Use of a multispecies probiotic to protect *Artemia* from the pathogenic effects of *Vibrio harveyi*

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Introduction

Vibrio harveyi is an important pathogen for a wide range of marine species, and their effects on the aquaculture industry have been extensively documented (Owens & Busico-Salsedo, 2006). This species has been considered an opportunistic pathogen but has been shown that some strains can cause devastating effects on aquaculture, especially in shrimp production. *Artemia* is considered as the main vector of *Vibrio* in shrimp larvae production, and some *Vibrio* species that proliferate during the hatching process can affect the survival rate of these organisms fed *Artemia*. Several studies have shown the beneficial effect of probiotics on the crops but some of them have failed in controlling the adverse effects of *Vibrio*. Thus, the aim of this study was to investigate whether a multispecies probiotic preparation could inhibit the growth of *V. harveyi* during *Artemia* hatching.

Methods

Colonization and effect of two *Vibrio harveyi* strains (14126 and EC11) on hatching rate was evaluated under gnotobiotic conditions. Briefly, axenic *Artemia* cysts (INVE ®) were placed in tubes with 10 ml of sterile seawater and inoculated with different doses of *V. harveyi* (1, 10.100, 1000 and 2000 µl of a *V. harveyi* suspension at 6×10^8 CFU·ml^{-1}). The tubes were placed for hatching in a rotator (Barnstead Thermolyne Labquake ®) at 28 °C under continuous light. The colonization was evaluated by counting the number of colonies on TCBS plates and the hatch and survival were recorded at 24 h.

A mixture of 10 bacterial strains belong to the genera *Bacillus*, *Lactobacillus* and *Lactococus* (2, 2 and 6, respectively) was selected due to its ability to adhere to the cysts and nauplii during the hatching process. Different concentrations of the bacterial mixture were added during the experimental infection of *Artemia* cysts with *Vibrio harveyi* EC11 strain at numbers close to the LD_{50}. The evaluation was performed by triplicate in 100 ml flasks with 100 cysts per flask. Temperature at 28 ^{o}C, continuous aeration and light were maintained during the hatching process. The survival of *Artemia* was evaluated at 24 h.

Results

There were marked differences in colonization ability among different *Vibrio* strains. The strain ATCC 14126 did not colonize the cysts, whereas the strain EC11 effectively colonized *Artemia* cysts, and the intensity of colonization was directly related to the dose used for infection. The highest recorded value was 1.12×10^9 CFU·cyst^{-1}.

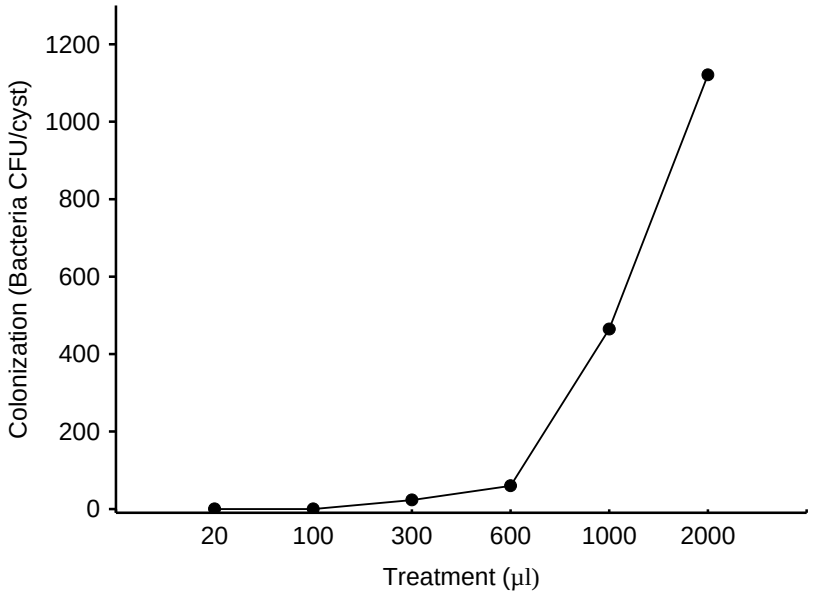

Figure 1. Colonization of *Vibrio harveyi* EC11 for the hatching of *Artemia* cysts from INVE ®.

