

What are Probiotics?

A great deal of recent research has identified the microbes making up the "microbiome" of animals and humans and has documented the benefits in assuring that certain types of bacteria are present in these microbiomes. Bacteria that can become established in the gut, on the skin, in oral cavities and other locations can significantly influence the health of the organism. Metabolic products produced by these microbes can enhance nutrition, interact beneficially with the immune system and suppress other harmful microbes. Bacteria that are introduced directly to an organism to provide health benefits are called probiotics.

Applications in Shrimp Farming

Farmed shrimp, particularly those reared in high density aquaculture, are especially vulnerable to disease. Antibiotics have been used to control disease outbreaks in shrimp aquaculture, but the combination of an increase in microbial resistance and consumer resistance to the use of antibiotics creates a need for alternatives for disease control in shrimp farming operations. As a result of the microbiome studies cited above, the use of specialized, preselected microbial additives to enhance productivity and quality in shrimp aquaculture is becoming increasingly common.

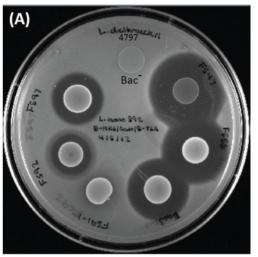
Development of Probiotic Cultures for Vibrio Control

Monera, Inc. and its research partners have been engaged for some time in developing naturally-occurring, specialized cultures for this purpose. The first step in the development process is to screen a variety of bacterial species and strains to find bacteria that can survive in the shrimp gut and/or in the aqueous environment of shrimp ponds. Then, strains that restrict the growth of harmful microbes are selected and evaluated for their

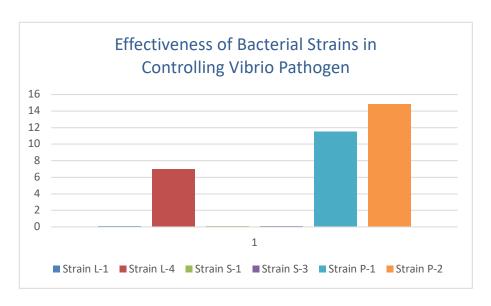


effectiveness in controlling the disease-causing microbe. One such harmful microbe is the pathogen *Vibrio harveyi*.

A common method for evaluating the effectiveness of candidate probiotic cultures uses agar plates containing nutrients on which both the candidate organisms and the target pathogen can grow. The pathogen is inoculated uniformly into the agar so that it will grow on the entire surface area of the plate. The candidate organism is inoculated at a point on the surface of the agar. When the candidate organism grows to form a colony, a zone



of inhibition around the colony is proof that the test organism prevents the growth of the pathogen. The larger the zone of inhibition around the colony, the more potent the candidate organism.



This example clearly shows that the P species are the most effective in controlling the Vibrio pathogen and that the P-2 strain is better than the P-1 strain.

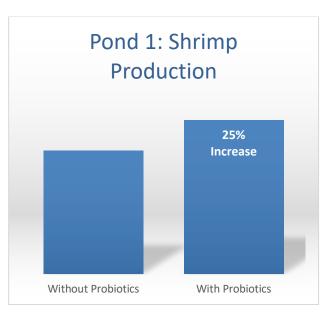
After selection of strains that demonstrate effectiveness in such tests in inhibiting a pathogen in the lab, one or more products are formulated with combinations of strains that <u>should</u> be beneficial in a variety of ways in shrimp growing ponds and other containments. Field evaluations are then run to see if the product is, in fact, effective under actual growing conditions that can vary widely depending on the intensity of the farming and the ambiental conditions.

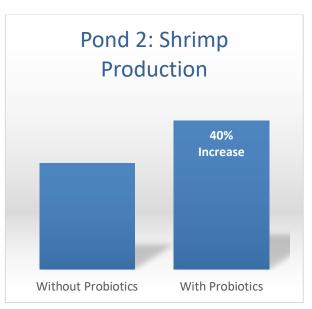


Field Testing – An Example

In one set of field evaluations, total shrimp production was determined in two parallel ponds. Each pond was divided into a control section and a treated section with the treated sections receiving the probiotic test mixture. Pond 1 showed a 25% increase in kilograms per hectare (kg/ha) of shrimp harvested due to probiotic treatment; Pond 2 showed a 40% increase in kg/ha.

Increase in Shrimp Production With Probiotics

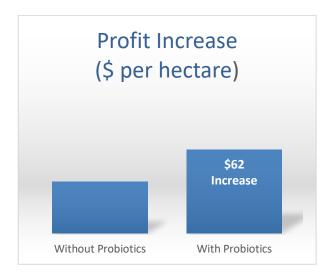


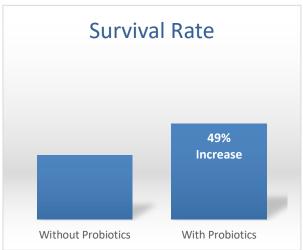


Multiple field trials of this type using the microbial strains developed as described above have shown increases in survival rate (10-50%) and in final weight of shrimp harvested (30-40%). Of course, the best measure of effectiveness is the increase in dollars returned to the grower per hectare. In two field trials, the probiotic treatment increased yield in the \$50-65 per hectare range.



Shrimp Production Increases With Probiotics







Application

In the Profloc Process, probiotics can be added directly to the shrimp feed or, alternatively, specialized formulations of microbes and other ingredients can be added directly to the water in the pond to simultaneously enhance and stabilize pond water quality.

Conclusions

This study shows that probiotics increased the production rate by 25-40%, the final weight of the shrimp by 30%, the survival rate by 49%, and increased the profit by \$62 per hectare.

