were transferred to new sterile 50-ml tubes that contained 20 ml of filtered and autoclaved artificial seawater. The pathogens were washed in filtered and autoclaved artificial seawater after incubation and the suspensions were diluted to an OD_{600} of approximately 0·1. Of the diluted suspensions, 20 μl was inoculated into the *Artemia* culture water (which is equivalent to approximately 10^5 CFU ml^{-1}) and 200 μl of a suspension of autoclaved LVS3 bacteria in filtered and autoclaved artificial seawater (OD_{600} of 1) was added as feed. Afterwards, the falcon tubes were put back on the rotor and kept at 28°C. *Artemia* cultures, to which only autoclaved LVS3 bacteria were added, were used as controls. The survival of *Artemia* was scored 2 days after the addition of the strains. All manipulations were carried out under a laminar flow hood to maintain sterility of the cysts and nauplii. Each treatment was performed in triplicate.

Statistics

Pearson correlations were calculated and independent samples *t*-tests were performed using the SPSS software, version 12.0 (SPSS, Chicago, IL).

Results

Luminescence of the isolates

All isolates were cultured in Marine Broth at 28°C with shaking, during which growth and luminescence were monitored. There was a substantial variation between the different isolates with respect to bioluminescence (Table 2). *Vibrio campbellii* LMG 21363 and *V. harveyi* BB120 were brightly luminescent and *V. campbellii* strains LMG 22888, LMG 22889 were dim. *Vibrio campbellii* strains LMG 21361 and LMG 22890 were dim in one of the experiments and the other strains were dark in all three experiments. Statistical analysis revealed that luminescence of strains LMG 21363, BB120, LMG 22888 and LMG 22889 was significantly different from background luminescence ($P < 0·01$). Luminescence of these strains was generally maximal during late exponential and early stationary phase (data not shown).

Table 2 Bioluminescence (mean ± standard deviation of three independent experiments) of late exponential phase cultures of the *Vibrio campbellii* and *Vibrio harveyi* isolates

Strain	Luminescence [log(RLU*)]
V. campbellii strains	
LMG 21361	3·5 ± 1·9
LMG 21362	1·2 ± 0·2
LMG 21363	7·5 ± 0·3**
LMG 22888	2·4 ± 0·5**
LMG 22889	2·8 ± 0·2**
LMG 22890	3·5 ± 1·8
LMG 22895	0·8 ± 0·1
V. harveyi strains	
BB120	7·8 ± 0·5**
LMG 22891	0·7 ± 0·2
LMG 22893	1·1 ± 0·3
LMG 22894	0·8 ± 0·1
VIB 571	0·7 ± 0·2
VIB 645	0·9 ± 0·2
Background luminescence	0·8 ± 0·2

LMG, Laboratory of Microbiology collection (Ghent University, Belgium).

*RLU is the relative unit of luminescence reported by the Lumac Biocounter M2500 luminometer.

**Significantly different from the background luminescence ($P < 0·01$).

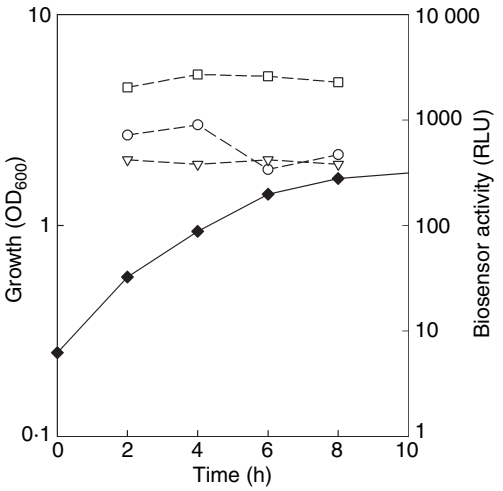

Figure 1 Growth of *Vibrio harveyi* BB120 in Marine Broth and detection of autoinducers by the reporter strains JAF375 (Cholerae autoinducer 1), JMH597 (autoinducer 2) and JMH612 (harveyi autoinducer 1) in cell-free culture fluids. Note that RLU is the relative unit of luminescence reported by the Lumac Biocounter M2500 luminometer (◆, growth; ○, CAI-1; ▽, AI-2; □, HAI-1).

