AQUAINTECH Inc.

True probiotics for aquaculture? Fact or fiction?

As a consumer you probably know what probiotics are. Living bacteria are present in all of the food we eat. The consumption of some of these bacteria are purported to be beneficial to us by aiding in digestion, stimulating immunity, providing important nutrients, etc. One of the properties widely claimed is that some types of bacteria bind to the walls of our intestines preventing other potentially harmful bacteria from binding to the same sites protecting us from their harmful effects. However, unfortunately, the science that supports these claims is far from universally agreed upon. In fact, only a few claims have been validated in the traditional peer reviewed manner that the scientific method requires. For agriculture, there is only one USFDA registered probiotic. This is for chickens and contains almost 30 strains of viable bacteria. For aquaculture, there are no registered probiotics in the US and one in Europe.

Over the years, the definition of probiotics has evolved with the times. While there is no universally accepted definition the term *probiotic* has been defined by the Food and Agriculture Organization (FAO) and World Health Organization (WHO) as "***live microorganisms*** which when administered in adequate amounts confer a health benefit on the host." Typically this benefit is postulated to occur via the gut and is assumed to be as a result of bacterial species colonizing the gut of the host and bringing about a change in the bacterial composition of the gut that in some way benefits the health of the host. The mechanisms involved are not well defined so they could be via a variety of different routes such as the production of nutrients including vitamins and critical cofactors, colonizing the gut and preventing other bacteria that can cause disease from attaching themselves to the occupied sites on these cells or the production of antibiotic type substances that inhibit other bacteria, etc. Examining the myriad of definitions for probiotics, one can conclude that an effective probiotic should be:

1. Living
2. Impacting the animal through the gut
3. Have a beneficial effect on the host health
4. Be benign

Many companies sell products that they claim are probiotics for use in aquaculture. A true probiotic would more than likely have to be delivered in the feed. This route allows for a number of potential impacts on animals that do not require the bacteria to stably colonize the gut. One such product is currently approved in the EU. The vast majority of products that are sold for use in aquaculture with a probiotic claim are however not used this way. They are added to the pond water.

There are many companies that sell either liquid or dry formulations of various types of bacteria for addition to the ponds. These products, if they work, would likely have a mechanism of action that

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involves an effect on the environment. The use of bacteria to impact various aspects of the environment is a widely established and routine practice that is used in a variety of industries. Probably the industry where one should be looking to is the waste treatment industry where bacteria are routinely used to reduce the organic load in sludge.

It is my contention that in reality most of the products that are being offered for sale to aquaculturists labeled as probiotics, when they are effective, likely act by reducing the sludge and lowering ammonia levels. They are not probiotics as defined above, and in my opinion they should not be sold as probiotics because of the misleading nature of this term.

In considering the compositions of these products one has to take into account the bacterial species and the quantity of bacteria in them. Production ponds are heavily populated by bacteria. They are dispersed throughout the water column and the sediments. A number of studies have shown that the culturable bacterial species are very diverse and range as high as several million per ml of water or gram of sediment. A one ha pond one meter deep, with an average of one million CFU (colony forming units- refers to the bacteria that grow on agar plates with the assumption that one CFU is equivalent to one bacteria) contains roughly 10 quadrillion bacteria (10^{16}) in the water. The sediment, at one million CFU per gram to a depth of one cm contains 100 trillion bacteria (10^{14}). A good quality average product being sold for addition to ponds contains approximately 1 billion CFU/ ml or gram (one trillion CFU or 10^{12} per kg). If one assumes that all of these bacteria will grow in the pond (a dubious assumption at best), then each kg of product added to a one ha pond would result in the numbers of bacteria shown in the table below.

