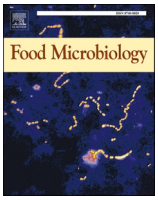
Food Microbiology 92 (2020) 103600

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm



Vibrio parahaemolyticus control in mussels by a *Halobacteriovorax* isolated from the Adriatic sea, Italy.

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ARTICLE INFO

Keywords:
Bdellovibrio and like Organisms (BALOs)
Decontamination experiment
marine Environment
Mytilus galloprovincialis
Vibrio spp.
V. parahaemolyticus

ABSTRACT

This study evaluated the application of a *Halobacteriovorax* isolated from water of the Adriatic Sea (Italy) in controlling *V. parahaemolyticus* in mussels (*Mytilus galloprovincialis*). Two 72 h laboratory-scale *V. parahaemolyticus* decontamination experiments of mussels were performed. The test microcosm of experiment 1 was prepared using predator/prey free mussels experimentally contaminated with *Halobacteriovorax*/*V. parahaemolyticus* at a ratio of 10^3 PFU/10^5 CFU per ml, while that of experiment 2 using mussels naturally harbouring *Halobacteriovorax* that were experimentally contaminated with 10^5 CFU per ml of *V. parahaemolyticus*. For experiment 1, was also tested a control microcosm only contaminated with 10^5 CFU per ml of *V. parahaemolyticus.*. Double layer agar plating and pour plate techniques were used to enumerate *Halobacteriovorax* and *V. parahaemolyticus*, respectively. 16 S rRNA analysis was used to identify *Halobacteriovorax*. For both experiments in the test microcosm the concentration of prey remained at the same level as that experimentally added, i.e. 5 log for the entire analysis period. In experiment 1, V. parahaemolyticus counts in mussels were significantly lower in the test microcosm than the control with the maximum difference of 2.2 log at 24 h. Results demonstrate that *Halobacteriovorax* can modulate *V. parahaemolyticus* level in the mussels. The public impact of *V. parahaemolyticus* in bivalves is relevant and current decontamination processes are not always effective. *Halobacteriovorax* is a suitable candidate in the development of a biological approach to the purification of *V. parahaemolyticus* in mussels.

1. Introduction

1.1. *Vibrio parahaemolyticus*

Vibrio parahaemolyticus is a marine microorganism native of estuarine waters throughout the world (Letchumanan et al., 2014). *V. parahaemolyticus* strains producing thermostable direct haemolysin (TDH) and/or TDH-related haemolysin (TRH) are recognized as a cause of diarrhoeal diseases worldwide, with bivalves, eaten raw or undercooked being the most common sources of infection (Letchumanan et al., 2014; Potasman et al., 2002). In Italy mussel farming (*Mytilus galloprovincialis*) has always played the most important role in marine aquaculture for productivity, areal exploitation and number of farms (Prioli, 2008). In previous studies in the Adriatic Sea we have reported a prevalence of *V. parahaemolyticus* in mussels <25% and in subsurface seawater levels never exceeding 105 CFU per mL (Masini et al., 2007; Ottaviani et al., 2005; Ottaviani et al., 2010a). However, the prevalence of *trh*-positive *V. parahaemolyticus* was higher respect to that reported from other European and Extra-European countries (Ottaviani et al., 2013). Moreover, we have reported illness due to *V. parahaemolyticus* with mussels or seawater of the Adriatic as the source of infection (Ottaviani et al., 2010b).

Abbreviations: BALOs, *Bdellovibrio* and like organisms; CFU, colony forming unit; PFU, plaque forming unit; TDH, thermostable direct haemolysin; TRH, TDH-related haemolysin.

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https://doi.org/10.1016/j.fm.2020.103600
Received 23 January 2020; Received in revised form 3 July 2020; Accepted 21 July 2020
Available online 26 July 2020

1.2. Depuration of bivalves

Depuration is a controlled process that relies on the ability of bivalves to purge their gastro-intestinal contents by filtering clean seawater. Depuration of bivalves depends on variables such as the level of microbial contamination, the microorganism type, the physiological state of the bivalve, temperature, salinity, chemical or physical sterilization system of the plant (Baker, 2016; Lee et al., 2008). It is a very effective process for the elimination of faecal bacteria (Baker, 2016) but it is less effective against naturally occurring *Vibrio* spp. (Croci et al., 2002; Martinez-Albores et al., 2020; Shen and Su, 2017; 2019). If on one hand conventional approach to the depuration of bivalves is ineffective in reducing *V. parahaemolyticus*, on the other, innovative post-harvest treatments are expensive, kill bivalves and do not satisfy those consumers who prefer live bivalves (Baker, 2016). To increase the efficacy of conventional approach to purification towards *V. parahaemolyticus*, it could be integrated with forms of biological control.

