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Microbial intervention for better fish health in aquaculture: the Indian scenario

A. Panigrahi · I. S. Azad

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Abstract Aquaculture has evolved as the fastest growing food-producing sector and developed as an important component in food security. To keep a sustainable growth pattern, health management strategies must go beyond antibiotics and chemotherapeutics, which create resistant bacteria and immunosuppression in the host. Besides development of drug resistant bacteria and pathogens, the adverse effect of antibiotics is caused by their influence on the aquatic microflora, and the retention of harmful residues in aquatic animals. On the other hand, the microbes with their unique structure and cell wall components can trigger immunity, and thus exposure plays an important role in the evolution. Microbial intervention through an environmentally friendly approach is an alternative method of health management. India is endowed with a bounty of varied climatic conditions, microbial diversity and fish fauna and aquaculture systems offering challenges in biological and environmental pursuits. Producing about 4.4% of world's fish and ranking third in global fish production, India trades about 2.4% in global fish market with the annual export earning being over $1311 million. Use of microbes for beneficial purposes is increasingly recognized as a valuable input for sustainable and responsible aquaculture. Microbial intervention in aquaculture can be broadly water/environment based through bioaugmentation, biostimulation, biocontrol measures, or (to generate) host response through probiotics, immunostimulants, and vaccines. Also, application of molecular methods such as polymerase chain reaction (PCR) and nucleic acid techniques are making increasing inroads into aquatic microbiological research in India. This paper elucidates all these aspects of microbial intervention in aquaculture, high-lighting Indian research and accomplishments.

Keywords Bioaugmentation · Biocontrol · Biostimulation · Immunostimulants · Probiotics · Vaccines

A. Panigrahi (✉) · I. S. Azad
KRC of CIBA (Central Institute of Brackishwater Aquaculture), Kakdwip, West Bengal 743-347, India
e-mail: apanigrahi2k@yahoo.com

I. S. Azad
Mariculture and Fisheries Department,
Kuwait Institute of Scientific Research,
P.O. Box 1638, Salmiya 22017, Kuwait

Introduction

Agriculture and production of food by man during the course of civilization were probably the first human interventions that resulted in the development of various specialized branches of food production. Aquaculture has emerged as one of the important branches of food production. Sustained and enhanced productivity are the major goals of aquaculture. Adoption of various measures and technologies such as intensive aquaculture, spatial and temporal

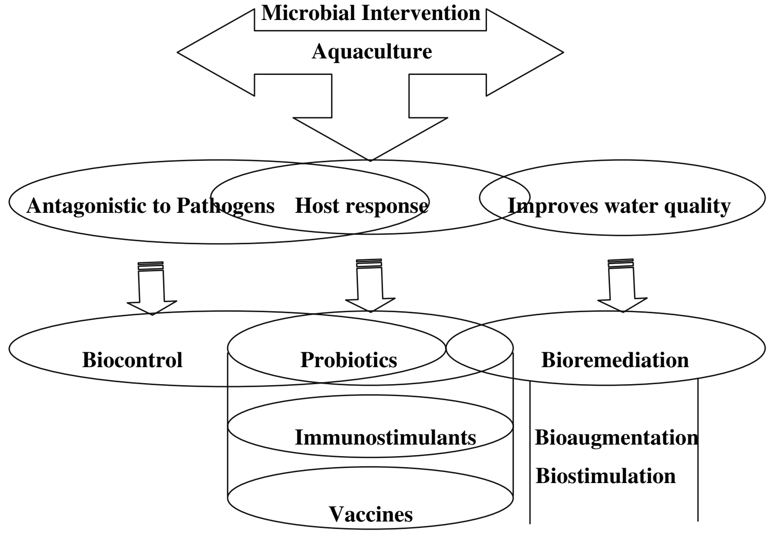

Fig. 1 Microbial intervention in aquaculture acts either as biocontrol (antagonist to pathogens), for host response (protective and physiological) or improves water quality (bioremediation). Different degrees of crossover show the overlapping and/or interrelationship between the interventions

