

Review Article

The Bed-Rock of Probiotics in Aquaculture-A Review

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Abstract

One major impediment restricting great expansion of aquaculture development, domestication and expansion are diseases which occur in all stages of production and species of fish. The global desire now is the use of probiotics which have some multi-dimensional benefits with generally regarded safe to the animals and the public health. The positive effects of beneficial bacteria on aquatic organisms have sparked extensive research, wide-ranging use in aquaculture, and innovative applications. The actions of probiotics in aquatic animals have garnered significant research attention in recent years. This paper reviewed retrospective view of probiotics including the foundation, discovery, evolution and potential bacteria with benefits used as a bedrock of probiotics. This paper also lay emphasis on extent of successful application of probiotics in aquaculture as growth promotion, immune modulation, improvement of water quality in both earthen and concrete ponds, and potentials on disease control and prevention. This review employed literature searches of databases and internet search engines such Google scholar, Pub Med, and Science Direct to identify peer-reviewed articles, reports and other resources relevant to probiotics in aquaculture. This review work will reveal some useful information on probiotics.

Keywords: Bed-rock, History, Evolution, Probiotics, Benefits, Aquaculture

1. Introduction

Aquaculture has been ranked the fastest growing food-producing sector in the world that has accounted for over 50% of annual world's fisheries production with 110 million tons of fish in 2006 that is used for food (Ngwu, et al. 2011). The rapid growth of the aquaculture sector in the tropics and the world wide at large has been driven by

variety of factors such as the growing world population that has been estimated to be 9 billion by 2050, the economic growth and the expanding exportation opportunities (Souza, 2018). The rapid growth of aquaculture is not far-fetched from its contribution to the world food production (FAO, 2010), which made the need

for aquaculture to increased globally. It is also because the harvest from many wild fisheries has picked up all over the world making the aquaculture to widely be recognized as an effective way to meet the sea food demand of the growing population. In addition, fish products are the cheap source of protein and an important cash crop improving the livelihood of both rich and peasant in many regions of the world (FAO, 2009, 2016) that has contributed for 1% of all global trade in value terms (Bhatnaga and Davi, 2013).

The application of beneficial (probiotic) bacteria which control pathogens through variety of mechanisms of action is globally viewed to be the best alternative to antibiotic treatment. There are many potential advantages of probiotics over conventional therapy, which include: stimulation of appetite, improvement of nutrition by production of vitamins, detoxification, and break down of indigestible compound (Irianto and Austin, 2002) which are not obtainable with antibiotics (Biradar, et al., 2004). These probiotics have been used in both animals and humans for ages without any known pathology.

The usual conventional approaches adopted to prevent and control disease occurrence in aquaculture is the use of antimicrobial drugs (Alderman and Hasting 1998), but Sojjid (1991), Al-Marzouk and sahib, (2012) had observed that limited success had been achieved. Disease treatments are often ineffective, costly and result in development of drug resistant pathogens, product and environmental contamination (Senok et al., 2005). While application of vaccine most especially through parenteral route would have brought success, very few vaccines exist in fish industry, and administration to small size fish or adult fish in large numbers is in practicable. Furthermore, only a few therapeutic/chemicals are approved for disease treatment in aquaculture (USDA, 2005). The indiscriminate use of antibiotics has led to an increase frequency of drug resistant microorganisms and multiple antibiotics

resistance (Alderman and Hasting, 1998). There have been serious growing concerns on the public health threat and not only in human medicine, but also in aquaculture (Verschuere, et al. 2000). The development and spread of resistance factor among bacteria has attracted awareness that antibiotics should be used with great care which has resulted in reduced confidence in antimicrobial agents. The problem created from using antibiotics are enormous that made some government and organizations to place embargo or tighter restrictions on their use in food producing animals. For example, between 1997 and 1999 European Union banned ovapicin and virginiamycin, while spiromicin, tylosin and bacitramycin and Chloramphenicol were banned in Thailand (Aditya, et al. 2007). Even though some countries like Nigeria have less antibiotic control pressure, but through foreign restrictions is likely becoming a global target.

