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The impact of the multichannel quorum sensing systems of *Vibrio tasmaniensis* and *Vibrio crassostreae* on virulence towards blue mussel (*Mytilus edulis*) larvae

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Abstract :

Mussel culture in Europe is currently suffering from mass mortality events with mortality rates up to 100%. Vibrios belonging to the Splendidus clade have recently been implicated in these events. In this study, we demonstrate that V. tasmaniensis LGP32 and V. crassostreae J2-9, two model pathogens of oysters, are also pathogenic to blue mussel larvae in an immersion challenge test. After five days, LGP32 and J2-9 had killed 93 and 73% of challenged mussel larvae, respectively. Because quorum sensing, bacterial cell-to-cell communication with small signal molecules, has been demonstrated to control the virulence of various vibrios, we further investigated whether it had an impact on the virulence of V. tasmaniensis and V. crassostreae. We identified the components of a multichannel quorum sensing system (as also found in many other vibrios) in the genomes of both species. Knock out of selected components of this pathway revealed that in general quorum sensing in V. tasmaniensis LGP32 and V. crassostreae J2-9 has no impact on motility, protease activity, biofilm formation and virulence towards mussel larvae. Finally, the quorum sensing inhibitor cinnamaldehyde did not protect mussel larvae from these pathogens. Together, these data indicate that the multichannel quorum sensing systems of V. tasmaniensis LGP32 and V. crassostreae J2-9 have no impact on virulence of the bacteria towards blue mussel larvae. Hence, quorum sensing controlling virulence is not a general feature in vibrios as it has different outcomes on virulence in different species.

Highlights

► Splendidus clade vibrios are major pathogens of bivalves. ► *V. tasmaniensis* and *V. crassostreae* contain multichannel quorum sensing systems. ► These systems have no impact on virulence to blue mussel larvae. ► The link between quorum sensing and virulence is different for different vibrios.

Keywords : Bivalve, Splendidus clade, Larviculture, Immersion challenge, Quorum sensing

1. Introduction

Mussels are the most important aquaculture species in Europe based on live weight. The two main cultivated species are the blue mussel (*Mytilus edulis)* and the Mediterranean mussel (*Mytilus galloprovincialis)* (FAO, 2017). Recently, mussel culture in Europe has suffered from mass mortality events with mortality rates up to 100%. In addition to environmental and genetic factors (Benabdelmouna and Ledu, 2016; Polsenaere et al., 2017), bacterial pathogens have been linked to mussel mortality (Eggermont et al., 2014; Ben Cheikh et al., 2016). Vibrios belonging to the *Splendidus* clade (including *V. crassostreae* and *V. tasmaniensis*) are amongst the major bacterial pathogens of marine bivalves (Beaz-Hidalgo et al., 2010; Travers et al., 2015; Le Roux et al., 2016; Dubert et al., 2017) and *V. splendidus* strains have also been implied in mussel mortality events (Ben Cheikh et al., 2016; Oden et al., 2016; Eggermont et al., 2017). The implication of vibrios in mortality events in mussels is a very recent observation and the pathogenicity mechanisms are currently unknown.

Quorum sensing, bacterial cell-to-cell communication with small signal molecules, is known to control the virulence of many bacteria, including vibrios, and inactivation of these systems often decreases the virulence of these pathogens (Milton, 2006; Defoirdt, 2014). Vibrios usually contain multichannel quorum sensing systems, in which multiple signal molecules determine the output of the system (**Figure 1**). Three types of signal molecules can be produced by vibrios (although not all vibrios produce all types): acylated homoserine lactones (AHLs), autoinducer-2 (AI-2), and cholerae autoinducer-1 (CAI-1) (Milton, 2006). The concentrations of the signal molecules determine the cellular level of the quorum sensing master regulator (a homolog of *V. harveyi* LuxR), which in turn controls the expression of many genes (van Kessel et al., 2013). Virulence-related phenotypes that are controlled by this kind of systems include motility, biofilm formation, and the production of lytic enzymes such as proteases (Sultan et al., 2006; Tian et al., 2008; Natrah et al., 2011; Defoirdt, 2014; Yang and Defoirdt, 2015). Moreover, multichannel quorum sensing systems have been documented to be required for full virulence of vibrios towards various host organisms (Ye et al., 2008; Bjelland et al., 2012; Defoirdt and Sorgeloos, 2012), and inhibitors of these systems (such as cinnamaldehyde, brominated furanones and brominated thiophenones) protect aquatic animals from vibriosis (Defoirdt et al., 2006; Brackman et al., 2008; Yang et al., 2015).

