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USE OF PROBIOTICS IN AQUACULTURE

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Abstract
Antibiotics have been widely used in aquaculture in sterilization of equipments and in treatment of fish diseases, and control of bacteria infections in fish hatcheries. However, the negative effects associated with the use of antibiotics such as development of resistant bacteria which constitute potential hazard to the environment and the people has led credence to the use of probiotics. Probiotics are live harmless bacteria that help the well being of the host animal and contribute directly or indirectly to protect the host animal against harmful bacteria pathogens. This paper reviews the types of natural and synthetic/commercial probiotics available for aquaculture; the selection criteria for probiotics; some techniques or parameters used in the assessment of the performance of probiotics and specific effects of some probiotics on aquaculture. The findings showed that probiotics are useful in reducing anti-nutritional factors (tannin, phytic acid, mimosine) in non-conventional fish feed ingredients; in improving water quality and the growth of fishes and in immune stimulation in fishes; in increasing the population of native non-pathogenic bacteria and digestive enzyme activities; and in inhibition of pathogenic organisms and reduction in incidence of diseases. Probiotics are also environmentally friendly therefore their use in aquaculture is indispensable.

Key words: Antibiotics, probiotics, aquaculture

Introduction

What are probiotics

Probiotics are simply described as harmless bacteria that help the well being of the host animal and contribute, directly or indirectly to protect the host animal against harmful bacteria pathogens. Fuller (1989) defined probiotics as live microbial feed supplements which beneficially affects the host animal by improving its intestinal microbial balance. Tannock (1997) also explained probiotics as living microbial cells administered as dietary supplements with the aim of improving health. WHO (2001) similarly described probiotics as live microorganisms which when administered in adequate amounts confer a healthy benefit on the host. A beneficial effect by application of certain beneficial bacteria in human, pig, cattle and poultry nutrition has been well documented (Gilliland, 1979; Conway, 1989; Jong, 1993). But the use of such probiotics in aquaculture is a relatively new concept (Kozasa, 1986). Presently the interest and awareness is growing daily. Most probiotics are supplied as live supplements in food, which must have the ability to survive passage through the intestinal tract (Fuller, 1992). The benefit to host may arise as a nutritional effect, whereby the bacteria are able to break down the toxic or otherwise nutritious components of the diet, which the host can then digest (Smoragiewicz *et al*., 1993). Alternatively, the probiotics may prevent the potential pathogens from colonizing the gut by production of antimicrobial compounds, or by out competing them for nutrients or mucosal space (Smoragiewicz *et al*., 1993). Gatesoupe (1997) stated that probiotic microorganisms should be non-pathogenic and non-toxic in order to avoid undesirable side effects when administered to fish.

Why probiotics

Prevention of fish diseases is essential to the success of any large-scale, intensive production of fish in culture. Bacterial infections, the major causes of mortality in fish hatcheries and adult fish production systems (Austin and Stobie, 1992) are controlled prophylactically, and therapeutically based on oral administration of antibiotics. Such treatment may cause the development of resistant bacteria (Aoki *et al*., 1985) and can lead to potential hazard to the public health and the environment. Besides, the normal beneficial microbial flora in the digestive tract of fish may also be killed or inhibited (Sugita *et al*., 1991). Amabile-Cuevas *et al*. (1995) stated that vaccines are also being developed, but cannot also be a universal disease control measure in aquaculture. A new approach that is gaining acceptance within aquaculture industry is the use of probiotic bacteria to improve disease resistance, water quality and/or growth of cultured fishes (Verschuere *et al*., 2000). Probiotics are defined as live microbial feed additive, which gives a good effect on the host animal by improving the microflora of their gastrointestinal tract (Fuller, 1989) via the production of nutrients enhancing immune responses and improving the water quality. In order to make aquaculture products safe for human consumption, it is very important to develop an alternative to the use of antibiotics by using living microbial cells as additives in fish feed, to control pathogens and to ensure improved growth and immunity of the fishes. Probiotics enhancement of the natural flora in the gut of organisms will enable the natural intestinal flora to participate in a more war-like activity, and actively produce substances that may inhibit or kill the pathogens (Sugita *et al*., 1996).

