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Chalee's Way in Thailand
Understanding Media Filtration

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Next Gen Shrimp Farmers Talk



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At Delta Marine in Sumbawa, Indonesia; Rizky Darmawan and Chodpipat Limlertwatee with Chaimate Chuenchom. P16.

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Tailoring probiotic strategies for *Vibrio* management in shrimp larviculture

Strategic probiotic use can dramatically reduce pathogenic bacteria while accelerating larval development in Pacific whiteleg shrimp

By Siriwan Khidprasert, Chanadda Kasamechotchung, Rungkarn Suebsing, Kallaya Sritunyalucksana, Olivier Decamp, Andrew P. Shinn, Ratchakorn Wongwaradechkul and Barbara Hostins



In the high-stakes world of shrimp aquaculture, where disease outbreaks can devastate entire production cycles, hatchery managers face a constant battle against pathogenic *Vibrio* bacteria. New research on a commercial probiotic formulation from INVE Aquaculture offers promising evidence that the right probiotic strategy can significantly reduce these disease-causing organisms while simultaneously improving larval development without compromising shrimp health or survival.

The *Vibrio* problem

For shrimp producers worldwide, *Vibrio* species represent an ever-present threat. These opportunistic pathogens that are responsible for devastating diseases including acute hepatopancreatic necrosis disease (AHPND) and translucent post larvae disease (TPD), can cause mortality rates exceeding 70-100% in affected facilities. The larval development period is particularly vulnerable, as larval and juvenile shrimp are highly susceptible to changes in environmental conditions, feed quality, and bacterial infections - all factors that can lead to poor development, mass mortalities, and significant economic losses.

Antibiotics are no longer used in shrimp aquaculture because of concerns over antimicrobial resistance, residue contamination, and export restrictions that demand antibiotic-free production. Concerns regarding antimicrobial resistance and sustainability have driven the industry to seek alternatives. Probiotics - beneficial bacteria that can improve water quality, maintain healthy gut microbial communities, and inhibit pathogenic bacteria - have emerged as a leading candidate. Yet questions remain about optimal dosing strategies and the benefits producers can expect at different application rates.

Evaluating probiotic dosage strategies in shrimp hatcheries

A recent investigation conducted in Thailand assessed the impact of multiple concentrations of Sanolife® MIC (S-MIC), a commercial probiotic formulation comprising *Bacillus subtilis*, *B. licheniformis*, and *B. pumilus*, on Pacific whiteleg shrimp, *Penaeus vannamei* development from the nauplius stage to post larval stage 15 PL15. This 23-day period represents the most critical phase of larval growth.

Researchers tested three probiotic concentrations against an untreated control group (T1), using twelve 300L- tanks with 50,000 nauplii each. The concentrations represented different industry scenarios.

a) 1g/m^3/day which is the manufacturer's recommended routine dosage (T2);

b) 5g/m^3/day, commonly used during challenge conditions, particularly when managing *Vibrio* outbreaks (T3); and,

c) 30g/m^3/day based on usage reports from Latin American hatcheries facing severe disease pressure (T4).

The breakthrough finding: *Vibrio* control

The most striking and consistent result emerged from microbial analysis: all three probiotic concentrations dramatically reduced *Vibrio* bacterial counts in both culture water and shrimp larvae across all developmental stages. At the crucial PL15 stage, when many post larvae shipments are transferred to grow-out farms, the difference was striking: *Vibrio* levels in probiotic-treated groups were 2.1 to 4.6 times lower than in the untreated control.



"Lower *Vibrio* loads at the end of the hatchery phase mean reduced risk of disease transmission when larvae are transferred to grow-out."

This reduction was not isolated to a single developmental phase. From the zoea stage through mysis and into post larval development, probiotic supplementation consistently suppressed *Vibrio* populations. At the mysis I stage, the highest probiotic concentration (30g/m^3/day) showed significantly lower *Vibrio* counts than the lower concentrations. By PL1 stage, both the 5g/m^3/day and 30g/m^3/day groups demonstrated significantly lower counts compared to untreated controls.

The implications are profound. Lower *Vibrio* loads at the end of the hatchery phase mean reduced risk of disease transmission when larvae are transferred to grow-out systems. Since disease outbreaks often originate from contaminated post larvae, this prophylactic reduction in pathogenic bacteria could significantly decrease the incidence of devastating diseases like AHPND and TPD in production facilities.