expansion of aquaculture activities, introduction of new species for aquaculture, improved health management strategies, and so forth, are responsible for the steep rise in aquacultural production and productivity. Diseases have become an integral part of intensive aquaculture necessitating the use of chemicals, drugs and antibiotics in health management. Although these measures produced enhanced productivity, continual use of chemicals and fertilizers are known to have deleterious effects on the environment and sustained productivity (World Health Organization antimicrobial resistance fact sheet 194, http://www.who.int/inf-fs/en/fact194.html). Some of the insecticides and biocides used in aquaculture are known to accumulate and concentrate in aquatic organisms, while antibiotics may well induce resistance in pathogens through mutagenesis and plasmid mediated gene transfers (Toranzo et al. 1984). In recent years, attention has been focused on the interactions between host and microbial molecules, which may determine the quality and quantity of the host's immune responses (Wilson et al. 1998). Non-specific defense is the first line of defense against microbial infections in vertebrates. Microbial interventions were initiated to make aquatic production more sustainable and disease management measures more environmentally friendly. In aquaculture, this may be achieved by the maintenance of balanced populations of bacteria and by the use of defined probiotics in a number of ways such as enrichment of larval food, inclusion in the diet, or addition to the water, as a remediation agent. Microbial intervention (e.g., by probiotics and prebiotics or through biocontrol and bioremediation measures (Fig. 1)) offers an alternative to health management in aquaculture and improves the aquatic environment.

Awareness campaigns have been organized by various agencies to explain the harmful effects of antibiotics in aquaculture and "special weeks" are organized that advocate good farming practices, including the regulated use of antibiotics and drugs. Use of antibiotics disturbs the microbiological balance of gut flora eliminating most of the beneficial flora. The use of antibiotics is discouraged as it has led to the appearance of drug-resistant bacteria, immunosuppression in animals besides harmful effects on the environment and concerns on food safety. Moreover, aquacultural products are sometimes banned due to rejection of export consignments. Hence, usage of probiotics is propagated to counter the effect of viral and bacterial infections in commercial aquaculture. It is reported that fish ingest only 20–30% antibiotics and the remaining reach the environment. Even the antibiotics ingested by aquatic animals may be excreted as such or as metabolites, which might be harmful to the animal as well as to human consumers. Pathogens such as *Vibrios* and *Aeromonas* can develop resistance to antibiotics very quickly. Biomagnification of antibiotics through the food chain before it is discharged in the ecosystem is another topic to be studied. So there is a definite need for the alternative health management strategy, which can be accomplished by microbial intervention.

Microbial interventions in aquaculture can broadly be divided into the following headings:

- Bioremediation (bioaugmentation and biostimulation)
- Probiotics
- Immunostimulation
- Vaccination

Bioremediation

Traditional aquaculture system of pokkali paddy-cum-fish of Kerala in India is probably the best example of a microbial intervention. These traditional farms are more productive than similar production systems due to the enrichment of aquatic medium and enhancement of primary productivity. Unlike in agriculture where plants use nutrients from the soil for primary productivity, aquatic organisms such as fish and shellfish depend on the primary producers, the plankton, for growth and development. Uptake of nutrients by plants is rendered more efficient through microbial interventions in the form of microbial biofertilizers (Nagaraju et al. 2001). Studies have shown that similar nutrient utilization occurs naturally in aquatic ecosystems too. Of the two important nitrogen-fixing bacteria, *Azotobacter* and *Azospirillum*, the former is more abundant in the freshwater aquatic ecosystems because of its aerobic nature (Das and Ayyappan 1998). An in vitro evaluation of these two bacterial species revealed that *Azotobacter* helps in raising PO_4-concentrations of the water phase and hence, phosphorus was not limiting for plankton productivity (Tripathy and Ayyappan 2005). Similar studies on the nitrogen fixing bacteria (Garg and Bhatnagar 1999) and optimization of exploitation of nutrients by bacteria through manipulation of CN ratios (Jana et al. 2001) were carried out that helped in achieving enhanced fresh water aquaculture production.

