Control of *Vibrio parahaemolyticus* in the White shrimp *Litopenaeus vannamei* by phage therapy

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Introduction

Vibrio parahaemolyticus is considered the principal cause of gastroenteritis by seafood consumption in humans and was associated with vibriosis in marine organisms (Gopal *et al*, 2005). Its effects are more evident in aquaculture where serious economical losses are attributed to these bacteria. Antibiotic therapy was the common strategy to control their negative effects; however, since bacterial antibiotic-resistance became a major problem, it was necessary to develop alternative therapies (Alagappan *et al*, 2010). Phage therapy is a potential alternative to control the effects of pathogenic bacteria, which is based in the ability of phages to kill bacteria. Apparently, their use does not mean the adverse effects of antibiotics, such as the selection of resistance mechanisms and transference to human pathogens. Phage therapy has already been used successfully to treat bacterial infections in fish and other aquatic organisms (Park *et al*, 2000, Vinod *et al*, 2006). In the present study we evaluated the therapeutic use of two lytic *V. parahemolyticus* bacteriophages during experimental infections of *Litopenaeus vannamei* larvae.

Methods

The phage therapy was evaluated during challenge test with shrimp larvae. The phages used in the present study (A3S and vpms1) were previously isolated from invertebrate samples and were selected on basis of their lytic effect on *V. parahaemolyticus*. The conditions for the challenge test were previously standardized; briefly, shrimp larvae at nauplii stage 1 (N1) were obtained from a commercial hatchery and acclimated in our laboratory at 30 °C until reach the nauplii stage 5. At this stage the larvae were disinfected in a bath of 100 ppm ClO_2 during 5 minutes, the residues were neutralized using sodium thiosulfate and the larvae were washed thoroughly with sterile artificial seawater (ASW). The larvae were dispensed in the experimental units with 100 ml of ASW at a rate of 100 larvae per unit. The experimental units were infected with *V. parahaemolyticus* strain ATCC 17802 at ca. $2 \cdot 10^6$ CFU·mL^{-1} and treated with 100 µL of phage suspension at ca.$\cdot 10^9$ PFU·mL^{-1} to reach a multiplicity of infection (MOI) near to 5. The experiments were achieved by triplicate during 96 h, and during this time the units were maintained at 30 °C and feed daily with 10^5 cel·mL^{-1} *Chaetoceros calcitrans*. Controls without phage treatment and without bacteria were maintained simultaneously. The survival and signs of disease was recorded at 96 h and the data were analyzed using ANOVA and Tukey test.

Results and discussion

During our experiments, the use of phage therapy was effective to eliminate the adverse effects of *V. parahaemolyticus* on shrimp larvae. Significant differences were found between phage treated and the untreated controls ($P<0.05$): in the controls without *V. parahaemolyticus* a survival of 87 % was recorded, while the infected with *V. parahaemolyticus* reach a survival of 59 %. No differences were observed between the experimental units treated with A3S and Vpms1 phages and the uninfected controls ($P>0.05$). They reach a survival of 77 % and 80 % respectively (Figure 1). Vinod *et al*. (2006), found similar beneficial effects when a mixture of phages (lytic on *Vibrio harveyi*) was directly supplied on larvae cultures of *Penaeus monodon*. Considering that phage are highly specific, the phage therapy is also a highly specific strategy to control of bacteria, their success is hardly compromised with the species or strains involved in an infectious process. In our study the phages used were very specific for *V. parahaemolyticus* and their effects are predicable in terms of their ability to kill the infecting bacteria.