Detection of autoinducers in cell-free culture fluids

Quorum sensing has been studied extensively in *V. harveyi* strain BB120 and production of the autoinducers in this strain has been shown to be maximal during late exponential and stationary phase. In a preliminary experiment, we aimed at detecting autoinducer production in this strain during late exponential growth. To this purpose, the strain was grown overnight in Marine Broth and diluted to an OD_{600} of approximately 0·2 in fresh medium. The diluted culture was incubated at 28°C with shaking and every 2 h, cell-free culture fluids were prepared, which were then assayed for the presence of autoinducers with the reporter strains. Luminescence of the reporter strains was measured every hour and the background signal (i.e. interference by endogenous autoinducer production of the reporters) was found to be minimal between 3 and 4 h. Consequently, this time point was chosen for monitoring the effects of autoinducers in the cell-free culture fluids. Luminescence in the three reporters was found to be significantly induced by the cell-free culture fluids of strain BB120, with only minor differences between the different samplings (Figure 1).

All strains showed similar growth profiles during late exponential phase (data not shown) and therefore, we decided to follow the same procedure as used for strain BB120 to study autoinducer production by the strains. Strains were grown to high density, diluted and regrown to an OD_{600} of 1. Cell-free culture fluids were prepared and assayed for the presence of autoinducers. Fresh medium was analysed as a negative control. The culture fluids of all isolates significantly induced bioluminescence in all three reporter strains ($P < 0·05$; Table 3). There was no significant correlation between the levels of induction of the different reporters ($P > 0·5$). Background luminescence was 45%, 2% and 10% for the CAI-1, AI-2 and HAI-1 reporter, respectively. The high background luminescence of the CAI-1 reporter can be explained by the early activation of the CAI-1 channel of the quorum sensing system (see Discussion). An independent repetition of the CAI-1 detection experiment yielded similar results, with levels of induction between 62% and 115% and a background luminescence of 43 ± 5% (data not shown). From these observations, we conclude that all isolates produce the three autoinducers. We also performed the CAI-1 detection assay with a 10-fold lower initial concentration of the CAI-1 reporter strain to decrease the background luminescence. However, in this case, the luminescence signal was too low for a good detection.

Virulence of the strains towards gnotobiotic *Artemia* nauplii and relation between virulence and luminescence and autoinducer production

All isolates caused significant mortality in gnotobiotic *Artemia* nauplii when compared with unchallenged nauplii ($P < 0·01$). Most isolates caused between roughly

Table 3 Induction of bioluminescence in *Vibrio harveyi* reporter strains by cell-free culture fluids from the *Vibrio campbellii* and *V. harveyi* isolates (mean ± standard deviation of three replicates). Cell-free culture fluids from *V. harveyi* BB120 were used as a positive control and the level of BB120 stimulation was normalized to 100%

	Induction of bioluminescence in reporter (%)		
Strain	JAF375 (CAI-1)	JMH597 (AI-2)	JMH612 (HAI-1)
V. campbellii strains			
LMG 21361	147 ± 3**	49 ± 4**	35 ± 4**
LMG 21362	107 ± 8**	115 ± 15**	34 ± 1**
LMG 21363	95 ± 4**	68 ± 5**	50 ± 3**
LMG 22888	65 ± 7*	98 ± 9**	22 ± 5*
LMG 22889	81 ± 5**	98 ± 4**	42 ± 4*
LMG 22890	71 ± 7*	58 ± 9**	49 ± 3**
LMG 22895	74 ± 6**	54 ± 8**	37 ± 2**
V. harveyi strains			
LMG 22891	123 ± 7**	83 ± 8**	24 ± 6*
LMG 22893	62 ± 5*	77 ± 8**	41 ± 4**
LMG 22894	88 ± 7**	112 ± 20**	63 ± 1**
VIB 571	70 ± 7**	36 ± 9**	60 ± 11**
VIB 645	62 ± 9*	102 ± 5**	99 ± 7**
Controls			
BB120	100 ± 3**	100 ± 13**	100 ± 7**
Medium	45 ± 8	2 ± 1	10 ± 2

LMG, Laboratory of Microbiology collection (Ghent University, Belgium); CAI-1, cholerae autoinducer 1; AI-2, autoinducer 2; HAI-1, harveyi autoinducer 1.
Significantly different from bioluminescence of the same reporter induced by medium (*$P < 0{\cdot}05$; **$P < 0{\cdot}01$).

