ISBN: 978-93-90847-97-6

1. Aquaculture Microbiology

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1.1 Introduction:

Aquaculture is an age-old traditional practice of farming aquatic animals (e.g., fish, crustaceans, molluscs, algae etc.) by regulating the rearing process to increase their productivity. In the era of global food crisis, aquaculture stands as a sustainable approach to restore biodiversity and ensure nutritional security to a large populace. It is the most vibrant sector contributing to livelihood, food security, and rural employment.

Southern Asia, one of the biodiversity hotspots, is native to many indigenous freshwater fish species. Fish farmers use various conventional fishing gears and techniques to collect fishes from waterbodies.

They use *Ghuni* (Used to catch bottom dwelling fishes like *Trichogaster chuna, Anabas testudineus* etc.), *Palu'i* (Used to catch fishes in slime), *Dughārē* (Used to catch small indigenous fishes in shallow river), Baṛaśi (Used to catch small and medium sized fishes), *Cāp Jāl* (Used to catch small fishes), Drag net (Used to catch carps, small indigenous fishes of a pond), *Ghuṇ Jāl, Phām̐di, Wheel chip, Jhim chip, Ṭōgi, Jaṭā* (Used to catch *Clarias orientalis, Monopterus cuchia, Clarias magur etc.*), *Laṛkā* (Used to catch *Channa. striata*), Kēm̐cā (Used to catch fishes like *Channa. striata*) and other fishing devices.

1.2 Limnological Parameters:

Aquaculture pond is a dynamic equilibrium which exhibits constant fluctuation due to prevailing physical and chemical processes. Water is a matrix of dissolved gases, inorganic substances, and organic matters.

The physico-chemical properties (e.g., pH, temperature, ammonia-, nitrate- and nitrite content, dissolved oxygen, biological oxygen demand, salinity, alkalinity, water depth) of water generally govern the life of aquatic organisms living in it.

Aquatic animals need to adapt to variable environment due to sudden fluctuation in water quality. The leftover feed, fecal matter of fishes and decomposition of organic material accumulate nitrogenous substances that are detrimental to aquaculture practices.

Industrial effluents, chemical fertilizers, pesticides and other anthropogenic activities in adjacent areas concomitantly pollute the aquatic environment.

In addition, pathogenic infections and algal bloom cause deterioration of water quality and depletion of aquatic diversity.

Aquaculture productions are thus depended upon maintenance of a steady state of those limnological parameters.

Proper exchange of gaseous substances, reduction of nitrogenous wastes, balancing of planktonic diversity and microbial population are crucial for fish growth and metabolism.

1.3 Aquaculture:

Aquaculture can be conducted through following approaches:

(1) Extensive: It is the farming of aquatic animals in their natural or semi-natural condition (e.g., large ponds, beels, rivers, lakes).

It is the most cost effective technique but the productivity generally remains relatively low compared to other approaches.

(2) Intensive: A fish farming procedure in which all the parameters are properly monitored to get the maximum yield in a specified time. It is generally conducted in small waterbodies or tanks maintaining high stocking density.

Fish are fed only with formulated feeds. However, the cost of investment remains very high.

(3) Semi-intensive: It is the most sustainable approach which maintains a delicate balance between the natural & artificial farming. It is also conducted in small waterbodies or tanks but with moderate level of stocking density.

Fish are generally fed on natural feeds with added feed supplements. It requires a modest level of monitoring system and hence becomes cost-effective. It generates a significant level of productivity.

However, intensification of aquaculture practices is often accompanied with many challenges and obstacles.

Massive urbanization, exploitation of natural habitats, uncontrolled introduction of allied exotic fishes, high- stocking density and microbial infections often poses a threat to the aquaculture sector. Abrupt use of pesticides in the adjacent agriculture fields also has made the situation more hostile.

1.4 Microbes and Aquaculture:

The production in aqua-sector is largely allied with its surrounding microbiota. Microbes possess both beneficial and harmful effects to its host organism (Figure 1.1). The harmful ones, known as pathogens, are involved in imparting infections to the susceptible species. While, the beneficial ones are regarded as probiotics. The utilization of probiotics in aqua-farming assists aquatic organisms in boosting the immune system against pathogenic diseases. The significance of probiotics in aquaculture is not just constrained to the gastrointestinal tract; but also it plays a key role in enhancing an organism's health quality, by stimulating growth, preventing diseases, enhancing immunity, and improving water quality by altering the microbiota of water and associated sediments.

