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***Research Article***

# USE OF PROBIOTICS AS BIOLOGICAL CONTROL AGENTS IN AQUACULTURE FOR SUSTAINABLE DEVELOPMENT

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## ABSTRACT
Aquaculture plays an important role in global food production. Due to changes in human dietary habits, fish consumption has been increasing, with more people changing to a healthier diet with an appropriate nutritional profile. Aquaculture is one of the fastest developing growth sectors in the world and Asia presently contributes about 90% to the global production. However, disease outbreaks are constraint to aquaculture production thereby affects both economic development of the country and socio-economic status of the local people in many countries of Asia-Pacific region Aquaculture has become an important source of fish available for human consumption. Antibiotics are very useful additions to any fish-health manager's toolbox, but they are only tools and not 'magic bullets "Survival of the fittest" holds good for men and animals as also for bacteria. the use of such expensive chemotherapeutants for controlling diseases has been widely criticized for their negative impacts like accumulation of residues, development of drug resistance, immunosuppressants and reduced consumer preference for aqua products treated with antibiotics and traditional methods are ineffective against controlling new diseases in large aquaculture industry has been achieved by following different methods using traditional ways, synthetic chemicals and antibiotics. One of the alternative method is use of probiotics is one of such method that is gaining importance in controlling potential pathogens. This review provides a summary of the criteria for the selection of the potential probiotics, their importance and future perspectives in aquaculture industry. Resistance mechanisms can arise one of two ways: chromosomal mutation or acquisition of plasmids. Chromosomal mutations cannot be transferred to other bacteria but plasmids can transfer resistance rapidly. Several bacterial pathogens can develop plasmid-mediated resistance.

***Keywords:*** *Aquaculture, Antibiotics, Chemicals, Diseases, Ineffective, Probiotics, Controlling Pathogens*

## INTRODUCTION
Global consumption of fish has doubled since the early 1970s and will continue to grow with population, income, and urban growth in the developing world in the golden age of the discovery of antibiotics, innumerable lives were saved. These highly potent "miracle" drugs are no longer as effective as they were a half a century ago. Globally, tones of antibiotics have been distributed in the biosphere during an antibiotic era of only about 60 years duration Antibiotics are drugs of natural or synthetic origin that have the capacity to kill or to inhibit the growth of micro-organisms. Antibiotics that are sufficiently non-toxic to the host are used as chemotherapeutic agents in the treatment of infectious diseases of humans, animals and plants.

Aquaculture is currently the fastest growing food production sector in the world, but diseases especially bacterial infections remain primary constraints to its continued expansion (Abd *et al.,* 2009; Pieters *et al.*, 2008; El-Haroun *et al.,* 2006). Micro-organisms have been implicated in this problem and its control in aquaculture is a challenge (Ringo and Birkbeck, 1999). Thus heavy reliance on vaccines and antibiotics to combat these diseases is inevitable and their use for decades.

According to the UN Food and Agriculture Organization, aquaculture is growing more rapidly than all other animal food-production sectors (www.fao.org). Its contribution to global supplies of several species of fish, crustaceans and mollusks increased from 3.9% of total production by weight in 1970 to 33% in 2005. It has been estimated that fisheries and aquaculture supplied the world with about 110 million metric tons of food fish per year (FAO, State of World Fisheries and Aquaculture, 2010), providing a per capita supply of 16.7 kg (live weight equivalent). Of this supply, 47% is derived from aquaculture production. Intensive aquaculture (shrimp and fish farming) has led to growing problems with bacterial diseases, the treatment of which now requires the intensive use of antimicrobials.

***The Present Scenario in India***

Antibiotics and antibacterial substances are indiscriminately being used in India in shrimp farms in Andhra Pradesh, Tamil Nadu, Kerala & Karnataka. Maximum ~ in Andhra Pradesh. A recent study in Central Institute of Fisheries Technology, Cochin showed that out of the 2086 samples of farmed shrimp (Tiger prawn. White prawn and Freshwater scampi) Tetracyclines were detected in 434 samples and Chloramphenicol in 58 samples. Recently, Chloramphenicol and Nitrofurans were detected in Indian farmed shrimp exported to Spain, Netherlands and UK in ppb levels. All the consignments were confiscated and destroyed. Indian Exporters lost nearly Rs.lOO crores. Note: Farmed shrimps from China. Vietnam & Philippines are banned in EU countries for the presence of Chloramphenicol. The Government of India, Ministry of Agriculture vide notification No.33035/4/2002-Fy(T2) dated July 5, 2002 has banned the use of 24 antibiotics and pharmacologically active substances in aquaculture.

