

Microbiome manipulation in shrimp: Fact or Fiction?

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Life depends on microbes (this includes fungi, bacteria, viruses, archaeobacteria, etc.). Science is just beginning to unravel how complex this relationship is. Among the discoveries is the observation that more than 80% of bacteria cannot be cultured using conventional approaches. Thus many of the conclusions we have drawn from what we thought were well designed experiments are not based on a complete picture, likely leading to misleading conclusions. Using high throughput sequencing technologies we can now gain a much better idea about how complex the microbiome is and what can impact it (1).

It is widely held that the ability to reproducibly manipulate the microbial ecology of the digestive tract will offer some promise in controlling the impact of infectious diseases on animal health, improving stress tolerance as well as digestibility and optimum utility of feed components among many other possible benefits (2). We have only just begun to figure this puzzle out.

Focusing on shrimp farming, perhaps one of the areas of greatest interest involving the use of microbes is the use of what has been coined as probiotics. The widely agreed upon definition of probiotics (3) is “live microorganisms which when administered in adequate amounts confer a health benefit on the host.” This fails to recognize that many organisms are immunogenic (whether they are dead or alive) and the term health benefit is vague. Since shrimp

constantly forage and bacteria are an important natural component of their diets, this further muddies the applicability of the term for use in shrimp.

This definition requires that the bacteria be alive at the time of application. They must survive ingestion and through any possible unspecified mechanism have an impact on animal health. There are some schools of thought that also believe that the definition should include the ability to colonize the digestive tract in a manner where there is a measurable change in the composition of the bacteria in the gut and a measurable impact on animal health.

The vast majority of products marketed as probiotics into shrimp farming do not fit this definition. In fact, there is little evidence to support that most even act directly on the animal at all. They are in

reality tools for bioremediation.

Historically most of the bacterial strains in these products were being used to degrade the organic matter in septic tanks. They act on the environment and the impacts that they have on animals are a result of this. The most common organisms used are the gram-positive spore-forming rods, members of the *Bacillus* species. Members of this genus are widely employed for their enzymatic abilities in many commercial processes. They form spores that are shelf and heat stable. Spores, while alive, are not the same as actively metabolizing vegetative cells.

There is little to no evidence to support that idea that the microbiome in farmed shrimp in production environments is stable (4). More than likely it is constantly changing in response to changes in composition that occur

naturally as water quality, population dynamics, pond ecology and feed composition and microbial make up varies.

Even though there is no reason to believe that stable changes in the microbial composition in the guts of shrimp are readily achievable, in our work with bioremediation we have seen meaningful impacts in shrimp hatcheries. One of our products is a tableted blend of *Bacillus* spores (PRO 4000X) that we designed and field-tested more than a decade ago. We have found that this approach has a meaningful and reproducible impact on shrimp when used in hatcheries (Table 1). Among the impacts observed are changes in the composition and levels of vibrios, cleaner tanks, increased survivals and more robust animals. These are widely

Table 1. Impact of PRO4000X on hatchery reared shrimp

Observations	Comment
Control of heterotrophic bacterial loads	Plate counts of production tanks consistently show much lower levels of vibrios and other types of bacteria.
Growth rate is better.	PLs grow faster in tanks treated with PRO4000X tablets and are larger at harvest.
Animals feed more aggressively.	No molting problems in treated tanks contrasted with control tanks.
Fewer mortalities	Higher survivals as a result of lower pathogen loads, less stress and better water quality
Controlled fouling issues	Lower accumulated organics equals less food for bacteria and fungi that cause fouling
Less accumulated organics	The primary benefit from using the product
Very low levels of metabolites	Ammonia and its breakdown products are controlled by the <i>Bacillus</i> species in the product.
No other bacteria added or needed.	Only the use of PRO 4000X is required.

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reported for our product and for some others. Unlike approaches that require activation of the bacterial spore mixtures prior to use the tablets dissolve in the tank and the spores germinate through out the tank. There is no requirements for field users to culture before use thus lessening the chances of adding something unwanted that could grow during an extended activation.

By the definition above, microbes that act on the environment and thus impact the health of the shrimp would be considered to be probiotics. Since the coiners of the definition were focused on ingestion (such as *Lactobacillus* species in yogurt), there likely would not have been any thought that this term would apply to bacteria that impact the environment. Will we be able to feed shrimp bacteria that have the same impact? This will depend on the ability of the material to withstand the rigors of milling into feed or the use of top dressing mechanisms that ensure that sufficient numbers are ingested. Most of the spores will pass through the gut, as the gut transition time is less than the typical spore germination time and enter the environment through the feces. If these are re-ingested as living cells are they a probiotic? It is apparent that regardless of what we call the products, their use can have a positive impact on production in shrimp hatcheries. Maybe someday we will have products that act on the microbiome and we can equate activity with the presence of specific strains. There is much work to do.

References

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