The increase in the virulence of *Vibrio parahaemolyticus* for crustaceans is mediated by phages

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Introduction

Different species of *Vibrio* are frequently reported as responsible during high mortality in the culture of marine organisms. However, their role is still uncertain due to the fact that some species are reported as pathogenic and at same time, as probiotics (i.e. *Vibrio alginolyticus*) (Direkbusaram *et al*, 1998; Vandenberghe *et al*, 2003). This difference in the ability to induce mortality can be explained by the presence and expression of virulence genes in the pathogenic strains and by the absence of them in related strains (Hall y Collis, 1995; Kutter y Sulakvelidze, 2005). *Vibrio parahaemolyticus* is an opportunistic bacterium which is a part of the shrimp microbiota and is common in the culture systems, where it can no exhibit perceptible effects on the shrimp, can delay its development or can be a cause of massive mortalities. This condition suggests that bacteriophages can be implicated in the transformation of this bacterium in the culture facilities. So in this study we evaluated the effects of temperate phages, isolated from the shrimp culture on the virulence of *V. parahaemolyticus* during challenge tests with *Artemia* and white shrimp nauplii.

Methods

A group of 10 temperate phages (α, 2, 5+++, 5c, 5s, γ, E2, W, S y Ese) previously isolated from the intensive shrimp culture, were used in the present study. The virulence of *V. parahaemolyticus* was evaluated via challenge tests. Briefly *Artemia* nauplii were axenically hatched and distributed in the experimental units with 100 mL of sterile seawater at a density of 100 nauplii per unit and were infected with 600 µl of *V. parahaemolyticus* suspension (O.D. = 1, 560 nm), also 100 µl of phage suspension were added at each unit. The units were incubated for 48 hrs at 30°C and then the survival of *Artemia* nauplii was recorded. Simultaneously, the virulence of *V. parahaemolyticus* with phages was evaluated on *Litopenaeus vannamei* nauplii, which were collected in experimental units with 100 ml of artificial seawater and infected with 2 ml of *V. parahaemolyticus* suspension (O.D. = 1, 560 nm) and 100 µl of phage suspension. The units were incubated as described above and the organisms were maintained with daily doses of *Chaetoceros calcitrans* at density of 100 000 cel•ml^{-1}, survival rates were evaluated after 96 h. The metabolic changes induced by each bacteriophage in *V. parahaemolyticus*, were evaluated as the enzyme production and carbon source usage by using the multi-test strips API-ZYM and BIOLOG GN2 respectively. The obtained data was analyzed via ANOVA and multiple comparisons test using Statistica®.

Results y discussion

Some of tested bacteriophages were responsible for induction of the increment in the mortality caused by *V. parahaemolyticus* on *Artemia* nauplii and white shrimp larvae (Figs 1 and 2). In the case of *Artemia*, intestinal lesions and lethargy was observed; while in shrimp larvae, the phage induce anorexia and appendages malformations. The tested bacteriophages induced an increase in lipase production and changes in the profile of carbon sources usage, providing the possibility of use of new substrates and mediating advantages over other bacteria.