In this study, we aimed at identifying multichannel quorum sensing systems in these pathogens, and at determining the impact of these systems on the virulence of *V. tasmaniensis* and *V. crassostreae* towards blue mussel larvae.

2. Materials and methods

2.1. Bacterial strains and growth conditions

V. tasmaniensis and *V. crassostreae* strains used in this study are described in **Table 1**. The bacteria were grown in marine LB broth (m-LB; tryptone 10 g/l, yeast extract 5 g/l, Instant Ocean synthetic sea salt 35 g/l) at 18°C with shaking (100 rpm). Cell densities were determined spectrophotometrically at 600 nm.

2.2. Identification of multichannel quorum sensing system genes in *V. tasmaniensis* and *V. crassostreae* and confirmation of the activity of the quorum sensing system

GenBank was screened for homologs of components of the *V. campbellii* multichannel quorum sensing system in *V. tasmaniensis* and *V. crassostreae* by performing BLAST searches at the website of the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov).

The activity of the quorum sensing system of the vibrios was assessed by introducing the cosmid pBB1 (Bassler et al., 1993) into natural rifampicin resistant mutants through conjugation using the donor strain *E. coli* SM10 (λpir). The pBB1 cosmid contains a tetracycline resistance gene and the *V. campbellii lux* operon under control of its native promoter and is activated by homologs of the *V. campbellii* quorum sensing master regulator LuxR. Hence, vibrios with an active quorum sensing system will produce quorum sensing-dependent bioluminescence. Transconjugants containing the pBB1 cosmid were selected on agar containing rifampicin (100 mg/l) and tetracycline (20 mg/l).

2.3. Construction of *V. tasmaniensis* and *V. crassostreae* quorum sensing mutants

Deletion of selected genes was performed by allelic exchange using pSW7848T (Val et al., 2012), a R6K γ-*ori*-based suicide vector. This vector encodes the *ccdB* toxin gene under the control of an arabinose-inducible and glucose-repressible promoter, P_{BAD} (Le Roux et al., 2007). Briefly, two 500 bp fragments flanking the gene to delete were amplified and cloned into pSW7848T using the Gibson assembly method according to the manufacturer's instructions (New England Biolabs, NEB). The construct was transferred from an *E. coli* donor strain to the *Vibrio* LGP32 or J2-9 by conjugation as described previously (Le Roux et al., 2007). Subsequently, the first and second recombination leading to pSW7848T integration and elimination was selected on chloramphenicol + glucose and arabinose media, respectively. After re-isolations, the gene deletion was confirmed by PCR using external primers.

2.4. Motility, protease and biofilm formation assays

Swimming motility was assessed as described previously by Yang and Defoirdt (2015). Briefly, 5 µl aliquots of the isolates (OD_{600} = 1) were inoculated in the centre of LB_{35} soft agar (0.2 % agar) plates. Plates were incubated upright at 18°C, and motility halos were measured after 24 h.

Caseinase and gelatinase activities were determined according to Natrah et al. (2011). For each assay, overnight grown cultures were diluted to an OD_{600} of 0.5, and 5 µl aliquots of the diluted cultures were inoculated to the centre of the test plates. Caseinase and gelatinase activities were assessed on agar plates containing 2% skimmed milk powder and 0.5% gelatin, respectively. Colony and clearing zone diameters were measured after 4 and 7 days incubation at 18°C for caseinase and gelatinase, respectively. In order to visualise the clearing zones in the gelatinase assay, saturated ammonium sulphate (80% solution in distilled water) was poured over the plates.

Biofilm formation was measured by crystal violet staining as described by Brackman et al. (2008). Briefly, overnight grown cultures were diluted to OD_{600} of 0.5 and inoculated into the wells of a 96-well polystyrene plate. The plates were incubated for 24h. After incubation, the OD_{600} of the wells were measured. After this, the wells were rinsed 3 times with tap water, and stained for 20 min with a 0.1% crystal violet solution. The stain was removed, wells were rinsed 3 times with tap water, and air dried. The dye bound to adherent cells was redissolved in 95% ethanol, and absorbance was measured at 570 nm and expressed relative to the OD_{600}.