***Traditional Methods of Disease Control in Aquaculture***

**Use of chemicals:** Prophylactic measures are taken to disinfect the pond and the gears. The nets and other tools are routinely disinfected with benzalkonium chloride solution. Ponds having fish and threatened with disease outbreak are disinfected with either potassium permanganate (0.5 g/100 L) or benzalkonium chloride solution (600 ppm) or calcium cyanamide. Formalin (40% formaldehyde) is a traditionally tried chemical. It is very effective at concentrations of 167 to 250 mg $L^{-}$) against parasitic infections of protozoan ectoparasites (*Costia*) and trematodes like *Discocotyle*. Organophosphates are useful for such ectoparasites where formalin fails. These are effective against crustacean parasites like *Lernaea* and *Argulus* and such trematodes as *Gyrodactylus*. Malachite green is the next most tried chemical, especially effective against fungal infections including *Saprolegnia*. It is used at a concentration of 1-2 mg $L^{-1}$. Copper sulphate is effective against bacterial infections but it is not favored for reasons of its toxic effects. Quaternary ammonium compounds are very effective against bacterial infections. These are generally used at concentrations of 1-4 mg $L^{-}$). Furazolidane, a nitrofuran, popular in veterinary therapy, is used in USA for incorporation in fish food to control/prevent bacterial infection; the recommended dose is 11 g/100 kg fish/day for about a week. Sulphadiazine, a sulphonamide, mixed with trimethoprim, is also found to be equally effective against bacterial diseases when administered through diet; the recommended dose is $5^{1}/_{2}$ g/100 kg fish/day for a week These chemicals are, however, non specific with low efficacy, that is why, these are less reliable. Epson salt (magnesium sulphate) mixed with diet when given is found very useful in treatment of protozoan parasites of the gut. Di-n-butyl tin oxide also administered via diet with a dose of 25 g/100 kg fish/day for three days is effective against most parasites of the gut other than protozoans.

**Use of antibiotics:** Earlier, the control of fish diseases was focused on the use of chemotherapeutants, such as erythromycin and deoxycycline. Oxytetracycline an antibiotic used in veterinary therapy is commonly used for bacterial therapy of vibriosis and ulcer disease in fishes; the recommended dose is $7^{1}/_{2}$ g/100 kg fish/day for one to two weeks. But now focus is increasingly being shifted to vaccination. Combined with the problem of antibiotic contamination of aquaculture facilities and livestock, the indiscriminate worldwide use of antibiotics in aquaculture has led to the development of drug-resistant bacteria which are becoming increasingly difficult to control and eradicate; ). To keep a sustainable growth pattern, health management strategies must go beyond antibiotics and chemotherapeutics which create resistance in the 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 (Subsequently, certain antibiotics such as chloramphenicol have been banned in many countries.

**Hygiene maintenance:** Hygiene maintenance is the most important part of prevention of fish diseases. In traditional methods, pond hygiene used to be the first priority of pond management. Pond must be periodically dried and then refilled with abundant water of good quality. Pond should be well maintained to avoid silting and weeds are controlled. High stocking densities, over feeding and pollution are avoided.

Entry of wild fish or restocking of ponds with unhealthy or sub quality eggs and young fish are prevented. Too frequent handling, transfer or transport of pond fish is avoided. The condition of gills must be periodically checked in random samples of the selected fishes. As soon as the onset of a disease is noticed, the seriously affected, moribund or the dead fish must be removed from ponds and buried with quicklime.

**Use of probiotics in aquaculture:** Pro: favor, Bios: life. An antonym of antibiotic, probiotics involves in multiplying few good/useful microbes to compete with the harmful ones, thus suppressing their growth. These include certain bacteria and yeasts that are not harmful on continued use for a long time Administration of beneficial organisms to animals started in the 1920's and the name "probiotics" was introduced by Parker when the production of bacterial feed supplements began on a commercial scale. A growing concern for the high consumption of antibiotics in aquaculture has initiated a search for alternative methods of disease control. One of the methods gaining recognition for controlling pathogens within the aquaculture industry is the use of beneficial or probiotic bacteria). Improved resistance against infectious diseases can be achieved by the use of probiotics.

