PROBIOTICS IN MARICULTURE - APPLICATIONS AND FUTURE PROSPECTS

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In present day culture regimes, animals are subjected constantly to intense management practices which tell upon their ability to remain healthy as they are driven to achieve high production standards. The system of the body most likely to succumb to such stressful conditions is the intestinal tract which constitutes a doorway for pathogenic microorganisms. Traditionally, farmers have used electrolytes and antibiotics to combat diseases and infection, but pressure is mounting for much greater control over the use of antibiotics and farmers are thus forced to look for alternative methods for healthier and quicker growth and disease control. Invasion from such micro organisms and the resulting disease can be circumvented by the administration of probionts in order to reinforce the beneficial intestinal microflora. Though many theories have been proposed about the use of probiotics, still many doubts their existence with as regard to their safety and efficiency.

Disadvantages of using antibiotics as feed additives

Though antibiotics have been used as feed additives, the associated toxicity, allergy, residues in food and resistance obtained after long term administration of low doses makes their use worthy on second thought. Also the indiscriminate use of broad-spectrum antibiotics may alter the normal gut flora by suppressing its growth and cause an overgrowth of pathogenic bacteria.

Because of decreased production of lactic acid and other fatty acids by the normal gut microflora, the growth of yeasts, fungi, *Enterobacteriaceae* and other pathogens gains prominence. They ascend into the normally and sparsely colonised small intestine and change their characteristics through gene transfer processes, and a state of intestinal dysbiosis is created. The ensuing diarrhoea is unresponsive to antibiotic therapy. These undesirable side effects are not encountered in the use of probiotics.

Definition and development of probiotics

The word 'probiotic' is derived from the greek meaning 'for life' and originally referred to a phenomenon observed when two organisms were cultured together, in which substances produced by one organisms stimulated the growth of the other. These growth promoting substances were referred to as 'probiotics'. The term was subsequently used to describe living preparations of microbal cells, that could be administered to animals, including humans with the aim of promoting the health of the consumer. This latter concept is derived from the observations of Elie Metchnikoff (Box 1). In the case of farm animals, faster weight gain for the same amount of food consumed (growth promotion, feed efficiency) has been of primary importance. Fuller (1989) redefined probiotics as 'A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance'. This revised definition stressed the need for a probiotic to be viable. The principle of a probiotic product containing viable bacteria is that these bacteria become associated with the epithelial cells and are then trapped within the mucous layer (glycocalyx) and can grow and multiply.

Box 1. ***Elie Metchnikoff (1845-1916) and the elixir of life***

Elie Metchnikoff, Nobel Laureate (for his discovery of phagocytosis) of the Institut Pasteur, Paris was interested in the scientific basis of ageing. According to Metchnikoff, the large bowel harboured microorganisms that produced substances that were toxic to the vascular and nervous systems. The toxic substances, as a result of absorption into the bloodstream, contributed to the ageing process. Thus, intestinal microorganisms were the aetiological agents of 'autointoxication', because they produced ammonia, amines and indole as a result of protein hydrolysis (putrefaction) in the digestive tract. Metchnikoff's remedy for autointoxication was radical; he advocated surgical removal of the large bowel. However, a more acceptable remedy was to modify the intestinal microflora by replacing or diminishing the number of putrefactive microorganisms in the intestine. This could be accomplished, it was suggested by enriching the microflora with bacterial populations that obtained energy by the fermentation of carbohydrates rather than hydrolysis of proteins. Lactic-acid producing bacteria were favoured as fermentative microorganisms for this purpose, because it had been observed that the natural fermentation of milk by these microorganisms prevented the growth of non-acid-tolerant microorganisms, including those with proteolytic activity. If the lactic fermentation prevented the putrefaction of milk, would it not have the same effect in the digestive tract if appropriate microorganisms were used. The inhabitants of Eastern European countries, some of whom were apparently extremely long-lived, consumed fermented milk as a constant part of their diet. Thus, yogurts were introduced to Western Europe as health-related foods.

Source: Tannock. G.W. (1977)

Composition of probiotic preparations

Metchnikoff and his colleagues worked with Bulgarian bacillus an organism closely related to lactobacillus, starter of yoghurt (*L. delbreuckii* subsp. *bulgaricus*) and to this day lactobacilli have remained the most commonly used probiotic organisms. Currently available probiotic preparations contain *L. delbreuckii* subsp. bulgaricus, *L. brevis, L. cellobiosus, L. lactis* and *L. reuteri.* Of the bifidobacteria currently being used as probiotics are *Bifidobacterium adolescentis, B. animalis, B. bifidium, B. infantis, B. longum* and *B. thermophilum.* The yoghurt starter *S. salivarius* subsp. *thermophilus* is still a common probiotic organism (Table 1). Many commercial probiotic products currently on the market abroad also contain lactic-acid producing organisms (Table 2).