2.5. Challenge tests with blue mussel D-larvae

Challenge tests were performed as described previously (Eggermont et al., 2017). Wild-caught mature blue mussels were stimulated to spawn by thermal shocks in sterile sea water at 5°C and 20°C until gametes were released. Spawning males and females were transferred to sterile plastic cups containing 50 ml sterile sea water and allowed to spawn for 15 minutes. Sperm and eggs were collected and gently mixed at a 10:1 ratio in a beaker containing 1 l of sterile sea water. After the appearance of polar bodies, the eggs were gently rinsed with sterile sea water using a sterile 30 µm sieve to remove excess sperm. Fertilized eggs were incubated in 2 l of sterile sea water (max 100 eggs/ml) containing chloramphenicol, nitrofurazone and enrofloxacin (each at 10 mg/l). After two days of incubation, D-larvae were harvested on a sterile 60 µm sieve. The larvae were washed gently with sterile sea water to remove the antibiotics. Rinsed D-larvae were transferred to a beaker containing 1 l of sterile sea water and distributed uniformly using a plunger. Subsamples were taken to calculate the larval density, and the density was corrected in order to obtain a final concentration of 250 larvae/ml. All manipulations were performed under a laminar flow hood.

One ml aliquots of the larval suspension in synthetic sea water supplemented with 10 mg/l tryptone and 5 mg/l yeast extract were subsequently transferred to 24-well plates. The final larval density was 200 larvae/ml. Vibrios were inoculated into the rearing water at 10^5 cells/ml. In the experiment with cinnamaldehyde, 10 µM of cinnamaldehyde (Sigma) was added to the wells (Pande et al., 2013). Larvae to which no bacteria were added and that were otherwise treated in the same way as challenged larvae, were used as controls. Each treatment was performed in 24 replicates. The plates were incubated at 18°C. Each day, four replicates per treatment were stained with lugol (5% (v/v)), and stained larvae were counted under a binocular microscope (*Nikon Eclipse E 200, Nikon Instruments Europe*). Larvae were considered alive when stained black by lugol, death if only parts of the larvae were stained or if shells were empty.

2.6. Statistics

All statistical analyses were performed using the SPSS software, version 24. A significance level of 1% was used in all analyses.

3. Results and discussion

3.1. Virulence of *V. crassostreae* J2-9 to blue mussel larvae

V. tasmaniensis LGP32 and *V. crassostreae* J2-9 are well-known pathogens of oysters, that have been used as model strains to explore virulence mechanisms and immune responses in oysters (Duperthuy et al., 2010; Duperthuy et al., 2011; Toffiano-Nioche et al., 2012; Lemire et al., 2015; Vanhove et al., 2015; Le Roux et al., 2016; Bruto et al., 2017). Presence of genetically related strains has also been documented in mussels (Vezzulli et al., 2015). We previously reported that *V. tasmaniensis* LGP32 is also pathogenic to blue mussel larvae (Eggermont et al., 2017). In order to determine pathogenicity of *V. crassostreae* J2-9 to blue mussel larvae, we used the same immersion challenge test with sterile D-larvae (Eggermont et al., 2017). *V. crassostreae* J2-9 showed to be pathogenic to the larvae, leading to significant mortality from day 2 onwards when compared to the unchallenged control (independent samples t-tests, $P < 0.01$) (**Figure 2**).

3.2. Identification of components of a multichannel quorum sensing system in the genomes of *V. tasmaniensis* and *V. crassostreae* and confirmation of the activity of the system

Similar to many other *Vibrio* spp., vibrios belonging to the *Splendidus* clade have recently been suggested to contain multichannel quorum sensing systems (Zhang and Lee, 2021). Based on the genome sequences that are available in GenBank (Le Roux et al., 2009; Wang et al., 2013; Lemire et al., 2015), we identified homologs of the components of the *V. campbellii* multichannel quorum sensing system in the genomes of *V. tasmaniensis* and *V. crassostreae* (**Table 2**). This suggests that these two species also contain a multichannel quorum sensing system, with AHL, AI-2 and CAI-1 as signal molecules and a signal transduction cascade with LuxU, LuxO and LuxR, and is consistent with detection of AHLs, AI-2 and CAI-1 in *Splendidus* clade vibrios (including *V. splendidus, V. chagasii* and *V. pomeroyi*) by using signal molecule reporter strains (Yang et al., 2011). Recently, Girard et al. (2017) reported that *V. tasmaniensis* LGP32 produces various AHLs, which were identified by mass spectrometry.

