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HATCHERYFEED

Advances in feeding early life stage and broodstock aquatic species

ADVANCES IN MICROBIAL MANAGEMENT

Probiotic Use in Hatcheries

New Line of Live Feed Disinfectants

Advances in Shrimp Microdiets

Genetic Editing and Management

Probiotic use in hatchery production systems to increase PL stress resistance

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The shrimp industry depends on hatchery production of post larvae (PL). For farmers to achieve successful production, they need access to quality PL that are stress-resistant and disease-free. PL quality is so critical, the FAO (Food and Agriculture Organization of the United Nations) has designated access to healthy, disease-free PL as one of the top issues facing the industry today.

Production cycles can be ruined by unpredictable stressors such as sudden environmental changes in temperature, salinity and pH. These environmental stressors coupled with abundant disease outbreaks are leading the industry to look for novel strategies to combat these issues. Production managers are increasing the use of post hatchery-controlled nurseries to reduce grow out time and increase stocking densities, while also recognizing the need for added biosecurity. Hatcheries are continuing to provide disease-free assurances while implementing new strategies such as developing alternative strains of PL that are faster growing and less susceptible to stress.

Probiotics in hatchery production

Probiotic technology is an increasingly important tool in hatchery production with the potential to address many of these issues. In the context of shrimp production, the WHO (World Health Organization) defines probiotics as live microorganisms which, when administered in adequate amounts, confer a health benefit on the PL. Probiotics may be administered through feed, either in-feed or top coated onto feed, to provide significant benefits, for example, to gut health. They may also be delivered into the water column to help maintain water quality.

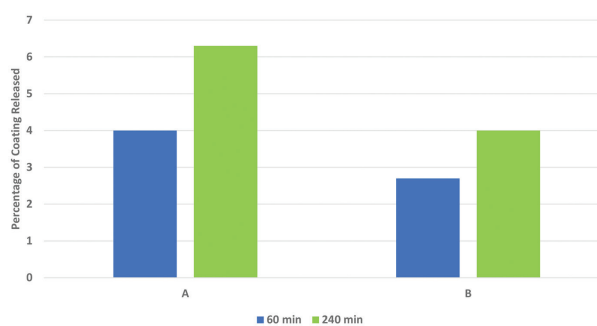


Figure 1. When coated onto 1 mm feed, BiOWiSH® MultiBio 3PS provides a stable, coated product with high retention, greater than 94-97% retention in water (Treatment A: ambient temperature (21 °C) feed coated with BiOWiSH® MultiBio 3PS; Treatment B: 40 °C feed coated with BiOWiSH® MultiBio 3PS).

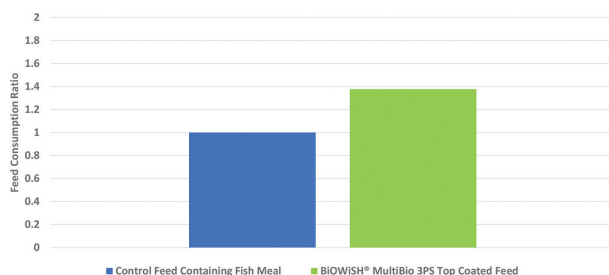


Figure 2. Shrimp feed attractability testing shows BiOWiSH® MultiBio 3PS increases rate of feed consumption by 38% when top-coated onto commercial feed (34% protein/21% fish meal).

In order to provide sustained economic value to the industry, however, probiotics must overcome historical issues such as inadequate stability, variable performance, and poor production quality. To address these issues, different microbial species have been introduced into probiotics products. In addition, the probiotics industry is developing more rigorous standards for optimizing species and strain selection, product formulation levels, and dosages for different

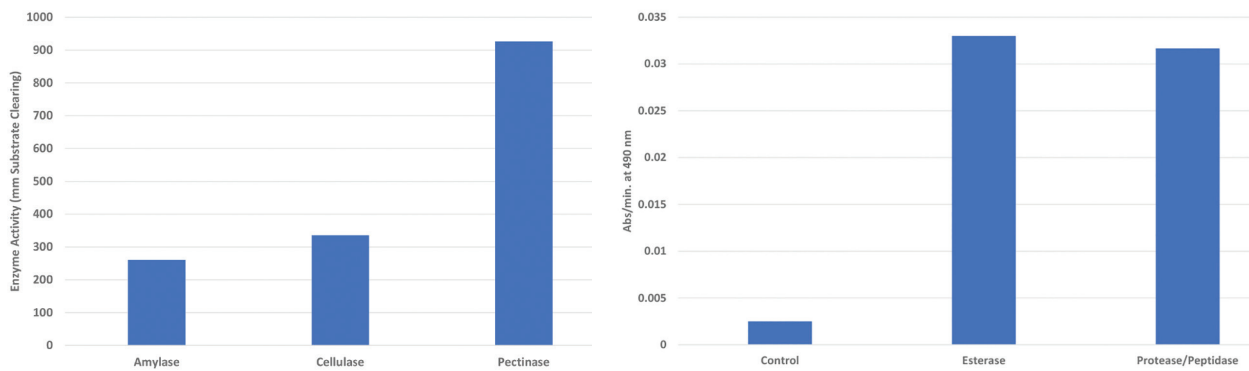


Figure 3. Relative activities determined versus controls on dyed starch, cellulose, and pectin substrates via plate clearing zone assays (left). Protease and esterase activities determined via diacetyl fluorescein hydrolysis assay (right).

use conditions. Building a technical foundation for the use of probiotics in hatchery production is critical if the industry is to overcome previous challenges and begin to adopt probiotics as a sustainable aspect of hatchery management.