Probiotics are live microorganisms administered in adequate amounts as feed or food supplements which have beneficial effects on the intestinal microbial balance of the host. These are emerging as significant microbial food supplements in the field of. In aquaculture, the term Aprobiotics@ is often loosely used to describe a microbial formulation responsible for biocontrol or bioremediation of pathogens. The term probiotics comes from the Greek word "pro bios" meaning "for life". The original definition of probiotics given by Lilly and Stillwell (1965) "substances produced by one protozoan that stimulated the growth of another" was expanded from an agricultural perspective and redefined as "a live microbial feed supplement which beneficially affects the host animal its intestinal microbial balance" (Fuller, 1989). Probiotics are commonly defined as mono- or mixed cultures of live microbes that, when applied to animal or human, generate a beneficial effect on health of the host. These beneficial effects include disease treatment and prevention as well as improvement of digestion and absorption in the host.

However, according to Salminen *et al.*, (1999), probiotics include microbial cell preparations or microbial cell components. Gatesoupe (1999) redefined probiotics for aquaculture as microbial cells that are administered in such a way so that these could enter the gastrointestinal tract of the aquatic animal to be kept their alive, with the aim of improving its health. Various bacteria commercially used as probiotics are: *Lactobacillus* species viz. *L. acidophilus, L. casei, L. fermentum, L. gasseri, L. johnsonii, L. lactis, L. paracasei, L. plantarum, L. reuteri, L. rhamnosus, L. salivarius*; Bifidobacterium species viz. *B. bifidum, B. breve, B. lactis, B. longum* and *Streptococcus* species viz. *S. thermophilus*. Likewise, non-bacterial sources of probiotics are yeast. The yeast, ***Saccharomyces cerevisiae***, has also been commonly identified as probiotic. It stimulates immune system of fish for the production of inhibitory substances against foreign antigen Yeasts are not affected by antibiotics. This is advantageous in probiotic preparations used for preventing disturbances in the normal microflora in the presence of antibacterial metabolites. Strains of ***Saccharomyces cerevisiae*** and *Dermocystidium hansenii* have been shown to attach and grow in the intestinal mucus of fish.

Probiotics protect their host against neighboring or invading pathogens by interfering with their cellular functions. Probiotics may protect their host from pathogens by producing metabolites which inhibit the colonization/growth of other microorganisms or by competing with them for resources such as nutrients or space.The addition of potentially probiotic microorganisms to culture water in larval fish systems is a means of biocontrol. It is possible that some of these may be ingested and has a probiotic effect on the host animal.

Probiotics are marketed in two forms a) Dry forms: the dry probiotics that come in packets can be given with feed or applied to water and have to be brewed at farm site before application Each kit of dry probiotics contains a packet of dry powder and a packet of enzyme catalyst. Brewing has to be done in clean disinfected water after emptying the packets and blending thoroughly. Usually, it is brewed at 27–32°C for 16 to 18 hours with continuous aeration. The finished products must be used within 72 h. Maximum aeration is required in semi-intensive culture ponds. If aeration is less, the application of

probiotics has to be spread for two consecutive days, applying 50% of the dose each time. Liquid forms: The hatcheries generally use liquid forms which are live and ready to act. These liquid forms are directly added to hatchery tanks or blended with farm feed. The liquid forms can be applied any time of the day in indoor hatchery tanks, while it should be applied either in the morning or in the evening in outdoor tanks. Liquid forms give positive results in lesser time when compared to the dry and spore form bacteria, though they are lower in density. There are no reports of any harmful effect for probiotics but it is found that the BOD level (biological oxygen demand) may temporarily be increased on its123
therefore it is advisable to provide subsurface aeration to expedite the establishment of probiotics organisms. A minimum dissolved oxygen level of 3% is recommended during probiotics treatment.