The activity of the quorum sensing system was investigated by conjugating the cosmid pBB1 into the vibrios. This cosmid contains the *V. campbellii lux* operon under control of its native promoter and is activated by homologs of the *V. campbellii* quorum sensing master regulator LuxR. We could confirm activity of the *V. tasmaniensis* quorum sensing system as *V. tasmaniensis* LGP32 pBB1 produced the typical V-shaped bioluminescence pattern that is the hallmark of quorum sensing-regulated bioluminescence in vibrios (**Figure 3**). Unfortunately, it was not possible to introduce cosmid pBB1 into *V. crassostreae* J2-9 as this strain is naturally resistant to tetracycline, the antibiotic required to select pBB1 carrying bacteria.

3.3. Impact of quorum sensing on motility, protease activity and biofilm formation of *V. tasmaniensis* and *V. crassostreae*

To explore their role in QS-related phenotypes, the *luxM, luxS* and *luxR* genes were successfully deleted in *V. tasmaniensis* LGP32, and the *luxM, luxS* and *cqsA* genes were deleted in *V. crassostreae* J2-9. We determined the impact of these knock outs on phenotypes that have been previously associated with quorum sensing-regulated virulence of other vibrios, i.e. motility, protease activity and biofilm formation (Defoirdt, 2014). Both *V. tasmaniensis* LGP32 and *V. crassostreae* J2-9 were highly motile (**Table 3**). There were no significant differences between wild types and mutants, except for the *luxM* deletion mutant of *V. tasmaniensis* LGP32 (which was more motile than the wild type) and the *cqsA* deletion mutant of *V. crassostreae* J2-9 (which showed lower motility than the wild type). The higher and lower motility of the *luxM* and *cqsA* deletion mutant of *V. tasmaniensis* and *V. crassostreae*, respectively, might be explained by a (yet unknown) signal transduction pathway that does not involve the known shared signal

transduction pathway (using LuxU, LuxO and LuxR). A similar effect has been previously observed for the *vhh* hemolysin gene in *V. harveyi*, which was differentially expressed in a *luxS* deletion mutant, whereas inactivation of other components of the multichannel quorum sensing system had no effect (Ruwandeepika et al., 2011). Another explanation for the higher motility of the *luxM* mutant of *V. tasmaniensis* is that both the production of AHLs and motility are energy-consuming processes (McCarter, 2001; Keller and Surette, 2006) and consequently, inactivation of the AHL synthase LuxM might result in increased cellular energy levels which in turn might lead to higher motility. Further, the strains tested positive for caseinase and gelatinase activities, with no significant differences in these activities between wild types and quorum sensing mutants. Finally, all strains were capable to form biofilms on polystyrene (**Table 3**). The quorum sensing mutants of *V. tasmaniensis* showed lower biofilm formation than the wild type. However, the difference was only significant for the *luxS* deletion mutant. There were no significant differences between wild type and mutants of *V. crassostreae*.

3.4. Impact of the multichannel quorum sensing system of *V. tasmaniensis* and *V. crassostreae* on virulence towards blue mussel larvae

We further compared mortality in blue mussel larvae caused by wild types and quorum sensing mutants of *V. tasmaniensis* and *V. crassostreae*. For *V. tasmaniensis*, there were no significant differences in virulence between wild type and quorum sensing mutants (**Figure 4A**). For *V. crassostreae* a slightly (but significantly) increased mortality was observed for larvae challenged with the *luxS* deletion mutant when compared to larvae challenged with the wild type from day 2 onwards (independent samples t-test, $P < 0.01$) (**Figure 4B**). Survival of larvae challenged with the *luxM* and *cqsA* deletion mutants was similar to that of larvae challenged with the wild type.

In a last experiment, we used a chemical biological approach to block the multichannel quorum sensing systems of *V. tasmaniensis* and *V. crassostreae* by adding cinnamaldehyde to the rearing water. Cinnamaldehyde has been shown before to block the multichannel quorum sensing in vibrios by inhibiting the DNA-binding activity of the quorum sensing master regulator LuxR, and the compound blocked the virulence of *V. harveyi* towards brine shrimp and giant river prawn larvae (Brackman et al., 2008; Pande et al., 2013). However, no significant differences in survival of mussel larvae challenged with *V. tasmaniensis* or *V. crassostreae* were observed after treatment with cinnamaldehyde (**Figure 5**). Together, our data indicate that inactivation of the quorum sensing system of *V. tasmaniensis* and *V. crassostreae* does not attenuate their virulence. A multichannel quorum sensing system has been reported to be required for full virulence in several *Vibrio* species, such as *V. alginolyticus, V. campbellii* and *V. salmonicida* (Ye et al., 2008;

Bjelland et al., 2012; Defoirdt and Sorgeloos, 2012), whereas it has no effect on the virulence for *V. tasmaniensis* and *V. crassostreae*, which is similar to what has been reported for *V. anguillarum* (Li et al., 2018).