Table 4 Percentage mortality of *Artemia* nauplii (mean ± standard deviation of three replicates) after 48 h challenge with the *Vibrio campbellii* and *Vibrio harveyi* isolates

Strain	Mortality in *Artemia* nauplii (%)*
V. campbellii strains	
LMG 21361	57 ± 8
LMG 21362	68 ± 8
LMG 21363	82 ± 8
LMG 22888	52 ± 8
LMG 22889	50 ± 5
LMG 22890	62 ± 6
LMG 22895	48 ± 10
V. harveyi strains	
BB120	48 ± 8
LMG 22891	38 ± 8
LMG 22893	45 ± 5
LMG 22894	52 ± 8
VIB 571	50 ± 9
VIB 645	38 ± 3

LMG, Laboratory of Microbiology collection (Ghent University, Belgium).
*Mortality in unchallenged nauplii was 15 ± 5%.

35% and 55% mortality (Table 4). However, there were significant differences between the different isolates, with *V. campbellii* LMG 21362 and LMG 21363 being the most virulent strains.

There was no significant correlation between luminescence of the strains and mortality caused in *Artemia* nauplii ($P = 0{\cdot}064$). In addition to this, an independent samples *t*-test learned that there was no significant difference between luminescent and dark strains with respect to virulence towards *Artemia* nauplii ($P = 0{\cdot}35$).

It is not possible to calculate correlations between virulence and autoinducer detection as autoinducer detection assays using bioluminescence as reporter are qualitative, but not quantitative (Turovskiy and Chikindas 2006). However, both low and high virulent strains were shown to significantly induce bioluminescence in the reporters, indicating that they all produce the three signal molecules. Therefore, the lower virulence of some strains is not due to a lack of autoinducer production.

Discussion

Although *V. harveyi* and related bacteria are commonly denoted as luminescent vibrios, we found a large difference between different strains with respect to bioluminescence. In fact, only two out of 13 tested strains were visibly luminescent. Similar differences in luminescence between different strains of *V. harveyi* have been reported earlier (Gomez-Gil *et al.* 2004; Nakayama *et al.* 2006a). Interestingly, we found no difference between luminescent and nonluminescent strains with respect to virulence towards shrimp larvae *in vivo*. This is consistent with the report of Nakayama *et al.* (2006b), who showed that dark strains produced high levels of haemolytic activity *in vitro*. The reason why some *V. harveyi* strains are luminescent and others are dark is yet unknown. Nakayama *et al.* (2006b) hypothesized that because luminescence is regulated by quorum sensing, dark strains might be able to express luminescence in the presence of high concentrations of autoinducers. However, in this work, we showed that there were no significant differences between luminescent and dark strains with respect to autoinducer production and therefore, an inability to produce autoinducers is not the reason for the dark strains to be dark. The nonluminescent phenotype is probably caused by defects in one of the genes in the *lux* operon or genes involved in luminescence regulation (such as the quorum sensing signal transduction). Bacteria with such defects have been reported for the cold-water fish pathogenic species *Vibrio salmonicida* and have been referred to as cryptically luminescent (Fidopiastis *et al.* 1999).

A second series of experiments aimed at detecting autoinducers in cell-free culture fluids of the different

