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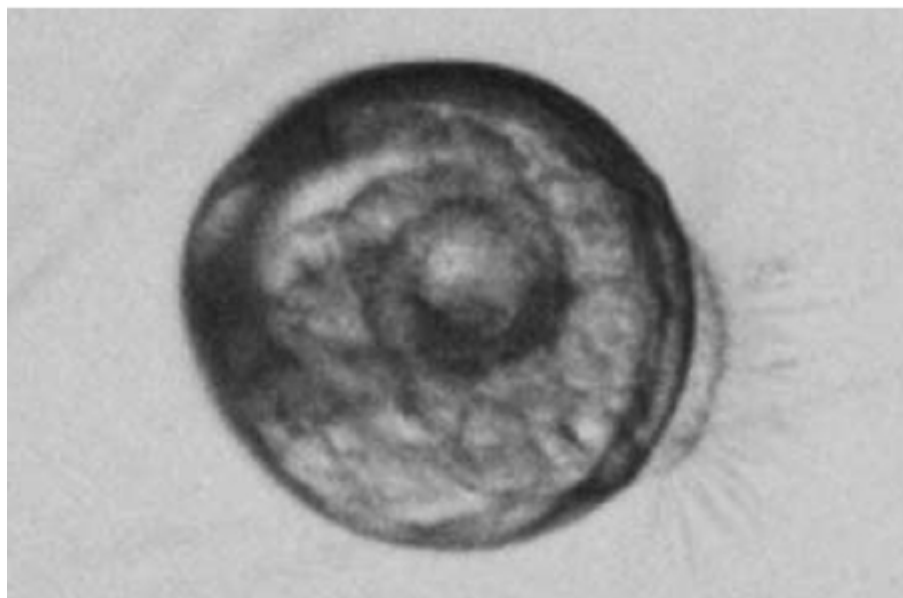
Australian test studies potential of probiotics in oyster hatcheries

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1 June 2003 Cheok Keong Tan, B.Sc. Lewis F. Gibson, Ph.D. Anthony M. George, Ph.D.



A. media and *V. alginolyticus* can be easily cultured and administered



Oyster larva challenged by *Vibrio tubiashii* treated with *Aeromonas media*.

A common threat to oyster hatcheries around the world is the occurrence of infectious bacterial diseases. Prevention and control of these bacterial diseases traditionally has involved the use of chemicals such as antibiotics and disinfectants. However, the chronic and/or prophylactic use of antibiotics for disease control encourages the emergence and proliferation of resistant bacteria. Furthermore, these resistant bacteria can also transfer their resistant genes to other bacteria.

An alternative to antibiotics is the use of probiotics, a single or mix culture of nonpathogenic bacteria that improve the health and survival of the host organism. They can be applied directly in the feed and/or culture tank as preventive agents.

Experimental setup

The authors recently conducted a study at the University of Technology, Sydney to evaluate two potential probiotics, *Aeromonas media* and *Vibrio alginolyticus*, that are active against the *Vibrio tubiashii* pathogen that affects the larvae of Sydney rock oysters (*Saccostrea glomerata*).



Oyster larva challenged by *Vibrio tubiashii* treated with *Vibrio alginolyticus*.

The trial used 2-day-old Sydney rock oyster larvae cultured in 2-l flasks with a stocking density of 5 larvae per millileter. Water was exchanged every two days using 1- μ filtered seawater. *Aeromonas media*, *V. alginolyticus*, and *V. tubiashii* were inoculated at the start of the trial and after each water exchange.

The potential probiotics were inoculated at a concentration of 10⁴ cells per millileter one hour prior to the addition of *V. tubiashii*, which had a concentration of 10⁵ cells per millileter. Larvae were fed daily with a combination of Tahitian *Isochrysis sp.* (T-Iso) and *Pavlova lutheri*.

