PROBIOTICS

An emerging concept in aquaculture nutrition and disease control

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INTRODUCTION

The word probiotics is often used as an opposite of antibiotics, i.e., as a promoter of life. In animal nutrition, antibiotics and chemotherapeutic agents are widely used as feed additives to curb pathogenic bacteria. Indiscriminate use of antibiotics will lead to drug-resistant strains. For example, many shrimp hatcheries introduce antibiotics starting from the first day of operation. Unfortunately, the use of chemicals and drugs reduces the natural defence mechanism of the larvae. The use of the drug may have no apparent adverse effect on the larval production in the hartchery. However, such shrimp larvae often become very weak after stocking in grow-out ponds and succumb to stress fairly easily.

Antibiotic resistance by fish and shrimp bacterial pathogens has been reported in all areas of aquaculture, mainly due to the indiscriminate use of antibiotics. Deceased efficacy has been documented in many antimicrobial drugs regardless of their mechanism of action. Such resistance emerges by two known genetic mechanisms, mutation on the bacterial chromosome, or extra-chromosomal transfer mediated by plasmids.

In live-stock nutrition, growing public disquiet over the use of antibiotics in feed additives has encouraged commercial interest in probiotics as an alternate therapy. A number of commercial preparations are available for poultry and livestock which aim to promote colonisation of desirable bacteria in the gut by application of live microorganisms from both indigenous and exogenous sources. However, in aquaculture, the use of probiotics is not as widespread and prevalent.

PROBIOTIC CONCEPT

The term probiotics is defined as 'organisms and substances which contribute to intestinal microbial balance'. In other words, they are natural intestinal bacteria which after oral administration in effective doses are able to establish, eventually colonize in the digestive tract, and to keep or increase the natural flora of the digestive tract and to prevent colonization of pathogenic organisms and to promote optimal utilization of the feed. The concept of microfloral manipulation was first appreciated by Metchnikoff in 1907, who viewed the consumption of yoghurt by Bulgarian peasants as conferring in long span of life. Currently, the most commonly used probiotic in animal nutrition are (LAB) lactic acid bacteria (*Lactobacillus acidophilus* and *Streptococcus faecium*) and some strains of Bacillus . The search for new probionts with additional benefits is continuing in many laboratories around the world.

MODES OF ACTION

Several mechanisms have been suggested to explain the mode of action of probiotics, however, many of them are still speculative.

* Probiotics inhibit the proliferation of pathogenic bacteria by producing organic acids and antibiotic substances or by reducing the pH.

* They produce hydrogen peroxide (H_2O_2) and prevent the adhesion of pathogenic bacteria.

* They produce metabolites which are able to neutralize bacterial toxins *in situ.*

* By their own enzymes, they increase the digestive utilization of feeds or detoxify injurious metaboloites from the flora.

* They stimulate the non-specific immune system of the host and also promote the production of vitamins and increase brush border cell activities of lactase, sucrase and maltase.

* They proliferate in the digestive tract and compete with pathogenic bacteria.

* Further, LAB also facilities improved lactose utilization (in lactase mal-digesters), inhibits certain forms of cancers and helps in control of serum cholesterol levels.

All these beneficial effects of probiotics have recieved considerable attention of animal nutritionists in recent years. Some of those possibly advantageous to aquaculture are detailed below.

PROTECTION AGAINST ENTERIC PATHOGENS

Several mechanisms have been investigated whereby lactic acid bacteria (LAB) could inhibit colonisation of the intestine by coliforms and other enteric pathogens. These include (1) adhesion of LAB to the digestive tract wall to prevent adhesion by pathogens; (2) neutralization of toxins - several experiments with piglets, rats and calves have supported the anti-enterotoxic activity of LAB, though the neutralizing substance is yet to be identified and (3) bactericidal activity - primarily through the production of organic acids (lactic, acetic and formic acids) which can reduce the pH of stomach contents thereby preventing the growth of many pathogenic bacteria. Further many strains of LAB are known to produce hydrogen peroxide which is bactericidal for gram-negative bacteria. Besides, LAB also produces different types of bacteriocins which characteristically have a proteinaceous active site and inhibits both gram-positive and gram-negative bacteria.