4. Conclusions

In this study, we identified the components of a multichannel quorum sensing system in the genomes of the bivalve model pathogens *V. tasmaniensis* LGP32 and *V. crassostreae* J2-9. We found that motility is controlled by HAI-1 in *V. tasmaniensis* and by CAI-1 in *V. crassostreae* and that biofilm formation is controlled by AI-2 quorum sensing in *V. tasmaniensis,* whereas the multichannel quorum sensing systems of *V. tasmaniensis* and *V. crassostreae* have no effect on protease activity, and on virulence of the pathogens towards blue mussel larvae. Hence, since quorum sensing controlling virulence is not a general feature in vibrios, it is not possible to extrapolate the impact of quorum sensing on virulence of vibrios from one species to another.

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Tables

Table 1. Strains used in this study.

Strain	Genotype	Reference
V. tasmaniensis LGP32		
LGP32 WT	Wild type from which the *luxM, luxS* and *luxR* deletion mutants were derived.	Le Roux et al. (2009)
LGP32 *luxM*	Deletion of *luxM* (AHL synthase gene)	This study
LGP32 *luxS*	Deletion of *luxS* (AI-2 synthase gene)	This study
LGP32 *luxR*	Deletion of *luxR* (quorum sensing master regulator)	This study
V. crassostreae J2-9		
J2-9 WT	Wild type from which the *luxM, luxS* and *cqsA* deletion mutants were derived.	Lemire et al. (2015)
J2-9 *luxM*	Deletion of *luxM* (AHL synthase gene)	This study
J2-9 *luxS*	Deletion of *luxS* (AI-2 synthase gene)	This study
J2-9 *cqsA*	Deletion of *cqsA* (CAI-1 synthase gene)	This study

Table 2. Homologs of components of the *V. campbellii* ATCC BAA-1116 (Vca) multichannel quorum sensing system identified within the genomes of *V. tasmaniensis* LGP32 (Vta) and *V. crassostreae* J2-9 (Vcr), and nucleotide identities between the homologs.

Gene	Function	Locus tag[1] Vca (chromosome)	Locus tag[1] Vta (chromosome)	Locus tag[1] Vcr	% identity Vca-Vta (% coverage)	% identity Vca-Vcr (% coverage)	% identity Vta-Vcr (% coverage)
luxM	AHL synthesis	M892_17055 (chr I)	VS_RS16040 (chr II)	VCR9J2v1_1350180	70 (95)	72 (96)	77 (94)
luxN	AHL detection	M892_17050 (chr I)	VS_RS16035 (chr II)	VCR9J2v1_1350181	73 (95)	76 (96)	80 (95)
luxS	AI-2 synthesis	M892_13670 (chr I)	VS_RS12075 (chr I)	VCR9J2v1_700111	81 (100)	81 (100)	89 (100)
luxP	AI-2 detection	M892_24610 (chr II)	VS_RS16480 (chr II)	VCR9J2v1_110050	68 (80)	68 (81)	84 (100)
luxQ	AI-2 detection	M892_24605 (chr II)	VS_RS16485 (chrII)	VCR9J2v1_110051	64 (57)	64 (63)	81 (99)
cqsA	CAI-1 synthesis	M892_21495 (chr II)	VS_RS08055 (chr I)	VCR9J2v1_720342	76 (90)	75 (90)	83 (99)
cqsS	CAI-1 detection	M892_21490 (chr II)	VS_RS08060 (chr I)	VCR9J2v1_720343	72 (98)	73 (98)	83 (99)
luxU	Signal transduction	M892_16185 (chr I)	VS_RS04560 (chr I)	VCR9J2v1_50013	69 (56)	71 (26)[2]	83 (100)
luxO	Signal transduction	M892_16180 (chr I)	VS_RS04555 (chr I)	VCR9J2v1_50012	76 (95)	77 (95)	88 (100)
luxR	Master regulator	M892_13795 (chr I)	VS_RS11960 (chr I)	VCR9J2v1_700132	77 (86)	79 (86)	89 (100)

[1]locus tags refer to the genome sequences in Genbank: Vca chr. I: CP006605; Vca chr. II: CP006606; Vta chr. I: NC_011753; Vta chr. II: NC_011744; Vcr: CCJY00000000.1
[2]60% identity at amino acid level (70% coverage)

Table 3. Motility, caseinase and gelatinase activities and biofilm formation of *V. tasmaniensis* LGP32 and *V. crassostreae* J2-9 wild types and quorum sensing mutants (average ± standard deviation of three independent experiments).

