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SHORT COMMUNICATION

Expression profiles of heat shock protein 70 and high-mobility groups box-1 protein in gnotobiotic brine shrimp challenged with different virulence levels of isogenic *Vibrio harveyi* strains

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KEYWORDS
Artemia, hmgb1, hsp70, phenotypic switching, *Vibrio*

1 | INTRODUCTION

Vibrio harveyi is a ubiquitous, bioluminescent marine bacterium that causes vibriosis in both marine vertebrates and invertebrates, leading to significant losses in the global aquaculture industry. Pathogenic *Vibrio* spp. evade host defensive mechanisms by using different strategies. Recently, a few studies have indicated that pathogenic bacteria evade host defense mechanisms during infection by limiting the translation of host mRNAs, which encode proteins that mediate defense responses (Fontana et al., 2011; McEwan et al., 2012).

The bacterial *V. harveyi* has been reported to regulate their pathogenicity by producing a wide variety of virulence factors, which are regulated by quorum-sensing signal molecules (Defoirdt et al., 2010; Natrah et al., 2011).

Hsp70 and Hmgb1 proteins are associated with both repair and immune mechanism to fight against pathogenic stressor by different ways such as acting as molecular chaparone for protein and DNA, respectively, functioning as danger-associated molecular pattern during inflammation and various cellular processes, and/or participating in the activation of cell surface innate immune receptors, thereby modulating many aspect of the host's immune responses (Norouzitallab et al., 2015).

In this study, using the gnotobiotic *Artemia* model organism (GART) system, we aimed to determine whether isogenic *V. harveyi* strains with different levels of virulence inflict damage and cause disease in *Artemia* by interfering with the mRNA translational machinery of the host defense system.

2 | MATERIALS AND METHODS

Live axenic nauplii at the instar II stage were collected, counted and then fed once with 10^7 cells mL^{-1} of autoclaved *Aeromonas* sp. LVS3 (Marques et al., 2004). The nauplii were challenged with luminescent (group 1) or non-luminescent (group 2) variants of *Vibrio* at 10^6 cells mL^{-1} (Table 1). *Artemia* nauplii that were non-challenged (group 3) were used as controls. Samples were harvested at 6 and 12 h post-challenge, rinsed in sterile distilled water, immediately frozen in liquid nitrogen and preserved at −80°C for further analysis.

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Total RNA was extracted using the SV Total RNA Isolation System (Promega, Leiden, the Netherlands) according to the manufacturer's instruction. First-strand cDNA was synthesized using the RevertAid™ H minus First-strand cDNA synthesis kit (Fermentas Gmbh, Germany). The expression of *hsp70* and *hmgb1* genes was analysed by qRT-PCR using the following two primer pairs (*hsp70*: forward—CGATAAAGGCCGTCTCTCCA, reverse—CAGCTTCAGGTA ACTTGTCCTTG; *hmgb1*: forward—GGATGAAAGCAAACCCCGTg, reverse—GTGCTCTTCTCTGCAAGTCTG). qRT-PCR was carried out following protocol: denaturation (10 min at 95°C); 40 cycles of amplification and quantification (15 s at 95°C, 30 s at 60°C and 30 s at 72°C); melt curve analysis (55–95°C with a heating rate of 0.10°C s^{-1} and a continuous fluorescence measurement) and finally cooling to 4°C. The expression was normalized to the endogenous control (elongation factor, $EF_{\alpha}1$: forward—GGTCGGGTTGAAA CTGGTAT, reverse—AGGGATTCGTGGTGCATTT) by calculating ΔC_T ($=C_{ttarget}-C_{tEF\alpha1}$) and expressed relative to calibrator sample by calculating $\Delta\Delta C_t$ ($=\Delta C_t-\Delta C_{tcalibrator}$). A sample of non-challenged *Artemia* at 6 h was used as a calibrator. The relative expression was then calculated as follows: relative expression $=2^{-\Delta\Delta C_T}$.

The extraction and analysis of Hsp70 and Hmgb1 proteins were followed the descriptions of Norouzitallab et al. (2015).

All statistical analyses were performed using the Statistical package for the Social Sciences (SPSS) version 22.0 using a significant level of 5%. All treatments were compared to the control and each of the other treatments individually using independent samples *t*-test for each time point. Per time point, a matrix was obtained with an overview of all the results from the independent samples *t*-test.

3 | RESULTS AND DISCUSSION

Inhibition of host translation is hypothesized to facilitate bacteria up take essential nutrients from the host. Disruption of host translation is central to cause disease in many pathogenic bacteria but also facilitates the host to detect and restrict pathogens by macrophages (Belyi, 2020; Copenhaver et al., 2015; Fontana et al., 2011).