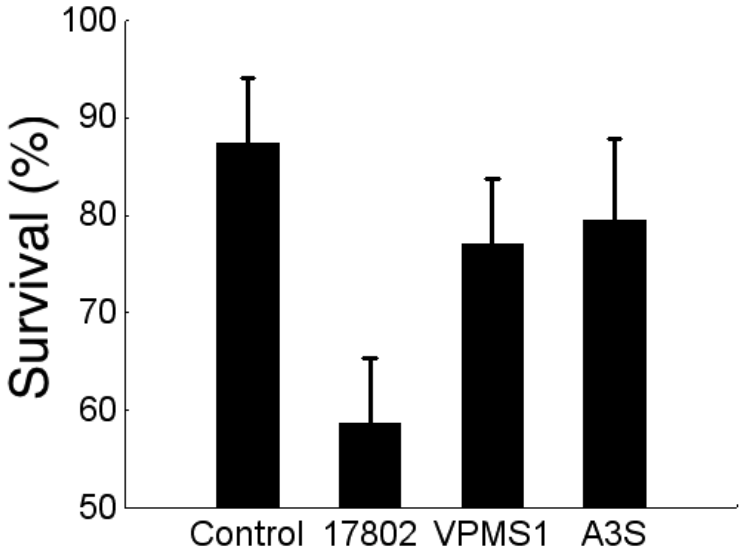

Figure 1. Survival of infected larvae with *V. parahaemolyticus* and treated with bacteriophages vpms1 y A3S.

A common effect attributed to *V. parahaemolyticus* is the reduction in the degree of development, however in our study we can't found evidence of that effect; the survivors of all treatments reach the same stage of development (Zoea II).

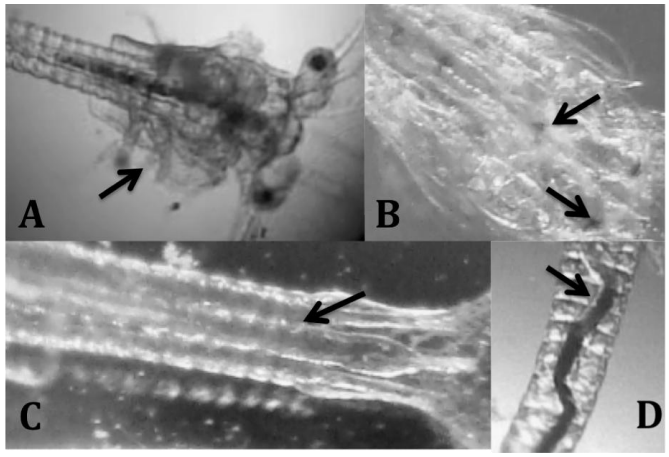

Figure 2. Signs of vibriosis detected in Zoea II stage larvae infected with *V. parahaemolyticus*. A) Lack o mutilation of maxillae and maxillipeds; B) Chromatophores excited; C) No feed digestive tract; D) Digestive tract malformed.

The recorded signs of infection were anorexia (empty digestive tract), lethargy, loss and deformation of the first and second antenna, loss of caudal spines and black spots in the surface due to stimulation of chromatophores (Figure 2). Controls without bacteria and treated with phages were very active swimming, few phage treated larvae show loss of spines from the upper appendages and feed discontinuous in the intestine (Figure 3B).

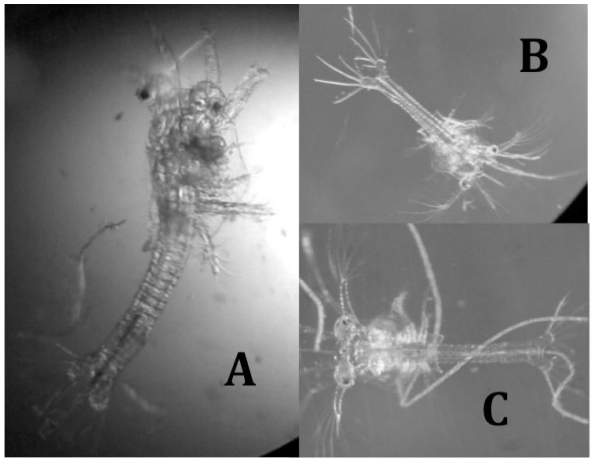

Figure 3. A) *V. parahaemolyticus* infected larvae; B) *V. parahaemolyticus* and vpms1 phage treated larvae; C) Uninfected larvae.

Conclusions

The use of A3S and Vpms1 phages during experimental infection was effective to prevent the mortality and to reduce the signs of vibriosis in the larvae. The phage therapy is an alternative to control of negative effects of *V. parahaemolyticus* in aquaculture. Their use could reduce the use of antibiotics. The use of phages could be extensive to the decontamination of seafood in order to prevent the human gastroenteritis.

Acknowledgements

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