THE ROLE OF PROBIOTICS AND THEIR MECHANISMS OF ACTION: AN AQUACULTURE PERSPECTIVE

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THE APPLICATION OF PROBIOTICS IN AQUACULTURE

The rise in bacterial antibiotic resistance and antibiotic residues in cultured aquatic animals due to extensive use of chemotherapeutic agents has become a global concern. Vaccination and immunostimulant treatment are ideal methods for preventing infectious diseases but their use remains very limited and rather uncommon in aquaculture, especially in Southeast Asia.

In recent years, attention has focused on the use of probiotics in the development of strategies for microbial control and to reduce the use of therapeutic chemicals and antibiotics, towards a more environmentally friendly and sustainable aquaculture (Pintado et al. 2010). Many aquaculture farms in Southeast Asia apply probiotics as a prophylactic measure to improve farm production yields (Fig. 1). The term “probiotic” was first introduced by Parker (1974) and the original definition was “organisms and substances which contribute to intestinal microbial balance.” The definition of probiotic was redefined as “live microorganisms which, when administrated in adequate amounts, confer a health benefit on the host” (FAO/WHO 2001). Most probiotics are supplied as live supplements in feed to benefit the host by inhibiting pathogenic microbes, improving immune response, improving survival and growth rates, enhancing digestion and increasing feed utilization, promoting antimutagenic and anticarcinogenic activity, and improving water quality in culture systems (Harikrishnan et al. 2010, Andani et al. 2012).

The mechanisms of action of probiotics are well defined: creation of a hostile environment for pathogens by production of inhibitory compounds (bacteriocins, lysozymes, proteases and hydrogen peroxide), competition for adhesion sites and essential nutrients and further improvement of immune responses of hosts, supplementation of essential nutrients, vitamins and enzymes, direct uptake of dissolved organic materials mediated by beneficial bacteria, and modulating interaction with the environment (Gatesoupe 1999, Gomez-Gil et al. 2000, Irianto and Austin 2002, Balcázar et al. 2006).

Most probiotics proposed as biological control agents in aquaculture are lactic acid bacteria (Lactobacillus and Carnobacterium) and representatives of the genera Vibrio (V. alginolyticus and V. parahaemolyticus), Bacillus, Pseudomonas, and others including Aeromonas and Flavobacterium (Balcázar et al. 2006, Das et al. 2006, Balcázar et al. 2007).

Unlike human probiotics, there are only a few key genera of probiotic bacteria, such as Lactobacillus and Bifidobacterium.

Identification of probiotic bacteria requires multiple screening steps to confirm its feasibility for field application. Molecular identification usually follows stringent screening steps to verify the identity of the beneficial bacteria (Figs. 2A and 2B).

MECHANISMS OF ACTION

Competitive Exclusion

Probiotics can bind to colonic cell lines and to mucin, which is thought to aid colonization of the animal gut system (Schiffrin et al. 1995, Ouwehand et al. 2000, Juntunen et al. 2001). The microflora of the gastrointestinal tract (GIT) of aquatic animals can be modified by ingestion of other microorganisms. However, the dominant bacteria in fish intestine are quite different from those observed in mammals (Gatesoupe 2005). The composition of microbial communities is greatly influenced by husbandry practices and environmental conditions, e.g. culture water and abiotic or biotic factors, which stimulate the proliferation of selected bacterial species. Therefore, addition of beneficial bacteria to culture water or via feed supplementation during initial egg fertilization or pre-larval stages could have a distinct advantage through the mechanism of competitive exclusion for attachment sites on egg surfaces or in the GIT (Irianto and Austin 2002). Competitive exclusion by potential probiotic bacteria can be evaluated by in vitro antagonistic screening against multiple strains of pathogenic bacteria (Figs. 3A and 3B).

Bacterial antagonism is a common phenomenon in nature. Probiotics may prevent opportunistic pathogens from colonizing (CONTINUED ON PAGE 20)
the surface of eggs or the GIT by producing antimicrobial compounds or by outcompeting them for nutrients or mucosal space (Bandyopadhyay and Mohapatra 2009). Certain pathogens produce proteolytic enzymes that can dissolve and digest the bacteria that approaches them, the most remarkable being *Streptococcus* and *Bacillus pyocyaneus* (Rettger 1905).