Biofilms and substrate based aquaculture

Biofilm and periphyton-based complex microbial systems have been intensively studied for their beneficial effects on the productivity of carp aquaculture by two independent groups working in India and Bangladesh. These investigations have shown that periphyton and biofilm-based complex microbial communities develop on the substrate provided in the production systems and results in enhanced overall productivity from such aquaculture systems (Shankar et al. 1998; Ramesh et al. 1999; Dharmaraj et al. 2002; Keshavanath et al. 2004; Mridula et al. 2005; Azim et al. 2003a, b, 2004; Uddin et al. 2006). These pioneering scientific studies involving the periphyton and biofilm-based microbial interventions gave rise to a substrate based low-cost aquaculture especially for omnivore and herbivore freshwater fish. Systematic investigations are lacking on the role of biofertilizers in marine and brackish water aquaculture.

Bioaugmentation involves seeding of bacteria that purify aquaculture water making it less stressful to the cultured organism. Depending on stocking density, total phosphorus, nitrogen and suspended solids in case of a shrimp farm were reported to be as high as 321, 668 and 215,000 kg/ha/cycle, respectively (Dierberg and Kiattisimkul 1996). The pond aquatic conditions can become toxic due to high total ammoniacal nitrogen (TAN). Under such systems, organic load in the pond water and discharge water has to be managed to avoid eutrophication. Mechanical treatment and effluent biofiltration systems involve a high capital cost, and other technical problems, making bioaugmentation a suitable alternative for aquaculture systems.

Nitrification takes place naturally by two groups of bacteria: *Nitrosomonas* and *Nitrobacter*. Both being slow growing species and the practice of water exchange does not allow them to get established in the pond and to perform in an efficient manner. Thus, measures of bioaugmentation by way of providing correct concentrations of bacteria on a stabilized substrate have to be implemented. Efficacy of commercial bioaugmentation products has been questionable due to the reasons of inadequate substrate, inter-specific competition, growth inhibition and insufficient acclimatization period (Stephenson and Stephenson 1992). Hence, considerable research on the isolation and the establishment of native beneficial bacteria on suitable substrates has to be carried out. Removal of TAN using enriched immobilized cultures of autochthonous nitrifying bacteria has several potential advantages: longer biomass retention in pond water, maintenance of high microbial cell density, optimization of microbial growth and metabolic rates and protection from inhibitory compounds (Travieso et al. 1995; Yang 1997). Cultures of indigenous nitrifying bacteria immobilized on clay pellets were found to remove TAN efficiently with capacities ranging between 0.5 and 3.2 mg TAN per day. Bagasse, the fiber-rich solid by-product obtained after the extraction of sugarcane juice, used in bagasse-assisted bioremediation of ammonia from shrimp farm waste water was studied by Krishnani et al. (2006). They indicated that the bagasse-assisted substrate supported by the rich bacterial biofilm could remove TAN to an extent of 95% in 144 h.

Bioaugmentation and bioremediation measures, and technologies are being developed in various institutes and laboratories in India. The National Centre for Aquatic Animal Health (NCAAH), comprising a team headed by Dr. Bright Singh, has ventured into these aspects with tangible research accomplishments. A bioreactor technology (patent yet to be granted) meant for nitrifying seawater before and after larval rearing was developed (http://www.cfddm.org). The team also claims to have developed a cyanobacterial system consisting of two species of genera *Synechocistis* for the control of *Vibrios* in aquaculture ponds. Work on the isolation and characterization of bacterial antagonists has been carried out in India by several active working groups. The NCAAH, Cochin; the Marine Biotechnology (MIRCEN) laboratory headed by Dr. Karunasagar at College of Fisheries, Mangalore; the Central Institute of Brackishwater Aquaculture (CIBA), Chennai are notable among them. Several non-pathogenic bacteria are known for their anti-pathogenic effects and hence, have tremendous potentials for application in managing the microbial balance in favor of the host and in reducing the pathogen load. Lactic acid bacteria (Gatesoupe 1994; Ajitha et al. 2004), *Caronbacterium* (Robertson et al. 2000), *Bacillus* (Gatesoupe 1991; Rengpipat et al. 1998; Vaseeharan and Ramasamy 2003), *Vibrio* (Austin et al. 1995) and *Pseudomonas* (Chythanya et al. 2002; Vaseeharan et al. 2003; Vijayan et al. 2006) are some of the bacterial species known for their pathogen antagonism of *Micrococcus* sp.

