Exploiting Microbial Communities Associated with Marine Fish: An Indispensable Approach to Sustainable Aquaculture

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Abstract: Improved aquaculture and fisheries has the capacity of increasing a country's gross domestic product, providing employment and food for the teeming population. The review is on exploiting microbial communities associated with marine fish: an indispensable approach to sustainable aquaculture. Disease outbreaks, high cost of diets and poor nutrition among others are serious bottlenecks to aquaculture. The intestinal microbial communities of fish are related to its health status. Imbalances caused by shifts in microbial species abundances can lead to disease outbreaks in fishes and subsequent proliferation of pathogens. To maintain fish health and adequate productivity, bio-based options favouring the ability of hosts' native intestinal microbiota to suppress pathogens must be adopted. Such options include probiotics, prebiotics, symbiotic and biocontrol agents. The principle underlying the afore-mentioned options is increasing the abundance and activity of host beneficial bacteria. However, one or group of microbes which are non-native to the host but known to effectively colonize the host intestines can also be used to achieve synergistic effects, thus improving aquaculture productivity. Probiotics have been shown to suppress proliferation, reproduction and stress tolerance in fish. Intestinal microbes can produce digestive enzymes which can be stepped up and used for industrial production. The enzymes can be added to fish feed to enhance digestion, thus ensuring better digestion of feed components, improving productivity and reduction of pollution of ponds.

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1. Introduction

Aquaculture make critical contributions to development by providing employment, food security, nutrition and trade (exports). It also provides raw materials for industrial and pharmaceutical use and aquatic organisms for stocking and ornamental trade.

According to FAO (2014) report, more than 800 million people suffer from chronic malnourishment and global population is expected to grow by another 2 billion to reach 9.6 billion people by 2050 – with a concentration in coastal urban areas. In view of this, we must meet the huge challenge of feeding our teeming population while safeguarding its natural resources for future generations. Aquaculture remains one of the fastest-growing food producing sectors.

Aquaculture is the farming of aquatic organisms by intervention in the rearing process to enhance production and private ownership of the stock being cultivated.

Compared to fishing, it allows a selective increase in the production of species used for human consumption, industry or sport fishing. Generally, Nigerian aquaculture has grown more slowly than other regions. Some bottlenecks to aquaculture in Nigeria include resource conflicts and difficulties in accessing credit, quality seed and feed, and information. Aquaculture is also vulnerable to diseases and adverse impacts of environmental changes.

In addition, there has been emerging concerns over several problems aquaculture has brought: environmental, pollution, entrophication, extravagant fishmeal consumption, and ecosystem disturbance by the escape of cultured animals into natural waters. However, major research themes and congress should gear towards an eco-friendly and energy efficient aquaculture systems, artificial fish feed improvement, and a cutting edge technology so as to ensure sustainable aquaculture. Improved postharvest processing facilities and appropriate national development policies and regulations favouring aquaculture should also be put in place.

Diverse microorganisms inhabit both the internal and external surfaces of fish such as the gills intestines and the skin. The microbial composition and species prevalence of a healthy fish differs from that of unhealthy fish, characterizing the species composition of a healthy microbiota may be a key step in identifying bacterial or physiological conditions that are present or absent in an unhealthy microbiota. The ultimate aim of this re view is to assess the effective manipulation of intestinal microorganisms as vital approach to promoting animal health and improving aquaculture productivity.

1.1 Intestinal Microorganisms in Fish

The gastrointestinal tract of fish contains quite complex microbiota which encompasses bacteria, yeast, viruses, archeans and protozoans. The presence, prevalence and complex interactions of these microbes with one another and with the epithelial cells influence various host functions. These functions include development, digestion nutrition, disease resistance and immunity. Previously, conventional culture based methods have been employed in studying the microbiota of the gastrointestinal tract of fishes (Cahill, 1990, Ringo and Birbeck, 1999) but now culture-independent (molecular) methods are being used.

Such culture independent methods include 16SrRNA sequencing, rDNA sequencing, denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE), single – strand conformation polymorphism (SSCP), Amplified ribosomal DNA restriction analysis (ARDRA), Ribosomal intergenic spacer analysis (RISA), Terminal – Restriction fragment length polymorphism (T-RFLP), fluorescence in-situ hybridization (FISH) and DNA microarray technologies. Most bacteria from intestinal microbiota are strict anaerobes and thus are difficult to culture.