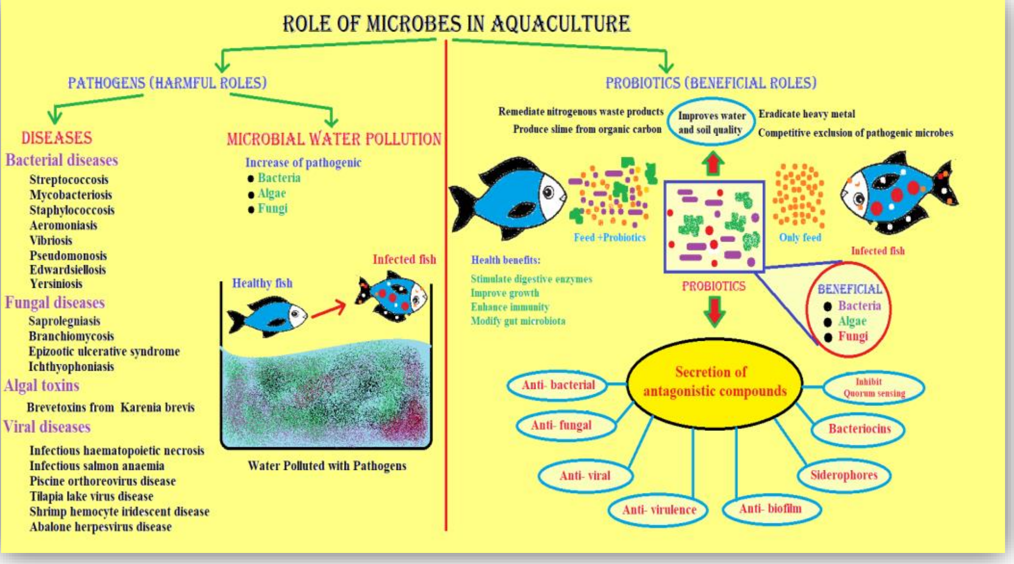

Figure 1.1: Role of microbes in aqua-sector

1.4.1 Microbial Diseases in Aquaculture:

Disease outbreaks are a major stumbling factor to the aquaculture sector and it acquire primacy owing to the ability to wipe out fish stocks, through spread of diseases.

Diseases in aquaculture are caused by complex interplay among the host, pathogen, and extrinsic stressors like environmental deterioration due to altering climate, eutrophication in native habitat, and inefficient fish monitoring systems.

Furthermore, increased stocking density exacerbates the prevalence and severity of infections.

Disease outbreaks have been considered as a major constraint in aquaculture sector. The epidemic diseases often result in massive financial damages.

Again, the convergence of persistent and emergent infirmities eventually constitutes a potential danger to the aquaculture sector, necessitating its quick management. Various microbial diseases allied with aquaculture are shown in Table 1.1.

Table 1.1: Microbial diseases in aquaculture sector

Pathogen		**Host**	**Disease**
Viral pathogens	*Herpesviruses*	*Cyprinus carpio, Anguilla* sp., *Acipenser* sp., *Gadus* sp.	Herpesvirus Diseases
	Cyprinid herpesvirus 3 (CyHV3)	*Cyprinus carpio*	Herpesvirus Diseases
	Channel Catfish Virus (CCV)	*Ictalurus punctatus, Clarias gariepinus, Hemibagrus gracilis, Pangasius hypophthalmu*s.	Herpesvirus Diseases
	Iridovirus	*Epinephelus sp.*	Iridovirus Diseases
	Betanodavirus	*Epinephelus fuscoguttatus, Epinephelus lanceolatus, Trachinotus blochii, Lates calcarifer*	Viral Nervous Necrosis, Viral Encephalopathy and Retinopathy
	Tilapia Lake Virus	*Oreochromis* sp., *Barbonymus schwanenfeldii*	Syncytial hepatitis
	White Spot Syndrome Virus	*Penaeus monodon, Penaeus merguiensis*	White Spot Syndrome
	Macrobrachium rosenbergii nodavirus	*Macrobrachium rosenbergii*	White tail disease
	Infectious Hypodermal and Haematopoietic Necrosis Virus (Parvoviridae)	*Penaeus stylirostris, Penaeus vannamei, Penaeus monodon, Macrobrachium rosenbergii*	Infectious hypodermal and haematopoietic necrosis disease
	OMV	*Oncorhynchus masou, O. nerka, O. keta*	Oncogenic along with skin ulcerative condition with hepatitis

Pathogen		**Host**	**Disease**
	Spring viremia of carp virus	*Cyprinus carpio, Ctenopharyngodon idella, Carassius auratus, Leuciscus idus, Tinca tinca, Hypophthalamicthys, molitrix Danio rerio,* *Lebistes reticulates, Catostomus commersonii*	Spring viremia of carp
	Viral nervous necrosis virus	*Epinephelus akaara, Gadus morhua, Epinephelus coioides, Lates calcarifer, Oplegnathus fasciatus, Dicentrarchus labrax,* *Pseudocaranx dentex*	Nervous necrosis
	Walleye dermal sarcoma virus	*Stizostedion vitreum*	Sarcoma
Bacterial pathogens	*Vibrio harveyi, V. parahaemolyticus, V. alginolyticus, and V. anguillarum*	*Lates calcarifer, Epinephelus fuscoguttatus, Epinephelus coioides, Lutjanus* sp.*, hybrid grouper (E. fuscoguttatus* x *E. lanceolatus), Oreochromis* sp.*, Pangasius hypophthalmus, Scylla serrate, Portunus pelagius, Paphia textile, Meretrix meretrix, Cerithidea obtuse, Pseudosciaeana crocea*	Vibriosis
	Yersinia ruckeri	*Oncorhynchus mykiss,* Salmonids, *Anguilla reinhardtii,* Goldfish, Sole, Sturgeon and *Psetta maxima*	Yersiniosis, Enteric redmouth disease (ERM)
	Photobacterium damselae	*Chromis punctipinnis, Psetta maxima, Oncorhynchus mykiss, Trachinotus ovatus, Anguilla*	Photobacteriosis, skin ulcers