V. campbellii and *V. harveyi* strains. *Vibrio harveyi* mutant strains that are defective in one of the autoinducer receptors are commonly used as reporters to detect signal molecule production. The HAI-1 receptor mutant BB170, for instance, is commonly used as AI-2 reporter (Bassler *et al.* 1997; Ren *et al.* 2001; McDougald *et al.* 2003). However, those mutants are not specific as they are still responsive to two of the three signals produced by *V. harveyi*. Consequenntly, in this study, the autoinducer receptor double mutants JAF375 (sensor HAI-1$^-$, sensor AI-2$^-$, sensor CAI-1$^+$), JMH597 (sensor HAI-1$^-$, sensor AI-2$^+$, sensor CAI-1$^-$) and JMH612 (sensor HAI-1$^+$, sensor AI-2$^-$, sensor CAI-1$^-$) were chosen as reporters for CAI-1, AI-2 and HAI-1, respectively, as these strains are only responsive to one of the signals. Although these strains allow for a more specific detection of autoinducers than the single mutants, their sensitivity is significantly lower. Indeed, when compared with the single mutants, the relative background luminescence (i.e. the difference in luminescence between positive and negative control) of these double mutants is higher. This can be explained by the fact that the *V. harveyi* quorum sensing system is a coincidence detector, which results in a much higher induction of luminescence in the presence of two autoinducers when compared with only one (Henke and Bassler 2004b). The commonly used single mutant reporter strains can still detect two of the three autoinducers, whereas the double mutants can only detect one. Moreover, the CAI-1 channel of the *V. harveyi* quorum sensing system is activated already at quite low cell densities (Henke and Bassler 2004b) and because of that, the background luminescence of the double mutant CAI-1 reporter JAF375 is quite high. However, the signal molecule receptor double mutants are the only reporters currently available that allow for a specific detection of the different autoinducers produced by vibrios. We performed an independent repetition of the detection assay and analysed the data statistically to compensate for the low sensitivity of the reporters (especially in case of the CAI-1 reporter JAF375). We could conclude that the cell-free culture fluids of all strains significantly induced luminescence in the CAI-1, AI-2 and HAI-1 receptors, from which we deduce that all isolates produce the three different autoinducers. These results are in accordance with previous reports mentioning CAI-1, AI-2 and HAI-1 production by luminescent vibrios (Bassler *et al.* 1997; Henke and Bassler 2004b; Defoirdt *et al.* 2006).

There was no relation between virulence (as assessed by mortality in *Artemia* nauplii) and the level of induction of the different reporters by cell-free culture fluids of the strains, indicating that the lower virulence of some of the strains is not due to a lack of autoinducer production. Previous work at our laboratories has shown that a functional quorum sensing system is essential for pathogenicity of luminescent vibrios towards different hosts (Defoirdt *et al.* 2005, 2006; Tinh *et al.* 2007). The results obtained in this study suggest that there is a difference between different strains of these bacteria with respect to the induction of virulence by quorum sensing signals. This is consistent with our previous observations that there is a difference between different strains in the level of protection offered by the natural quorum sensing-disrupting furanone (*5Z*)-4-bromo-5-(bromomethylene)-3-butyl-2(*5H*)-furanone in challenge tests with brine shrimp (Defoirdt *et al.* 2006). The pathogenicity mechanisms of *V. harveyi* and closely related bacteria still have to be fully elucidated, although several different virulence factors have been shown to be involved (Austin and Zhang 2006). These virulence factors include a bacteriocin-like substance, lytic enzymes, toxins, lipopolysaccharide and siderophores (Owens *et al.* 1996; Harris and Owens 1999; Montero and Austin 1999; Prasad *et al.* 2005; Austin and Zhang 2006). Not all strains produce all of these virulence factors and different strains are also known to produce different levels of virulence factors (Soto-Rodriguez *et al.* 2003). We hypothesize that the difference in the quorum sensing regulation of virulence can be explained by differences in the relative amounts of virulence factors produced by different strains and by the fact that probably not all virulence factors are quorum sensing regulated. In fact, of the known virulence factors, only siderophore and extracellular toxin are yet known to be regulated by quorum sensing (Lilley and Bassler 2000; Manefield *et al.* 2000). Hence, it will be important to further investigate which of the virulence factors produced by *V. harveyi* and related species are regulated by quorum sensing to better understand the pathogenicity mechanisms of these bacteria. Finally, it would be highly interesting to study quorum sensing regulation of virulence *in vivo* (i.e. during infection of a host) as this can be quite different from the *in vitro* regulation (i.e. during growth in growth medium) (Defoirdt *et al.* 2005). In this respect, it would be of significant interest to construct autoinducer reporter strains that enable *in vivo* detection of signal molecule production. All this knowledge will be important with respect to possible practical applications of quorum sensing disruption to control luminescent vibriosis in aquaculture.

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