Analysis based on microscopic examination has shown that 60-70% of the intestinal bacteria cannot be cultured (Rigottier-Gois *et al*., 2003). The colonization of the gut begins at birth and is characterized by a succession of microbial consortia. The microbial composition of the intestine is influenced by changes in diet, environment, temperature and life events. Dietary components that escape digestion by endogenous enzymes become available as substrates for intestinal microbiota. Changes in diet can alter the relative abundance and composition of the taxa that are already present in the community (Spor *et al*., 2011; Louise *et al*., 2007). Microbial speciation to diet substrates probably underlies the high species diversity of the gut microbiota as bacterial species partition the nichespace according to their substrate preference and use, and as a result modulation of the diet composition alters the relative abundances of the taxa that are present.

1.2 Frequently observed Bacterial Genera and Phyla in Fish

Nayak (2010) reviewed common intestinal bacterial flora of fresh water and marine fish to include the following genera: Vibrio, Aeromonas, Flarobacterium, Plesiomonas, Psendomonas, Enterobaceriacece, Micrococcus, Acinetobacter, Clostridium, Fusarium and Bacteroides. Using culture-independent techniques, identified genera include Mycoplasma, Arthrobacter, Brochotrix, Jeotgailbacillus, Ochrobactrum, Psychrobacter and Sejongia.

Ringo *et al.* (1995), asserts that intestinal microbial populations and genera in salmonids vary with NaCl concentration antibiotics, chronic oxide, diet and dietary components such as linoleic acid, and display day-to-day fluctuations. *Enterobacter* spp., *Acinetobacter* spp. and *Psendomonas* spp. are regarded as antochthonous microorganisms in *Oncorhynchus* species, while *Aeromonas* spp., *Flarobacterium* spp., *Pseudomonas* spp. and *Lactobacillus* spp. are suggested as autochthonous in Arctic charr, *Salvelinus alphinus*.

Navarrete *et al.* (2009) observed that the predominant species in the three different compartments of the digestive tract of juvenile farmed Atlantic salmon was *Pseudomonas* spp. when assessed by directly extracting DNA from each gut compartment and analyzing the 16SrDNA using temporal temperature gradient gel electrophoresis (TTGE) method. In contrast using conventional culture methods, *Shewanella* spp. is the most prevalent isolate. This discrepancy can be explained by preferential cultivation of *Shewanella* spp. under the experimental conditions. Navarrete *at al.* (2012) assessed the effects of host genetics and diet on the intestinal microbiota of rainbow trout and observed that the microbial composition of the intestines may be influenced by the host.

In addition the effect of diet on the microbiota composition was dependent on the trout family. Romero *et al.* (2014) in his review pointed out that environment also influences the occurrence and dominance of certain microbial species in the gut. Kim *et al.* (2006) analyzed the microbial community of intestinal contents and mucosal layer of rainbow trout by means of culture – dependent and culture independent molecular techniques. They observed that the microbial diversity of the intestinal mucosa is different from that of intestinal contents.

Aeromonadaceae, Enterobacteriaceae and Pseudomonadaceae representatives were the predominant cultured bacteria. Culture independent techniques revealed the dominance of proteobacteria. However the phyla Bacteroides and Fusobacteria which were observed in the intestinal content were not found in the mucosal content. Pond *et al.* (2006) described the intestinal microbiota of rainbow trout, using a clouring approach, to correspond to clostridium and *Aeromonas* species. In wild Salmon (entirely carnivorous), the abundance of mycoplasm was 96% of the clones analyzed.

The low microbial diversity observed in the wild salmon is widely attributable to the carnivorous diet it consumes. Omnivorous fish exhibits greater microbial diversity than carnivorous fish (Romero *et al.* 2014; Ley *et al*., 2008; Ward *et al.*, 2009; Smirga *et al.* 2010). With regards to microbiota composition, culturable bacteria may be dominated by *Vibrio* spp. in some fishes. Romero *et al.* (2014) also asserted that some fish gut-associated bacterial phylotypes are specific symbiouts. Zebraperch (Hermosillia azurea), which exclusively feeds on macroalgae and has a long digestive tract with an enlayed hindgut and an associated blind caccum, has a microbiota composition dominated by the Proteobaeteria, Interovibrio and Desulfovibrio; other minor components were Bacerioides and Faecalibacterium from Bacteroidetes and Firmicutes phyla respectively. Moran *et al.* (2005) found the intestinal microbiota of the herbivorous kyphosus sydneyanus, a species from the same family as Hermosilla azurea, to be different being dominated by Clostridium (Firmicutes). Data on intestinal microbial composition in fish is controversial comparison of data and subsequent interpretation is further compounded by the fact that the researchers use different techniques and also distinguished between autochthonous and allochthonous organisms. Fig. 1 shows the distribution of aerobic microbes grouped into Gram-negative (A) and Gram-positives (B) as they have been observed in the G1 tract of marine or freshwater fish.