1.3. Bdellovibrio and like organisms (BALOs)

Bdellovibrio and like organisms (BALOs) are Gram-negative, aerobic bacteria which prey upon other Gram-negative bacteria (Bratanis et al., 2020; Stolp and Starr, 1963; Williams and Baer, 2005). Recent studies reported that marine BALOs were capable of containing *V. parahaemolyticus* levels in seawater (Richards et al., 2012, 2016; Williams et al., 2015). Since it has been widely demonstrated that BALOs cannot grow in eukaryotic cells they do not represent a specific risk for human safety (Bratanis et al., 2020; Shatzkes et al., 2017; Williams and Baer, 2005). Moreover, the BALOs' capability to parasitize bacteria organized in biofilms or in viable but nonculturable forms (Markelova, 2010; Williams et al., 2009), make them not susceptible to those common mechanisms of competition or defense that pathogens can activate on molluscs. *Halobacteriovorax* genus includes the marine members of BALOs (Koval et al., 2015). In the Adriatic Sea of Italy our group has found *Halobacteriovorax* in subsurface seawater at levels never higher than 103 PFU per mL (Ottaviani et al., 2018, 2020). Moreover, we characterized an indigenous *Halobacteriovorax* strain that we named HBXCO1, which showed high predatory efficiency towards a wide range of pathogenic vibrios, including *V. parahaemolyticus* (Ottaviani et al., 2018). *Halobacteriovorax* was also found from subsurface seawater of the Atlantic and Pacific Oceans although at natural levels lower than those of the Adriatic Sea (Richards et al., 2013). To date, only Li et al. (2011) had tested *Halobacteriovorax* ability to contain *V. parahaemolyticus* in oysters, using 107 PFU per mL of predator. However, as far as we know, such high natural *Halobacteriovorax* concentration has never been reported in seawater. Furthermore, the effects that *Halobacteriovorax* could have on the mussel microbiome are not known, especially when these are contaminated by such high level of predator.

1.4. Aims of this work

This work had two aims. The first was to investigate the potential role of *Halobacteriovorax* as a natural modulator of *V. parahaemolyticus* in the mussels of the Adriatic Sea. The second to assess at laboratory level whether *Halobacteriovorax* could control the growth of *V. parahaemolyticus* on mussels by testing predator concentrations similar to those naturally occurring in seawater. This represents the first step with the ultimate goal of standardizing a biological protocol using *Halobacteriovorax* to decontaminate mussels from *V. parahaemolyticus* that could integrate conventional approaches of depuration.

2. Materials and methods

2.1. Reference strains

V. parahaemolyticus NCTC 10885 strain was used as prey. Fresh enrichments were prepared from a stock culture grown on 3% NaCl Luria-Bertani broth until prey reached an OD_{600} of 0.20 (~1.8×10^8 CFU per mL). *Halobacteriovorax* strain HBXCO1 (GeneBank accession number MG 770616), was used as predator (Ottaviani et al., 2018). To prepare the attack-phase of the predator, 20 μL of HBXCO1 stock culture and 200 μL of prey enrichment were added to 2 mL of 30 ppt salinity sterilized artificial seawater (ASW) and incubated at 26 °C. Three-day enrichments of HBXOC1 (approximately 1×10^6 PFU per mL) were filtered through a 0.45- m-pore-size Millex HV syringe filter (Millipore Corp., Billerica, MA) to remove primary prey.