Types of probiotics

Two types of probiotics are natural and synthetic or commercial probiotics. Probiotic bacteria can be isolated from the gastrointestinal tract (GIT) of a fish. That is from the intestines, stomach, gill, kidney and the gonads. They can also be isolated from the internal organs of other animals. After isolation, depending on requirement, the target ones can then be cultured or multiplied. And these groups constitute the natural sources. On the other hand, the commercial sources consist of those already synthesized and are available on the shelf for immediate use. The most frequently used probiotic bacteria are those from Lactobacillus or Bifidobacterium species. Some of the commercially available probiotics are listed below.

Lactobacillus species	**Bifidobacterium species**
Lactobacillus acidophilus	*Bifidobacterium bifidum*
Lactobacillus casei	*Bifidobacterium breve*
Lactobacillus fermentum	*Bifidobacterium lactis*
Lactobacillus gasseri	*Bifidobacterium longum*
Lactobacillus lactis	Streptococcus species
Lactobacillus plantarum	Streptococcus thermophilus
Lactobacillus salivarius	Saccharromyces species
Lactobacillus rhamnosus	*Saccharromyces boulardii*
Lactobacillus johnsonii	*Streptococcus cremoris*
Lactobacillus paracasei	Bacterial mixture (Add-B)
Lactobacillus reuteri	
Lactobacillus helveticus	
Lactobacillus bugaricus	

Selection criteria for probiotics

The purpose of probiotics is maintenance or re-establishment of favourable relationships between friendly and pathogenic microorganisms that constitute the flora of intestinal or skin mucus of a fish. Successful probiotics are expected to have certain qualities mentioned below. In order to have a beneficial effect on the form of growth promotion or to protect fish against bacteria pathogens, the strains should also have the ability to colonize the fish by adhesion (Olsson *et al*., 1992), and to produce important substances, like vitamins. Adhesion is one of the most important selection criteria for probiotic bacteria, because it is considered a pre-requisite for colonization (Beachey, 1981). Therefore the microorganisms must be viable for long periods under storage and in field conditions (Fuller, 1989), although non-viable bacteria are able to adhere to tissue culture cells indicating adhesion without viability (Hood *et al*., 1988; Coconier *et al*., 1993). Probiotic microorganisms must have to be non-pathogenic and non-toxic to avoid undesirable side-effects when administered to fish. Tests of antagonism, adhesion and challenge tests in vitro are essential to select among the probiotic species. Challenge experiments where fish treated with friendly bacteria are subjected to pathogens are also needed (Gatesoupe, 1999). They must also have the ability to produce vitamins for example, the bacterial strain *Rhodospirillum rubrum* produces considerable amount of vitamin B_{12} (Hirayama and Katsuta, 1988). Vitamin B_{12} is an important vitamin for several functions involved in digestion in fish (Sugita *et al.*, 1991). Besides, many probiotics appear to improve the activity of beneficial bacteria species already present in the digestive tract of fish.

Methods of administration of probiotics

Probiotics can be administered orally or as additive in animal feeds or added in water used in culturing fishes. When orally administered with diet or supplied to rearing water, they could attach themselves to the surface of gastrointestinal tract and colonize it. Also when added to the rearing water, they can enter fish gastrointestinal tract and colonize it. They can also be incorporated in live fish feeds.

Mechanism/benefits of probiotics

There are several mechanisms by which probiotics may protect the host from intestinal disorders. In general, the processes by which bacteria inhibit colonization by other strains is called colonization resistance. The mechanisms on how probiotic bacteria may protect the host against intestinal diseases are given as follows. Probiotic bacteria produce a variety of substances that are inhibitory to both gram-positive and gram-negative bacteria. These inhibitory substances include organic acids, such as acetic, lactic acids, hydrogen peroxide and bacteriocins. These compounds may reduce not only the number of viable cells but may also affect bacterial mechanism or toxin production. For instance, *Lactobacillus acidophilus* has been used to produce substances between the molecular weights of 200 and 6,200, some of which are sensitive proteases that can inhibit Staphylococcus, Streptococcus, *Escherichia coli* and Salmonella species. *Lactobacillus rhamnosum* strain GG (LGG) produces a broad spectrum of low molecular weight centimicrobial peptide which is plasmid mediated and has activity against Staphylococcus, Streptococcus, *Escherichia coli,* Mycobacterium, Clostridium and Listeria species.