***Benefits of Probiotics in Aquaculture***

1. Antiviral activity: Some bacteria used as candidate probiotics have antiviral activities. Though the exact mechanism by which these bacteria do this is not known, laboratory tests indicate that the inactivation of viruses can occur by chemical and biological substances, such as extracts from marine algae and extracellular agents of bacteria. It has been reported that strains of Pseudomonas sp., Vibrios sp., Aeromonas sp., and groups of coryneforms isolated from salmonid hatcheries, showed antiviral activity against infectious hematopoietic necrosis virus (IHNV) with more than 50% plaque reduction. Girones *et al.,* reported that a marine bacterium, tentatively classified in the genus Moraxella, showed antiviral capacity, with high specificity
for poliovirus.

2. Production of inhibitory compounds: Probiotic bacteria release a variety of chemical compounds that are inhibitory to both gram-positive and gram-negative bacteria. These include bacteriocins, siderophores, lysozymes, proteases, hydrogen peroxides etc. Lactic acid bacteria (LAB) are known to produce compounds such as bacteriocins that are inhibitory to other microbes.

3. Influence on water quality: Probiotics also help improve the water quality in aquaculture ponds. This is due to the ability of the probiotic bacteria to participate in the turnover of organic nutrients in the ponds. However, there are few scientifically documented cases in which bacteria have assisted in bio-augmentation, with the notable exception of manipulating the NH3 /NO2
/NO3 balance [51] in which nitrifying bacteria are used to remove toxic NH3 (and NO2).

Fish expel nitrogen waste as NH3 or NH4+ resulting in rapid buildup of ammonia compounds which are highly toxic to fish [52]. Nitrate, in contrast, is significantly less toxic being tolerated in concentrations of several thousand mg perlitre. Several bacteria e.g. Nitrosomonas, convert ammonia to nitrite and other bacteria e.g. Nitrobacter, further mineralize nitrite to nitrate. Nitrifying bacteria excrete polymers allowing them to associate with surfaces and form biofi lms. Recalculating systems must employ biofilters to remove ammonia, and Skjolstrup et al demonstrated a 50% reduction in both ammonia and nitrite in an experimental fluidised biofilter in a rainbow trout recirculating unit. Sulfur-reducing bacteria oxidize organic carbon using sulfur as a source of molecular oxygen. The hydrogen ion released when organic carbon fragments are oxidized is combined with sulfate to form sulfide which is less toxic to the aquatic animals. Methane-reducing bacteria use carbon dioxide as a source of molecular oxygen. Methane diffuses into the air and thereby improves the water quality.

4. Enhancement of immune response: The non-specific immune system can be stimulated by probiotics. It has been demonstrated that oral administration of Clostridium butyricum bacteria to rainbow trout enhanced the resistance of fish to vibriosis, by increasing the phagocytic activity of leucocytes Rengpipat et al reported that the use of Bacillus sp. (strain S11) has provided disease protection by activating both cellular and humoral immune defenses in tiger shrimp (Penaeus monodon). Balcazar ()[1] demonstrated that the administration of a mixture of bacterial strains (Bacillus and Vibrio sp.) positively infl uenced the growth and survival of juveniles of white shrimp and presented a protective effect against the pathogens Vibrio harveyi and white spot syndrome virus. This protection was due to a stimulation of the immune system, by increasing phagocytosis and antibacterial activity. In addition, Nikoskelainen *et al.,* () showed that administration of a lactic acid bacterium Lactobacillus rhamnosus (strain ATCC 53103) at a level of 105 cfu g–1 feed, stimulated the respiratory burst in rainbow trout (Oncorhynchus mykiss).