Table 1. ***Examples of microorganisms used in probiotic products***

Products for human	Products for farm animals
Lactobacillus acidophilus	*L. acidophilus*
Lactobacillus casei shirota strain	*L. casei*
Lactobacillus delburecki subspecies *bulgaricus*	*L. delibrueckii* subspecies *bulgaricus*
Lactobacillus johnsonii	*L. plantarum*
Lactobacillus reuteri	*L. reuteri*
Lactobacillus rhamnosus	*Bifidobacterium bifidum*
Products for humans	**Products for farm animals**
Bifidobacterium adolescentis	*Bacillus subtilis*
Bifidobacterium bifidum	*Streptococcus thermophilus*
Bifidobacterium breve	*Pediococcus pentosaceus*
Bifidobacterium longum	*enterococcus faecium*
Bifidobacterium infantis	*Saccharomyces cerevisiae*
Streptococcus thermophilus	*Aspergillus oryzae*
Saccharomyces, boulardii	*Torulopsis* spp.

Source: Tannock G.W. (1997)

Table 2. ***Probiotic products currently on the market***

Manufacturer	Trade name	Lactic-acid producing organism
Microbial Genetics	Probiocin	*Lactobacillus acidophilus*
West Des Moines, Iowa		*Lactobacillus plantarum*
		Lactobacillus casei
		Streptococcus faecium
Bio-Ceutic Division	Micro-Vet Eubiotic Gel	*Lactobacillus acidophilus*
Boehringer Ingelheim Animal Health, Inc. St. Joseph, Mission	Micro-Vet Eubiotic Feed Additive	*Streptococcus faecium*
Anchor Division	Feed - Mate Boviload	*Lactobacillus acidophilus*
Boehringer Ingelheim Animal Health, Inc. St. Joseph, Mission	Eubiotic Feed Additive Feed-Mate Reload Eubiotic Gel Feed-Mate 68	*Streptococcus faecium* Feed Mate 68 also contains *Lacto-bacillus plantarum*
Conklin Agricultural products	Fastrack	*Lactobacillus acidophilus*
Shakopee, Minnesota		*Streptococcus faecium*

The move towards intestinal isolates resulted in the use of *Enterococcus faecium.* Other species of streptococci used as probiotics are *S. lactis, S. cremoris, S. diacetilactis* and *S. intermedius.* Probiotics also contain bacteria belonging to the genera *Leuconostoc, Pediococcus, Propionibacterium* and *Bacillus.* Yeasts *(Sacharomyces cerevisiae* and *Candida pintolopesii)* and moulds (*Aspergilus niger* and *A. oryzae*) are used in animal products. Probiotics may contain one or several (upto nine) strains of microorganisms and may be presented to the animal in the form of powder (loose or in capsules), tablets, granules or pastes (Table 3). They may be administered by direct insertion into the mouth or by inclusion in the food or water. Moreover, to achieve best results it is better to select an organism that is a natural inhabitant of the gut. Bacterial genera commonly detected as components of the human intestinal microflora are listed in Box 2.

Table 3. ***Probiotics and their concentration***

Presentation	Microbial standard
Bolus (ruminants)	5.0×10^6 CFU/g
Dispersible powder (ruminants, horses, swine)	1.0×10^7 CFU/g
Gel (horses)	1.0×10^7 CFU/g
Gel (ruminants)	1.0×10^7 CFU/g
Granules (ruminants, swine, horses, poultry)	1.0×10^7 CFU/g
Oral suspension (swine)	5.0×10^6 CFU/g
Soluble (swine)	5.0×10^7 CFU/g
Gel (ruminants)	not less than 10^{10} CFU/10 ml
Live dry (ruminants)	9.0×10^{10} CFU/1b
Gel (ruminants)	Not less than 1.0×10^{10} CFU/10 ml
Live dry (ruminants)	9.0×10^{10} CFU/1b
Live dry (swine, calves, foals, poultry)	12.6×10^6 CFU/g
Dispersible liquid (calves, poultry, young pigs)	4.0×10^{11} CFU/1b
Microfeeds (livestock)	
Paste (ruminants)	
Paste (nonruminants)	
Pellets fish	5.0×10^7 - 5.0×10^9 CFU/g
Pellets shrimp	1.0×10^6 CFU/g

Source: Tannock. G.W. (1977)

Inspite of careful selection of strains, permanent establishment of the probionts may not be achieved in the intestinal tract and multiple dosing is essential if the full probiotic effect is to be obtained.