Strain BB120NL is as virulent as BB120L in *Artemia* challenge test (Hong et al., 2015). Results of this study showed that BB120L strain seems to up-regulate the expression of both the *hsp70* and *hmgb1* genes (Figures 1 and 3) with no associated increase in protein (Figures 2 and 4). It is possible that just before or during infection non-luminescent cells switch back to luminescent cells. Yet Tuan et al. (2016) reported that *Vibrio campbellii* cells with different morphologies were observed after phagocytosis by shrimp hemocytes. Assuming that such a phenotypic switch would indeed be necessary for infection, then non-luminescent might infect *Artemia* with an unknown amount of delay (relative to luminescent cells) because of the (short) time needed to make that switch.

In another case, BB120L and JMH634L are virulent and non-virulent to *Artemia*, respectively. *Artemia* exposed to BB120L seems to reduce the protein production of hmgb1 while the gene expression is not affected (Figures 3 and 4). Probably, inhibition of host protein synthesis might be one of the virulence strategies of pathogenic bacteria to survive and cause disease after infection. This phenomenon has also been revealed in many recent studies (Chakrabarti et al., 2012; Chauhan & Shames, 2021). For example, the secreted ligand, Upd3 was not produced in *Drosophila* infected

Strain	Relevant feature
BB120 RR-L	Wild type
BB120 RR-NL	Wild type
JMH634 RR-L	Mutation in LuxM (AI-1 synthase), LuxS (AI-2 synthase) and CqsA (CAI-1)
JMH634 RR-NL	Mutation in LuxM (AI-1 synthase), LuxS (AI-2 synthase) and CqsA (CAI-1)

TABLE 1 *Vibrio harveyi* strains (Hong et al., 2015).

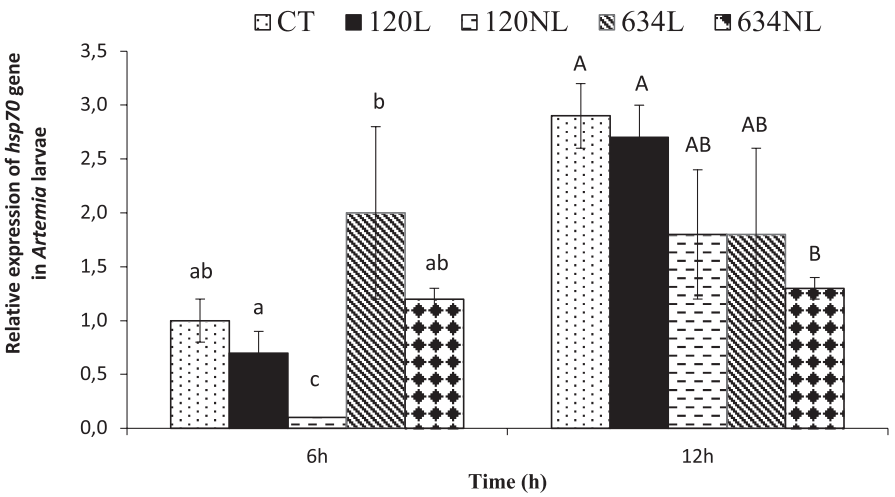

FIGURE 1 Relative expression of the *hsp70* gene in *Artemia* larvae. Different letters (small and capital letters for 6 and 12 h, respectively) were used for significant differences among groups ($p < .05$).

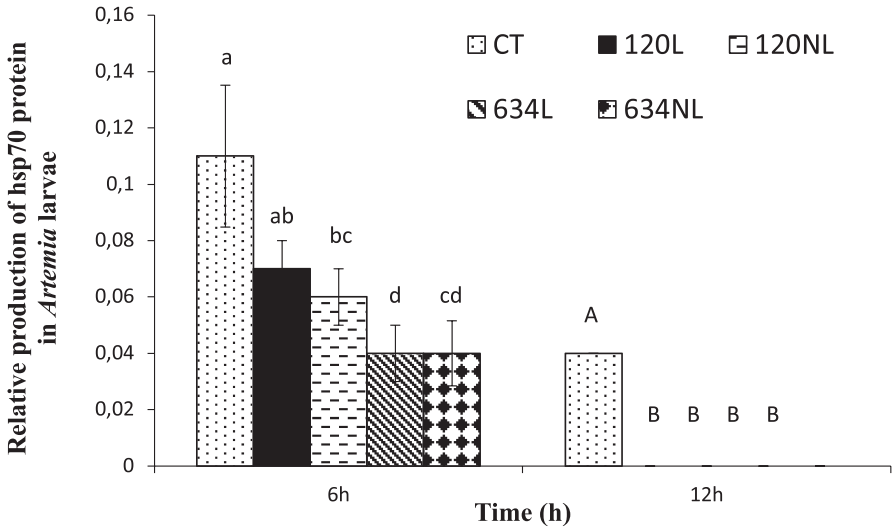

FIGURE 2 Relative production of hsp70 protein in *Artemia*. Different letters (small and capital letters for 6 and 12 h, respectively) were used for significant differences among groups ($p < .05$).