The use of probiotics in human (Anukam, 2007, Sang, et al. 2012), aquaculture (Irianto and Austine 2002), poultry (Dham,et al., 2011), small ruminants (Abd El-Tawab, et al. 2016), large ruminants (Yutaka, et al., 2015) and monogastric animals (Martin and Barbar, 2002) have been documented and is currently focused on the probiotics ability to improve the immune response and nutritional parameters through efficiency and feed conversion ratio (Rini, et al. 2014). There are many potential advantages of probiotics over conventional therapy, which include: stimulation of appetite, improvement of nutrition by production of vitamins, detoxification, and breakdown of indigestible compound (Irianto and Austin, 2002) which are not obtainable with antibiotics. The probiotics do produce natural antibiotic like substances called bacteriocin which help to eliminate unwanted organisms (Biradar, et al., 2004). These beneficial bacteria produce inhibitory substance or create an acidic environment that helps to eliminate unwanted organisms or inhibit intestinal growth of pathogenic bacteria, yeast and even fungi

(Biradar, et al., 2004) in favor of the host without any harm nor public health hazard. The most widely accepted and used definition that embraced all applications of life was provided by joint committee of FAO/WHO in 2001 'as live microbial which when administered adequately or appropriate confers health benefits to the host (FAO/WHO, 2001). The aim of this review is to assess the foundation of probiotics and its evolution in aquaculture.

2. Brief History of Probiotics

The history of probiotics is so long that could be traced hundreds of year's back which started even pre-Christian period where fermented milk was transferred to fresh milk with the mindset of maintaining fermentation (Fuller, 1992). There was no idea of the health benefits derived from the ingestion of the microorganisms responsible for the fermentation, since the motive was only the preservation of the milk to extent its life. However, the 28 years of Eli Metchnikoff, a Russian Scientist who worked at pasture institute, Paris was able to, and the first to established that lactic acid bacteria (LAB) were beneficial to the intestinal health of human that they could suppress the activity of harmful germs. The knowledge of the LAB increased so much that he proposed a theory after noting the long life of Bulgarians Peasant linking their longevity of life to the constant intake of large amount of fermented yoghurt. Even Abraham in the bible, his exceptional long life was also attributed to his constant consumption of sour milk in Genesis 18:8 which today are called as yoghurt (Ho and Prasad, 2013). After proving the reality of the link between human system and the intestinal bacteria, the idea that certain bacteria help to fight off infectious disease became an eye opener. In the ancient past, infections were often treated with moldy bread and fermented milk products (Fuller, 1992). This means that the use of live microorganism in disease control and diet has a long

history and is one of the oldest methods for controlling diseases and preserving food (Gary, 2001).

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3. Yoghurt: The foundation of probiotics

Probiotics was introduced to the world though fermented milk (yoghurt). Yoghurt with live bacteria (probiotic) in olden days was inserted into the rectum of patient with the use of enemas (Kellogg, 2014). Dairy products such as Kefir, koumiss, leben and dahi were often used therapeutically before the existence of microorganisms (Verschuere et. al., 2000). These dairy products were mentioned in the Bible and the sacred book of Hinduism in early 1900BC (Irianto and Austin, 2002). Genesis, 18:1-8 suggests that "Abraham owed his longevity to the consumption of sour milk and even agrees to the fact that

Abraham offered to God fermented milk (Gogineni, et al. 2013). The consumption of fermented milk product called Laban Rayeb and Laban Khad started as far back as 7000 BC. Even though the people of the ancient taking the fermented milk knew not that fermented milk were a powerful remedy to treat gastrointestinal issues, it was until the 19th century that it came to understand why it helped (Ezema, 2013). The collection of different names of fermented milk in various parts of the world is presented in the table 1 below.