Box 2. *Bacterial genera that are commonly detected as components of the intestinal microflora of humans*

Bacteroids

Gram-negative, non-spore-forming bacilli. Obligate anaerobes. Metabolic products include combinations of acetic, succinic, lactic, formic or propionic acids. If N-butyric acid is produced, isobutyric and isovaleric acids are also present.

Bifidobacterium

Gram-positive, non-spore-forming, nonmotile bacilli, sometimes with club-shaped or spatulate extremities. Obligate anaerobes. Acetic and lactic acids are produced primarily, in the molar ratio 3:2. Glucose is degraded exclusively and characteristically by the fructose-6-phosphate 'shunt' metabolic pathway.

Clostridium

Gram-positive bacilli that form endospores. Obligate anaerobes.

Enterococcus

Gram-positive cocci. Facultative anaerobes. Lancefield group D. Can grown in 6.5% NaCl broth and in normal broth at pH 9.6.

Eubacterium

Gram-positive bacilli, non-spore-forming. Obligate anaerobes. Produce mixtures of organic acids including butyric, acetic and formic acids.

Fusobacterium

Gram-negative, non-spore-forming bacilli. Obligate anaerobes. N-butyric acid is produced, but isobutyric and isovaleric acids are not.

Peptostreptococcus

Gram-positive cocci. Obligate anerobes. Can metabolize peptone and amino acids.

Ruminococcus

Gram-positive cocci. Obligate anaerobes. Amino acids and peptides are not fermented. Fermentation of carbohydrates produces acetic, succinic and lactic acids, ethanol, carbon dioxide and hydrogen.

Lactobacillus

Gram-positive bacilli, non-spore-forming. Grow best under anaerobic conditions. Lactic acid is a major product of glucose fermentation.

Escherichia coli

Gram-negative rods, facultatively anaerobic. Citrate not utilized. Carbohydrates fermented to lactic, acetic and formic acids. Part of formic acid is split by a complex hydrogenase system to give equal amounts of carbon dioxide and hydrogen. Lactose is fermented by most strains, but fermentation can be delayed or absent. Motile by means of peritrichous flagella or nonmotile.

Possible modes of action of probiotics

Three possible mechanisms have been proposed for the mode of action of probiotic preparations. These are summarize below:

1. Suppression of viable count by :
   a) production of organic acids, hydrogen peroxide and CO_2
   b) production of antibacterial compounds
   c) competition for nutrients or nutrient depletion
   d) competition for adhesion sites
2. Alteration of microbial metabolism by :
   a) increased enzyme activity
   b) decreased enzyme activity
3. Stimulation of immunity by :
   a) increased antibody levels
   b) increased macrophage activity

Though there is some experimental data to support these modes of action, there has been in most cases, conflicting experimental data attributable to technical difficulties.

Characteristics of an effective probiotic

An effective probiotic is required to operate under a variety of different environmental conditions and to survive in many different forms and therefore should have the following characteristics.

1. It should be prepared as a viable product on an industrial scale.
2. It should remain stable and viable for long periods under storage and field conditions.
3. It should have the ability to survive (not necessarily grow) in the intestine.
4. It must produce a beneficial effect in the host animal.
5. It should be non-pathogenic with no toxin production.

Beneficial claims made for probiotic supplementation are numerous and include :

1. Improved growth rate of farm animals due to suppression of a sub clinical infection with a growth depressing microorganism.
2. Improved utilisation of food which may be achieved by increased efficiency of existing digestive processes or by promoting the digestion of previously undigestive substances.
3. Improved health which includes resistance to infectious diseases either by direct antagonism or by stimulation of immunity.
4. Increased egg production in poultry animals. There have been reports of increases in number of eggs produced and also individual egg weights.
5. Improved milk production by dairy cattle. This is obtained particularly with fungal supplements such as *S. cerevisiae* or *A. oryzae* and may be manifested as an increased yield or an increase in fat content which may be a consequence of the effect on rumen metabolism.