Blocking of adhesion sites

Competitive inhibition for bacterial adhesion sites on intestinal epithelial layers is a mechanism of action of probiotics. Consequently some bacteria strains are selected for their ability to adhere to epithelial cells. Studies have shown that Lactobacillus can prevent adherence of *Escherichia coli,* Klebsiella species and *Pseudomonas aeruginosa* to intestinal cells.

Competition for nutrients

In competing for nutrients, probiotics can out-compete the pathogens by consuming the nutrients that would otherwise been consumed by pathogenic microorganisms. This mechanism would limit the existence of the pathogens in the intestinal cells because without nutrients the organisms can not survive. .

Degradation of Toxin Receptor

The mechanism by which *Saccharomyces boulardii* protects animals against *Clostriodum difficile* intestinal disease is through degradation of the toxin receptor on the intestinal mucosa. By this process the toxins does not accumulate on the cells, so there will be no means of pathogenic activities.

Stimulation of Immunity

Stimulation of specific and non-specific immunity may be another mechanism by which probiotics can protect against intestinal diseases. The underlying mechanisms of immune stimulation are not well understood but specific cell wall components or cell layers may act as adjuvant and increase humoral immune response.

Non Specific Immune Systems

Specific effects of probiotics on aquaculture

The use of probiotics in aquaculture is a recent event because of the environmental health problems associated with the use of antibiotics. Studies have concentrated on the use of probiotics in fish juveniles, but more attention is now on larvae of fish, shell fish production and on live food organisms. The overview of literature reports on probiotics as biological agents in aquaculture is presented in Table 1.

Table 1. Effects of probiotics on aquaculture production

Probiotics	Application	Administration Method	Observations	Mode of action	References
Rhodospirillum & *Rhodopseudomonas*	Polluted	Culture water	Improvement		Kamal et al. (1990)
Vibrio alginalyticus	Atlantic Salmon		Reduce diseases	Antagonism	Austin et al.(1995)
Bacteria strains of turbot	Turbot *Scophthalmus maximus*		Suppress growth of *Vibrio anguillarum*		Olsson et al.(1992)
Streptococcus faecium	Israeli carp	Added to feed	Improved growth and feed efficiency	Improved nutritional Value	Noh et al. (1994) Bogut et al. (1988)
Bacillus spp	Channel catfish	Added to rearing water	Increased survival and production		Queiroz and Boyd (1998)
Lactic *ac*id bacteria (LAB) *Lactobacillus rhamnosus*	Rainbow trout	Added to feed	Higher immunoglobulin Elevated lysozyme activity	Immune stimulation	Panigrahi et al. (2004)
Lactobacillus plantarum	Halibut larvae	Added to culture water	Increase in survival of Halibut larvae		Olafsen (1998)
Lactobacillus sp	Turbot larvae	Enrichment of rotifers & Artemia	Increase in survival of Turbot larvae		Gatesoupe (1994)
	Pacificoyster (*Crassostreagigas*) larvae	Addition to feed	Improved in survival & production	Nutrient enrichment & Antagonism	Douillet & Langdon (1994)
Vibrio alginalyticus	*Artemia nauplii*	Addition to culture water	Increased survival	Immune stimulation	Gomez-Gil et al. (1998)
Bacillus subtilis	Channel catfish	Addition to pond water	Increased survival and net production	Immune stimulation & nutrient enrichment	Queiroz & Boyd (1998)
Vibrio alginolyticus	Atlantic salmon	Bathing in bacterial suspension	Increased survival	Immune stimulation	Austin et al. (1995)
Fluorescent Pseudomonad F19/3				Antagonism	Smith & Davey (1993)
Camobacterium Strain K1		Intestinal mucus & faecal extract	Growth inhibition of *V. anguilarum* & *A. salmonicida*		Joborn et al. (1997)
Lactic acid bacteria	Atlantic cod	Addition to diet (*Gadus morhua*)	Increased survival & inhibition of *V. anguillarum*		Gildberg & Mikkelsen (1998)
Microbially matured water	Turbot & Halibut larvae	Added to culture water	Increase in growth rate	Nutrient enrichment	Skjermo et al. (1997)
Carnobacterium sp	Atlantic salmon	Added to diets	Suppression of *Aeromonas ydrophilia*; *A. salmonicida; Streptococcus milleri*	Antagonism	Robertson et al. (2000)

