

Relative Contribution of *Halobacteriovorax* and Bacteriophage to Bacterial Cell Death under Various Environmental Conditions

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Introduction

The role of protists and bacteriophages in bacterial predation in the microbial food web has been well studied. There is mounting evidence that Bdellovibrio and like organisms (BALOs) also contribute to bacterial mortality and, in some cases, more so than bacteriophages. A full understanding of the ecological function of the microbial food web requires recognition of all major predators and the magnitude of each predator's contribution. Here we investigated the contribution of *Halobacteriovorax (HBx)*, one of the BALOs, and bacteriophages when incubated with their common prey, *Vibrio vulnificus*, in a seawater microcosm.

Results and Discussion

We observed that *Halobacteriovorax* was the greatest responder to the prey, increasing 18-fold with a simultaneous 4.4 log reduction of *V. vulnificus* at 40 hours, whereas, the bacteriophage population showed no significant increase (Fig1). In subsequent experiments to formulate a medium that would support the predatory activities and replication of both predators, low nutrient media favored the predation and replication of the *Halobacteriovorax*, whereas higher nutrient media enhanced phage growth. Greatest prey reduction and replication of both Halobacteriovorax and phage were observed in media with moderate nutrient levels. Additional experiments show that the predatory activities of both predators were influenced by environmental conditions, specifically, temperature and salinity. The two predators combined exerted greater control on *V. vulnificus*, a synergism that may be exploited for practical applications to reduce bacterial populations.



Fig.1 Kinetics of the lysis of prey cells (a) and growth dynamics of Halobacteriovorax (HBx) and phage on Vv prey (b) over a 40-h period in test (with HBx+phages+Vv) and control (with either predator or no predators) microcosms. F1 (HBx+phages+Vv) designates the microcosm with both Halobacteriovorax and phage predators. F2 and F3 are the microcosms consisting of Vv and either Halobacteriovorax or phages, respectively. F4 is the microcosm with prey Vv only. Predator and prey counts were obtained in triplicates. Error bars are standard errors from three independent experiments.

Conclusions

These findings suggest that along with bacteriophage and protists, *Halobacteriovorax* has the potential to have a prominent role in bacterial mortality and cycling of nutrients, two vital ecological functions

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References

[1] Chen H., et al., mBio, 9(4):e01202-18. (2018)