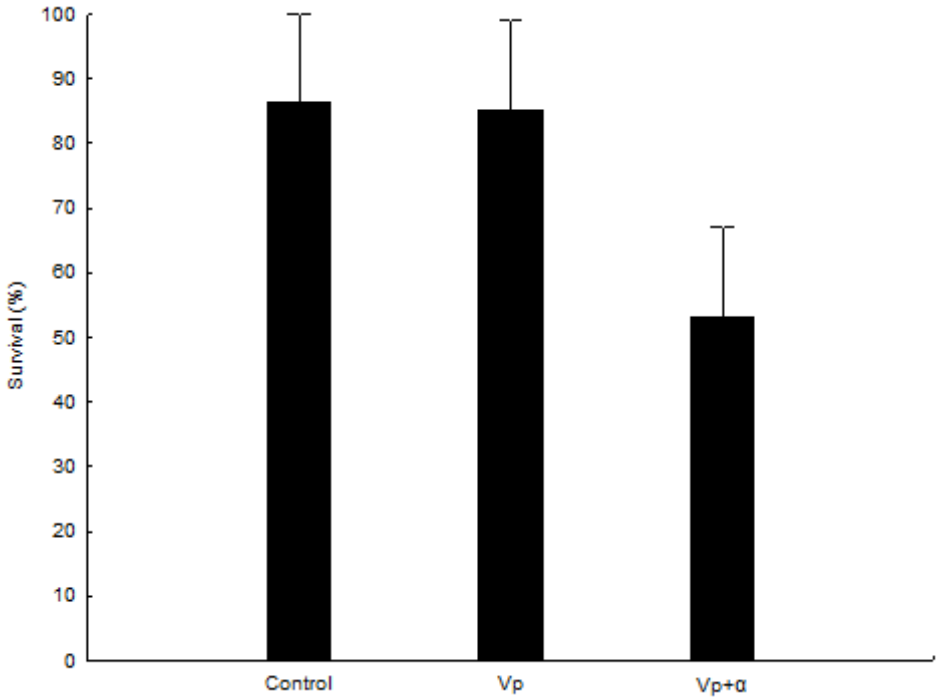

Figure 1. Survival of *Artemia* nauplii at 48 hrs post-infection with *V. parahaemolyticus*, and *V. parahaemolyticus*-phage α.

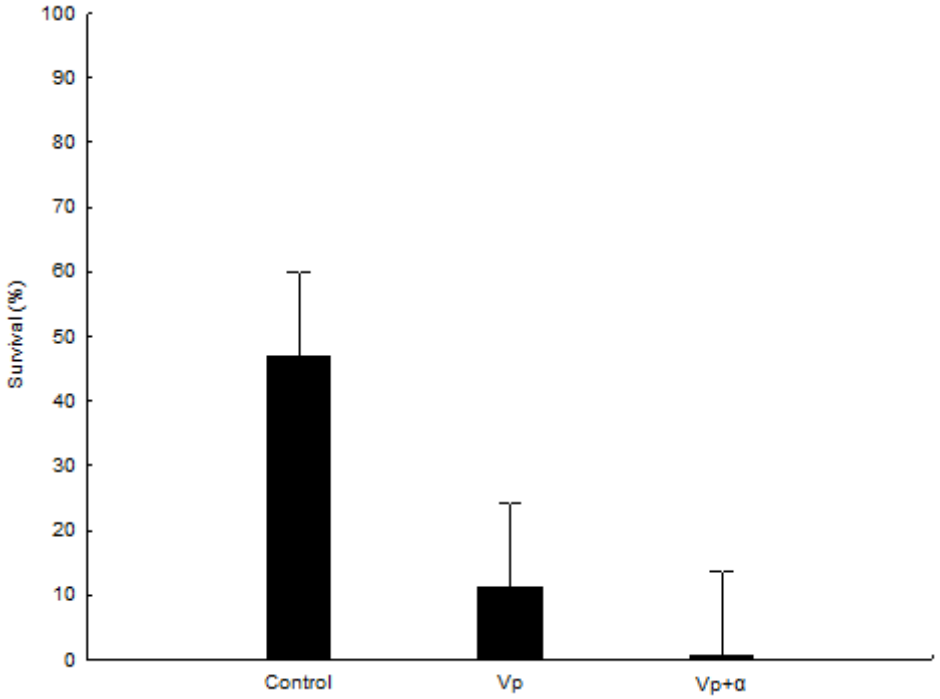

Figura 2. Survival of shrimp larvae exposed to *V. parahaemolyticus*, and *V. parahaemolyticus*-phage α.

Conclusions

The virulence of *V. parahaemolyticus* is modified by some bacteriophages that occur in the shrimp culture system, it can explain the variability of this bacterium in the crustaceans cultures.

Acknowledgements

This research was supported by the projects SIP 20100865 and CONACyT 085033.

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