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REVIEW ARTICLE **OPEN ACCESS**

Probiotics and its applications in aquaculture

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Abstract

The age old practice of the use of curd to maintain healthy gut microbiota is well established. The good bacteria namely Lactobacillus sp present in curd is mainly responsible to maintain healthy gut microflora. These microorganisms reduce the overgrowth of pathogenic bacterial colony in our gut. Later on incorporation of other beneficial microorganisms in medicine as probiotics proves helpful to reduce the harmful bacterial load and subsequently bacteremia in animal body. In the recent past the application of naturally occurring beneficial microorganisms in the supplementary food materials in animal husbandry as well as in pisciculture proves effective with respect to their productivity. However, the judicial use of good microorganisms in animals feed needs the approval of a regulatory body to prevent transfer of antibiotic resistance genes from probiotics to pathogenic microorganisms and subsequently to the environment. The present review highlights the effect of probiotics in the overall productivity as well as hazards and future approach in this field of research.

Keywords: probiotics, prebiotics, microorganisms, productivity, antimicrobial resistance *etc.*

Introduction

Besides nature human body contains trillions of microorganisms and this microbiome constitute about 1-3 percent of our body mass. Their biodiversity differ from person to person and they live in harmony with us. Human beings capitalize good microorganisms as 'probiotics' for their wellbeing as well as for increasing productivity of their livestock and in pisciculture in terms of growth, immune modulation and resistance to pathogenic microorganisms. Probiotics, as defined by Fuller in the year 1992, are viable microorganisms that have beneficial effect on the intestinal microflora. WHO in the year 2001 defined probiotics as "live microorganism, which when administered in adequate amounts confer a health benefit on the host". We mainly consume Lactobacillus sp as probiotics in our daily life from yogurt and other fermented milk products. Other specific strains of the genera like *Bifidobacterium*, *Enterococcus*, *Streptococcus*, *Pediococcus*, *Leuconostoc*, *Bacillus*, *Escherichia coli* and Yeast namely *Saccharomyces cerevisiae* are also used as probiotics.

As pisciculture is an important industry and contributing World's GDP, the judicious use of probiotics in this field may be a boon in the near future.

Necessity:

The occurrence of mortality due to microbial infection is a huge loss to the industry. The uses of antibiotics to control diseases are causing the emergence of antibiotic resistant and /or multidrug resistant bacteria in our ecosystem (Aghamohammad *et al.*, 2022; Munita JM and Arias CA, 2016). It is proven fact that the use of probiotics helps to control diarrhea, constipation, irritable bowel syndrome, colitis in human beings (Dooheon Son *et al.*, 2023; Hungin *et al.*, 2018; Sanders *et al.*, 2018). Instead of giving antibiotics at the time of infection of enteropathogenic bacteria, it is always better to maintain optimum level of beneficial microorganisms in the gut (Silva, D.R. *et al.*, 2020). This approach not only reduces the chances of infection by enteropathogenic bacteria, it also minimizes the use of antibiotics and its subsequent environmental hazards. The use of good microorganisms as Probiotics or symbiotic proves effective by increasing the feed efficacy through stimulating digestive enzymes, absorption of nutrients, improving gut barrier, releasing of antimicrobial substances and competitive exclusion of

enteropathogenic bacteria (Raheem A. *et al.,* 2021). Thus increase the productivity, immunity in pisciculture (Shariffuzzaman and Austin, 2017), poultry (Upadhaya *et al.,* 2016; Ogbuewu IP *et al.,* 2022) and animal husbandry (Wisener *et al.,* 2014). Different types of teleost fishes having commercial value are studied by giving prebiotics to increase their productivity (Wee W *et al.,* 2022).

Selection of probiotics:

Type of beneficial microorganisms present in the gut varies significantly among species inhabiting different environmental condition. It is always better to identify beneficial microorganisms present inside gut microbiota, GIT mucosa and skin of a healthy species of interest (Newaz-Fyzul *et al.,* 2007; Boutin *et al.,* 2012; Fijan S, 2014). The widely used identification procedure for unknown microorganisms is the sequencing of the conserved region in the genome like 16S ribosomal DNA, DNA/DNA hybridization and pulse field gel electrophoresis (PFGE) in bacteria and ITS region of fungi and yeast (Reller *et al.,* 2007).