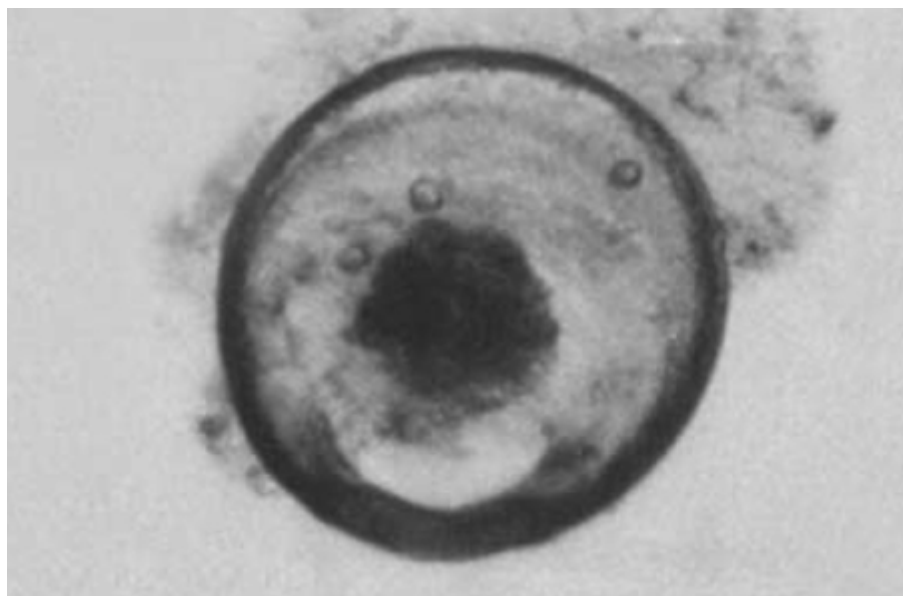
Results

No significant differences were found in the survival of oyster larvae treated with *A. media* or *V. alginolyticus* to that of the control, which had a mean larval survival of 48-60 percent. However, when the control larvae were subjected to a challenge with *V. tubiashii*, the mean larvae survival decreased to less than 10 percent. And the larvae that survived the challenge were, on average, smaller in size and less active than the unchallenged larvae.

Larvae challenged with *V. tubiashii* and treated with either *V. alginolyticus* or *A. media* showed a fourfold improvement in survival. They were also larger in size and more active than untreated, challenged larvae.

The concentration of *V. alginolyticus* increased over the first 24 hours and remained at this concentration for the next 72 hours. In contrast, the concentration of *A. media* decreased over the first 24 hours to 10² cells per milliliter and then plateaued. After 72 hours, *A. media* could not be detected in the larval culture – a promising fact, as it performed its function and then disappeared from the larvae, minimizing concerns about product safety and quality.

Additional tests



Larva not treated with probiotics.

A second strain of *V. alginolyticus* isolated from finfish, Val 2, was used as a negative control to determine if the improvement in larval survival was due to the presence of probiotics or simply the presence of bacteria. Val 2 was found to be nonpathogenic to the larvae, but it failed to provide any protection to the larvae when challenged with *V. tubiashii*.

The mean survival rate of the challenged oyster larvae was also less than 10 percent, similar to that of the nontreated challenged larvae. These findings suggested that both *A. media* and *V. alginolyticus* were improving larval survival, directly or indirectly.

The effects of the probionts on the growth and survival of *P. lutheri* and *T-Iso* were also investigated. The results showed that neither *A. media* nor *V. alginolyticus* had any detrimental effects on the viability and growth of the algae. Similarly, the viability of the probiotics was not affected by the presence of the algae. This suggested that the algae could be used as a vector for introducing the probiotics into the digestive systems of the larvae.

Mode of action

The mode of action of probiotics varies between different strains of bacteria. Some strains of probiotics produce inhibitory compounds, while others compete for nutrients and available energy. In vitro, *A. media* produces a bacteriocin-like inhibitory substance (BLIS) that acts against a number of known pathogens, including vibrios and aeromonads.

However, in vivo, BLIS could not be detected from the culture water. Hence, improvement of larval survival may or may not be related to BLIS. In contrast, no inhibitory substances were detected from *V. alginolyticus* in vitro. However, in vivo, *V. alginolyticus* has always been the dominant microflora in the culture system after 24 hours. This suggests its mode of action may possibly be the result of competition.

Prevention versus treatment

The emphasis in disease management should be on disease prevention, not treatment of symptoms. Prevention of disease can be achieved through good husbandry and management of the hatchery.

It is often difficult to predict when an outbreak will occur; hence, if these two probiotics can be established, either individually or together, as the dominant microflora in the culture system before pathogen invasion, the risks of

bacterial outbreaks should be reduced or eliminated. Furthermore, the use of these two probiotics is also likely to be cost-effective, as it can eliminate the use of chemicals, and these probiotics can be easily cultured and administered.

Conclusion

Studies found *A. media* and *V. alginolyticus* to be prime candidates as probiotics for use in oyster hatcheries. They are nonpathogenic to, and provide protection for the larvae against pathogens; do not affect the larval feed; and can be easily cultured and administered.

(Editor's Note: This article was originally published in the June 2003 print edition of the Global Aquaculture Advocate.)

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