Probiotics

Probiotics are defined as microbial cell preparations or components of microbial cells, which have a beneficial effect on the health and well being of the host (Salminen et al. 1999). Probiotics can help to build up the beneficial bacterial flora in the intestine and competitively exclude certain pathogenic bacteria. The Joint FAO/WHO Working Group Report on drafting guidelines for the "Evaluation of Probiotics" in Food (2002) defined probiotics as live microorganisms that confer a beneficial physiological effect on the host when administered in adequate amounts. During the past 80 years there have been numerous claims advocating therapeutic benefits of probiotics in human. Many studies elucidate the use of lactic acid bacteria as probiotics (Gatesoupe 1994, 1999; Ringø and Gatesoupe 1998; Robertson et al. 2000; Verschuere et al. 2000). Again, the probionts belonging to *Vibrionacea, Pseudomonads* and *Bacillus* are found to protect turbot, salmon, cod, prawns and oysters. Probiotic bacteria directly take up or decompose the organic matter or toxic material and improve the quality of water. The microbial cultures produce a variety of enzymes like amylase, protease, lipase, xylanase, and cellulase in higher concentrations compared to the native bacteria, which help to degrade waste. These bacteria have a wide range of tolerance for variations in salinity, temperature, and pH, which usually exist in aquaculture operations.

Probiotics is a big business today in Indian aquaculture, worth 109 million $, and most of them are imported. A survey in Andhra Pradesh, the leading State in India for aquaculture activities has revealed that farmers are using both water and feed probiotics (Rao et al. 1999). The water probiotics contain multiple strains of bacteria like *Bacillus acidophilus*, *B. subtilis*, *B. licheniformis*, *Nitrobacter* sp., *Aerobacter* sp., and *Saccharomyces cerevisiae* while feed probiotics contain *Lactobacillus* sp., *Bacillus* sp. or *Saccharomyces cerevisiae*. These are reported to give better survival and growth and improve the protective response especially in the larval stages. Regular use of probiotics in the feed of fish in United Kingdom and other European countries has been reported to have several health benefits. Atlantic salmon fed with probiotics showed reduced mortality caused by vibriosis, furunculosis and enteric red-mouth diseases. Moreover, fish showed enhanced appetite, grew better and had fewer problems with fin and tail rot. *Vibrio alginolyncus* introduced in larval rearing tanks caused a reduction in the incidence and severity of luminous vibriosis caused by *Vibrio harveyi* and improvement in growth of shrimp larvae.

Indian fish pathologists are also looking at probiotics as a potentially useful disease prevention measure in aqua farms and active research is continuing in this regard. The institutes involved in promoting this research include the National Institutes such as Central Marine Fisheries Research Institute, Kochi; the College of Fisheries, Mangalore, Karnataka; the National Institute of Oceanography, Goa; the Central Salt and Marine Chemicals Research Institute, Bhavnagar, Gujarat and the Cochin University of Science and Technology, Kochi, Kerala. Some of the important studies on the role of probiotics in aquaculture in India include the work done by Vasudevan (2000), Sridhar and Paul Raj (2001). Again the role of probiotics in health management of fish is studied by Karunasagar (2001), Azad et al. (2005) and Panigrahi et al. (2004, 2005, 2007).

A powerful spore forming bacteria acidifies the gut and therefore prevents the growth of different pathogens such as C*lostridium, Streptococci, Escherichia, Salmonella*, and *Vibrio* sp. These bacteria are also capable of releasing enzymes such as amylase, protease and lipase in substantial quantities along with B group of vitamins. This has a very positive effect on digestion of feed material. Several factors (such as poor diet, stress, antibiotics, aging) can tilt the scale in the direction of the pathogenic bacteria and perhaps probiotic bacteria can help in this situation.