2.2. Detection and count of halobacteriovorax

While the quantification techniques of *Halobacteriovorax* in seawater are sufficiently standardized (Ottaviani et al., 2018; Richards et al., 2012, 2013; 2016), those for their quantification within bivalves are not. The homogenates obtained from the body and intervalvular liquid of the mussels, due to their high viscosity, make the filtration extremely slow and some *Halobacteriovorax* adhering to fragments of tissue are retained by the filter. In this work we tested only the intervalvular liquid that can be treated in a similar way to sea water. However with this operating mode it was not possible to accurately quantify *Halobacteriovorax* within the mussels. *Halobacteriovorax* were then only qualitatively detected in mussels while they were counted in ASW. For *Halobacteriovorax* count from ASW a double layer agar plating assay was performed combining 7.5 mL of filtered ASW and its 10-fold serial dilutions in ASW with 1 mL of prey enrichment, to 7.5 mL of molten (48 °C) Pp 20 agar in tubes. The tubes were poured on top of the existing bottom layer, then incubated at 26 °C (Ottaviani et al., 2018). *Halobacteriovorax* was detected from mussels combining 7.5 mL of intervalvular fluid from 10 mussels with 1 mL of a *V. parahaemolyticus* enrichment to 7.5 mL of molten (48 °C) Pp 20 agar in tubes. The tubes were poured on top of the existing bottom layer and incubated at 26 °C.

2.3. Detection and count of V. parahaemolyticus

For *V. parahaemolyticus* count from mussels 10 g of body and intervalvular liquid, obtained from 10 mussels, were 1:10 diluted in physiological saline solution (ISO 6887-3, 2017), homogenized, and serially diluted in the same buffer. Then, 10 mL of each dilution were inoculated onto three plates of TCBS agar (3, 3 and 4 mL) and incubated at 37 °C for 24 h. *V. parahaemolyticus* count from ASW was performed in TCBS with 10 mL of undiluted ASW and its 10-fold serial dilutions in ASW. *V. parahaemolyticus* detection from mussels was performed from 25 g of body and intervalvular liquid, obtained from 10 mussels according to a standard procedure (ISO/TS 21872–1: 2017).

2.4. Preliminary study to evaluate the optimal predator/prey ratio to be used in decontamination experiments

Predator concentrations had to be similar to the natural levels reported for marine environment that were in the range from 10^0 to 10^3 PFU per mL (Ottaviani et al., 2018; Richards et al., 2013). BALOs encounter and attack prey by random collision (Stolp and Starr, 1963). For this reason, to optimize the attack rate of predator, we only tested 10^3 PFU per mL of HBXCO1. The concentration of prey greatly influences the efficacy of *Halobacteriovorax* in fact predatory activity is inhibited at prey levels below 104 CFU per mL (Williams et al., 2015). To choose the most effective concentration of prey, test flasks with 50 mL ASW containing 10^3 PFU per mL of HBXCO1 were inoculated with the following prey concentrations: 10^4, 10^5, 10^6, 10^7, 10^8 CFU per mL. For each test microcosm, the same prey concentration was inoculated into ASW, without HBXCO1, as control. Cultures were incubated at 17 °C on a shaker for 24 h. At 0 and 24 h the counts of the prey in the test and control microcosms were performed. Each experiment was replicated twice and the average of the data was calculated. The difference in the

prey counts of duplicate experiments was always within 0.5 log that was the limit of repeatability that we had defined on the basis of previous studies (Ottaviani et al., 2018, 2019). The highest prey reduction equal to 2.48 log was obtained with 10^3 PFU/10^5 CFU per mL predator/prey ratio (Supplementary Table 1).