Strain	Motility halo[1]	Caseinase activity[2]	Gelatinase activity[2]	Biofilm formation[3]
V. tasmaniensis LGP32				
LGP32 WT	62 ± 4	1.4 ± 0.1	2.6 ± 0.2	0.22 ± 0.03
LGP32 *luxM*	78 ± 2*	1.4 ± 0.0	2.7 ± 0.1	0.14 ± 0.03
LGP32 *luxS*	72 ± 5	1.6 ± 0.1	2.6 ± 0.2	0.11 ± 0.02*
LGP32 *luxR*	63 ± 3	1.4 ± 0.1	2.7 ± 0.2	0.18 ± 0.04
V. crassostreae J2-9				
J2-9 WT	72 ± 5	1.4 ± 0.0	2.5 ± 0.2	0.17 ± 0.04
J2-9 *luxM*	72 ± 4	1.4 ± 0.0	2.6 ± 0.2	0.13 ± 0.02
J2-9 *luxS*	75 ± 4	1.4 ± 0.0	2.7 ± 0.2	0.19 ± 0.01
J2-9 *cqsA*	56 ± 3*	1.3 ± 0.1	2.6 ± 0.2	0.22 ± 0.02

[1] Diameter of the motility zone (mm)

[2] Ratio between the activity zone and the colony diameter

[3] OD_{570} of crystal violet-stained biofilms on polystyrene multiwell plates/OD_{600}

* Significantly different from the wild type (independent samples t-test; $P < 0.01$)

Figures

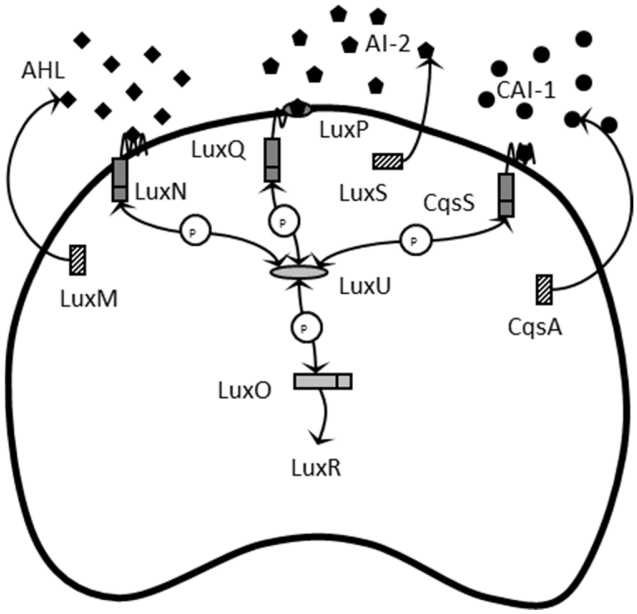


Figure 1. Generic scheme of multichannel quorum sensing systems of vibrios. The LuxM, LuxS and CqsA enzymes synthesise acylated homoserine lactone (AHL), autoinducer-2 (AI-2) and cholerae autoinducer-1 (CAI-1), respectively. These signal molecules are detected at the cell surface by the LuxN, LuxPQ and CqsS receptors, respectively. The receptors feed a shared phosphorylation/ dephosphorylation signal transduction cascade involving the LuxU and LuxO proteins. This cascade controls the production of the master regulator LuxR. The level of LuxR is proportional to the concentration of the signal molecules, and LuxR determines the transcription of the quorum sensing target genes. “P” denotes phosphotransfer. Based on Defoirdt et al. (2008).