Table 1. Theoretical CFU per ml of water or gram of sediment for each kg of a one trillion CFU per/kg product (assumes 100% viability)

CFU/ml	CFU/gram
100	2000

As one can see for every kg of the product the numbers of bacteria that would be added are far below the likely average number of bacteria already present. Even ten kgs does not significantly change the numbers. It just is not likely that 100 bacteria (assumes that all the bacteria in the product are viable) will reproduce to large enough numbers to compete against the much larger numbers of bacteria already present. From a strict numbers point there is little logic to this approach. It is widely held, based on a number of studies, that the bacterial composition of the guts of fish and shrimp is similar to what it present in the environment that they are living in. Since the pond bacterial composition is already relatively stable and orders of magnitude larger than what is being added it would be a stretch to assume that the bacteria that are being added colonize the fish and shrimp in the pond.

Furthermore, it is likely a stretch as well that there is going to be much if any effect on the environment at all

As an aside, it is important to understand the difference between correlation and causation. Statistically many things can be proven to correlate with each other in the absence of any scientific basis to explain why the observed correlation is scientifically valid. Many things are correlated with each other that have no possible way to be connected. The vast majority of observations that are being touted as proof of efficacy of these types of products are correlative in nature and may also rely on some manipulation of the numbers to strengthen the observed effect. Some studies have even gone so far as to changing the level of significance from the universally accepted $p < .05$ (the probability of two events being correlated with each other is more than 95% to $p < .10$-the probability is more than 90%). Few statisticians would concur that this is acceptable. What is important to remember is that in the absence of a plausible mechanism correlation statistics should always be viewed with a healthy degree of skepticism. This does not mean that they are useless, only that they should be questioned.

So the observations that there are correlations between adding a few hundred bacteria per ml to an environment with potentially tens of thousands of times the levels of bacteria already in the ponds, with impacts on animal health, growth, disease resistance , etc. should be viewed skeptically. There are no magic bullets and there are no super bacteria that will dramatically outgrow the bacteria that are already in the pond. The fact that these products all need to be added repeatedly further strengthens this argument. Furthermore since every pond is unique in terms of its chemical and biological makeup , adding a consistent amount of a given product makes little sense as well. To expect that the same relatively low numbers of bacteria added to a pond will always have the same impact regardless of what is going on in the pond already is not logical.

Aside from this number conundrum there is also a problem with the types of bacteria being added. Many different types of bacteria (and fungi) are being sold. The list is far too long for this short article but commonly includes Lactobacillus species, Bacillus species, Nitrosomonas, Nitrobacter, Saccharomyces species, photosynthetic bacteria such as purple sulfur bacteria, Pseudomonas species, etc. Each of these bacteria has specific properties and for many of them it precludes them being viable in a dried product (or in meaningful numbers in a liquid product).

The bacteria that most people think about when one thinks about probiotics are Lactobacillus species. Several studies suggest that when they are directly fed to shrimp or fish, one can see a measurable and reproducible impact, notably on disease resistance. Claims that these act as probiotics, i.e. binding to and stably colonizing the intestine are simply not supported by the data. The published evidence suggests that more than likely they are acting as non-specific immune stimulants, stimulating protective immunity by acting on the non-adaptive or innate component of the immune system. This does not even require live cells. Note that this is when fed. Currently there are no inexpensive technologies that allow for this genus of bacteria to be sold in a shelf stable reasonably long lived dried form.

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Lactobacillus species require refrigeration for shelf stability. This makes them unlikely candidates for addition to ponds in dried products.

Nitrogen fixing bacteria including Nitrobacter and Nitrosomonas species are natural inhabitants of terrestrial and aquatic ecosystems. These have very exacting nutrient requirements and are difficult and costly to culture to any high counts. Adding them to degrade ammonia makes little sense biologically as they are in balance with each other in aquatic environments and are inhibited by excess amounts of ammonia and ammonia breakdown products. Furthermore because of the high costs of production when they are actually present in inexpensive products they are typically present at very low levels. Shelf stability in a dried form is also problematic. Keeping in mind the problem with numbers, one can readily see that adding a few million of these bacteria to a pond is likely problematic.