To be a probiotic, a microorganism should have antipathogenic (Hai 2015, Korkea-aho *et al.,* 2011), colonization and stress tolerance potential (Hai, 2015; Kavitha *et al.,* 2018) as well as immune stimulatory properties (Nayak SK. 2010; Akhter *et al.,* 2015). After identification of species specific beneficial microorganisms it is necessary to propagate them inside gut (Hai, 2015; Krysiak K *et al.,* 2021; Markowiak P and Śliżewska K, 2018).

During selecting a probiotic strains, their safety, functionality and technological usefulness should be taken care of (WHO, FAO and EFSA, 2001). A probiotic should be of human or animal origin, gut friendly, should not be pathogenic and/or contain any antibiotic resistance property in their mobile genetic elements in past. It should withstand the acidic and alkaline environment, should be able to adherence to the gut and propagate there, should have the capability to competitively exclude pathogenic bacteria and above all withstand from the antimicrobial chemicals secreted by other commensal gut microbiota. The probiotic species of interest should multiply in sufficient numbers in the culture medium and persist in good numbers while keeping its properties during its subsequent processing to a marketable product.

Selection of prebiotics:

Selection of prebiotics for the optimum growth of the probiotics is necessary in vitro and subsequently in vivo (Ricke SC, 2018; Wee W *et al.,* 2022). Prebiotic should have long life and it should not be digested by host, rather consumed by selective intestinal microbiota for the benefit of its host (Wang Y, 2009; Markowiak P and Śliżewska K, 2018; FAO and WHO, 2006).

Application in aquaculture:

Aquaculture is a fast growing industry throughout the world. It has the potential to supply easily digestible cheap protein source and also generate employment. But the production of species in more quantity and quality is often hampering due to the infection of aquatic species by various pathogenic microbes. Excessive use of antibiotics for this reason although minimize the rate of infection but at the same time it is producing multidrug resistant varieties of microbes which has impact on health to all living eukaryotes.

Growth and hence the productivity of fish is directly correlated with their good health. A healthy fish is always best for its fecundity and flesh. After screening the microbial communities in the fish body we can manipulate beneficial microbes to promote fish health. Beneficial microorganisms inhabiting in the fish gastrointestinal tract increases their life span by stimulating the overall digestibility, nutrition uptake and immunity (Suguna T, 2020). Moreover, they act as a barrier against enteropathogenic microbes. The composition of gut microbiota varies in different fishes as well as in the same species depending on their life stages, food habit, seasons, trophic levels etc. (Liu *et al.,* 2016; Stephens *et al.,* 2016; Michl *et al.,* 2017; Wang *et al.,* 2018).

Normal gut microbiota found among indigenous fin fishes are generally the members belongs to *Lactobacillus, Lactococcus, Leuconostoc, Enterococcus, Streptococcus, Carnobacterium, Pediococcus, and Weissella* genera (Merrifield et al. 2014, Ringo *et al.,* 2018). As lactic acid bacteria (LAB) have abilities to stimulate host GI development, digestive function, mucosal tolerance, immune response and antipathogenic, they are regarded as potential probiotics by many investigators (Ringo *et al.,* 2018). Investigation in search of beneficial gut microbiota in fish has been reported by many scientists in the field of aquaculture. Sakata *et al.,* (1980) reported that Obligate anaerobes (*Bacteroidaceae*) were predominant over facultative anaerobes (*Vibrionaceae* and *Enterobacteriaceae*) Nile tilapia *Tilapia nilotica*, goldfish (*Carassius auratus*), and Ayu (*Plecoglossus altivelis*). Sugita *et al.,* 1991 discovered *Bacteroides* type a, producing vitamin B_{12} in Japanese eel (*Anguilla japonica*), carp *(Cyprinus carpio)*, goldfish (*Carassius auratus)*, Ayu (*Plecoglossus altivelis)*, tilapia (*Tilapia nilotica*) and channel catfish (*Ictalurus punctatus*). Sugita *et al.,* 1997 have demonstrated *Aeromonas*, *Bacteroidaceae* and *Clostridium* strains play an important role in the digestion of starch by producing amylase in Ayu

(*Plecoglossus altivelis*), carp (*Cyprinus carpio*), channel catfish (*Ictalurus punctatus*), Japanese eel (*Anguilla japonica*) and Tilapia (*Tilapia nilotica*).