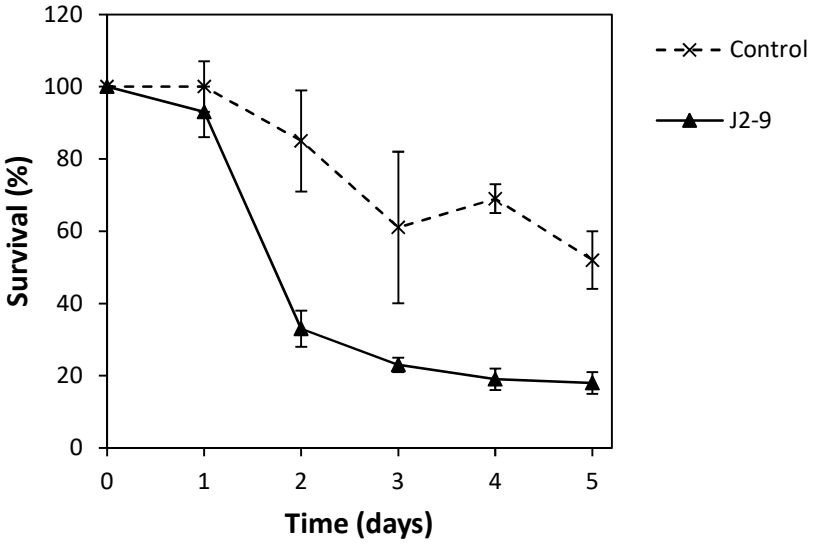


Figure 2. Survival of blue mussel D-larvae challenged with *V. crassostreae* J2-9. Error bars represent the standard deviation of four mussel cultures. "Control" refers to unchallenged larvae that were otherwise treated the same as the other larvae.

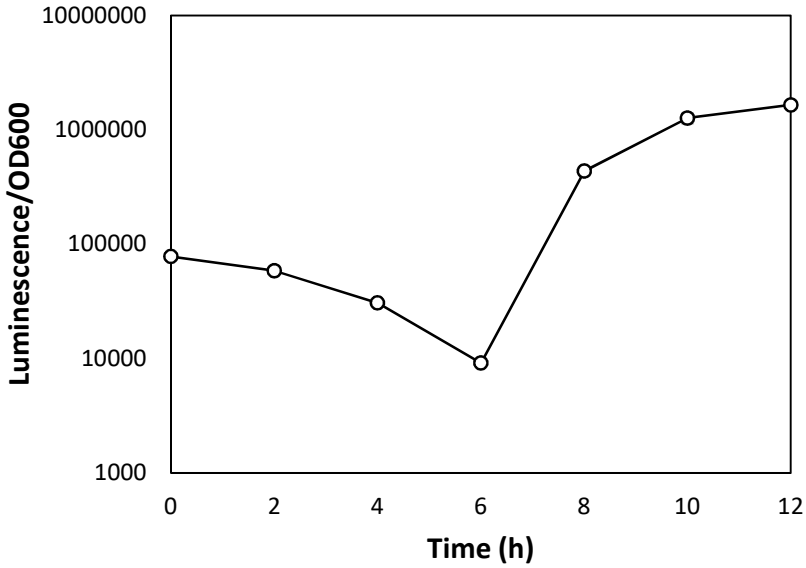
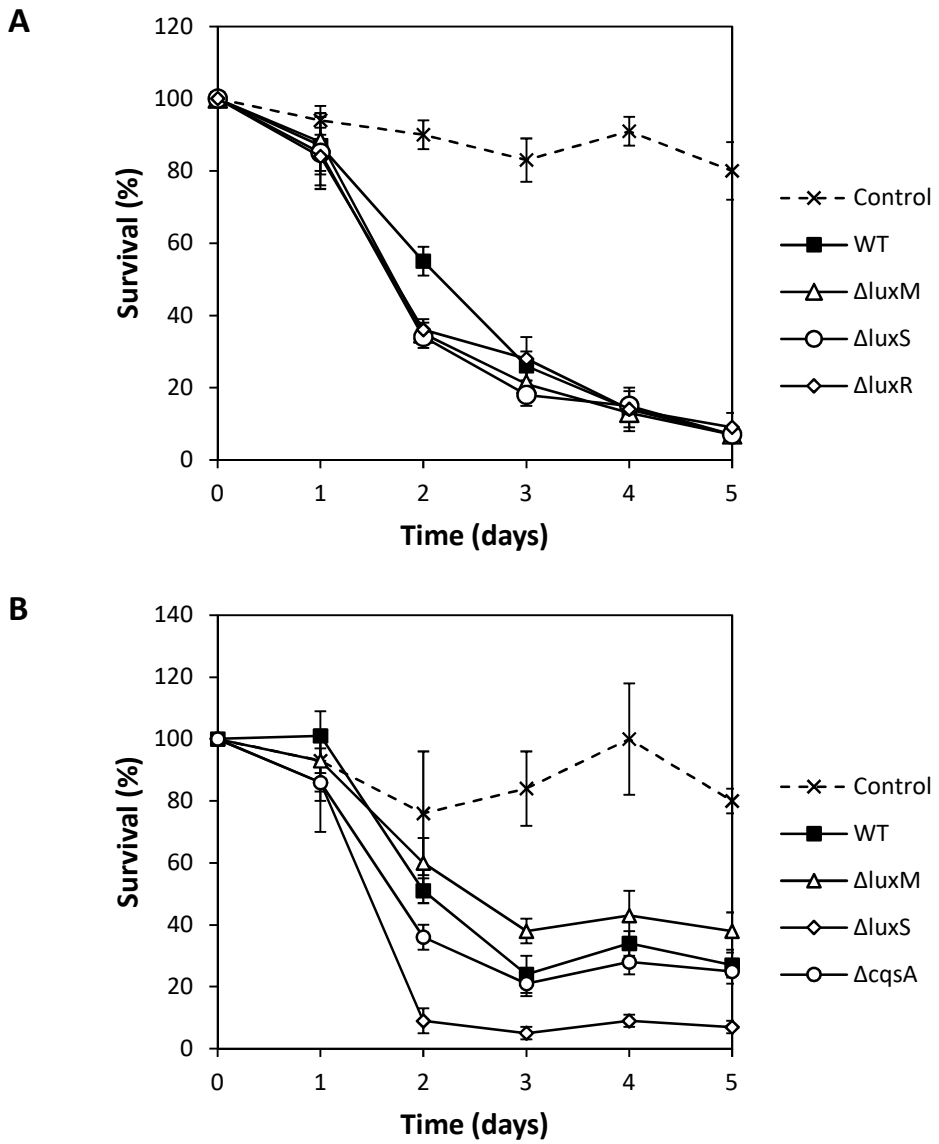


