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- Log In

- About
  - Who We Are
  - Our History
  - Our Team
  - Sustainable Development Goals
  - Careers
- Membership
  - Overview
  - Our Members
  - Corporate Membership
- Resources
- Certification
  - Best Aquaculture Practices
  - Best Seafood Practices

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Log In

- About
  - Who We Are
  - Our History
  - Our Team
  - Sustainable Development Goals
  - Careers
- Membership
  - Overview
  - Our Members
  - Corporate Membership
- Resources
- Certification
  - Best Aquaculture Practices
  - Best Seafood Practices
- GOAL Events
- Advocate Magazine
- Aquademia Podcast
- Blog
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Health & Welfare

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Selection of probiotics for marine larviculture

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1 August 2004 O. Decamp P. Makridis T. Vercauteren K. Dierckens K. Van Driessche

Bacterial cultures modify microbial communities, reduce pathogens and improve growth, survival

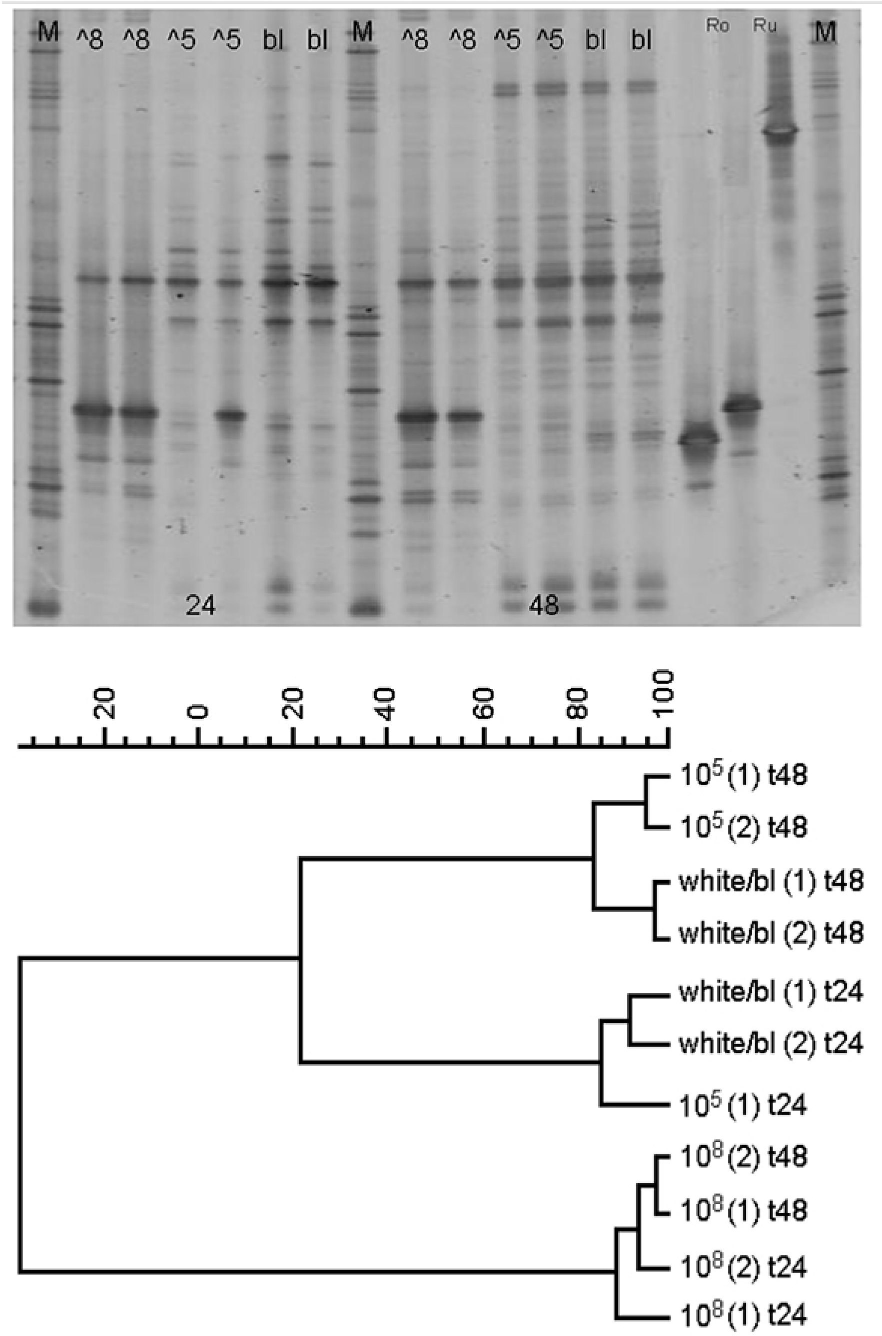

Fig. 1: Effect of a bacterial mixture on the genetic diversity of bacteria associated with Artemia following 24 and 48 hours of Artemia cyst incubation. Evaluated by denaturing gradient gel electrophoresis and comparative sequence analysis of PCR-amplified 16S rDNA genes. In this case, related bacterial communities were clustered together, indicating they were affected by an application of a bacterial cocktail at 1 x 108 cfu/ml but not 1 x 105 cfu/ml.

Most marine hatcheries encounter bacterial problems that negatively affect production. Live feed, which is still essential in the larval rearing of fish and shrimp, is also a significant source of bacterial contamination in larval-rearing tanks. For example, the concentration of bacteria in hatching tanks for artemia can reach 10^7 bacteria per milliliters water after 24 hours of incubation, with a high proportion of potentially hazardous *Vibrio* sp.

For this reason, hatcheries apply water treatments such as ultraviolet irradiation, ozonation, membrane filtration and the addition of antibiotics and disinfectants to reduce bacterial loads in the culture water. However, these approaches disturb the balance of microbial communities and favor uncontrolled growth of opportunistic and potentially pathogenic bacteria. Furthermore, the prophylactic use of antibiotics in aquaculture may lead to the selection of bacteria that are drug-resistant or more virulent, and should be avoided.

Probiotics offer a treatment alternative. These bacterial cultures modify the microbial communities in water and sediment, reduce or eliminate pathogenic microorganisms, and generally improve the growth and survival of the targeted species.