Vibrio alginolyticus	*Penaeus vannameri* larvae		Increased growth & reduction in diseases	Nutrient enrichment & antagonism	Garriques and Arevalo (1995)
Carnobacterium sp	Salmonids (Atlantic salmon and rainbow trout)	In vitro	Produced inhibitory compounds in intestinal mucus	Antagonism	Joborn et al. (1997)
Clostridium butyricum	*Miichthys miiuy*	Added to feed	Improved growth performance Significant improvement in lysozyme serum & skin mucus activity Improved total immunoglobulin (IgM) in serum Significant improvement in serum acid phosphatase activity	Nutrient enrichment Immune stimulation Antagonism	Song et al. (2006)
Bacillus subtilis	Leucaena leaf meal leucaena leaf meal	Innoculated in anti-nutritional factors	Significant reduction in (tannin, phytic acid, mimosine)	Nutrient enrichment	Bairagi et al. (2004)
Bacillus subtilis	Rohu carp (*Labeo rohita*)	Added to feed	Significant increase in growth performance, protein digestibility, á-amylase activity	Nutrient enrichment	
Live yeast *Debaryomyces hansenii* CBS 8339	Leopard grouper (Mycteroperca rosacea) & Gilthead sea bream (*Sparus aurata*)		Enhancement of growth	Nutrient enrichment, antagonism against *Amyloodinium ocellatum* & *Aeromonas hydrophila*	Bacerril et al. (2008)
Bacillus subtilis	Sea cucumber		Increased survival, growth & intestinal microflora	Nutrient enrichment, immune stimulation & antagonism against *Vibrio splendidus*	Mai et al. (2008)
Live yeast isolated from red claw crayfish gut	Red claw crayfish (*Cherax quadricarinatus)*		Increased survival & growth rate	Nutrient enrichment & immune stimulation	Olvera-Novoa et al. (2008a)
Lactic acid bacteria isolated from Nile tilapia intestinal microflora	Nile tilapia		Increased growth performance at the same level with antibiotic	Nutrient enrichment (Oxytetracycline)	Olvera-Novoa et al. (2008b)

Summary
The accelerated growth of aquaculture industry has been accompanied by severe outbreaks of diseases caused by wide range of pathogens (Olvera-Novoa *et al*. (2008b). Gatlin *et al*. (2008) reported that inspite of expansion in the production of hybrid striped bass, *Morone chrysops* x *M. saxatilis* in the United States, that pathogenic organisms such as *Streptococcus iniae* have resulted in economical losses of several millions dollars annually. Application of accurate, enough, target and safe dosage of probiotics could be a saving grace to most of the disease problems in aquaculture. More so, that probiotics are environmentally friendly and could eliminate the disease resistant problems in the environment and people associated with the use of antibiotics. Besides, as probiotics can improve the quality of non-conventional feed ingredients by denaturing their anti-nutritional factors to liberate the bound nutrients and make them more digestible, it can be applied in the development of low-cost fish feeds. Successful application of probiotics in fish larval rearing and development has been reported. Yufera *et al*. (2003) described that microcapsules including probiotics can be used as a vehicle to administer specific substances that offer positive responses in larval growth and development. This may form a break through to massive production of marine fish larvae as very high mortality is usually recorded at that stage of development. That will also invariably lead to reduction in the cost of marine fish production. Also as the probiotic bacteria can be isolated from the GIT of the particular animals concerned, it could reduce the cost of aquaculture management in terms of animal growth enhancement and disease preventions.

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