5. Competition for nutrients: Probiotics utilizes nutrients otherwise consumed by pathogenic microbes. Competition for nutrients can play an important role in the composition
of the microbiota of the intestinal tract or ambient environment of the cultured aquatic organisms Hence, successful application of the principle of competition to natural situation is not easy and this remains as a major task for microbial ecologists.
6. Source of nutrients and enzymatic contribution to digestion: Some researchers have suggested that probiotic microorganisms have a beneficial effect in the digestive processes of aquatic animals. In fish, it has been reported that Bacteroides and Clostridium sp. have contributed to the host's nutrition, especially by supplying fatty acids and vitamins. Some microorganisms such as Agrobacterium sp., Pseudomonas sp., Brevibacterium sp., Microbacterium sp., and Staphylococcus sp. may contribute to nutritional processes in Arctic charr (Salvelinus alpines L.). In addition, some bacteria may participate in the digestion processes of bivalves by producing extracellular enzymes, such as proteases, lipases, as well as providing necessary growth factors. Similar observations have been reported for the microbial flora of adult penaeid shrimp (Penaeus chinensis), where a complement of enzymes exists for digestion and synthesis compounds that are assimilated by the animal. Microbiota may serve as a supplementary source of food and microbial activity in the digestive tract may be a source of vitamins or essential amino acids.
7. Competition for adhesion sites: Probiotic organisms compete with the pathogens for the adhesion sites and food in the gut epithelial surface and finally prevent their colonization Adhesion capacity and growth on or in intestinal or external mucous has been demonstrated in vitrofor fish pathogens like Vibrio anguillarum and Aeromonas hydrophila.
8. Interaction with phytoplankton: Probiotic bacteria have a significant algicidal effect on many species of microalgae, particularly of red tide plankton Bacteria antagonistic towards algae would be undesirable in green water larval rearing technique in hatchery where unicellular algae are cultured and added, but would be advantageous when undesired algae species are developed in the culture pond.

***Limitations***

Probiotics can be used in advance as prevention tools. They can prevent the disease rather than treatment of the disease. They can be established well in static or low water exchange systems (re-circulatory system). They are effective if applied as soon as the water medium is sterilized before contamination with other microbes In the process of application of probiotics, no other chemical or drug should be used for treating other diseases like fungal and protozoan diseases caused by those other than bacteria. These probiotics can easily be destroyed by any other chemical or drug which generally interferes with the establishment of useful microbes.

## CONCLUSION

Diseases take a heavy toll of the aquaculture production. Traditionally, these diseases are controlled by using chemical compounds. The use of antimicrobials is a common practice in practically all types of hatcheries. The latter can result in the development of resistant strains of bacteria. Such resistance can be readily transferred to other strains. Therefore, the need of alternative measures to control these diseases is of prime importance. In recent years probiotics have a center stage and are used as alternative measures to control the aquatic diseases. Probiotics inhibit pathogenic microorganisms and have been used therapeutically to treat a variety of gastrointestinal and even systemic disorders. The use of probiotics will prove a new ecofriendly alternative measure for sustainable aquaculture.

## REFERENCES

**Abd El-rhman AM, Khattab YAE and Shalaby AME (2009).** Micrococcus luteus and Pseudomonas species as probiotics for promoting the growth performance and health of Nile tilapia, Oreochromis niloticus. *Fish and Shellfish Immunology* **27** 175-180.

**Adamek Z, Hamackova J, Kouril J, Vachta R and Stibranyiova I (1996).** Effect of ascogen probiotics supplementation on farming success in rainbow trout (*Oncorhynchus mykiss*) and wells (*Silurus glanis*) under conditions of intensive culture. *Krmiva (Zagreb)* **38** 11-20.

**Agouz HM and Anwer W (2011).** Effect of biogen® and Myco-ad® on the growth performance of common carp (*Cyprinus carpio*) fed a mycotoxin contaminated aquafeed. *Canadian Journal of Fisheries and Aquatic Sciences* **6** 334-345.

**Austin B, Stuckey LF, Robertson PAW, Effendi I and Griffith DRW (1995).** A probiotic strain of *Vibrio alginolyticus* effective in reducing diseases caused by *Aeromonas salmonicida, Vibrio anguillarum* and *Vibrio ordalii*. *Journal of Fish Diseases* **18** 93-96.

**Bader JA, Shoemaker CA and Klesius PH (2003).** Rapid detection of columnaris disease in channel catfish (*Ictalurus punctatus*) with a new species specific 16S rRNA gene based PCR primer for *Flavobacterium columnare*. *Journal of Microbiological Methods* **52** 209-220.

**Balcazar JL (2003).** Evaluation of probiotic bacterial strains in *Litopenaeus vannamei*. Final Report, National Center for Marine and Aquaculture Research, Guayaquil, Ecuador.

**Bandyopadhyay P and Mohapatra PKD (2009).** Effect of a probiotic bacterium *Bacillus circulans* PB7 in the formulated diets: On growth, nutritional quality and immunity of *Catla catla* (Ham.). *Fish Physiology and Biochemistry* **35** 467-478.