Supplementing the diet with bacterial growth nutrients like fermentable sugars, yeast extract, peptides, buffers and trace minerals have proved to improve the hosts response to probiotics. Simultaneous incorporation of direct-fed microbials and antibiotics have also been suggested in cases where the probiotic bacteria may not be as effective if the gut contains high concentrations of pathogenic microbes or if the probiotic bacteria are unable to displace the microbes. In addition, there is some thought that probiotics may also help in preventing the development of antibiotic resistance.

Bacterial interactions in the gut

Microbial interactions represent the main force which contributes to the homeostasis of the bacterial flora in the gut. This flora forms an ecosystem with its host, comprising (a) biotic components *e.g.* indigenous and transient microbes, and gastrointestinal epithelial cells which delimit the biotope; (b) abiotic components of dietary origin, namely those that have not been digested during their course through the small intestine and (c) endogenous components coming from saliva, gastric, pancreatic, hepatic and intestinal secretions or excretions, including enzymes, hormones, mucus, bile salts, urea, immunoglobulins, peptides and several other unknown components. All these components interact and the result of such interactions is compatible with the healthy survival of the host. When gastro intestinal disorders arise the ecosystem becomes destabilized. This emphasises the importance of maintaining microbial interactions in a way that maintain the stability of the ecosystem and the optimum health for the host.

Metabolic interactions in the gut

The most important way in which a probiotic organism can exert a beneficial effect on its host is by modifying the metabolic processes of the gut which could be achieved in theory by a variety of mechanisms :

1. Suppression of reactions which result in the generation of toxic or carcinogenic metabolites.
2. By stimulating the enzymic reactions involved in detoxification of potentially toxic substances either ingested or formed endogenously.
3. By stimulating mammalian enzymes involved in the digestion of complex nutrients, or where such enzymes are absent (due to genetics or disease) providing a bacterial source of these enzyme.
4. By synthesizing vitamins and other essential nutrients which are not provided in sufficient quantities in the diet.

Strong evidence exists that at least certain strains of *Lactobacillus* can modify intestinal bacterial metabolism and the biological and toxicological significance of the changes seen has been established and indicates that ingestion of such probiotic organisms has beneficial effects.

Application of probiotics in mariculture

Probiotics are widely used in human and animal nutrition, but their advent into aquaculture and mariculture in particular are still in the stages of infancy with very little work having been carried out on the effects of potential probiotic strains on marine finfishes and shellfishes. However, it is known that bacteria account for over 80% of the total biosurface in seawater and bacteriovory is widespread among a number of marine larvae (Azam *et al.*, 1984). A series of studies were carried out by Tanasomwang and Muroga (1990) on the intestinal microflora of larval and juvenile stages of important species such as red sea bream (*Pagrus major*), black sea bream *(Acanthopagrus schlegeli)*, japanese flounder *(Paralichthys olivaceus)*, rockfish *(Sebastes schlgeli)*, tigerpuffer *(Takifugu rubripes)* and red grouper (*Epinephelus akaara*) in relation to the microflora of ambient water and feeds. Newly hatched larvae and low bacterial populations which increased in direct proportion to the size of the fish upto 10^5 CFU/fish after commencement of feeding with live feeds. Feeding with artificial feed and minced fish caused a decrease. Average bacterial counts were 10^4, 10^7-10^5 and 10^4 CFU/ml or gin water, live diets, minced fish and artificial feed respectively. The bacterial genera recovered from the intestine were *Vibrio, Pseudomonas, Acinetobacter, Moraxella, Cytophaga* and *Alcaligenes*.

Based on these observations studies on finfishes have undertaken feeding the animals with the probiotic both directly and indirectly. Indirect feeding being carried out with the help of live food organisms like artemia nauplii and rotifers reared on probiotics (Gate Soupe 1989, 1991). In oral feeding studies, carried out with rainbow trout fingerlings using seven strains of bacteria isolated from the gut, superior growth and feed conversion efficiency was observed in animals fed probiotic diets as compared to control (Sridhar and Austin 1998).

Garriquers and Arevalo (1995) have reported on the beneficial effects of nutritional probiotics in developing shrimp of high immunity. Mohamed (1996) used live heterotrophic bacteria as 50% replacement to microalgae in the diet of *P. monodon* larvae and reported marked improvement in survival and growth rate. Sridhar and Chandrasekar (1996) in evaluating the effect of feeding five strains of bacterial biomass to

larvae of *P. indicus* also revealed the beneficial effects of probiotics on growth and survival of shrimp larvae. Also, mantis shrimp, feed with wheat bran fermented with *B. licheniforms* and *Beauveria* sp. fungi, fed to postlarvae of *P. indicus* showed enhanced growth and survival. Probionts may therefore provide growth and survival. Probionts may therefore provide growth factors and inhibit the proliferation of pathogen by stimulating the nonspecific immune response. The studies of Garriques and Arevalo (1995) and Gatesoupe (1994) also agree with these observations and recommend the use of probionts in increasing the resistance to disease of animals.