Figure 3. Quorum sensing-dependent bioluminescence of *V. tasmaniensis* LGP32 pBB1. The data points are averages of three replicates. Error bars are too small to be visible on the graph.



519 **Figure 4.** Survival of blue mussel D-larvae challenged with *V. tasmaniensis* LGP32 (panel A) and *V.*
520 *crassostreae* J2-9 (panel B) wild type (WT) and quorum sensing mutants. Error bars represent the standard
521 deviation of four mussel cultures. "Control" refers to unchallenged larvae that were otherwise treated the
522 same as the other larvae.
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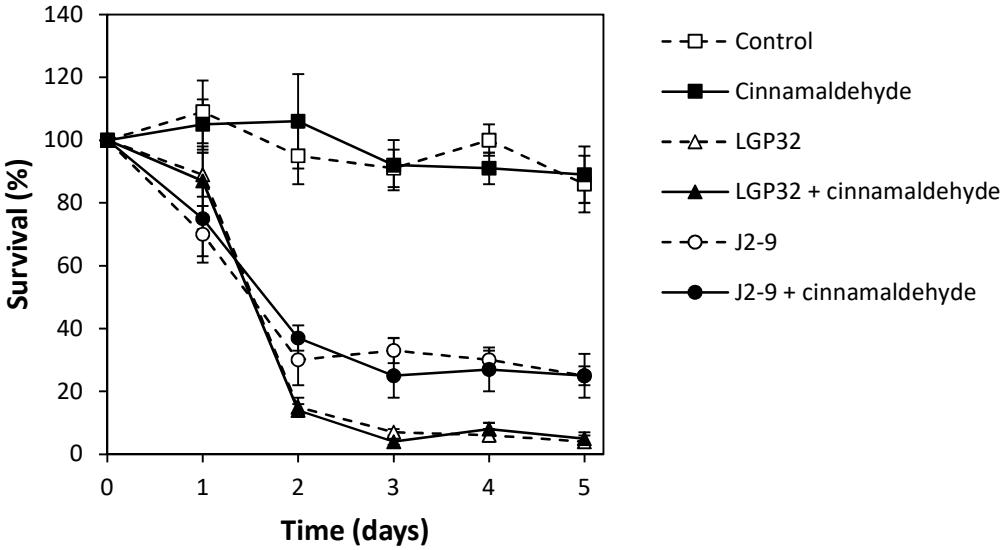

528
529 **Figure 5.** Survival of blue mussel D-larvae challenged with *V. tasmaniensis* LGP32 or *V. crassostreae* J2-9,
530 with and without the quorum sensing inhibitor cinnamaldehyde (added at 10 µM to the rearing water at
531 the start of the experiment). Error bars represent the standard deviation of four mussel cultures. "Control"
532 refers to unchallenged larvae that were otherwise treated the same as the other larvae.
533
534