The principal probiotic yeast now in use is *Saccharomyces boulardi (Saccharomyces cerevisiae)*, a non-pathogenic yeast. The aquaculture industry uses many probiotics, most of which do not stand scientific evaluation but attract farmers for the so called best feed assimilation, restricting *Vibrio*, or other pathogenic forms resolving loose shell, blue shell, antenna cut and white gut, and so forth.

Lactobacillus GG was one of the first and best scientifically documented probiotic strains with significant health effects. Probiotic bacteria and their health benefits became the focus of intensive research in European Union, Japan and United States. For industrial production, they need very fast growth rate, and survival in product and during processing of preparations. Besides these requirements, safety of these strains is very important. Though there is no perfect system to quantify the level of safety of these strains, the important factors looked for are: mucus non-degradative, non-toxigenic, non-infectious, less active or inactive in genetic material transfer.

The bacterial strain is the core of the probiotic action; it is mandatory for the future to have a more in depth knowledge of each single strain used as probiotic supplementation. Another area of research will be the molecular ecology of the intestinal tract. Combining these two areas, we will be able to obtain information on the molecular mechanisms used by probiotic bacteria to exert the observed beneficial effects, an area which is still a black hole. Some pioneering research work pertaining to aquaculture by the author (Panigrahi et al. 2005, 2007) are significant.

Prebiotics

The concept of prebiotics in feed is fairly recent. Prebiotics are basically food for probiotics. They are supposed to be resistant to attack by endogenous enzymes and hence can reach the site of action to promote the proliferation of gut microflora. Some of the prebiotics, that are currently used in animal feed are mannan-oligosaccharides (MOS), fructo-oligosaccharide (FOS), and mixed oligo-dextran. Bacteria have lectins (glycoproteins) on the cell surface that recognize specific sugars and allow the cell to attach to that sugar. Binding of *Salmonella, E. coli, and Vibrio* sp. has been shown to be mediated by a mannose-specific lectin like substance present on the bacterial cell surface. In our experiments on immunomodulation, FOS along with different probiotics are used for their advantage in aquaculture.

Mechanism of action of probiotics

The three essential mechanisms proposed for the mode of action of probiotics are described in this section,

Antagonism (antimicrobials, Competition for nutrients, Competition for adhesion sites)

Probiotic strains alter the properties of indigenous microflora by affecting the balance between pathogenic and harmful microbes and beneficial microbes. They compete for adhesion sites and nutrients with other microbes in the niches. They also induce the host defence by inducing the production of antimicrobial proteins like defensins. To give all the said beneficial effects, probiotic strains are required to have certain important characters such as tolerance to gastric acidity and bile salts, resistance to digestive enzymes, adherence to enterocytes, production of antimicrobial substances and ability to grow and survive in the gastrointestinal environment.

Alteration of microbial metabolism (Enzymes)

Probiotics antagonise other microbes by producing antimicrobial substances like organic acids, bacteriocins, bacteriocin-like substances, hydrogen peroxide, non-proteinaceous inhibitory metabolites such as diacetyl, reuterin and other compounds.

Stimulation of immunity (Antibodies, Macrophages)

Other protective mechanisms include antitumorogenic, hypocholesteremic, antimutagenic, anticarcinogenic properties, and so forth.

Immunomodulation

Probiotics help improve immune activity of host by improving barrier properties of mucosa, sampling and modulating production of cytokines. Viable live probionts are better than the non-viable heat killed probionts in inducing a higher immune response in rainbow trout especially in the head kidney leucocyte phagocytosis, serum complements, etc (Panigrahi et al. 2005). In recent years, a number of in vivo and in vitro studies have investigated the interaction between dietary probiotics and immunocompetence. By increasing the host's specific and non-specific immune mechanisms, lactic acid bacteria (LAB) can protect the host against infection by enteric pathogens, and tumor development. Immunological mechanisms behind the probiotic action may include:

- Stimulation of specific antibody secreting cell response (Kaila et al. 1992).
- Enhancement of pathogen phagocytosis (Panigrahi et al. 2004, 2005).
- Modification of cytokine production (Panigrahi et al. 2007).

