Newsletter

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> Editorial

The manipulation and control of the intestinal microbiota has been identified as an important area for future developments in aquaculture. It is well documented that the gut microbiota is essential for growth and health of the host, since the microbiota has impacts on intestinal development entailing good resorption, homeostasis and protection against pathogenic challenges. The gut also represents the natural interface between intestinal microbiota and the host. To the contrary of terrestrial animals, in aquatic species the structure and composition of the gut microbiota is additionally influenced by its surrounding environment. Colonization of intestinal mucosal surfaces with beneficial bacteria has a positive impact on immune regulatory functions of the gut. On the other hand, an imbalanced microbiota may contribute to the development of diseases. Thus, the management of the gut flora is important for the ability to prevent infections with pathogenic bacteria and to guarantee a well-functioning and effective digestion of nutrients. Beneficial bacteria, probiotics, contribute to a more stable gut microflora and assist in returning a disturbed microbiota to its normal beneficial composition.

Therefore, in this newsletter, attention will be given to the role of probiotics supplemented in aquaculture applications, their selection criteria, management and manufacturing of probiotic products!

Elisabeth Mayer

BIOMIN Aqua Products

Establishing a healthy gut is the key to animal health and performance. Probiotic solutions are a sustainable alternative to antimicrobial compounds, as the latter have led to the development of resistance among bacterial populations. Great care must be taken in the selection of probiotic strains, since it is essential to ensure that bacteria are beneficial to the host and able to thrive in the gut and aquatic environment. Lactic acid producing bacteria, e. g. *Enterococcus faecium*, have been the focus of much interest. Combined with other beneficial bacteria, multi-species products have proven that it is possible to provide synergistic bacteria with complementary modes of action to enhance health and zootechnical performances of cultured aquatic species. But why is there still doubt?

Probiotics in aquaculture Do they work?

Probiotics and their benefits

There is growing interest in the use of beneficial bacteria, probiotics, as an alternative strategy to antimicrobial compounds for disease prevention and control in aquaculture. These naturally occurring bacteria exert their beneficial effects on the host by modifying the microbial community associated with the host, by ensuring improved use of the feed or enhancing its nutritional value, or by enhancing the host response towards disease. At the animal level, probiotics improve the growth and survival of fish and shrimp by modifying the host-associated or ambient microbial community.

Feed application of probiotics for intestinal health

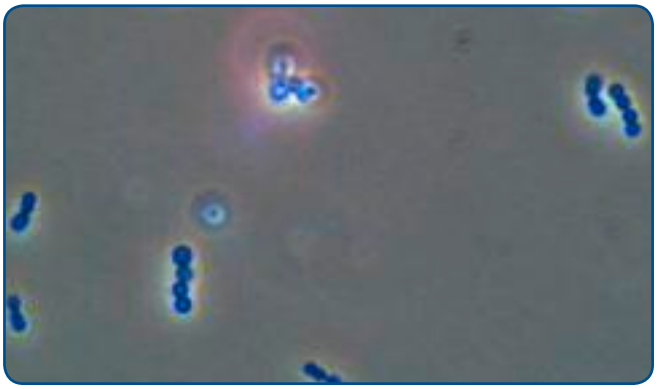
A well-established intestinal microflora is crucial for the growth and health of the animal, since the microflora has impacts on:

- *nutrition,*
- *the prevention of pathogenic infections,*
- *the integrity and function of digestive organs,*
- *the development of the immune system*

Thus, the management of the gut flora is important for the ability to prevent infections with enteric pathogens and to guarantee a well-functioning and effective digestion of nutrients that result in good growth performance parameters.

Probiotic bacteria modulate the gut microflora towards a favorable composition. Hence, selection criteria of probiotics for aquaculture should be based on their antagonism towards pathogens (through competitive exclusion), their growth, attachment to intestinal mucus and production of beneficial compounds (Vine *et al.*, 2004). Since aquatic species are cultured under different conditions salinity and optimum temperature range should also be considered for selection of the right probiotic strain. Another important factor is that probiotics lack pathogenicity. After all, probiotics should contribute to efficient production in a sustainable way, promoting healthy and robust animals (Brittain *et al.*, 2002).