Many studies have addressed the use of probiotics in aquaculture, such as the improvement of the survival of portunid crab larvae *Portunus trituberculatus* through the application of probiotic *Thalassobacter utilis* in the rearing water (Nogami and Maeda 1992, Nogami et al. 1997). Lactic acid bacteria, notably *Lactobacillus* sp., *Bifidobacterium* sp. and *Streptococcus* sp. are effective against diseases caused by *Vibrio* sp. (Gatesoupe 1994, Olsson et al. 1998). *Bacillus subtilis* BT23 has inhibitory effects against vibriosis in tiger shrimp *Penaeus monodon* (Vaseeharan and Ramasamy 2003). Application of a *Bacillus* strain to the common snook *Centropomus undecimalis* increased larval survival, promoted better growth and decreased the number of suspected pathogenic bacteria in the fish gut (Kennedy et al. 1998). *Alteromonas* sp. can reduce the infection of *Vibrio* sp. in Pacific oyster *Crassostrea gigas* larviculture (Douillet and Langdon 1994). Some *Pseudomonas fluorescens* strains reduce the mortality of rainbow trout *Oncorhynchus mykiss* fingerlings infected with a pathogenic *V. anguillarum* (Gram et al. 1999).
Lymphoid tissues (GALT) (Famularo et al. 2007). The immune response is largely specialized to gut-associated epithelial cells, alongside intestinal cellular components, and is critical for nutrient and immune system function. In many animal studies, the intimate association of probiotics with the intestinal mucosa, alongside intestinal cellular components, appears to improve immunological function in the GIT (Klaenhammer et al. 1997). Some fish gut microbiota may participate directly in the digestion processes of fish. Enzyme-producing microbiota such as *Bacillus* and *Enterobacteriaceae* (Acinetobacter sp., *Aeromonas* sp., *Flavobacterium* sp., *Photobacterium* sp., *Pseudomonas* sp., *Vibrio* sp., *Microbacterium* sp., *Staphylococcus* sp., *Streptococcus* sp., *Micrococcus* sp., and *Staphylococcus* sp.) can contribute to the host’s nutrition, especially in supplying fatty acids and vitamins to host cells (Sakata 1990, Rings et al. 1995). Some fish gut microbiota may participate directly in the digestion processes of fish. Enzyme-producing microbiota such as *Bacillus* and *Enterobacteriaceae* (Acinetobacter sp., *Aeromonas* sp., *Flavobacterium* sp., *Photobacterium* sp., *Pseudomonas* sp., *Vibrio* sp., *Microbacterium* sp., *Staphylococcus* sp., *Streptococcus* sp., *Micrococcus* sp., *Staphylococcus* sp.), and some unidentified anaerobes and yeasts are potential contributors (Ray et al. 2012).

The metabolic and physiological roles of fish gut microbiota have been the subject of several studies. These microbiota are able to stimulate gut epithelial differentiation and proliferation, gut motility, protein uptake, nutrient metabolism, and innate immunity (Rawls et al. 2004, Rawls et al. 2006, Bates et al. 2006). However, these functional roles are mostly limited to the initial stage of fish fry. In bivalves and crustaceans, microbiota in the gut facilitate digestion by producing extracellular enzymes such as proteases and lipases, as well as providing necessary growth factors (Prieur et al. 1990, Wang et al. 2000). Despite numerous studies demonstrating that microbial activity in the digestive tract may be an important source of nutrients and enzymes to the host, it is difficult to attribute the exact contribution of gastrointestinal microbiota in exothermic animals because of the complexity and variable ecologies of the digestive tracts of different fish species (Ray et al. 2012).

**Immune Response Enhancement**

Numerous studies using in vitro models and rats indicate that probiotics have potential benefits to the non-specific immune system. As in many animal studies, the intimate association of probiotics with the intestinal mucosa, alongside intestinal cellular components, appears to improve immunological function in the GIT (Klaenhammer 2007). The immune response is largely specialized to gut-associated lymphoid tissues (GALT) (Famularo et al. 1997). Certain infections are protected by serum, an effect called humoral immunity. The humoral immunity reaction is mediated by circulating antibodies known as immunoglobulins (Ig). Five immunoglobulins (IgA, IgD, IgE, IgG and IgM) play different roles in the humoral immune response system (Devereux 2002). Similar immune responses also have been observed in aquatic animals, when the disease protection mechanism is activated by both cellular and humoral immune defenses (Rengpipat et al. 2000).