The EC11 dose directly influenced the hatching and survival of *Artemia*. A significant reduction in the hatching and survival was observed at the lowest dose used, and the value of LD50 was estimated between 1 and 10 µl (ca. between 0.5×10^5 and 5.6×10^6 CFU·ml, respectively). (Fig 2)

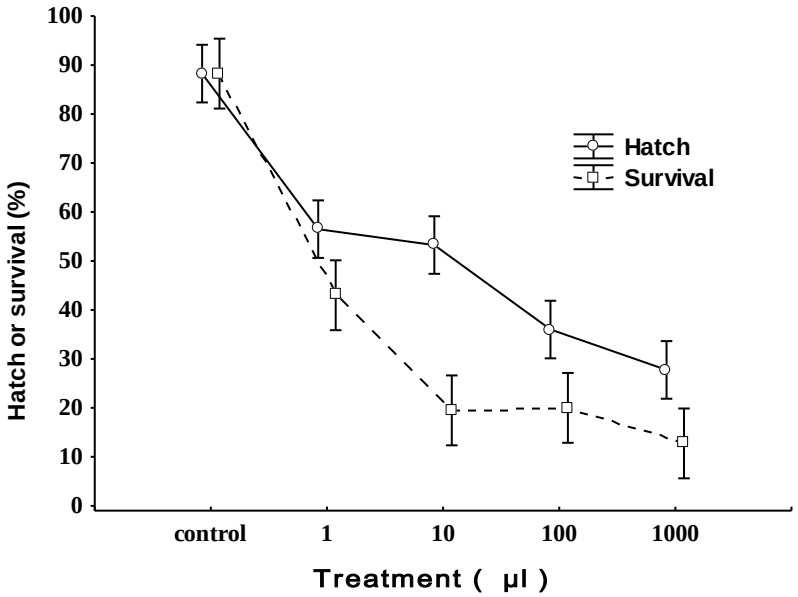

Figure 2 Percentage of hatching and survival of *Artemia franciscana* during experimental infection with different doses of *Vibrio harveyi* strain EC11 at 24h

The results also demonstrated that probiotics were able to reduce the mortality rate and the protective effect was correlated to the dose used. The survival at doses of 1 and 10 µl was not significantly different from the control infected with *V. harveyi*, while doses of 100 and 1000 µl were not significantly different from the axenic controls.

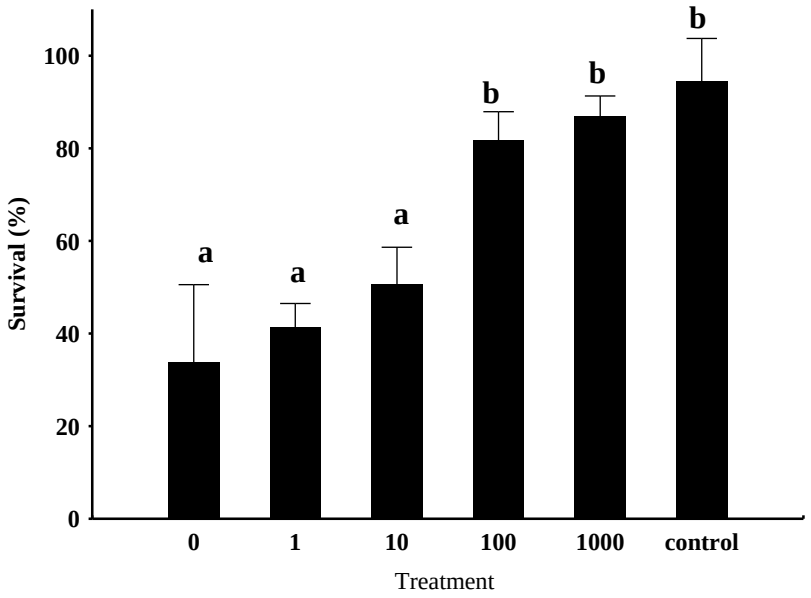

Figure 3. Survival of Artemia nauplii infected with *V. harveyi* 5.5×10^6 CFU ml EC11 and treatment with different doses of probiotic (equivalent to 1×10^7 CFU/ml) and axenic control.

Conclusions

Manipulation of the bacterial community during the *Artemia* hatching has a direct impact on the survival rate. *Vibrio harveyi* strain EC11 reduced severely the hatching percentage and the survival of *Artemia*. In contrast, the probiotic bacteria showed competence with *V. harveyi*, because of their ability to replace it and remain as a part of the microbiota, reducing the negative effects of this pathogen. The Artemia colonization with probiotic bacteria could be a strategy to break the Vibrio transmission from the hatching containers to shrimp tanks.

Acknowledgements

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

References

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