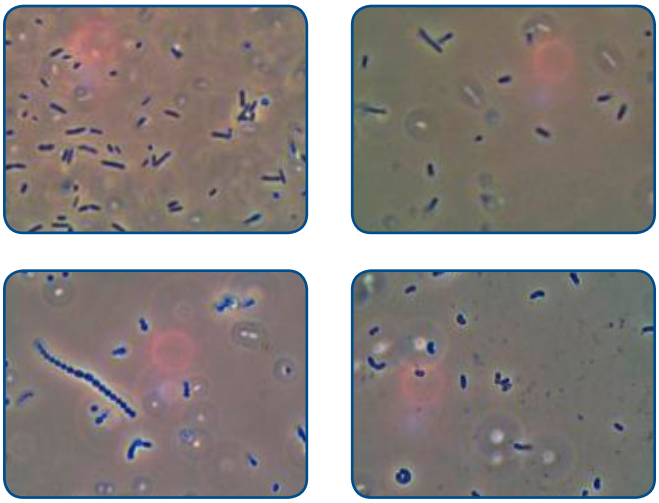
Lactic acid bacteria (LAB) are potential probiotic candidates in aquaculture and are also known to be present in the intestine of healthy fish (Balcázar *et al.*, 2008). *Enterococcus faecium* is one of the most commonly used lactic acid producing bacteria in animal nutrition and has become a focus of attention for use in commercially farmed aquatic species. Enterococci are Gram-positive, facultative anaerobic bacteria which are widely distributed in nature.



Picture 1: *Microscopic picture (1000-fold magnification) of Enterococcus faecium*

The use of the bacteriocin-producing *E. faecium* with probiotic properties alone or in combination with other beneficial intestinal bacteria was investigated in different studies.

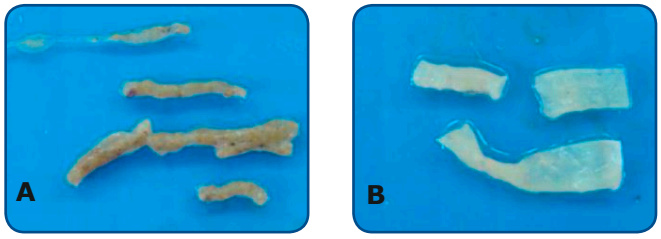
In vitro studies using the agar spot method (Rosskopf, 2010) have shown that *Enterococcus faecium* (strain IMB 52, BIOMIN) has inhibition properties against a wide spectrum of aquatic pathogens including *Yersinia ruckeri*, *Vibrio harveyi*, *Streptococcus agalactiae* and *Aeromonas veronii*. Similar observation was made by Swain *et al.* (2009) who proved the inhibitory activity of *E. faecium* isolated from brackishwater fish against *V. harveyi* and *V. parahaemolyticus*. This demonstrates the potential applications of *E. faecium* from fish intestine for controlling pathogenic vibriosis in shrimp culture.



Picture 2: *Microscopic pictures (1000-fold magnification) of Vibrio harveyi, Yersinia ruckeri, Streptococcus agalactiae and Aeromons veronii*

Panigrahi *et al.* (2007) examined immune modulation including cytokine gene expressions of rainbow trout (*Oncorhynchus mykiss*) and demonstrated that these parameters were improved by probiotic feeding of freeze-dried *Lactobacillus rhamnosus*, *Enterococcus faecium* or *Bacillus subtilis* (10^9 CFU/g) after 45 days. Particularly the fish fed the *E. faecium* strain showed better performance which could possibly be linked to the suitable ambient temperature conditions of this strain. Temperature is a major environmental factor controlling microbial growth and the ideal conditions differ among microorganisms. *E. faecium* was found to be more psychrotolerant than the other two bacteria, growing well at temperatures ranging from 12 to 30 °C. Rosskopf (2010) showed that the optimum temperature range for *E. faecium* (strain IMB 52, BIOMIN) reaches as far as to 37 °C.

In vitro studies were conducted by the Faculty of Natural Resources, Prince of Songkla University, Thailand, to demonstrate that *E. faecium* (strain IMB 52, BIOMIN) is also able to populate the intestine of Nile tilapia (*Oreochromis niloticus*) (*Picture 3* and *Table 1*) and induce a positive impact on bacterial ecology of the gut by inhibiting *Vibrio* spp. through competitive exclusion. *E. faecium* was even detected in the fish gut and faeces 10 days after product administration.