2.5. Laboratory scale decontamination experiments

Two decontamination experiments of mussels were carried out in an experimental aquarium (Adriatic Sea international, Italy) with 3% salinity ASW, maintained at 17 °C and constantly aerated. A ration of Instant Algae Shellfish Diet 1800) (Reed Mariculture, Campbell, CA, USA) consisting of four inactivated algae (Isochrysis, Pavlova, Thalassiosira weissflogii and Tetraselmis) was also added at the rate of 1×10^6 algae per mL of seawater (Ottaviani et al., 2017; Richards et al., 2012). Mussels (*Mytilus galloprovincialis*) from an authorized harvesting area of the Central Adriatic Sea (Italy) were used. Experiment 1 was conducted using predator/prey free mussels experimentally contaminated with *Halobacteriovorax*/*V. parahaemolyticus* at a ratio of 10^3 PFU/10^5 CFU per ml. Experiment 2 was performed using mussels naturally harbouring *Halobacteriovorax* that were experimentally contaminated with 10^5 CFU per ml of *V. parahaemolyticus*. The experimental design has been detailed in Fig. 1. Prior of each experiment, 250 healthy mussels of average size (5–7 cm in length) were placed in a 250 L tank containing ASW (1 L/mussel) and allowed to adapt for 72 h. Immediately on arrival 10 mussels were analysed to detect *V. parahaemolyticus* and indigenous BALOs. *V. parahaemolyticus* was never detected from mussels. Plaques of indigenous BALOs were detected after 72 h prior of experiment 2. For experiment 1, mussels (indigenous predator free) were contaminated by adding freshly prepared prey enrichment to the ASW in the tank to achieve a final concentration of 1×10^5 CFU per mL. ASW was mixed to ensure a homogeneous distribution of the bacterium. Then a TCBS pour plate assay was performed with ASW to count the prey. Prey level corresponded to that expected (data not shown). Mussels were allowed to accumulate *V. parahaemolyticus* for 6 h, the exposure time being based on previous experiments (Croci et al., 2002). Then, mussels were evenly divided and distributed in other 2 tanks, randomly assigned to 2 groups. One group was treated as control microcosm and HBXCO1 was not added, the other group as test microcosm, with the addition of attack phase HBXCO1 to achieve the final concentration of about 1×10^3 PFU per mL. The tanks were maintained constantly aerated for 72 h. Then, *V. parahaemolyticus* and HBXCO1 counts were performed from mussels and ASW, respectively, at 0, 6, 24, 48, 72 h. Simultaneously, 10 mussels from test microcosm were taken to detect HBXCO1. For experiment 2 the mussels (naturally contaminated with *Halobacteriovorax*) were transferred into another 250 L ASW tank where freshly prepared prey enrichment was added to achieve a final concentration of 1×10^5 CFU per mL. Then, a pour plate assay on TCBS was performed with ASW to count the prey. Prey level corresponded to that expected (data not shown). Mussels were allowed to accumulate *V. parahaemolyticus* for 6 h, as for the experiment 1. Counts of *V. parahaemolyticus* and indigenous *Halobacteriovorax* from mussels and ASW, respectively, were performed at 0, 6, 24, 48, 72 h. For both experiments, analogous test (with predator and prey) and control (with prey alone) microcosms, without mussels, were prepared and analysed.

2.6. BALOs identification

Plaques of presumptive *Halobacteriovorax* isolated from mussels prior of experiment 2 were molecularly identified by PCR (forward primer Bac676 F atttcgcatgtaggggta and reverse primer Bac1442 R gccacggcttcaggtaag). Furthermore, sequencing analysis was performed on a single purified PCR product of 700 bp, randomly selected from those obtained during the first trial of experiment 2, according to a standardized protocol (Davidov et al., 2006; Ottaviani et al., 2018). We named this *Halobacteriovorax* strain DOGA9. DOGA9 was used as predator in the test microcosm without mussels of Experiment 2. A BLAST Search in NCBI GenBank was performed for 16 S rRNA gene sequence of DOGA9 and compared to phylotypes and clones described for similar sampling sites, as described by Pineiro et al. (2007).

2.7. Statistical analysis

Each of two experiments was repeated by three separate trials and each trial was carried out in duplicate (n = 6). The three trials of each experiment aimed to obtain replicates of the prey and predator trends starting from a unique predator/prey concentration that was 103 PFU/105 CFU per ml. Consequently, the mussels used for the three trials of each experiment had to be as homogeneous as possible with regard to their natural contamination. Therefore, for each experiment, the three trials were carried out with mussels by 3 different sampling taken in 3 consecutive weeks from the same area. Results of microbiological analyses were reported as mean values (log transformed) ± standard deviation and analysed for differences in response to *Halobacteriovorax* using Student's t-test with error probability (P) ≤ 95%.

3. Results and discussion

3.1. BALOs identification

The plaques of indigenous BALOs detected from mussels prior of the experiment 2 were confirmed by PCR as *Halobacteriovorax*. The 16SrRNA sequence of DOGA9 was submitted to GenBank with the accession number MN750624. The 16SrRNA sequence of DOGA9 showed a 100% identity to the 16SrRNA sequence of OC71 strains isolated from Great Salt Lake (GenBank accession number DQ536436) and 91.51% identity to the 16 S rRNA sequence of our HBXCO1 strain (GenBank accession number MG770616).