In pisciculture probiotics are growth promoter, antipathogenic, helps to digest food, maintain water quality, and increase stress tolerance in the host (Cruz *et al*., 2012). It is also reported that probiotics influence the reproduction performance and matured gametes in pisciculture (Gioacchini *et al*., 2011; Nadio 2015; Ekasari *et al*., 2015; Ayuningtyas *et al.*, 2020). Gioacchini *et al*., (2011) reported the role of *Lactobacillus rhamnosus* in increasing GSI (gonado somatic index) and fecundity of zebra fish *Danio rerio*. Probiotic *Bacillus subtilis* was reported to influence the reproduction performance of guppy *Poecilia reticulata* and platy *Xiphoporus maculatus* Cruz *et al*. (2012). Ayuningtyas *et al.,* (2020) reported the positive influence of *Bacillus* sp. NP5 in reproductive performance of catfish when given as probiotic through feed. Tsuchiya *et al.,* (2008) discovered vitamin B_{12} producing Bacteroides type a strains and Vancomycin-resistant bacteria such as *Cetobacterium somerae* from Goldfish (*Carassius auratus*), common carp (*Cyprinus carpio*) and Mozambique tilapia (*Oreochromis mossambicus*). Roeselers *et al.,* (2011) found *Aeromonas* spp., *Pseudomonas sp*., *Plesiomonas* sp., *Vibrio* sp., *Shewanella* sp. and *Cetobacterium* sp. inside the gut of Zebrafish (*Danio rerio*). Mandal and Ghosh, 2013 discovered that *Enterobacter asbura*, *Pichia kudriavzevii*, *Candida tropicalis* and *Candida parapsilosis* can produce tannase to overcome the antinutritional factors in the feedstuffs of Rohu (*Labeo rohita*), Mrigal (*Cyrrhinus mirgala*) and Tilapia (*Oreochromis mossambicus*). Banerjee and Ghosh reported (2014) the presence of *Pichia kudriavzevii* and *Candida rugosa*, extracellular enzyme producing yeasts from Mrigal (*Cyrrhinus mirgala* and Tilapia (*Oreochromis niloticus*).

Probiotics has been reported to modulate gastrointestinal microbiota, immune modulation and disease prevention in *Cyprinus carpio* (Chi *et al.,* 2014), *Ctenopharyngodon idella* (Wang, 2011; Wu *et al.,* 2015), *Catla catla* (Bandyopadhyay and Mohapatra, 2009; Das *et al.,* 2013) and *Labeo rohita* (Giri *et al.,* 2012). Probiotics also modulate hematological parameters (Michael *et al.,* 2019) and morphological structure of the intestine (Han et al., 2015). Hossain KM *et al.,* (2022) reported the changes in intestinal morphology, lamina propria, enterocyte width, goblet cells and intestinal mucosal fold by the application of probiotics mixture containing *Bacillus sp.* ($1x10^9$ cfu /mL) and *Lactobacillus sp.* ($1x10^{11}$cfu/mL) in *Cirrhinus cirrhosus*.

So, species specific extensive research work is necessary to identify probiotics to increase the productivity in aquaculture after ensuring that these probiotics are free from multi drug resistant genes in their mobile genetic elements.

Risk factor related to probiotics:

Use of probiotics in aquaculture is promising because of its many fold benefits. In the field of aquaculture the need of species specific probiotics should be given prime importance. As *Lactobacillus spp.* was reported (Wong *et al.,* 2015) to harbor multiple antibiotics resistant properties (glycopeptides: vancomycin, aminoglycosides: streptomycin and gentamicin, mono-bactams: aztreonam and fluoroquinolones: ciprofloxacin), it should be used carefully after proper screening, in the field of aquaculture. Previous work on *Lactobacillus* reveals the transfer of its antibiotic resistant properties to other pathogenic bacteria like *Staphylococcus* (Tannock *et al.,* 1994). For this reason next generation probiotic should be protective commensal bacteria (Buffie and Pamer, 2013; Pamer, 2016).

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