Since LAB are capable of producing more than one inhibitory system, the antagonistic action they exert toward intestinal pathogens is likely to be the result of the involvement of more than one particular inhibitor.

IMPROVED NUTRITIONAL VALUE OF FOOD

Besides protecting animals against enteric infections, feeding LAB preparations result in increased feed conversion efficiency and live weight gain. However, it is not clear whether the growth responses stem directly from improved digestive performances or indirectly consequent to the suppression of gut pathogens. There is evidence that microbial degradation of dietary nutrients occur in the stomach, especially by fermentation of simple sugars and complex carbohydrates.

STIMULATION OF NON-SPECIFIC IMMUNE RESPONSE

Recent research has shown that LAB not only constitute an integral part of the healthy gastrointestinal microecology, but are also involved in the host's protective mechanisms by increasing both specific and non-specific immune mechanisms. In mice, it has been shown that a diet supplemented with live LAB ameliorates the host's defences against *Salmonella typhimurium* by (a) increasing the antibacterial activity of the lymphocytes; (b) increasing the number of macrophages in sites of infection and (c) increasing the proliferative responses of the splenocytes. Further, LAB diet also results in strong stimulation of gammainterferon (V-IFN) production in the peripheral blood lymphocytes.

CURRENT STATUS OF PROBIOTICS IN AQUACULTURE

In spite of the above advantages, the use of probiotics in the diet of aquaculture organisms during the grow-out phase is not wide-spread, although many commercial establishments advertise its availability in the market. Furthermore, there is very little research data to support its use during the grow-out phase. However, results on its use in larviculture of fish, shrimp and bivalve molluscs have been more forthcoming. Many labortories have experimented with the use of probionts in larviculture with encouraging results. Some of these are detailed below.

(A) Live Food Production :

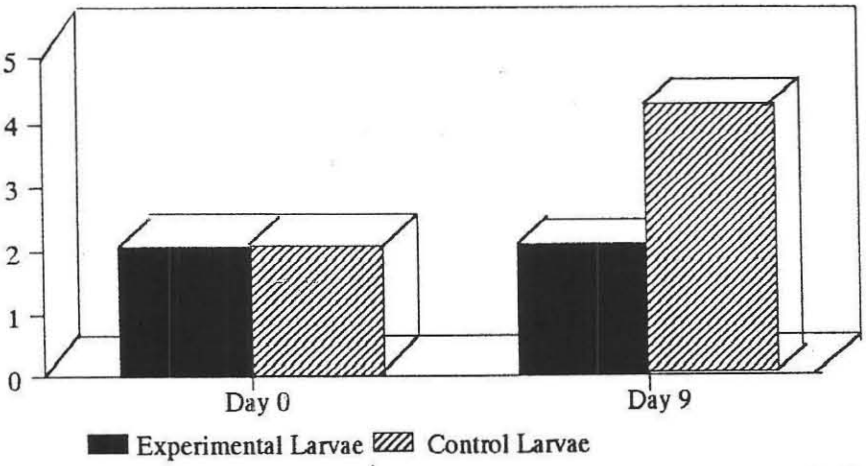
The production rate of rotifers (used as first feed for fish larvae) was significantly enhanced with the use of *Adjulact*, a spray-dried whey with live lactic bacteria (*Streptococcus thermophilus* and *Lactobacillus helveticus*). Another commercial preparation, *Acosil*, a spray-dried extract from sprouting cereal grain fermented with selected strains of lactic bacteria, limited the bacterial proliferation in rotifers during their overnight enrichment with fish oil emulsion. The use of LAB generally helped to reduce the counts of the dominant Vibrios, while spores of *Bacillus* sp (*Paciflor*) decreased the proportion of vibrios in the bacterial flora of rotifers. Another live food organism, the brine shrimp *Artemia*, have been grown to pre-adults on diets consisting of bacteria (*Flexibacter*) with significant increase in length and dry weight. The results of this study suggested that bacteria not only acted as food for *Artemia*, but also assisted in digestion of the algae.