Pathogen		**Host**	**Disease**
		reinhardtii, Sparus aurata, Dicentrarchus labrax, Seriola quinqueradiata, Pagrus Auriga, Diplodus sargus, Argyrosomus regius	
	Streptococcus iniae, S. agalactiae	Asian seabass, Hybrid tilapia	Streptococcosis
	Aeromonas hydrophila, A. veronii	Giant freshwater prawns, tilapia, catfishes	Motile Aeromonas Septicemia
	Mycobacterium marinum, M. fortuitum, M. chelonae	Wide range of Marine, brackish and freshwater fishes.	Piscine Tuberculosis
	Edwardsiella tarda	African catfish, tilapia, *Monopterus albus, Trichogaster pectoralis*, Asian seabass	Edwardsiellosis
Fungal pathogens	*Saprolegnia sp. (Saprolegnia parasitica, Saprolegnia diclina, Saprolegnia ferax)*	Carp, Catfish, Eels, Salmonids	Saprolegniasis
	Branchiomyces sanguinis, Branchiomyces demigrans	Carps, Tench, Pike, Largemouth bass, Striped bass	Branchiomycosis (gill rot)
	Aphanomyces invadans	*Cyprinus capio, Oreochromis niloticus, Chanos chanos*	Epizootic Ulcerative Syndrome (EUS), Red Spot Disease, Mycotic Granulomatosis, Ulcerative Mycosis
	Ichthyophonus hoferi	*Clupea harengus*, freshwater salmonids	Ichthyophoniasis, granulomatosis

Pathogen		Host	Disease
	Achlya sp.	*Puntius sophore, P. conchonius, P. ticto, Colisa fasciata, Chanda ranga, Labeo rohita (fingerlings), L. bata (fingerlings), Notopterus notopterus, Anabas testudineus, and Channa punctatus.*	Saprolegniasis

1.4.2 Prevention of Aquatic Diseases:

A. Antibiotics and chemical substances:

Prevention and control of aquatic diseases often focuses on the use of chemotherapeutic substances, immunostimulants and veterinary medicines that often make the situation more hostile. The emergence and vertical transfer of antibiotic-resistant genes has become a serious threat to public health. It happened most dramatically in the shrimp industry where massive increases in production, high- stocking density and overuse of antibiotics had led to the emergence of antibiotic resistant bacteria and production crashes in many Asian countries. There are many reports regarding sharp decrease in productivity due to abrupt use of anti-microbial drugs.

B. Probiotics:

Probiotics are live beneficial microorganisms which when consumed in adequate amount confer health benefit to the host. They are eco-friendly biocompatible substances that are alternatively used to prevent and control aquatic diseases in recent time. Aquaculture probiotics colonizes the gastrointestinal tract of aquatic species and confer protection against pathogens by limiting nutritional resources through the process of competitive exclusion. Probiotic isolates often secrete extracellular enzymes (e.g., amylases, proteases, lipases) or growth factors (e.g., vitamins, siderophores, fatty acids, amino acids) which can digest indigestible food components more efficiently and promote fish nutrition. The consortium of probiotics may also be effective and consistent than a single strain due to their synergistic nature.

Probiotic also enhances the quality of culture water through a cost-effective technology to monitor waste removal and reduce water pollution. Probiotics often exert signaling molecules to stimulate humoral or cellular immune response against pathogenic invasion. Application of probiotic *Lysinibacillus sphaericus* provided protection to *Clarias batrachus* against pathogenic *Vibrio harveyi*. Probiotics are used as functional feed additives to inhibit pathogenic infections, reduce biofilm formation, and to enhance fecundity.

C. Algal extracts:

Algae and algal extracts are enriched with proteins, vitamins, minerals and are used as feed ingredient in aquaculture sector. They often function as prebiotic supplement to provide nutrition to gut micro-flora. Potential algal extracts are also capable of producing secondary metabolites, expression of the pro-inflammatory genes (e.g., IL-1β and TNF-α) that might be used as immunotherapeutics against infection. For e.g., addition of a mixture of algal extracts (*Fucus vesiculosus*, *Ulva rigida* and *Nannochloropsis gaditana*) to the basal diet of Zebra fish increases C3b transcription that provides immunity against viral diseases in Zebra fish larvae.

D. Vaccination:

Vaccination can be an effective strategy to induce immunity in fish against viral infections. Several oral delivery techniques have been employed, including cholera toxin B molecular carrier, encapsulation of recombinant G protein with polyethylene glycol (PEG), and active *Aeromonas salmonicida* delivering the antigen. The benefit of live attenuated vaccination was first obtained against Channel Catfish Virus (CCV) of *Ictalurus punctatus*. An innovative live recombinant IHNV vaccine has been designed by French researchers by means of reverse genetics approaches.

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