Probiotic development

The development of suitable probiotics requires basic and applied fundamental research, full commercial-scale trials, and appropriate monitoring tools. As part of a European Union-funded research project, the authors investigated the potential of using selected bacterial strains for preemptive colonization of the culture environment for specific stages of larvae.

Selection steps

The selection procedure included the following steps:

1. The strains or mixtures of bacteria were screened *in vivo* with a focus on the ability of the probiotics to improve the performance of live food production or diminish the risk of transmitting pathogens to the fish larvae. This was accomplished by reducing the concentration of vibrios in the rearing water or colonizing the artemia per rotifers. In the latter case, the dose of probiotics applied to the rearing system to influence the bacterial community must be assessed (Fig. 1).
2. Optimal growth factors of the bacteria were considered in relation to the prevailing environmental conditions such as temperature, pH, and salinity in the aquaculture production systems in which the bacteria would be utilized.
3. The authors carried out a first estimation of the pathogenicity of the best-performing strains based on their molecular identification as well as published information. As a consequence, *Vibrio* strains and bacteria classified by the U.S. National Institutes of Health as “risk group 2 microorganisms,” agents associated with human disease that is rarely serious and for which preventive or therapeutic interventions are often available, were discarded.
4. The resistance of these bacterial strains to current human and animal therapeutic antibiotics was investigated using plating methods with different antibiotic concentrations to exclude antibiotic-resistant bacteria from future applications. Their safety toward the target organisms was obviously evaluated during the initial screening, and their lack of toxicity toward other aquaculture organisms was also assessed. For example, a probiotic cocktail leading to higher rotifer production could include bacterial strains that might be opportunistic pathogens to sea bream under particular conditions.
5. This was followed by field testing of the revised mixture of bacteria, taking into consideration that removing bacterial strains from a performing mixture might lead to poorer performance, since the functions of the individual strains were not always identified.
6. The final stage was registration. Regulations for the incorporation of microorganisms in animal feed have changed recently, and applications for fish are still to be drafted. Microorganisms for rotifer and artemia culture might remain free from specific regulation.