3.2. Decontamination experiment 1

The results are shown in Fig. 2 and in Supplementary Tables 2–7. In the microcosm with mussels 10^3 PFU per mL of HBXCO1 were counted at 0 h in ASW. Concentrations of HBXCO1 in water increased by about 2 log, from 10^3 to 10^5 PFU per mL within 6 h. It decreased by about 2 log, from 10^5 to 10^3 PFU per mL, between 6 and 24 h, it increased by about 0.5 log from 24 to 48 h, finally, it leveled off from 48 to 72 h. In the test microcosm without mussels concentrations of HBXCO1 in water increased by about 2 log within 6 h and it leveled off from 6 to 72 h. By comparing HBXCO1 concentrations in water at the different time points in test microcosms, with and without mussels they were significantly lower in the test with mussels than that without mussels at 24, 48, 72 h, with differences of about 2 log. This significant HBXCO1 decrease in water after 24 h in the microcosm test with mussels, probably reflects the passage of the predator from the water into the mollusk. To support this affirmation, detection of HBXCO1 from intervalvular fluid gave positive results at 24, 48 and 72 h, but not at 6 h. In the control microcosms with and without mussels, BALOs- *(Halobacteriovorax)* counts in water were always < 1 PFU per mL from 0 to 72 h. It is conceivable that *Halobacteriovorax* suspended in seawater can concentrate internally to mussels in a similar way to pathogens. In the test microcosm with mussels, the concentration of V. parahaemolyticus remained constantly around 5 log for the whole test period. In the control microcosm with mussels V. parahaemolyticus concentration remained constant between 0 and 6 h, it increased by about 2 log from 6 to 24 h, it leveled off from 24 to 48 h and finally, it decreased by about 1 log from 48 to 72 h. V. parahaemolyticus concentrations were significantly lower in the test than in the control microcosm at 24, 48, 72 h, with the maximum difference of 2.2 log at 24 h. In the test and control microcosms without mussels, the trends of *V. parahaemolyticus* were comparable to those obtained in the respective microcosms with mussels. This result suggests