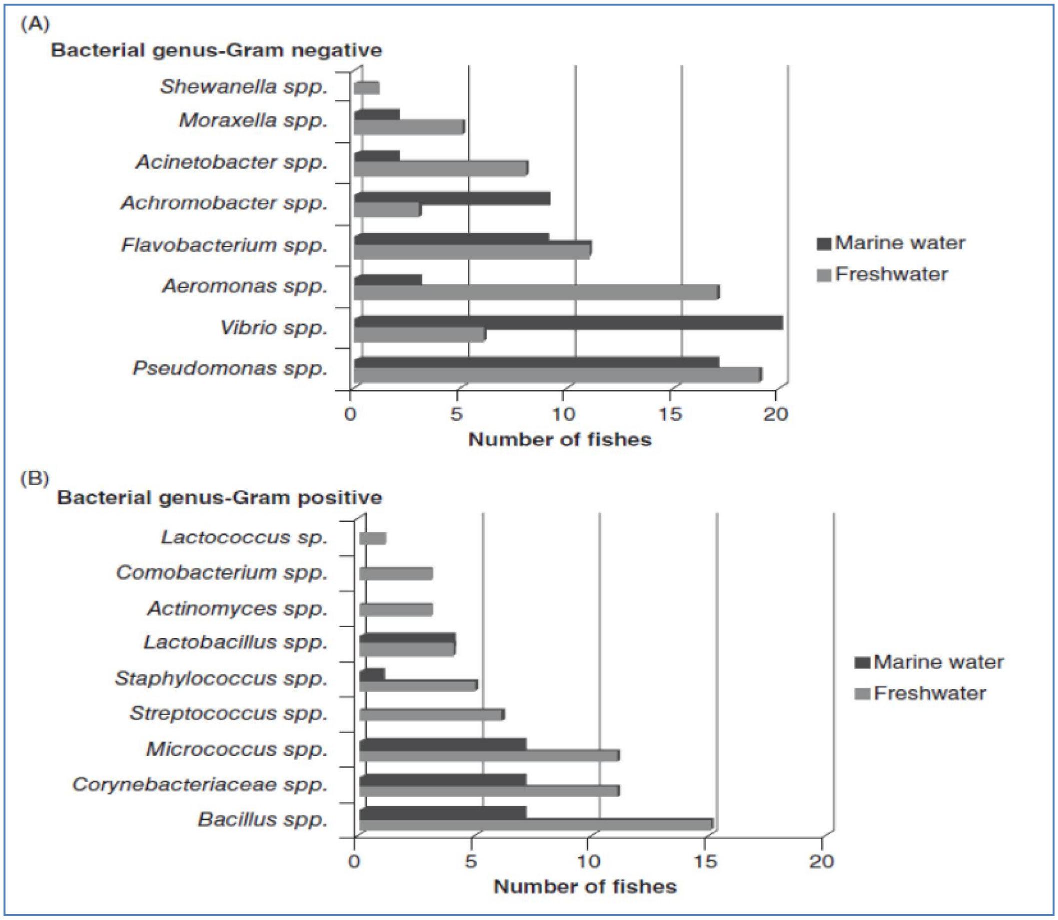

Fig. 1: Distribution of aerobic microbial grouped into Gram-negative (A) and Gram-positives (B) as they have been observed in the G1 tract of marine and freshwater fish.
Source: Nayak (2010).

According to Izvekova (2007), some bacterial genera such as *Vibrio*, *Pseudomonas*, *Bacillus* and *Micrococcus* spp. are more frequently observed. Some bacteria also show preference for particular environment, either marine or fresh water. Clostridium and bacteroides are the most frequent anaerobic bacteria genera reported in fishes (Fig. 2).