Consequently, probiotic bacteria may influence both specific and non-specific immune responses. Probiotics reverse the increased intestinal permeability induced by antigens.

Modification of cytokine production

The augmentation of the immune response by probiotic bacteria, a phenomenon similar to that of cholera toxin, may also occur in adherence with gut-associated lymphoid tissue (GALT) and may therefore directly affect leukocytes by stimulating phagocytosis (De Simone et al. 1987). Probiotic bacteria may also hydrolyze milk proteins, producing bioactive peptides, which may trigger gut immune responses (Sutas et al. 1996). Alternatively, probiotic bacteria can further induce receptor expression. Oral allergen challenge reduces production of interferon (IFN-γ) by peripheral blood mononuclear cells in hypersensitive subjects. Lipopolysaccharide (LPS) of gram- negative bacteria induces production of proinflammatory cytokines, tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6), as well as IL-10, which is known to inhibit the synthesis of the former two cytokines (Maxer et al. 1991). These cytokines contribute to defense mechanisms of the host (Peddie et al. 2002) in response to bacterial colonization or invasion, and when secreted in excess, they may induce immunopathological disorders. Many components of the gram positive bacterial cell wall, for example, capsular polysaccharides, peptidoglycans and lipoteichoic acids have been shown to be involved in cytokine induction (Miettihen et al. 1996; Secombes et al. 2001). Several live LABS (*Bifidobacterium longum, B. animals, Lactobacillus paracascei, L. acidophilus, Lactobacillus* GG, *Lactococcus lactis, Lactobacillus plantarum*) are potent inducers of cytokines such as TNF- α release. In a series of experiments (Panigrahi et al. 2007), an attempt was made to draw some relation between the different characteristics of probionts and the immune response they elicit in rainbow trout. We evaluated the ability of three different bacterial species to induce non-specific immune responses and the expression of certain cytokine genes, IL- β1 and 2, TNF- α1 and 2 and transforming growth factor (TGF-β). Temperature is a major environmental factor controlling microbial growth and the ideal conditions differ among microorganisms. A probiotic would be most effective when used in its optimum temperature range.

Acceptance of probiotic concept by regulatory bodies will be possible only if the mechanisms of action of probiotics is well explained. However, a lot more fundamental research has to be done to develop mechanisms to verify, models to certify and methods to quantify the beneficial effects of probiotics in aquaculture.

Immunostimulation

Immunostimulants are agents/factors that trigger the non-specific immune response and result in enhanced disease resistance. Several compounds have been reported to have immunostimulation properties. Many of these are derivatives or cellular components of bacterial, fungal, or animal origin. Laminarin, barley glucan, lactoferrin, levamisole, lipopolysaccharides, curdlan, scleroglucan, zymosan, inulin, chitosan, beta glucans, dextran, lentinan, krestin, saponins, herbal extracts, peptidoglycans, and so forth, are some of the examples of immunostimulants used in shrimp/fish aquaculture (Newman and Deupree 1994).

Aquatic animals, unlike their terrestrial counterparts, are constantly and intimately related with the composition of and changes in the surrounding environment. The aquatic environment supports their pathogens, which can reach densities sufficient to cause disease (Moriarty 1998). Opportunistic pathogens are more serious threats in aquaculture systems due to crowding and culture environment stress on the cultured species making it immuno-compromised. The early developmental stages of these organisms are naturally more susceptible to diseases due to the developing immune system and also because most larvae feed and filter huge amounts of micro particulates (Cahill 1990; Hagiwara et al. 1994; Ringø and Birkbeck 1999). Hence, crustaceans and the larval stages of fish depend on the nonspecific immune factors to fight against the invading pathogens (Ellis 1988; Vadstein 1997; Olsson et al. 1998). Several studies on the ontogeny of lymphoid organs and their maturation have been conducted in the last two decades.