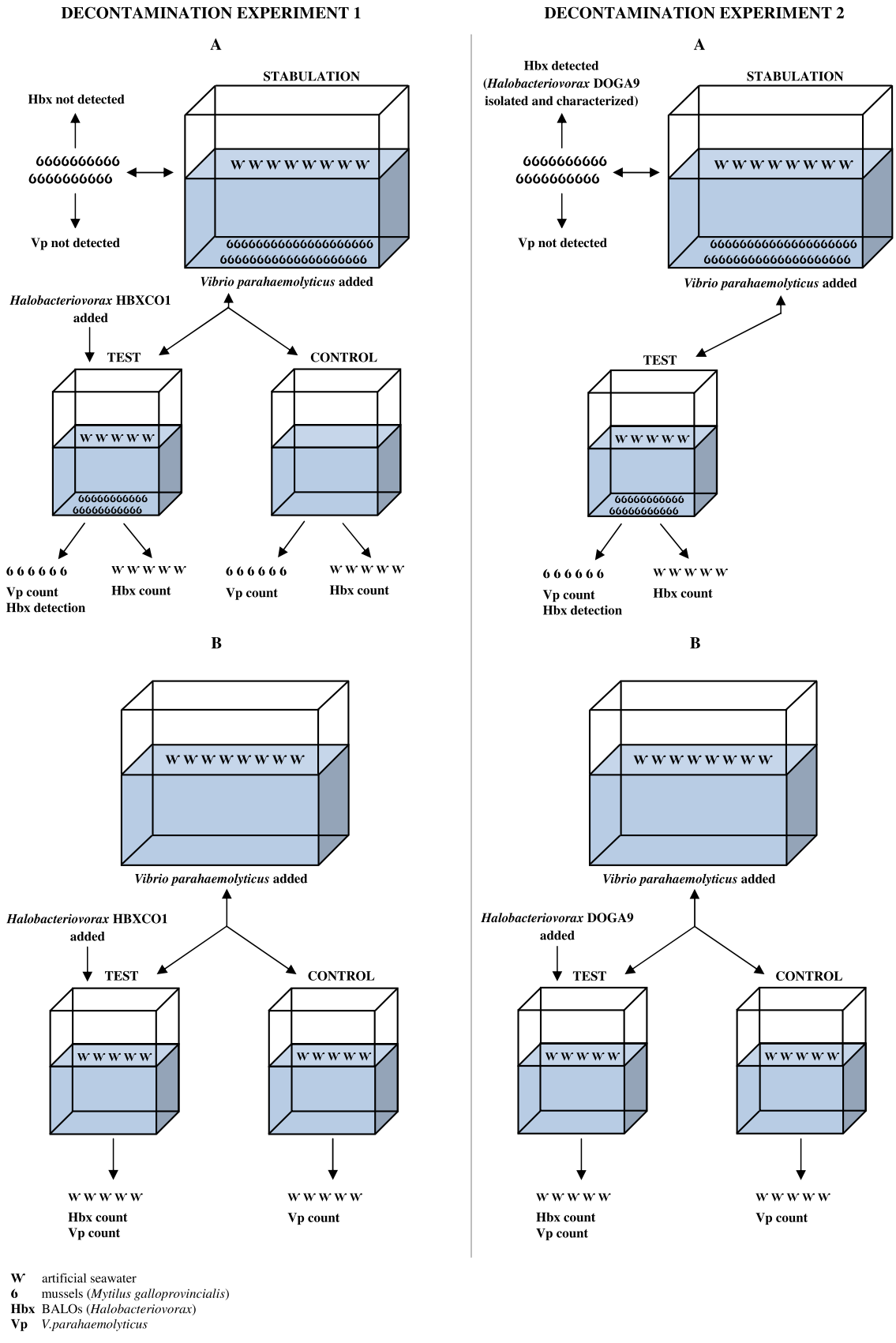

Fig. 1. A schematic flow chart of the experimental design used for the decontamination experiments. Decontamination Experiment 1: predator/prey free mussels experimentally contaminated with 10^3 PFU/10^5 CFU per ml of predator/prey; Decontamination Experiment 2: mussels naturally harbouring predator experimentally contaminated with 10^5 CFU per ml of prey; Test and control microcosms with mussels (A) Test and control microcosms without mussels (B).

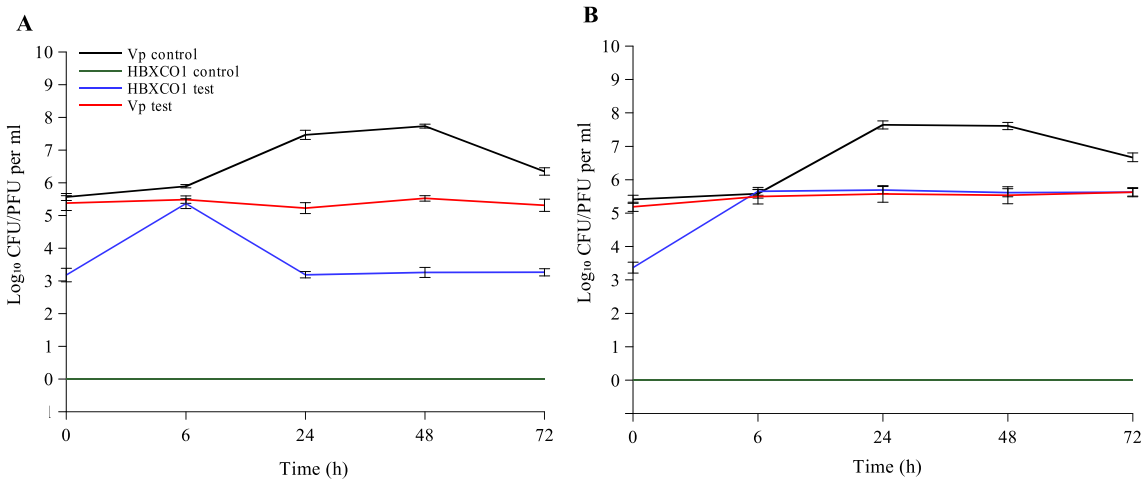

Fig. 2. Decontamination experiment 1. The population dynamics of HBXCO1 and *V. parahaemolyticus* (Vp) in test (with HBXCO1) and control (without HBXCO1) microcosms with mussels (A) and without mussels (B) — Vp control; — Vp test; — HBXCO1 control; — HBXCO1 test.

that the mollusk matrix does not reduce the effectiveness of HBXCO1 compared to that when it is in mussel-free water. This is probably related to the mussel filtration mechanism that creates a dynamic balance between prey and predator concentration in the water and inside the mollusk. For the same reason, the increase of *V. parahaemolyticus* inside the mussels in the control microcosm is probably due to the multiplication of the bacterium in water and its passage in the mollusk. This seems to be confirmed by the analogous trend of *V. parahaemolyticus* in the control microcosm without mussels. However, it cannot be excluded that *V. parahaemolyticus* may also multiply within mussels.