Table 1: Common names used for fermented milk in various parts of the world (Gogineni, et al. 2013)

Fermented Milk Product	Country
Kumiss	Mongolia
Kefir	Balkan
Taettemjolk	Scandinavia
Zabadi	Egypt
Doogh	Iran
Dahi	India
Koumiss	Russia
Cieddu	Italy

4. The discovery of probiotic bacteria

The first probiotic bacteria discovered was *Lactobacillus* species, (lactic acid producing bacteria) by Pastuer in 1857 (Rehana, 2003). They were thought to prevent colonization of the gut by other disease-causing bacteria. In 1889 Tisier also discovered *Bifidobacteria* sp. and in 1900 Moro discovered *Lactobacillus acidophilus* and presently the range of probiotics extend well beyond the *Lactobacillus* spp to include *Bacillus* sp, *Vibrio* sp, *Pseudomonas*, Yeasts and algae (Rehana, 2003) and so many. Among them the most highly researched today include *Lactobacillus acidophilus*, *L. bulgaricus*, *Bifidobacteria bifidum* *B. longum* and *B. infantis* (Irianto and Austin 2002) and *Bacillus* species. Following the

discovery of probiotic bacteria, the two foremost known bacteria characterized *Labctobacillos delbruickii* subsp *bulgaricus* and *Streptococcus thermophilus* (Rekha et al. 2023). Most probiotics discovered and identified fall into the group of lactic acid-producing bacterial (LAB), including *Lactobacillus* and *Bifidobacterium*, although there are non-lactic acid organisms. Between 1908 -1964 little or nothing was heard about microbial therapy until when Lilly and Stillwell attempted to define probiotics (Balcazar et al. 2006).

5. Evolution of the Word Probiotics

The actual word probiotics (for life) was first coined by Werner Kollath in 1953 as active bodies with essential functions with various health benefits and later used by Lilly and Stillwell in 1965 as contrast to the word antibiotics (against life) (McFarland, 2015). The word probiotics have been defined by several people over 100s of years with so many modifications and reviews on the first definition expressing different things depending on the environment and the intended use or purpose. Apart from the word probiotic other terms have been used in its place which includes: friendly bacteria, good bacteria, probiont and bio-agents. The review work of Stephen et al. (2009) showed that the original concept of probiotic was possibly formed by Eli Metchnikoff in 1907 who theorized that human health could be aided through ingestion of fermented milk. This idea therefore made him the grandfather of the modern probiotics which attracted him a Nobel prize in 1908 (Verschuere, et al. 2000). His thought was correct which laid the bedrock of probiotics, but he could not attempt to define that substance, only use as fermented milk.

The first review of the first definition was brought by Paker, (1974). He added a word to substance and re-defined it as organism and substance which contribute to the intestinal microbial balance of the host. After 15 years,

Fuller (1989), observed that the word substance, could be dead or live substance, so he removes the word substance and the definition became as live microbial supplement which beneficially affect the host by improving the microbial balance of the host.

Huis Int' Veld, et al. (1999) attempted to extend the definition as mono- or mixed culture of live microorganism which when applied to animal or man affect beneficially the host by improving the properties of the indigenous micro-flora. Since then, so many considerations have been put forward to use probiotic in all animals that generated many modifications of the definition of probiotics (Stephen, et al. 2009).

However, for probiotics in aquaculture practice, Nwogu, et al. (2011), Zorriehzahra, et al. (2016) considered the peculiarity of the aquatic environment, came up with definition of probiotics for aquaculture as live microbial supplement that is administered via feed or directly into rearing water which provide benefits to both aquatic and terrestrial animals. Verschuere, et al. (2000) and Merrifield, et al. (2010) also added that probiotics that is more applicable in aquatic environment should be live, dead or component of microbial cell that when administered via feed or to the rearing water benefits the host by improves the animal's disease resistance, feed utilization, stress response or general vigor which is achieved via improving the host microbial and the microbial balance of the ambient environment (Ringo, et al. 2010), enhancing the nutritional values, the host defense against diseases and improving water quality (Jutta, 2015).