(B) Fish Larvae :

Larval fish are generally fed probiotics through, their food viz., rotifers and artemia which act as bio-carriers. Several studies in France have reported the improved dietary value of probiotic-fed rotifers, which resulted in increase of mean weight and survival of turbot (*Scophthalmus maximus*) larvae. More encouraging results were observed when turbot larvae were fed with *Lactabacillus plantarum* enriched rotifers and the challenged with a pathogenic *Vibrio* on day 9. The probiotic-fed larvae proved to be more resistant to the pathogen (53% survival rate after 72 h challenge versus 8% for the infected control group without LAB). In another experiment in Norway with cod larvae, inoculation with

L. plantarum resulted in steep reduction in total bacterial flora in the larvae at day 9 (Fig.1). The effects of probiotics on fish larval rearing may be due to the occurrence of a vibriostatic agent in LAB. The deciding advantage of this treatment over standard antibiotics is that it works at a very low concentration and keeps the medium free of any drug.

Fig. 1. Log colony Forming Units

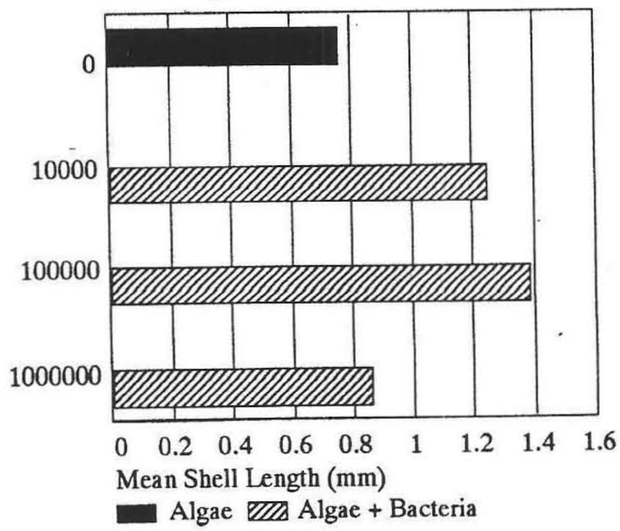


Total bacterial floar in Cod larvae at day 0 and day 9 after treatment with LAB (from Strom and Ringo, 1993)

(C) Bivalve Larvae :

The presence of bacteria in the rearing medium of bivalve larvae has been thought to be detrimental to the rearing process and considerable efforts are directed to maintain axenic cultures. However, recent research results suggest that inoculation of beneficial (probiotic) bacterial strains greatly enhance survival and growth of the larvae. For example, addition of bacteria (strain CA2) as a food supplement to xenic cultures of oyster (*Crassostrea gigas*) larvae consistently enhanced growth during different seasons of the year (Fig.2). This suggests that bacteria augmented oyster larval culture by providing essential nutrients not present in algal diets or improved their digestion by supplying digestive enzymes to the larvae.

Fig. 2. Bacterial cells added (per ml)



Mean shell length of Oyster spat in cultures fed Isochrysls supplemented with bacteria (Douillet & Langdon, 1994)

(D) Shrimp Larvae :

Relatively few studies have been made on the effect of probiotics on shrimp larviculture. Studies in India by the author has shown that *Penaeus monodon* larvae could be reared to PL-1 stage using live heterotrophic bacteria (*Pseudomonas* sp and *Micrococcus* sp) as 50% replacement diet instead of diatoms. Such larvae also showed faster metamorphosis and better survival. However, other beneficial effects like disease resistance were not studied. In a recent study in Ecuador, a non-pathogenic isolate of *Vibrio alginolyticus* added to *Penaeus vannamei* larval culture medium helped in controlling other pathogenic vibrios (*Vibrio parahaemolyticus*) and also improved the survival and average wet weight. These results indicate that under standard larval culture protocol, the inoculation of a non-pathogenic *V. alginolyticus* strain can increase survival and final wet weight of P. vannamei post-larvae as well as competitively exclude pathogenic vibrios and discontinue the use of antibiotic prophylaxis in hatcheries. However, the grow-out performance of such larvae need to be investigated.

CONCLUSIONS

It is clear from the above narrative that probiotics has immense potential for use in aquaculture. The advantages are varied and manifold. Its use in poultry and livestock diets has already proved beneficial. More research on its use in aquaculture, especially during larviculture when large scale mortalities take place due to opportunistic pathogens, should help to achieve better performances.