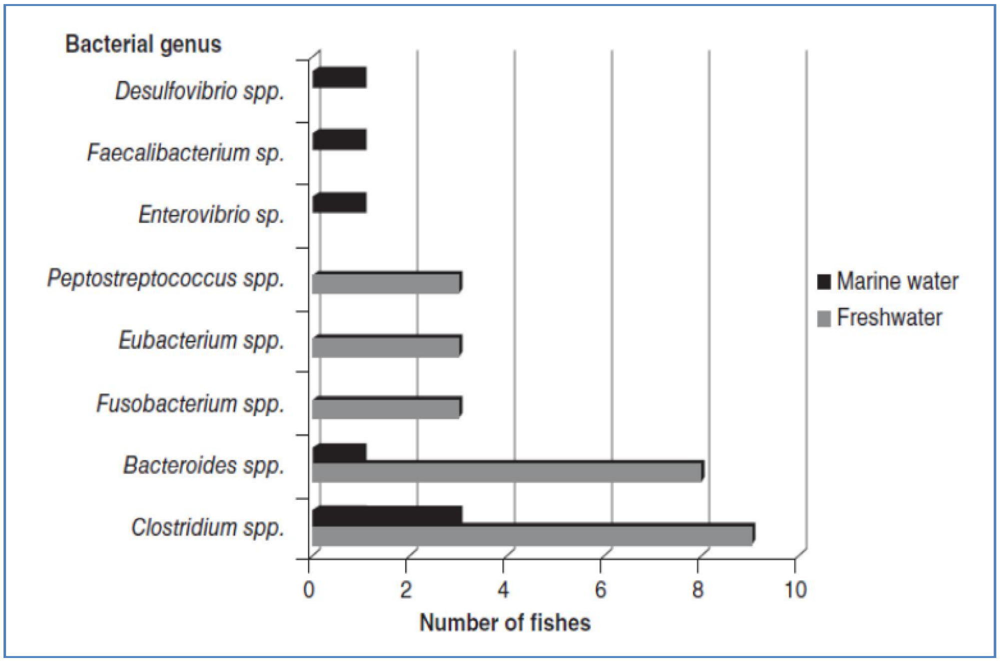

Fig. 2: Clostridium and bacteroides are the most frequent anaerobic bacteria genera in fishes
Source: Nayak (2010).

The predominant microbiota present in fishes belongs to five phyla: Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes and Fusobacteria. These phyla contribute to the host inner environment. Proteobacteria are known to induce important responses in the host and members can exploit environmental reservoirs outside their hosts to proliferate and persist in aqueous environments (Romero *et al.* 2014). This explains the high prevalence of these bacteria in the gastrointestinal tract of fish. Lactic Acid bacteria are an interesting bacterial group inhabiting the guts of fish. Merrifield *et al.* (2010) has reported a variety of lactic acid bacterial strains tested for their probioto potential. Some known fish pathogens include *Edwardsiella tarda*, *Aeromonas hydrophila*, *A. salmonicida*, *Flavobacterium psychrophilum*, *Photobacterium damselae* and *Vibrio* species.

1.3 Microorganisms that Produce Extracellular Enzymes

Characteristic microbial groups inhabit particular species of fish. These microbial groups are naturally distribution along different feeding habits, feed types environmental parameters (pH), and temperature**.** Most fish intestinal isolates are good producers of protease, amylase, cellulose, lipase and phytase liginolytic enzymes and chitinolytic enzymes.

Such microorganisms include members of the genera: Bacillus, Enterobacteriaceae, Acinetobacter, Aeromonas, Flarobacterium, Photobacterium, Pseudomonas, Vibrio, Microbacterium, Micrococcus and staphylococcus (Ray *et al*., 2012).

Production of these extracellular enzymes can be harnessed for use in industries, medicine and pharmaceuticals. Microorganisms producing these digestive enzymes can be incorporated into feeds as probiotics study of intestinal microorganisms inhabiting particular fish species capable of producing digestive enzymes helps in correct feed formulation at low cost.

1.4 Microorganisms used as Prebiotics

Probiotics are one or more microorganism with beneficial effects for the host, able to persist in the digestive tract because of its tolerance to acid and bile salts. Interest in probiotics has increased due to their potential in disease control. Antibiotics were used for a long time in aquaculture to prevent diseases. This however caused a lot of problems which include occurrence of antibiotic residues in animal tissues, acquisition of bacterial resistance mechanisms and imbalance in the fish intestinal microbiota, which affect fish health and productivity.