Other bacteria have issues as well. Some products are based on anaerobic bacteria. Since ponds are oxygenated and bacteria usually have to undergo metabolic shifts to accommodate new environments adding bacteria that are adapted to an anaerobic environment, even though they are termed facultative aerobes (meaning that they can grow in either an anaerobic or aerobic environment) requires the bacteria to adapt. This can involve large numbers of bacteria dying and a few survivors having adjusted their metabolisms surviving. Again the issue of their ability to effectively compete against what is already in the pond and the fact that these products are added regularly raises some questions.

The best types of bacteria for shelf stable products are the Bacillus species. This is a very large group of organisms with a wide range of metabolic activities. They have the ability in nutrient poor environments (and environments without adequate moisture) to form spores. These spores are extremely resilient and can survive for many years (centuries) in dry environments. Some species are naturally occurring inhabitants of the intestinal tracts of shrimp and fish in many cases. Although it is not likely when considering the issue of numbers that they act in this manner when used in the traditional manner of adding product to the water.

Even though they are the best candidate for stable bacterial products that are added to the water, they have the same number problems. On the other hand adding them in a format where they germinate (a term that refers to the changes that occur when spores become viable bacteria again), directly on the pond bottom delivers a much great number of bacteria to where the organic material in the pond accumulates. Delivery is problematic using the dried powdered products in the market place. These products are typically soaked to allow germination and then added to ponds requiring the bacteria to move through the water column to the sediment.

About 5 years ago we noted these problems and developed a tablet that contains high numbers of Bacillus spores that is added directly to the pond bottoms at the spots where management wants the bacteria to act. Each tablet contains approximately 52 billion CFU. One tablet is about 4 square cms or so and dissolves on the pond bottom within 30 minutes of being added to the ponds. The spores germinate and the bacteria act upon the substrate. In this manner very large numbers of bacteria are

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added per square cm. The potential bacteria from a single tablet create localized areas on the pond bottom where the Bacillus species in the tablets overwhelm the bacteria that are present. The bacteria that are contained within the tablets have been selected specifically for their ability to degrade organic material.

In large scale field trials a consistent impact has been noted. Farmers have noted much cleaner pond bottoms allowing shortened times between cycles and the use of less water exchange during the growout cycle. On a farm in Belize, Royal Mayan, they have been using the tablets for three years and have noted that the effects are consistent and reproducible. A few times in the last few years when for various reasons the tablets were not being used, water quality deteriorated with high blue green alga counts and the need to exchange more water returned. Other clients have observed substantial reductions in organic material as well. Some of have seen changes in the coloration of sediments from an anaerobic black color to a much healthier brown color with significant drops in the production of hydrogen sulfide. In general the effect seems to be one of impacting the amount of accumulated organic material with other impacts being related to this. In hatchery tanks, significant lowering of vibrio counts has been noted, likely as a result of competition of the bacteria in the tablets with the vibrios in the system for nutrients. Currently clients in Taiwan, India, Belize, Venezuela, Guatemala and Indonesia are using the tablets with additional trials ongoing in several other countries.

The bacterial strains that we use in the tablets are sold in our powdered products, AquaPro-B and AquaPro-EZ. These contain spores of our selected Bacillus species and nutrients that facilitate bacterial growth. AquaPro-B requires germination before use. Typically this involves soaking the material in clean water for a time before adding it to the ponds or tanks. AquaPro-EZ gets around this by the use of biodegradable bags that dissolved within moments of the product hitting the pond bottom. Observed benefits are the same. The issues relate to what we have discussed previously.

These are tools for improving the environment, not probiotics and are sold as such. The concept of targeted delivery to the pond bottom is a scientifically sound one and given the use of specific bacteria that have been selected solely for their ability to degrade organic matter has resulted in a product that works and for which there is a plausible mechanism of action. Those products that are in the market place that contains Bacillus species act by a similar mechanism although they are limited as discussed due to issues with numbers and delivery mechanisms.

If you have any questions, please contact us at any of the addresses below. Additional information on any of our products is available on line as well as by request.

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