6. Use of Probiotic Bacteria in Aquaculture

It was anticipated in 1980 that bacteria would be found to be useful both as food and as biological control agent of fish disease (Verschuere et al., 2000). The activity of probiotics in aquaculture may be similar to that observed

in terrestrial animals (Rafael and Luis, 2012), although the relationship of aquatic organisms with the environment is much more complex than one involved in the terrestrial animals. The complexes make the definition of probiotics previously insufficient for aquaculture. When looking for probiotic intended for and aquatic usage, it is important to consider certain influencing factors that are fundamentally different from the terrestrial based probiotics, because the terrestrial animals have a much closer relationship with their external environment (Table 2). The microorganisms present in the aquatic environment are in direct contact with the animals and their food supplied having easy access to digestive tract of the animal which are potentially pathogenic or opportunistic. These singular reasons therefore, the use of probiotics for aquatic organisms aims not only the direct benefit to the animals, but also their effects on the farming environment. Application of probiotic in all aspect of aquaculture has received a tremendous attention especially in develop countries of the world (Xiang-Hong et al., 2003).

A wide variety of micro-organisms have been found capable of producing substances which are inhibitory to other organisms. Those organisms that have the ability to produce substance that stimulate the growth of other organisms or inhibit the growth of another, are regarded as probiotics and in future may be utilized for biological control in aquaculture as in mammalian probiotics. Gomez-Gill et, al. (2000) listed the common probiotic bacteria employed in culture of different aquatic organisms which include, *Vibrio alginolyticus*; *V. harveyi*; *V. Pelagius*; *Thalassobacter utilis*; *Pseudomonas* sp.; *Nitrobacter* sp; *Nitrosomonas* sp., and *Bacillus* sp., *Bacillus toyoi* and *B.subtilis*, *B.lechiformi*, Lactic bacteria *Lactobacillus plantarum*, *L. helveticus*, *L. bulgaricus*, *Streptococcus lactis*, *Alteromonas* sp, *Aeromonas media*, *Rosebacter* sp (NS107). However, evidences indicate that probiotic effects are strain-specific,

Table 2: Different applications of probiotics in aquaculture (Cruiz, et al., 2012)