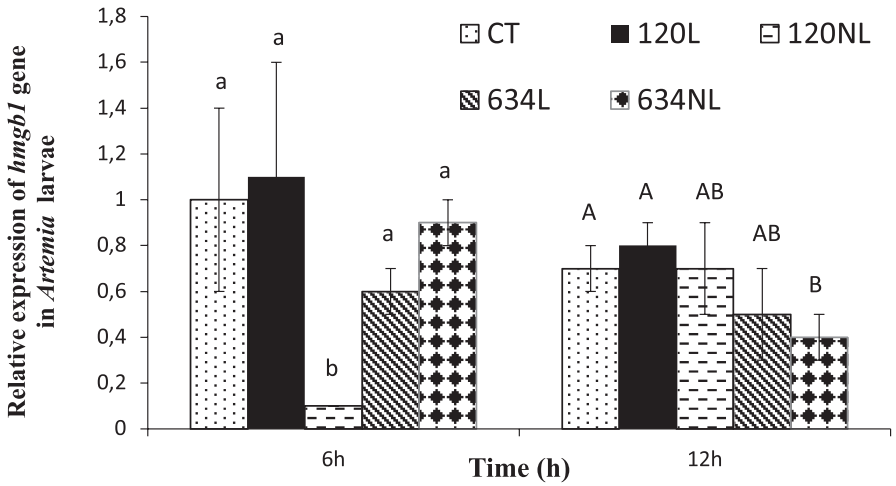

FIGURE 3 Relative expression of *hmgb1* gene in *Artemia* larvae. Different letters (small and capital letters for 6 and 12 h, respectively) were used for significant differences among groups ($p < .05$).

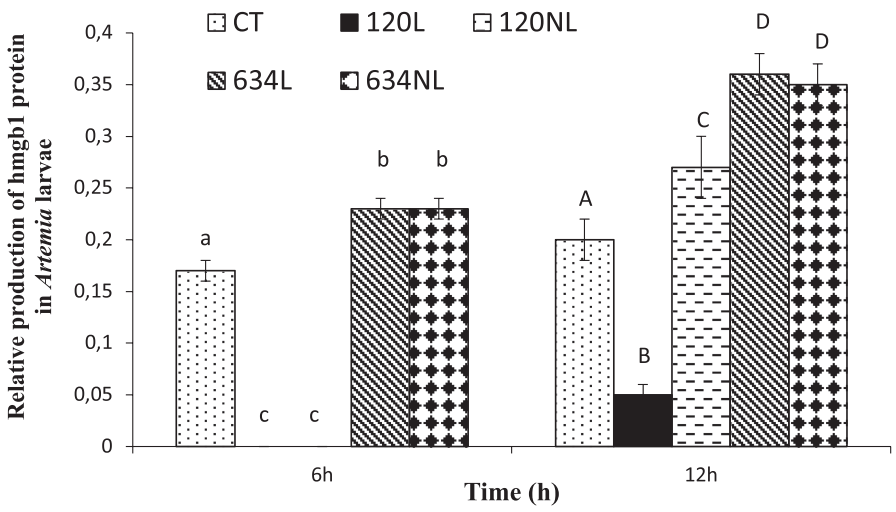

FIGURE 4 Relative production of Hmgb1 protein in *Artemia* larvae. Different letters (small and capital letters for 6 and 12 h, respectively) were used for significant differences among groups ($p < .05$).

with *Pseudomonas entomophila* despite the strong induction of the *upd3* gene. This study indicated that the uncoupling between protein and mRNA amount was indeed due to an inhibition of translation (Chakrabarti et al., 2012).

In conclusion, exposure of *Artemia* to *V. harveyi* might be associated with modulation of the synthesis of hsp70 and hmgb1 proteins. Since a battery of immune factors is involved in inducing resistance in the host against *V. harveyi*, further studies are warranted

to determine the effect of pathogenic factors on the translational machinery of the host defense system.

AUTHOR CONTRIBUTIONS

Nguyen Thi Xuan Hong: Conceptualization; data curation; investigation; validation; formal analysis; methodology; writing – original draft; writing – review and editing; visualization; software; funding acquisition. **Kartik Baruah:** Methodology; resources; writing – review and editing. **Nguyen Van Hung:** Investigation; methodology; resources. **Daisy Vanrompay:** Conceptualization; data curation; supervision. **Peter Bossier:** Data curation; supervision; writing – review and editing; conceptualization; funding acquisition.

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CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

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