The immune response of fish can be up-regulated through supplementation of probiotics, either in the form of monospecies or multispecies mixtures (Nayak 2010). Phagocytic, lysozyme, complement, respiratory burst activity, and the expression of various cytokines in fish can be stimulated by different probiotics (Nayak 2010). Phagocytic activity of leucocytes increased after oral administration of *Clostridium butyricum* bacteria to rainbow trout, which subsequently enhanced the resistance of fish to vibriosis (Sakai et al. 1995). Similarly, administration of a mixture of bacterial strains (*Bacillus* and *Vibrio* sp.) positively influenced the protective effect of white shrimp against the pathogens *V. harveyi* and white spot syndrome virus (WSSV) (Balcázar 2003). This protection is largely attributed to the stimulation of the immune system by phagocytosis and antibacterial activity. Administration of a lactic acid bacterium *Lactobacillus rhamnosus* (strain ATCC 53103) stimulates respiratory burst activity in rainbow trout *Oncorhynchus mykiss* (Nikoskelainen et al. 2003). Some bacteriocin-like inhibitory substances (BLIS) such as antimicrobial peptides, proteins, or protein complexes excreted/synthesized by probiotics are also effective to control several fish diseases, including *V. parahaemolyticus* (Carraturo et al. 2006), *Flavobacterium* sp. (Balakrishna and K eerthi, 2012) and *Aeromonas hydrophila* (Selvendran and Michael Babu 2013).

**Antiviral Effects**

Viral infection is one of the most common diseases in aquatic environments, seriously affecting aquaculture worldwide (Maeda 2001). Major viral diseases affecting aquaculture include White Spot Syndrome Virus (WSSV), Infectious Pancreatic Necrosis Virus (IPNV), Novirhabdovirus (VHSV), Rhabdovirus (IHNV), Nodavirus (CONTINUED ON PAGE 22)
(VNN), Hirame Rhabdovirus (HIRRV), Yellowtail Ascites Virus (YAV), Striped Jack Nervous Necrosis Virus (SJNNV), Ranavirus (EHN), Orthomyxovirus (ISA) and many others (Maeda 2001, Saravanan et al. 2012).

Beneficial bacteria able to suppress the growth of other bacteria may also potentially inhibit the growth of viruses because viruses have the ability to transfer through live cells (Maeda 2001). If the aquatic environment is dominated by bacteria infected with viruses, viral infection of fish may extend to a larger scale. Probiotic bacteria may be an alternative way to prevent viral diseases in aquaculture. Probiotics such as Pseudomonas sp., Vibrio sp., Aeromonas sp., and groups of coryneforms isolated from salmonid hatcheries have antiviral activity against infectious IHNV, with more than 50 percent plaque reduction (Kamei et al. 1988). Poliovirus infection is greatly reduced by the marine bacterium Moraxella sp. (Girones et al. 1989). Two strains of Vibrio sp. (NICA 1030 and NICA 1031) isolated from a black tiger shrimp hatchery had antiviral activities against IHNV and Oncorhynchus masou virus (Direkbusarakom et al. 1998). Although some probiotic bacteria may inhibit the transmission of viruses among fish in a population, the exact mechanism is still not well understood (Balcázar et al. 2006).

**Water Quality Improvement**

Accumulation of organic and nitrogenous wastes e.g. ammonia and nitrite, are a concern in aquaculture. Water quality is one of the criteria associated with fish disease outbreaks on farms. Toxic ammonia build-up in the water can cause environmental stress to farmed fish. In nature, these toxic substances are transformed into safer forms by ammonia-oxidizing bacteria (ammonia to nitrite) and nitrite-oxidizing bacteria (nitrite to nitrate) (Qi et al. 2009). However, during the peak of the production cycle, heavy loads of nitrogenous waste produced by cultured species in the water may accelerate water quality deterioration. Probiotics such as Bacillus sp. can improve pond water quality by decomposing organic matter to CO₂, especially during intensive production. In Malaysia, use of probiotics such as photosynthetic bacteria and Bacillus sp. can improve water quality, survival and growth rates of shrimp juveniles in commercial farms (Fig. 4). Use of Bacillus sp. can reduce the incidence of vibriosis on shrimp farms (Dalmin et al. 2001).

**Looking Ahead**

Recently research on probiotics has focused on identification of bioactive ingredients and compounds extracted from probiotic bacteria. This should help provide a clearer picture of the mode of action of probiotics in maintaining gastrointestinal health. However, much more work is needed to elucidate the potential benefits.

**Notes**

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