Application	Identity of the probiotics	Aquatic species applied on	Reference
Growth promoters	<i>Bacillus sp. S11</i> <i>Bacillus sp</i> <i>Carnobacterium divergens</i> <i>Alteromonas CA2</i> <i>Lactobacillus helveticus</i> <i>Lactobacillus lactis AR21</i> <i>Streptococcus thermophiles</i> <i>Streptomyces</i> <i>L. casei</i> <i>Bacillus NL 110, Vibrio NE 17</i> <i>Bacillus coagulans</i>	<i>Penaeus monodon</i> Catfish <i>Gadus morhua</i> <i>Crassostrea gigas</i> <i>Scophthalmus maximus</i> <i>Brachionus plicatilis</i> <i>Scophthalmus maximus</i> <i>Xiphophorus helleri</i> <i>Poeciliopsis gracilis</i> <i>Macrobrachium rosenbergii</i> <i>Cyprinus carpio koi</i>	Rengpipat, et al., 1998 Queiroz and Boyd, 1998 Gildberg, et al., 1997 Douillet and Langdon, 1994 Gatesoupe, 1999 Harzeveli, et al., 1998 Gatesoupe, 1999 Dharmaraj and Dhevendaran, 2010 Hernandez, et al. 2010 Rahiman, et al., 2010 Lin, et al., 2012
Pathogen inhibition	<i>Bacillus sp.</i> <i>Enterococcus faecium SF 68</i> <i>L. rhamnosus ATCC53103</i> <i>Micrococcus luteus A1-6</i> <i>Pseudomonas fluorescens</i> <i>P. fluorescens AH2</i> <i>Pseudomonas sp.</i> <i>Roseobacter sp. BS. 107</i> <i>Saccharomyces cerevisiae,</i> <i>S. exiguous, Phaffia rhodozyma</i> <i>Vibrio alginolyticus</i> <i>V. fluvialis</i> <i>Tetraselmis suecica</i> <i>Carnobacterium sp. Hg4-03</i> <i>Lactobacillus acidophilus</i> <i>Bacillus spp., Enterococcus sp.</i> <i>Lactococcus lactis</i>	Penaeids <i>Anguilla anguilla</i> <i>Oncorhynchus mykiss</i> <i>Oncorhynchus mykiss</i> <i>Oncorhynchus mykiss</i> <i>Oncorhynchus mykiss</i> <i>Oncorhynchus mykiss</i> <i>Oncorhynchus mykiss</i> <i>Scallop larvae</i> <i>Litopenaeus vannamei</i> Salmonids <i>Oncorhynchus mykiss</i> <i>Salmo salar</i> <i>Hepialus gonggaensis larvae</i> <i>Clarias gariepinus</i> <i>Farfantepenaeus brasiliensis</i> <i>Epinephelus coioides</i>	Moriarty, 1998 Chang and Liu, 2002 Nikoskelainen, et al., 2001 Irianto and B Austin, 2002 Gram, et al., 1999 Gram, et al., 2001 Spanggaard, et al., 2001 Ruiz-Ponte, et al., 1999 Scholz, et al., 1999 Austin, et al., 1995 Irianto and Austin, 2002 Austin, et al., 1992 Youping, et al., 2011 Abdullah, et al. 2011 Moreira, et al., 2011 Zhang, et al., 2012
Nutrient digestibility	<i>L. helveticus</i> <i>Bacillus NL 110, Vibrio NE 17</i> <i>Carnobacterium sp. Hg4-03</i> <i>Lactobacillus acidophilus</i> <i>Shewanella putrefaciens Pdp11</i>	<i>Scophthalmus maximus</i> <i>Macrobrachium rosenbergii</i> <i>Hepialus gonggaensis larvae</i> <i>Clarias gariepinus</i> <i>Solea senegalensis</i>	Gatesoupe, 1999 Rahiman, et al., 2010 Youping, et al. 2011 Dohail, et al., 2009 Tapia, et al., 2012
Water quality	<i>Bacillus sp. 48</i> <i>Bacillus NL 110, Vibrio sp. NE 17</i> <i>Lactobacillus acidophilus</i> <i>B. coagulans SC8168</i> <i>Bacillus sp., Saccharomyces sp.</i>	<i>Penaeus monodon</i> <i>Macrobrachium rosenbergii</i> <i>Clarias gariepinus</i> <i>Pennaeus vannamei</i> <i>Penaeus monodon</i>	Wang, et al., 2008 Rahiman, et al., 2010 Dohail, et al. 2010 Zhou, et al., 2009 Shishehchian, et al., 2009
Stress tolerance	<i>Lactobacillus delbrueckii</i> <i>Alteromonas sp.</i> <i>B. subtilis, L. acidophilus, S. cerevisiae</i> <i>L. casei</i> <i>Pediococcus acidilactici</i> <i>Shewanella putrefaciens Pdp11</i>	<i>Dicentrarchus labrax</i> <i>Sparus auratus</i> <i>Paralichthys olivaceus</i> <i>Poeciliopsis gracilis</i> <i>Litopenaeus stylirostris</i> <i>Makimaki</i>	Carnevali, et al., 2006 Varela, 2010 Taoka, et al., 2006 Hernandez, et al., 2010 Castex, et al., 2009 Tapia, et al., 2012
Reproduction improvement	<i>Bacillus subtilis</i> <i>L. rhamnosus</i> <i>L. acidophilus, L. casei,</i> <i>Enterococcus faecium,</i> <i>Bifidobacterium thermophilum</i>	<i>Poecilia reticulata,</i> <i>Xiphophorus maculatus</i> <i>Danio rerio</i> <i>Xiphophorus helleri</i>	Ghosh, et al., 2007 Gioacchini, et al., 2010 Abasali and Mohamad, 2010

Therefore a beneficial effect attributed to one strain cannot be assumed by another strain, even when they belong to the same species (Senok et al. 2005).