**Bansal GR, Singh VP and Sachan N (2011).** Effect of probiotic supplementation on the performance of broilers. *Asian-Australasian Journal of Animal Sciences* **5** 277-284.

**Bondad-Reantaso MG, Lumanlan SC, Natividad JM and Phillips MJ (1992).** Environmental Monitoring of the Epizootic Ulcerative Syndrome (EUS) in Fish from Munoz, *Nueva Ecija* in the Philippines. In: *Diseases in Asian Aquaculture*, edited by Shariff M, Subasinghe RP and Arthur JR, Fish Health Section, Asian Fish Soc., Manila, The Philippines 475-490.

**Cabello FC (2006).** Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. *Environmental Microbiology* **8** 1137–1144, doi: 10.1111/j.1462-2920.2006.01054.

**Chabrillon M, Arijo S, Diaz-Rosales P, Balebona MC and Morinigo MA (2006).** Interference of listonella anguillarum with potential probiotic microorganisms isolated from farmed gilthead seabream (*Sparus aurata*, L.). *Aquaculture Research* **37** 78-86.

**Chakrabarti NM (1994).** EUS Disease. IBS Delhi, India.

**Chinabut S, Roberts RJ, Willoughby GR and Pearson MD (1995).** Histopathology of Snakehead, *Channa striatus* (Bloch), experimentally infected with the specific *Aphanomyces* fungus associated with Epizootic Ulcerative Syndrome (EUS) at different temperatures. *Journal of Fish Diseases* **18** 41-47.

**Dahiya TP, Kant R and Sihag RC (2010).** Use of probiotics as an alternative method of disease control in aquaculture. *Biosphere* **2** 52-57.

**Das MK (1997).** Epizootic Ulcerative Syndrome (EUS) in fishes-its present status in India. CICFRI (ICAR), Bulletin No. 69 22.

**Delgado C, Wada N, Rosegrant M, Meijer S and Ahmed M (2003).** *Outlook for Fish to 2020: Meeting Global Demand* (Washington, DC: Int. Food Policy Res. Inst).

**El-Haroun ER, Goda ASAM and Kabir Chowdhury MA (2006).** Effect of dietary probiotic Biogen supplementation as a growth promoter on growth performance and feed utilization of Nile tilapia Oreochromis niloticus (L.). *Aquaculture Research* **37** 1473-1480.

**Evans JJ, Klesius PH and Shoemaker CA (2004).** Efficacy of *Streptococcus agalactiae* (Group B) vaccine in tilapia (*Oreochromis niloticus*) by intraperitoneal and bath immersion administration. *Vaccine* **22** 3769-3773.

**Fiorillo RL, Snart JE, Herias Gonzalez MV, Millsap KW and Newman KE (2002).** Effects of a lab-produced probiotic, a commercial probiotic, and a commercial prebiotic on broiler performance and fecal characteristics. M.Sc. Thesis, Mississippi State University, USA.

**Forestier C, De Champs C, Vatoux C and Joly B (2001).** Probiotic activities of *Lactobacillus casei rhamnosus*: *In vitro* adherence to intestinal. *Hydrobiology* **14** 67-70.

**Fuller R (1989).** Probiotics in man and animals. *Journal of Applied Microbiology* **66** 365-378.

**Gahlawat SK, Gupta RK, Sihag RC and Yadav NK (2006).** Latest Scenario of Fish Diseases in India. In: *Perspectives in Ecology and Reproduction*, edited by Gupta VK and Verma AK (Daya Publishing House) New Delhi, India 135-143.

**Gatesoupe FJ (1994).** Lactic acid bacteria increase the resistance of turbot larvae, *Scophthalmus maximus*, against pathogenic vibrio. *Aquatic Living Resources* **7** 277-282.

**Gibson LF, Woodworth J and George AM (1998).** Probiotic activity of *Aeromonas media* on the Pacific oyster, *Crassostrea gigas*, when challenged with *Vibrio tubiashii*. *Aquacult* **169** 111-120.

**Gullian M, Thompson F and Rodrýguez J (2004).** Selection of probiotic bacteria and study of their immunostimulatory effect in Penaeus vannamei. *Aquaculture* **233** 1–14.