Faster development at low doses

Beyond microbial control, the study revealed concentration-specific benefits for larval development. The lowest concentration (1g/m^3/day) significantly accelerated the progression from mysis stage to post larval stage 1 (PL1), a critical metamorphic transition. On day 9 of the trial, only 11% of larvae in the control group had reached PL1, compared to 17% in the 1g/m^3/day group—a statistically significant difference that persisted through day 10.

This acceleration has practical value for commercial operations. Faster development to post larval stages improves production efficiency and reduces the time larvae spend in vulnerable early stages. Every day saved in the hatchery translates to cost savings and reduced risk of disease outbreaks during the most critical development period.

The mechanism behind this accelerated development likely involves enhanced nutrient availability, as *Bacillus*-based probiotics are known to improve digestive enzyme production and nutrient absorption. Additionally, these beneficial bacteria produce antimicrobial compounds including bacteriocins, lipopeptides, and organic acids that can inhibit pathogens while promoting beneficial microbial communities.

Growth benefits at higher concentrations

While the lowest concentration excelled at accelerating development, higher concentrations showed different benefits. At the PL6 stage, larvae treated with the highest concentration (30g/m^3/day) were significantly heavier than those receiving the lowest dose. Although this weight advantage was not maintained through PL15, suggesting some compensatory growth in other groups over time, the early growth boost may still contribute to production efficiency by improving larval uniformity and reducing size variation within cohorts.

This pattern suggests a threshold effect: very low probiotic concentrations may be insufficient to stimulate significant weight gain during early post larval stages, while moderate to high concentrations (5-30g/m^3/day) provide measurable growth benefits.

Safety confirmed across all doses

Importantly, comprehensive quality assessments confirmed that probiotic supplementation posed no risks to larval health. No morphological deformities were observed in any treatment group. The muscle-to-gut ratio - a key indicator of nutritional status and development—showed no significant differences among groups, indicating that probiotics enhanced certain parameters without disrupting normal physiological proportions.

Survival rates at PL15 ranged from 85-87% across all groups, with no significant differences between treatments. When subjected to an industry-standard salinity stress test (transfer from 30ppt seawater to freshwater for 30 minutes), all groups exhibited exceptional tolerance with post-stress survival rates between 98-99.5% - well above the industry benchmark of 75% for high-quality post larvae.

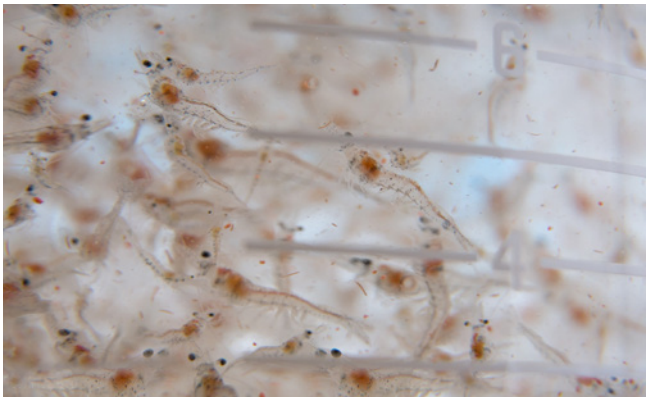
Oil droplet concentrations in the hepatopancreas, an indicator of nutritional status and metabolic health, were similarly consistent across all treatments (98-99.4%). These results demonstrate that S-MIC supplementation provides benefits without compromising the physiological robustness required for successful pond stocking.

Practical applications

The study's findings support flexible, objective-driven probiotic strategies in commercial hatcheries:

- For accelerated development: The 1g/m^3/day concentration effectively speeds progression to PL1 stage, improving production efficiency without additional costs associated with higher doses.
- For enhanced growth and maximum *Vibrio* control: Concentrations of 5-30g/m^3/day provide stronger growth benefits at PL6 and the most substantial reductions in pathogenic bacteria - particularly valuable during high-risk periods or in facilities with recurring vibriosis challenges.