Several studies on immunostimulation using microbial interventions have been conducted the world over. Use of bacterial preparations (bacterins) in crustaceans produced highly encouraging results (Itami et al. 1989, 1991; Karunasagar et al. 1996; Devaraj et al. 1998; Azad et al. 2002; Azad et al. 2005). Application of microbial interventions through whole bacterial cells delivered through feed is a practical, simple and effective way of inducing immunostimulation compared with the use of extracted or purified bacterial and fungal components. Lipopolysaccharide, the cell wall component of gram-negative bacteria, is known to activate shrimp immunity through transglutamase and phenol oxidase activation pathways (Albores et al. 1998).

Vaccines

Though microbial interventions during the larval stages of fish amount to immunostimulation rather than vaccination (as immunological maturity is age/time dependent during the larval development), the process through which specific pathogens are managed and the disease prevention strategies adopted, fall into the broader category of vaccines. Hence, we wish to cover these aspects of enhancing specific antibody-mediated immune enhancements under the head “vaccines”.

Ellis (1988) defined vaccine as a “preparation of antigens derived from pathogenic organisms, rendered nonpathogenic by various means, which will stimulate the immune system in such a way as to increase the resistance to disease from subsequent infection by a pathogen” The most striking feature of a vaccine is the induction of immunological memory so that future exposures to the primed antigens produce quick and intense immune response. The first reported effort of fish vaccination was made by Duff in 1942 and the next serious efforts in this field were made only during the 1970s and later (Evelyn 1997). Interestingly, this first vaccination effort was to orally vaccinate salmon against furunculosis (Duff 1942). Many of the vaccines developed for health management in aquaculture are whole cell preparations of bacteria. Schaperclaus (1954) was the first to attempt vaccination using *Aeromonas hydrophila* against bacterial hemorrhagic septicemia. Song and Kou (1981), Thune and Plumb (1982) tried vaccination of different fish species through various immunization routes against infection by the motile aeromonads. Contributions of Fryer and his group of researchers in the field of vaccination against vibriosis during the 1970s are note worthy (Fryer et al. 1976, 1978). Immersion, spray, injection and hyperosmotic infiltration have been different methods of vaccine delivery tested in many of the reported vaccination studies (Evelyn 1997).

Due to the ease, simplicity and practical applicability oral vaccination became the choice of antigen delivery. However, apart from Duff’s success of oral vaccination further studies were concentrated on immersion and injection vaccination. Oral vaccination attempts made during the 1970s and 1980s were either unsuccessful (Schachte 1978) or produced variable results (Fryer et al. 1976, 1978; Amend and Johnson 1981). These inconsistent and variable results obtained in oral vaccination trials were mostly due to un-availability of intact and sufficient antigens at the immune responsive hindgut of fish (Rombout et al. 1985). Several attempts were made to make the oral vaccines effective by means of protected antigen delivery. Oral or anal intubation of antigens (Davina et al. 1982; Rombout et al. 1986), encapsulated antigen microspheres (Piganelli et al. 1994; Dalmo et al. 1995) and bioencapsulation through live food organisms such as artemia and other planktons (Chair et al. 1994; Joosten et al. 1995) were some of the important studies carried out on improved oral antigen delivery.

Studies on fish vaccination in India were aimed mainly to design strategies of immunoprophylaxis against the motile aeromonad septicemia in freshwater carp culture. Whole cells of *A. hydrophila* were used in many of these investigations. The first attempt was made by Karunasagar and his team (Karunasagar et al. 1991). They used hemolysin negative mutant of *A. hydrophila* to vaccinate Indian major carps and recorded high protection to homologous and moderate protection to heterologous challenges. Investigations carried out using the biofilms of *A. hydrophila* in oral vaccination provided highly encouraging results. Induced biofilm mode of growth enables mimicking the polymer cover of synthetically encapsulated antigens. The glycocalyx cover of the bacterial biofilms was intended to serve the purposes of external capsule to resist gastric/enzymatic digestion of the antigens (Azad et al. 2000a, b). This mode of antigen delivery was found to deliver antigens in considerable quantities to the hindgut of carps as confirmed through monoclonal antibody-based antigen localization. Biofilm of *A. hydrophila* was also found to elicit higher serum anti-*Aeromonas* antibodies (Azad et al. 1999), contrary to the belief that oral antigens do not result in the production of serum antibodies (Fryer et al. 1976). This concept of biofilm antigens in oral vaccination was tested (Asha et al. 2004; Nayak et al. 2004) in Asian catfish (*Clarias batrachus*), the fish with strong gastric digestion unlike carps. They also noticed significant serum anti-*A. hydrophila* antibodies in biofilm antigen fed catfish. Further studies in this direction have to be carried out using other pathogenic bacterial species to make oral vaccination a method of choice in fish vaccinology against bacterial pathogens.