**Hung ATY, Su TM, Liao CW and Lu JJ (2008).** Effect of probiotic combination fermented soybean meal on growth performance, lipid metabolism and immunological response of growing-finishing pigs. *Asian Journal of Animal and Veterinary Advances* **3** 431-436.

**Irianto A and Austin B (2002).** Use of probiotics to control furunculosis in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases* **25** 333-342.

**Jhingran VG (1991).** *Fish and Fisheries of India,* 3rd edition (Hindustan Publishing Corporation) Delhi 525-533.

**Jiravanichpaisal P, Chuaychuwong P and Menasveta P (1997).** The use of *Lactobacillus* sp. as the probiotic bacteria in the giant tiger shrimp (*Penaeus monodon* Fabricius). *Proceedings of the Poster Session of the 2nd Asia-Pacific Marine Biotechnology Conference and 3rd Asia-Pacific Conference on Algal Biotechnology, May 7-10, Phuket, Thailand* 1-16.

**Jory DE (1998).** Use of probiotics in penaeid shrimp growout. *Aquaculture Magazine* **24** 62-67.

**Kumar R, Mukherjee SC, Prasad KP and Pal AK (2006).** Evaluation of *Bacillus subtilis* as a probiotic to Indian major carp *Labeo rohita*(Ham.). *Aquaculture Research* **37** 1215-1221.

**Lilly DM and Stillwell RH (1965).** Probiotics: Growth-promoting factors produced by microorganisms. *Science* **147** 747-748.

**Mona-Mori K and Matsumoto M (2008).** Effect of a probiotic Sacharomyces cerevies on hematological parameter on rainbow trout. *Fish Physiology and Biochemistry* **6** 78-80.

**Naylor R and Burke M (2005).** Aquaculture and ocean resources: raising tigers of the sea. *Annual Review of Environment and Resources* **30** 185–218.

**Naylor RL, Goldburg RJ, Primavera JH, Kautsky N, Beveridge MCM and Clay J *et al.*, (2000).** Effect of aquaculture on world fish supplies. *Nature* **405** 1017– 1024.

**Nikoskelainen S, Ouwehand A, Bylund G, Salminen S and Lilius EM (2003).** Immune enhancement in rainbow trout (Oncorhynchus mykiss) by potential probiotic bacteria (Lactobacillus rhamnosus). *Fish and Shellfish Immunology* **15** 443–452.

**Ouwehand AC, Kirjavainen PV, Gronlund MM, Isolauri E and Salminen SJ (1999).** Adhesion of probiotic micro-organisms to intestinal mucus. *International Dairy Journal* **9** 623-630.

**Pal J (1996).** The fish disease, epizootic ulcerative syndrome: The present state of research and its impact in North Bengal. *Proceedings of the National Workshop on Fish and Prawn Diseases, Epizootics and Quarantine Adoption in India*, (FPDEQAI'96), Central Inland Capture Research Institute, Barrackpore 95-98.

**Parker RB (1974).** Probiotics, the other half of the story. *Animal Nutrition and Health* **29** 4–8.

**Rengpipat S, Rukpratanporn S, Piyatiratitivorakul S and Menasaveta P (2000).** Immunity enhancement in black tiger shrimp (Penaeus monodon) by a probiont bacterium.

**Ringo E and Birkbeck TH (1999).** Intestinal micro flora of fish larvae and fry. *Aquaculture Research* **30** 73-93.

**Phianphak W, Rengpipat S, Piyatiratitivorakul S and Menasveta P (1999).** Probiotic use of Lactobacillus spp. for black tiger shrimp, Penaeus monodon. *Journal of Scientific Research Chulalongkorn University* **24**.

**Wang X, Li H, Zhang X, Li Y, Ji W and Xu H (2000).** Microbial fl ora in the digestive tract of tiger shrimp (Penaeus monodon). *Journal of General and Applied Microbiology* **43** 305–308.

**Yazid AM, Salina AB, Shuhaimi M, Osman H and Normah J (1999).** Inhibitory effects of probiotic bacteria against selected food-borne pathogens. *Pakistan Journal of Biological Sciences* **2** 660-663.

**Zhenming C, Zhiqiang L, Lingmei G, Fang G, Chunling M, Xianghong W and Haifeng L (2006).** Marine yeasts and their applications in mariculture. *Journal of Ocean University of China* **5** 251-256.