3.3. Decontamination experiment 2

The results are shown in Fig. 3 and in Supplementary Tables 8–12. BALOs-(*Halobacteriovorax)*, naturally present inside the mussels, were released in ASW at the concentration of 10^3 PFU per ml at 0 h. Predator concentration in water increased by about 2 log, within 6 h. It decreased by about 2 log between 6 and 24 h and it leveled off from 24 to 72 h. Regard the trend of *V. parahaemolyticus*, the concentration remained constantly around 5 log for the whole test period. In the test and control microcosms without mussels, the trends of *V. parahaemolyticus* and DOGA9 were comparable to those obtained in the respective microcosms of Experiment 1.

3.4. Correlations between the results of experiments 1 and 2

Predation trends towards *V. parahaemolyticus* of indigenous and exogenous *Halobacteriovorax* in test microcosm with mussels of experiment 1 and 2, respectively, were similar. For both experiments, prey concentration did not increase in mussels compared to the experimentally added level for the whole test period. Also predation trends of DOGA9 and HBXCO1 towards *V. parahaemolyticus* in test microcosm without mussels of experiment 1 and 2, respectively, were similar. This may be related to the fact that the same initial concentrations of predator and prey were in experiments 1 and 2 and to the high similarity between DOGA9 and HBXCO1 as was shown by the 16SrRNA sequencing analysis.

3.5. Halobacteriovorax to control V. parahaemolyticus

V. parahaemolyticus-specific *Halobacteriovorax* naturally present within the mussels was able to contain the level of *V. parahaemolyticus* added to laboratory scale. These results indirectly suggest that *Halobacteriovorax* could exert a physiological role in controlling the growth of *V. parahaemolyticus* and other vibrios in this marine ecosystem. We believe that *Halobacteriovorax* within mussels continues to parasitize vibrios and other potential pathogens as it does in sea water, when they are obtained from areas where *Halobacteriovorax* is present. It is known that the uptake and persistence of bacteria in mussel are dependent on

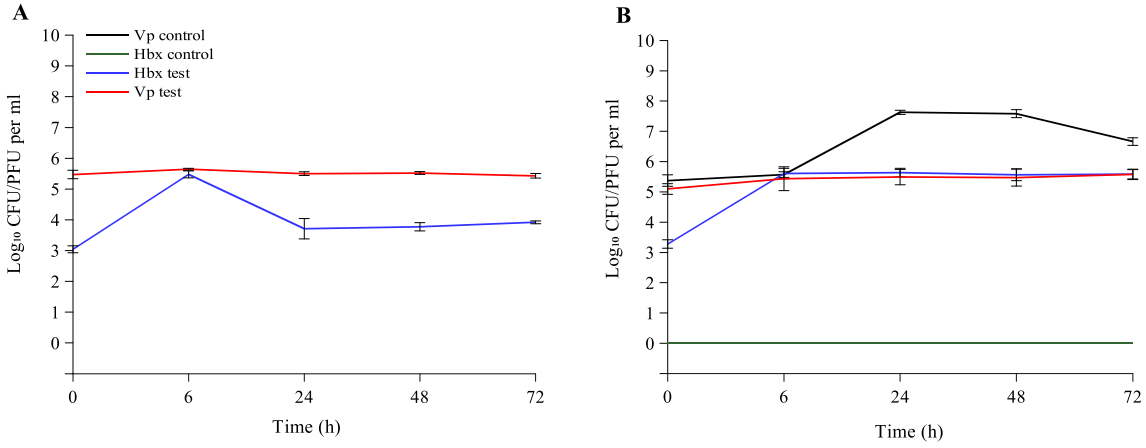

Fig. 3. Decontamination experiment 2. The population dynamics of indigenous *Halobacteriovorax* and *V. parahaemolyticus* (Vp) in the microcosm with mussels (A) and without mussels (B) — Vp control; —Vp test; — Hbx control; — Hbx test.