Anbarasu et al. (1998) found that formalin inactivated vaccines were superior to heat killed preparations, especially when the bacterins of *A. hydrophila* were injected with adjuvants. Whole cells of *A. hydrophila* and the extracellular proteases (ECPs) were administered as a polyvalent vaccine intraperitoneally to Indian major carps with a booster at 28 days post priming (Chandran et al. 2002). They also recorded very high relative survival (80–90%), following a challenge with live bacteria.

Unlike *Vibrio*, *A. hydrophila* was a difficult antigen to work with and many investigations carried out worldwide resulted in variable immune responses. Motile aeromonads are antigenically a very diverse group of bacteria making serodiagnostics and vaccine production a difficult issue to tackle. Antigenic diversity within this group was found to be caused by H and O somatic antigens. Ewing et al. (1961) described 12 O-antigen groups and 9 H-antigen groups. Each group was further divided into a number of additional serotypes. Antigenic diversity among the strains of motile aeromonads isolated from a single population of fish and from different organs of a single fish was recorded (Chodyniecki 1965) reflecting the variability in this group of bacteria. Probably these variations are essential to the complexity of the oral vaccination.

Microbial management in aquaculture

This is becoming the most important sector of research in Indian aquaculture. Some of the issues that should be urgently further investigated are:

- Deciphering functional diversity of bacteria for use in aquaculture
- Microbiological strategy for improving the productivity of aquatic system (developing BGA biofertilizer technology, use of asymbiotic nitrogen fixers)
- Organic aquafarming with microbial interventions- microbial utilization through biodynamic preparation, biodynamic composts, and yeast/bacteria based biopreparations

- Antagonists—used as antipathogens, detoxicants, nutrient solublizers and growth promoters in aquaculture
- Microbes for aqua-waste management/composting including initiation in vermicomposting
- Microflora associated with decomposing aqua-waste

Conclusion

Microbial interventions can play a vital role not only in aquaculture productions, but also in environmental protection and sustainability, which is so vital for survival of this industry. Rational selection and validation of promising microbial strains should be based on evidence obtained from experiments with in vivo and in vitro set up with a reliable predicted value or function. Genetically modified microbes can also improve water and soil quality by reducing levels of phosphorus and nitrogen in aquacultural waste and should be evaluated. Globally, the demand for quality aqua products will increase and with microbial intervention it would be possible sooner than later to meet this demand. As the ban on antibiotics or restricted use is being encouraged not only because of the risk of resistance of bacteria and associated health hazards, but also because the suppressing effects on the immunity in aquatic animals, intervention through probiotics and other beneficial bacteria will be the best alternative. With the existing body of knowledge, rich biodiversity, and low cost of production, India needs to strengthen its research and development efforts, quality control, and introduction of HACCP and ISO standards. Microbial management in both the hatchery system and grow-out system are becoming an integral part of aquaculture practices, adding values both in terms of quality and productivity. However, there is still not adequate and consistent scientific evidence to substantiate some of the proposed health benefits of microbial intervention with regard to aquaculture and hence greater effort in microbial intervention studies is imperative. The future of this unexplored field will be best justified by taking immediate steps in understanding the extent and magnitude of microbial diversity in India and identifying the aquaculturally important microorganisms, elucidating the metabolic potential of the microbial diversity and application in aquaculture. Expanding the frontier of knowledge in the strategy for microbial intervention in aquatic systems and their limits and strengthening the disciplinary crossover of biochemistry, genetics, nutritional studies, engineering and molecular biology will eventually help to evolve microbial aquaculture biotechnology.

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