Probiotic application

A similar screening method was applied for the development of INVE Sanolife PRO-1, a probiotic product for shrimp hatcheries. Daily applications of the mixture of bacteria led to a significant improvement in larval shrimp cultures in terms of survival rates and biomass production compared to the control treatments (Fig. 2).

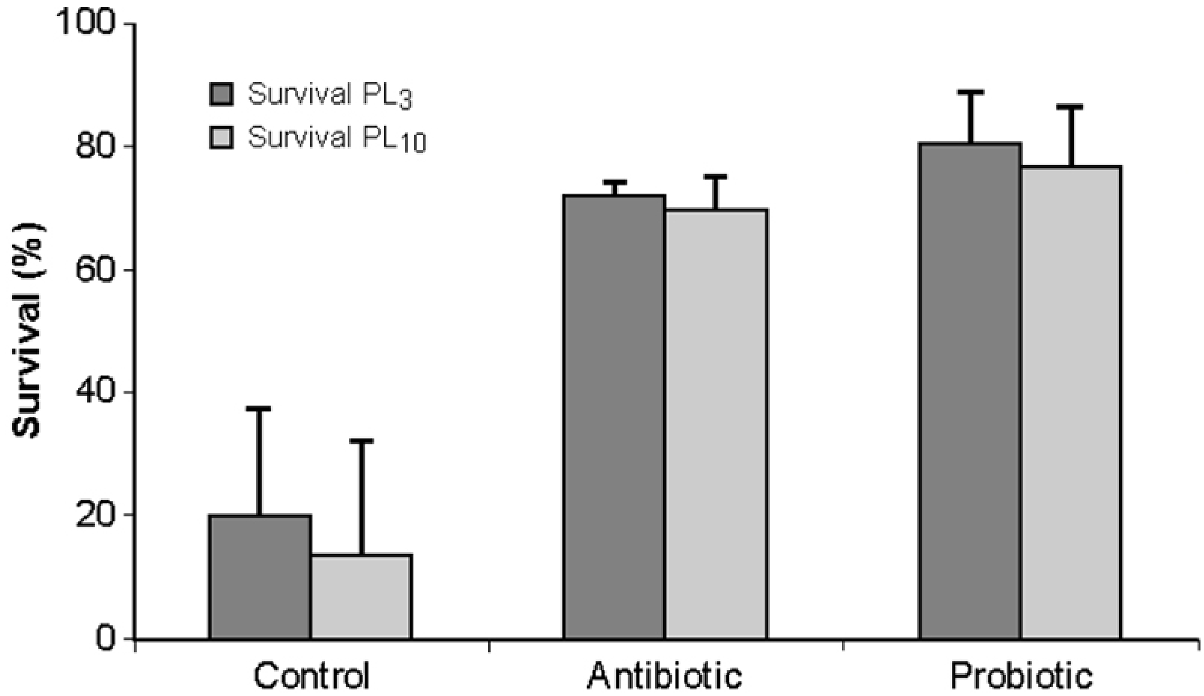

Fig. 2: Shrimp survival at PL3 and PL10 in rearing tanks receiving antibiotics or daily water application of a probiotic, compared to a negative control.

Conclusion

Concerns with respect to the use of microorganisms in animal rearing are growing. This new era for probiotics – one of registration and control, transparency, safety and efficacy – is to the advantage of farmers, producers and consumers.

(Editor's Note: This article was originally published in the August 2004 print edition of the Global Aquaculture Advocate.*)*

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