This necessitates the use of probiotics in disease control and for the inhibition of pathogens in aquaculture. Probiotic microorganisms release chemical substances exhibiting bactericidal or bacteriostatic effect on pathogens inhabiting the host’s intestine. Probiotics are used as growth promoters and to improve the health of fishes. New areas for application of probiotio include favourable effect on reproduction and stress tolerance, although this requires a more scientific development, improvement of water quality.

Probiotics can be used as a food additive either added directly to the culture tank or added to the feed. It can either be obtained as laboratory preparation of bacteria or commercially Queiroz and Boyd (1998) used a laboratory prepared probiotic called Biostart, a derived from *Bacillus* spp. to observe the effects of a bacterial inoculums in channel catfish ponds. They observed greater survival, productivity and improved water quality in probiotic treated ponds than in controls.

Probiotics administered to Tilapia Oreochromis mloticus increased non-specific immune response, and bactericidal activity, which improved the resistance of fish to infection by Edwardsiella tarda (Taoka *et al.*, 2006b). The probiotics used was a combination of *Bacillus subtilis*, *Lactobacillus acidophilus* and *Clostridium botyricum* and *Sacchanonyces cerevisiae*. *Enterococcus faecum*, a non pathogenic lactic acid producing bacterium and normal intestinal flora of humans, animals and fishes, has been used as probiotic to effectively use to control Edwardsiellosis, one of the most serious diseases in the culture of eels. Its causative agent is *Edwardsiella tarda*. *Enterococcus faecum* was also found to be effective against enteropathogens such as *Escherichia coli*, *Salmonellae* and *Clostridia* (Chang and Lici, 2002). *Bacillus toyoi* was also found to be effective against enterotoxigenic pathogens using Biogen (a combination of *Bacillus subtillis* and *hydrohytic* enzyme as probiotic Haroun *et al*. (2006) observed greater growth performance and feed utilization, and lowest total cost on Nile tilapia (*Oreochromis niloticus*) fingerlings fed diets treated with Biogen than those fed the control diet.

Thus addition of Biogen® as probiotic increased productivity. The lactic acid-producing bacteria, lactobacillus rhamnosus, (a human probiotic) was found effective in suppressing Aeromonas salmonicida, the causative agent for the fish disease, furunculosis. Mortality reduced from 52.6 to 18.9% when 10^9 cells g^{-1} of probiotic was added as a feed additive. Increasing the probiotic dose to 10^{12} cells g^{-1} caused mortality to increase to 46.3% (Nikoskelainen *et al.,* 2001). This shows that increasing dosage does not necessarily improve protection. A combination of several microorganisms applied as probiotics is effective in improving productivity and fecundity of fish. Abasali and Mohamad (2010) monitored the effect of a probiotic, primalac, on the reproductive performance of a freshwater ornamental species, the swordtail (Xiphophorus Helleri). Primalae probiotic is a combination of four microorganisms namely *Lactobacillus acidophilus*, *Lactobacillus casei*, *Enterococcus faecium* and *Bifidobacterium thermophilum*. Gonadosomatic Index (GSI) and growth was observed on the fish fed the probiotic treated diet than the fish fed the control diet.

Presently, commercial probiotics are available in liquid or powder presentations. Immobilization of probiotics using microencapsulation technologies has been developed. Microbial cells at high density are encapsulated using alginate, chitosan, carboxymethy cellulose, or pectin in order to physically and chemically protect the microbes from low pH and digestive enzymes. This ensures gradual release into the intestine and adequate colonization of the probiotic without any damage. Table 1 shows the different applications of probiotics in aquaculture.