Sridhar and Austin (1998) studied the resistance to infection by *A. Salmonicida* in fingerlings of rainbow trout and reported higher survival and resistance in animals fed with probiotic diets as compared to control.

Chandrika (1999) has recently found that *Bacillus* spp. can be successfully used as probiotics in feed preparations and also for the management of detritus in intensive aquaculture operations, to control the attack of microbial diseases thereby reducing high shrimp mortality.

Though limited in number the information generated from these studies does highlight the effect of probiotics in increasing the production efficiency and resistance to disease of aquaculture organisms. Further studies on the real effect and mode of action of various probiotic preparations for marine finfishes and shell fishes would definitely strengthen mariculture practices.

Future prospects

The use of probiotics in animal husbandry is an accepted practice and is on the increase. However, the interaction of a microbial feed supplement with the host animal and its gut microflora resulting in the expression of the probiotic effect is by its nature a very complex phenomenon. The factors affecting the probiotic response are :

1. Composition of the host animal gut flora
2. Dosing regime which may be continuous or as a single dose
3. Age and type of animal, since physiology and immune status of an animal change as the animal gets older
4. Quality assurance which is viability of the probiotic product and strain variation among species
5. Type of preparation : Because of the variety of different forms, one type may be more suitable than another for a particular animal
6. Production methods of the probiotic product may cause variations in response for the same organism

Therefore, the future development of probiotic products for mariculture purposes is dependent upon the availability of effective and reliable preparations which would give consistently positive results. With the development of such products, the mode of action of probiotic organisms would be understood and it would be possible to look for key biochemical features in the laboratory and select potential candidates for field trials. Genetic engineering allows us to have unlimited access to new strains and with detailed information on the mode of action of probiotics we may be able to introduce the probiotic effect into an organism which permanently colonizes the intestinal tract. It may also be possible to incorporate protective antigens from pathogenic bacteria into harmless intestinal commensals such as lactic acid bacteria and capitalize on their ability to stimulate the immune system. Genetic engineering would also aid to increase resistance to acid so that probiotics would survive passage through the stomach. Resistance to heat would also be an advantage enabling producers to include probiotics in feed without risking subsequent damage by the heat generated during the pelleting process.

An important development in probiotic research would be the production of non-viable probiotics. Once the biochemical basis of probiotic activity is known, it would be possible to produce the effect by feeding the substance responsible for the activity produce by the viable supplement. The yield can be improved by genetic manipulation without attending the problem of environmental release of genetically altered viable micro organisms.

Improved quality assurance of probiotic preparations should be maintained and their viability sustained throughout the stated shelf-life of the product. The live nature of probiotics creates unique features and problems compared with antibiotics and other drugs. Future research and development may enable us to identify the biochemical feature responsible for the probiotic effects and give rise to a second generation of probiotics which are nonviable.

Conclusion

The balance between normal and potentially pathogenic bacteria is altered in the intestine of animals subjected to stress. The result is domination by the pathogens giving rise to deteriorative changes like diarrhoea and decreased production performance in the host. Probiotics - products containing strains of lactic acid - producing / beneficial organisms - are biologic tools that promote digestive balance. Probiotics supplement intestinal microflora with beneficial bacteria and create conditions non-conducive to the growth of pathogens. Along with decreasing intestinal colonization by pathogens, probiotics improve production efficiency by increasing average daily weight gain, feed consumption and feed efficiency.

The use of probiotics such as food additives is preferred over the use of antibiotics, because they have no problems associated with antibiotics *viz.* toxicity, allergy, residues in food, bacterial resistance and indiscriminate suppression of intestinal microflora. However, probiotics should not be considered as alternatives to antibiotics in disease therapy, but as a complementary therapy for restoring balance to the intestinal flora.

The mechanism of action of probiotics which include depletion of nutrients, production of acids and antimicrobial substances, competition for adhesion receptors in the intestine and immunostimulation create an environment incompatible to the growth of pathogens.

The probiotic product selected should be capable of implanting itself in the gut and should inhibit pathogens and as far as possible it should be a normal inhabitant of the intestine capable of tolerating low pH levels and the effects of bile. The derivation of such efficaceous probiotics requires substantial research and development, especially at the level of fundamental science.

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