physical water conditions, nutrient availability, and mussel's ability to bioconcentrate and purge microbial contaminants (Phuvasate et al., 2012; Phuvasate and Su, 2013; Shen et al., 2019). However, we provide evidence that *Halobacteriovorax* likely play a direct role in modulating *V. parahaemolyticus* in seawater and mussel in the Adriatic Sea. The sequencing of the 16 S rRNA 700 bp fragment of DOGA9 showed >90% similarity with HBXCO1 and 100% similarity with a *Halobacteriovorax* strain of different origin. This suggests a high degree of genetic conservation for these microorganisms. In a future investigation, period and study area will be extended and all *Halobacteriovorax* isolates will be subjected to 16 S rRNA sequencing analysis. The aim of the study will be to assess whether stable strains of *Halobacteriovorax* are present in Adriatic Sea and, if so, their origin and seasonality. Moreover, marine BALOs have been applied to eliminate *V. parahaemolyticus* in oysters, at 25 °C laboratory-scale experiments, with a prey reduction in test respect to control of about 3.5 log after 7 days (Li et al., 2011). However, the prey trend was evaluated in conditions not in line with those of depuration plants that, at least in Italy, usually work for mussels at 17 °C for 24–48 h. Furthermore, Li et al. (2011) used a 107 PFU/108 CFU per mL predator/prey concentration. In the present study, work temperature and test times were chosen in line with those of a depuration plant and predator concentration was selected in order to be similar to that naturally occurring in seawater (Ottaviani et al., 2018). Despite the low temperature, 10^3 PFU per mL of HBXCO1 was able to prevent 10^5 CFU per mL of prey from increasing over time and kept it about 2 log lower than that of the control, from 24 to 48 h. We tested HBXCO1 with a non-toxigenic *V. parahaemolyticus* strain. However, it was shown that growth rates of pathogenic and non-pathogenic *V. parahaemolyticus* are similar (US FDA, 2001) and HBXCO1 showed predatory activity towards a large number of toxigenic *V. parahaemolyticus* strains (Ottaviani et al., 2018). The Center for Disease Control and Prevention stated that the average infectious dose for *V. parahaemolyticus* is 105 CFU per g (CDC, 2006). It is clear from our results that HBXCO1 did not eliminate *V. parahaemolyticus* from mussels. However, in mussels contaminated with a *V. parahaemolyticus* level at the limit of the average infectious dose, HBXCO1 was able to keep the concentration below that at risk, for at least 48 h. In contrast, in the control, prey exceeded the infectious dose after 6 h and remained above this limit for the whole test period. Although HBXCO1 was not able to eliminate *V. parahaemolyticus* from mussels, it could play an important role by integrating it with other chemical-physical strategies in a depuration plant. Previous researches reported that V. parahaemolyticus purge rates depended on bivalves stocking density, water temperature and salinity (Phuvasate et al., 2012; Phuvasate and Su, 2013; Shen et al., 2019). Moreover, even the addition of substances with natural antibacterial activities can increase the effectiveness of the *V. parahaemolyticus* depuration (Shen and Su, 2017). For their part, *Halobacteriovorax* and BALOs in general are resistant to disinfection systems commonly used in purification plants and are tolerant to a high range of salinity and temperature (Williams et al., 2009). In the light of these considerations, it would be possible to modify chemical and/or physical parameters of a depuration plant to increase its efficacy and integrate conventional approaches with biological ones, i.e. by *Halobacteriovorax*.

4. Conclusion

To the best of our knowledge, this is the first report on the role of *Halobacteriovorax* to control *V. parahaemolyticus* in mussel of the Adriatic Sea. In the short term further laboratory-scale decontamination experiments will be carried out with different predator/prey ratios to understand if the control efficacy is concentration-dependent. At the same time, the effects of the different *Halobacteriovorax* abundances on the mussel microbiome will also be evaluated. Moreover, we will further test HBXCO1 and other indigenous *Halobacteriovorax* to reduce *V. parahaemolyticus* in an experimental depuration system, using various combinations of salinity and temperature, different disinfection approaches and water-mussel ratio. Our ultimate goal will be to develop a new integrated post-harvest approach for the mussel depuration from *V. parahaemolyticus* and other potential pathogenic vibrios to be tested on industrial scale.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Acknowledgments

This work was supported by Italian Ministry of Health (grant RF 2013–02355019).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fm.2020.103600.

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