Bacillus probiotics

In regard to composition, probiotic products today may include a single species or a blend of microbial species with varying levels of activity. Typically, *Bacillus* probiotics are delivered in an inactive spore form, which is more stable. To be effective, these products require application conditions that can induce microbial germination. Germination can occur either in the water column or host animal. Probiotic microbial species that are not *Bacillus* typically exist as vegetative organisms. These may be susceptible to damage from heat exposure or other storage conditions. The latter is particularly important for how probiotics are applied in feed. Depending on feed process conditions and temperatures, probiotic products are more often top-coated onto processed feed.

BiOWiSH Technologies has identified several species of naturally occurring lactic acid bacteria and *Bacillus* microorganisms that are very beneficial in hatchery production. The company utilizes a standardized blend of these microorganisms to deliver probiotics for use in both water quality maintenance and feed enhancement.

BiOWiSH® MultiBio 3PS is a proprietary feed probiotic that can be blended into hatchery feed, or top-coated onto feed, depending on the feed type and stage of hatchery production. When applied to feed, it is stable and adheres well (Fig. 1). When coated onto feed, this

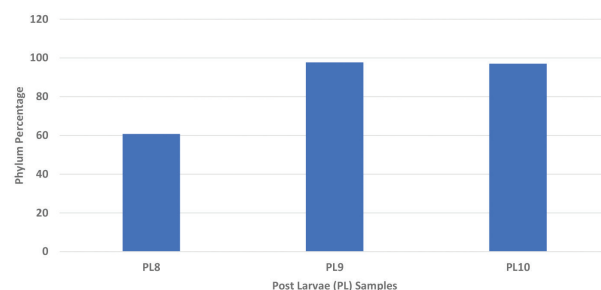


Figure 4. 16S Ribosomal RNA microbiome phyla analysis. As post-larvae develop, their microbiome changes/shifts. With the BiOWiSH® two-step approach, these changes include an increase in beneficial heterotrophs such as Firmicutes phyla containing *Lactobacillus* and *Bacillus* with no detectable *Vibrio*.

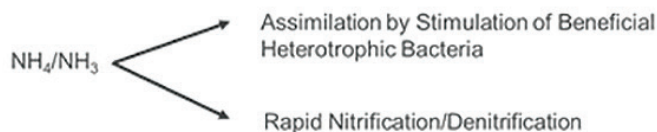
probiotic assemblage delivers three major performance benefits in hatchery production.

First, it provides an additional element of feed attractability, which increases feed consumption rates (Fig. 2). If feed sits in tanks unconsumed, vital nutrients can be leached out, reducing the nutritional value of the feed. Undigested feed can also contribute to an increasing organic waste and ammonia load during production, diminishing water quality and causing additional stress on PL. Increasing feed consumption rates helps ensure that the feed being consumed is higher in nutritional value to PL. Higher feed consumption rates help reduce the amount of undigested feed in the water column.

Second, when activated it produces a broad range of hydrolytic enzymes (Fig. 3) that can breakdown complex carbohydrates and protein present in feed. This helps improve digestibility and optimize nutrient availability, which is important to PL that have a short feed retention time. Lastly, a transcriptome analysis of PL during growth from PL8 – PL10 (Fig. 4) shows that this feed probiotic

Undigested Feed/Organic Waste Protein $\xrightarrow{\text{Ammonia-producing Enzymes}}$ Produces Ammonia (NH_3) / Nitrites (NO_2)

BiOWiSH® stimulates multiple metabolic pathways in Heterotrophic Bacterial Populations (HBP) that rapidly remove the build up of ammonia, nitrites, and organic carbon waste loads in aquaculture systems to maintain optimal water quality conditions.



helps ensure development of a healthy microbiome in PL during hatchery production. This benefit has been confirmed further in the shrimp gut when used in the later stages of nursery and grow-out production.

To help reduce chemical stress and maintain optimum water quality, BiOWiSH® AquaFarm added routinely to the tank water column reduces ammonia and nitrite buildup (Fig. 5). This proprietary formula induces a healthy beneficial heterotrophic bacteria population in the water column. These microorganisms maintain tank water quality through efficient nitrification of ammonia and a rapid, novel form of aerobic denitrification that reduces the levels of these harmful materials even as feed rates increase as PL grow.

The combination of these two products helps ensure successful production with increased PL size and uniformity. It also produces PL that are more resistant to unexpected environmental stressors during later stages of the production cycle. When applied the two product approach also helps reduce dependency on chemical additives which are becoming increasingly problematic for the industry.

Summary

Probiotics and other microbial technologies are demonstrating viable and beneficial strategies to overcome several challenges the hatchery industry faces. Building a stronger technical foundation for their application and mode of action, while ensuring consistent performance and product quality, is critical to their continued use in hatcheries and overall shrimp production. As with all new technologies, the outlook for their continued sustainability in hatchery management and value in the market will depend on their ability to deliver quality PL and successful production with a viable economic value proposition.

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NEXT ISSUE

Coming in December

Specialty feeds: feeding for niche species; targeted outcomes (such as smoltification, maturation)

Plant-based ingredients: phytonutrients, plant proteins, algal products