Table 1: Different Applications of Probiotics in Aquaculture

Application	Identity of the probiotic	Applied to aquatic species
Growth promoter	*Bacillus* sp. S11	*Penaeus monodon*
	Bacillus sp.	Catfish
	Carnobacterium divergens	*Gadus morhua*
	Alteromonas CA2	*Crassostrea gigas*
	Lactobacillus helveticus	*Scophthalmus maximus*
	Lactobacillus lactis AR21	*Brachionus plicatilis*
	Streptococcus thermophilus	*Scophthalmus maximus*
	Streptomyces	*Xiphophorus helleri*
	L. casei	*Poeciliopsis gracilis*
	Bacillus NL 110, *Vibrio* NE 17	*Macrobrachium rosenbergii*
	Bacillus coagulans	*Cyprinus carpio* koi
Pathogen inhibition	*Bacillus* sp.	Penaeids

Application	Identity of the probiotic	Applied to aquatic species
	Enterococcus faecium SF 68	*Anguilla Anguilla*
	L. rhamnosus ATCC53103	*Oncorhynchus mykiss*
	Micrococcus luteus A1-6	*Oncorhynchus mykiss*
	Pseudomonas fluorescens	*Oncorhynchus mykiss*
	P. fluorescens AH2	*Oncorhynchus mykiss*
	*Pseudomonas*sp.	*Oncorhynchus mykiss*
	Roseobacter sp. BS. 107	Scallop larvae
	Saccharomyces cerevisiae, S. exiguous, Phaffia rhodozyma	*Litopenaeus vannamei*
	Vibrio alginolyticus	Salmonids
	V. fluvialis	*Oncorhynchus mykiss*
	Tetraselmis suecica	*Salmo salar*
	Carnobacterium sp. Hg4-03	*Hepialus gonggaensis* larvae
	Lactobacillus acidophilus	*Clarias gariepinus*
	Bacillus spp., *Enterococcus*sp.	*Farfantepenaeus brasiliensis*
	Lactococcus lactis	*Epinephelus coioides*
Nutrient digestibility	*L. helveticus*	*Scophthalmus maximus*
	Bacillus NL 110, *Vibrio* NE 17	*Macrobrachium rosenbergii*
	Carnobacterium sp. Hg4-03	*Hepialus gonggaensis* larvae
	Lactobacillus acidophilus	*Clarias gariepinus*
	Shewanella putrefaciens Pdp11	*Solea senegalensis*
	Bacillus sp. 48	*Penaeus monodon*
	Bacillus NL 110, *Vibrio* sp. NE 17	*Macrobrachium rosenbergii*
Water quality	*Lactobacillus acidophilus*	*Clarias gariepinus*
	B. coagulans SC8168	*Pennaeus vannamei*
	Bacillus sp., *Saccharomyces* sp.	*Penaeus monodon*
	Lactobacillus delbrueckii	*Dicentrarchus labrax*
	Alteromonas sp.	*Sparus auratus*
Stress tolerance	*B. subtilis, L. acidophilus, S. cerevisiae*	*Paralichthys olivaceus*
	L. casei	*Poecilopsis gracilis*
	Pediococcus acidilactici	*Litopenaeus stylirostris*
	Shewanella putrefaciens Pdp11	Makimaki
Reproduction improvement	*Bacillus subtilis*	*Poecilia reticulata, Xiphophorus maculates*
	L. rhamnosus	*Danio rerio*
	L. acidophilus, L. casei, Enterococcus faecium, Bifidobacterium thermophilum	*Xiphophorus helleri*

Source: Cruz *et al.* (2012).

Conclusion

The current economic recession, growing population and poverty in the country necessitates quick response from all sectors of the economy including food production sustainable aquaculture will meet the growing demand for fresh water food and seafood.

A good knowledge of intestinal microbial populations and subsequent metabolic functions helps in maintaining fish health and correct feed formulation since the occurrence and prevalence of intestinal microorganism is directly related to host health, manipulation of the intestinal microbiota can help restore fish health on incidence of disease condition.

Intestinal microorganisms can be used as probiotics for improving health growth reproduction and suppressing pathogens. These benefits will increase aquaculture yields. Intestinal microorganisms can also be utilized in the industrial production of enzymes and bioactive products.

Recommendations

It is very important that government funds research in different areas of aquaculture. Research on characteristic gut microbiota and effective probiotic use in fish species of high biological and economic potentials should be conducted. Fish farmers should be intimated on the latest research discoveries so as to adopt the best technology and practices in aquaculture. Microcredits should also be made available to prospective fish farmers. Efforts should be made to bring aquatic pollution to the barest minimum.

Government should make efforts to provide at least one facility where molecular techniques such as PCR (Polymerase Chain reaction), FISH (fluorescent in-situ hybridization), and DGGE (denaturing gradient gel electrophoresis) will be carried out in order to facilitate research.

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