The dose-dependent nature of *Vibrio* control gives hatchery managers flexibility to adjust concentrations based on historical disease pressure and risk assessment. During periods of heightened disease risk, higher concentrations can provide additional protection, while routine operations may achieve adequate benefits with



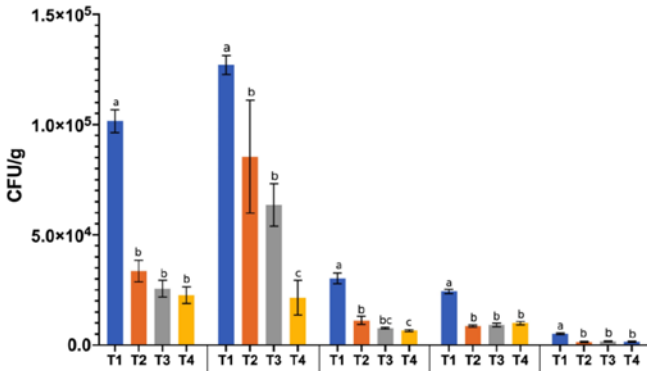


Figure 1. Comparison of total *Vibrio* counts in *Penaeus vannamei* larvae reared in culture water supplemented with different concentrations of Sanolife® MIC, relative to an unsupplemented control, throughout larval development. Treatments: T1 = control (no probiotic); T2 = 1g/m³/day; T3 = 5g/m³/day; T4 = 30g/m³/day.

lower, more economical dosing.

Understanding the mechanism

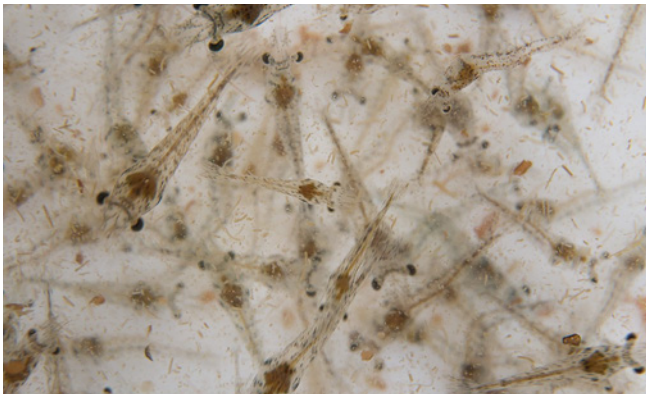
How do these *Bacillus*-based probiotics achieve such consistent results? The answer lies in multiple complementary mechanisms. *Bacillus* species produce diverse antimicrobial compounds that directly inhibit pathogenic bacteria. They also compete with harmful organisms for nutrients and colonisation sites in the larval gut and surrounding water.

Perhaps most importantly, *Bacillus* probiotics stimulate both innate and adaptive immune responses in shrimp, enhancing phagocytic activity, phenoloxidase activity, and antimicrobial peptide production. This immune stimulation, combined with antimicrobial compound production, provides comprehensive protection against disease while supporting overall larval quality.

The probiotics' spore-forming capability offers practical advantages as well: greater survival under varying environmental conditions and longer shelf life compared to non-spore-forming bacterial supplements.

Looking forward

This research provides strong evidence supporting the benefits of probiotic supplementation in shrimp hatcheries, demonstrating how targeted probiotic strategies can enhance larval performance and reduce pathogen pressure. Beyond improving hatchery outcomes, probiotic use also supports a reduction in antibiotic dependence - an increasingly important goal as the aquaculture industry aligns with global efforts to combat antimicrobial resistance and meet consumer demand for responsibly and sustainably produced seafood.



Conclusion

This research demonstrates that strategic probiotic supplementation with Sanolife® MIC offers shrimp hatcheries a practical, safe, and effective tool for improving production outcomes. The dramatic reduction in *Vibrio* bacteria - 2.1 to 4.6 times lower at the critical PL15 stage - addresses one of aquaculture's most persistent challenges. Combined with accelerated development at low doses and enhanced growth at higher concentrations, these benefits can be achieved without compromising survival rates, stress resistance, or physiological health.

For an industry seeking sustainable alternatives to antibiotics while maintaining biosecurity and production efficiency, probiotics represent more than just a supplement – they are a strategic tool for breaking the cycle of disease pressure that has long challenged shrimp aquaculture. The key lies in understanding that different concentrations serve different purposes, allowing producers to tailor their probiotic strategy to their specific operational needs and disease risk profiles.





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