Picture 3:
A) Faecal materials were removed from the intestine
B) Intestinal epithelium without faecal material

Table 1: *E. faecium in tilapia´s intestine and faeces at day 1 and day 10 after stopping to feed probiotics including this probiotic strain*

Experimental group	*E. faecium* in tilapia´s intestine (x 10^8 cell/g intestine)		
	Day 1	Day 10	
	: Intestine with faecal materials	: Faecal material	: Intestine w/o faeces
Control	0	0	0
Probiotic	1.37 ± 0.85	1.52 ± 0.98	1.33 ± 0.28

Means ± SD. Using Fluorescent in situ hybridization (FISH), 5 - 15 microscopic fields were counted

Why is still doubt?

Although there is experimental evidence that the prophylactic use of beneficial bacteria can improve health and performance of cultured aquatic species, there are still some doubts. Results can be affected by improper management methods and product quality, such as:

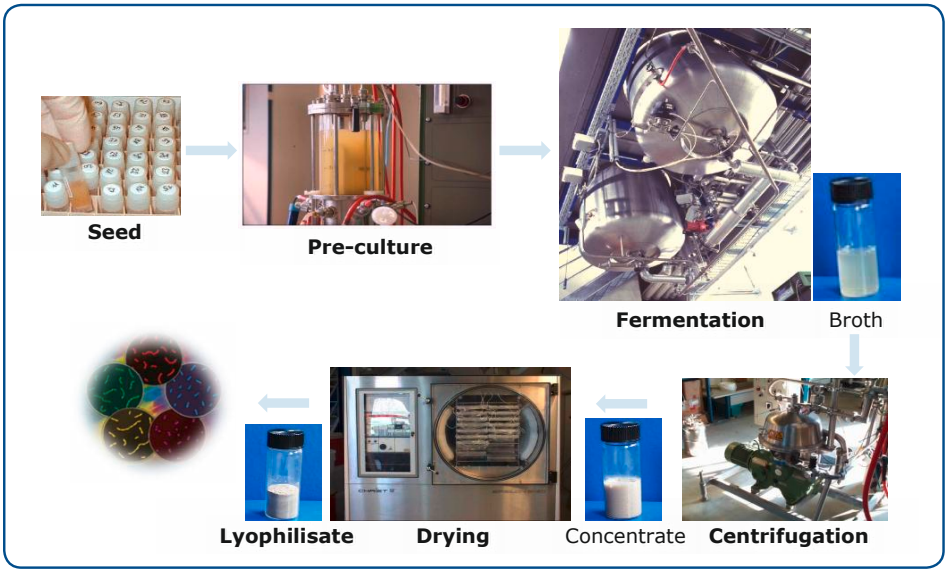
- incorrect application methods
  (e. g. simultaneous use of chemicals or antibiotics)
- incorrect claims of probiotics, which can´t be fulfilled
  (e. g. for white spot syndrome)
- too low bacterial concentrations
  (colony forming units should be above 10^8/g)
- poor bacterial stability during production and storage

High levels of viable organisms and stability during production and storage are important criteria for the selection of suitable strains. The safety of strains must be carefully assessed, as well, and transmission of antibiotic resistance or virulent plasmids must not take place. Of further great importance are the survival and growth of beneficial bacteria in culture conditions, and their ability to colonize the gut of the aquatic animals.

Additionally to the improper management of probiotics, the use of "fake" probiotics, which have unconventional manufacturing processes and/or an undefined blend of bacteria, cultivated in the "backyard" (*Picture 4*), can lead to doubts about significant effects and benefits of probiotics.

Picture 4: *backyard production of "probiotics"*

Continuous research has been undertaken to develop new products for modern and sustainable aquaculture. For the production of a probiotic product in conventional production facilities, the seed should come from a respective cell master bank stored at -80 °C which serves as a standardized source as inoculum for a working cell bank. The entire fermentation process has to be performed under fully sterile conditions. *Picture 5* shows the major production steps of single fermentation processes, from the bacteria seed to the dried lyophilisate. AquaStar®, BIOMIN´s probiotic product line for aquaculture, is a well-defined, multi-strain probiotic product, consisting of a blend of single-strain fermented bacterial strains.



Picture 5: *Major production steps of probiotic processing*

CONCLUSION

There is plenty of evidence that probiotics supplemented in feed and water are effective in aquaculture application. However, the success of probiotic supplementations depends on the strains, concentrations and management used. Effective probiotics are based on selected strains and controlled production conditions. Poor results are many times related to low-quality and inadequate probiotics.

It has been shown that well-defined probiotic strains, e. g. *Enterococcus faecium*, are able to reduce pathogenic bacteria, thus improving gut health, and enhancing performance and efficiency in production of aquatic species.

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