Extent of successful application of probiotics in the tropics
The application and adoption of probiotics in different ways for different purposes has been on increase with success. The success of application of probiotics in aquaculture in terms of disease prevention, growth promotion, improving digestibility, boosting immunity and improving water quality for cultured organisms have been reported (Zorriehzahra, et al., 2016, Abareethan and Amsath, 2015). The increase in several investigations on probiotics for several purposes across animals and humans all over the world on their role on every spheres of life indicate acceptability of probiotics globally. If the application of probiotics has not been with success, then the story on probiotics would have been on decrease and fading away. Probiotic researches suggest a range of potential health benefits to host organisms and its environment. While probiotics have been used in fermented food products for centuries organisms such as *Lactobacillus acidophilus*, *Saccharomyces cerevisiae*, and *Bacillus* species have been used in humans (Sang, et al., 2012). A probiotic containing multiple strains of bacteria like *Bacillus acidophilus*, *B. subtilis*, *B. lechiformis*, *Nitrobacter* and *Nitrosomonas species*, *Aerobacter* spp and *S. cerevisiae* have been applied to fish culture water to control water quality with great success (Ashiraf, 2000). George, et al. (2016) developed and evaluate the efficacy of a local probiotics (*Lactobacillus plantarum*) in comparison with commercial probiotics, MY-500 containing *Saccharomyces boularch* at different levels using 40% protein diet reported a better significant growth performance in all the diets supplemented with probiotics. They concluded that growth performance and nutrient utilization were significantly better in treatment groups where commercial and developed probiotics are added

than the control group. Krishman 2014 reported the successful inhibitory effect of *Bacillus* species against *Aeromonas hydrophila* and *Vibrio haveyi*. So many probiotic bacteria have been developed and packaged in various forms such as liquid or powdered form and applied on various aquatic and terrestrial animals with commendable results. The table 2 extracted by Cruz, et al. (2012) show many probiotic bacteria that have been reported by various researchers across the globe applied for various purposes on different aquatic organisms with great success.

7. Probiotics for Growth Promotion in aquaculture

Applications of probiotic bacteria to improve weight in aquatic and non-aquatic environment have been demonstrated by Krishman, (2014) and Rekha et al. (2023). In the study of Santhash (2017), the probiotic-supplemented diets successfully increase the final weight of shrimps showing that the addition of probiotics increased the growth performance of the shrimps (Table 3). Similarly, Bachruddin, et al. (2018) recorded highest values of growth in a pond compared other treatments in the shrimp growth with low mortality. Positive effects of probiotic administration to fish growth and immunity response, disease resistance and survival rate of aquatic animals are well documented also (Balcazar et al., 2006). The feed conversion ratio (FCR) value was observed to be higher with probiotic Biogen® supplemented pellet compared to control group which suggested that addition of probiotics improve feed utilization which corroborated with the report of Khattab, et al, (2004) when probiotics was used in diet for same species of fish.

Sayed, et al. (2011) conducted a trial research to investigate effects of 3 probiotics such as Super Bobus, Bio-yeast and Stop-Stress Gold, all consisting of *Lactobacillus acidophilus*, *L. casei*, *Bifidobacterium*

Table 3: List of different probiotic bacteria with their source and growth-promoting role (Rekha et al. 2023)

Bacteria	Source Organisms/Medium	Fish growth promoting Role	Reference
<i>Bacillus Sp.</i>	Common carp, <i>Cyprinus carpio</i> ponds	Increase Survival rate Protease activity Lipase activity	(Yanbo and Zirong, 2006)
<i>Bacillus spp.</i>	Intestines of <i>Penaeus monodon</i> and shrimp pond sediment	Increase Growth and survival Beneficial microbial probiotics	Nimrat et al., 2011
<i>Bacillus sp.</i> (HFH4, CPF3) <i>Bacillus licheniformis</i> (CPH6, CPF4)	The gastrointestinal tract of <i>Channa punctatus</i> and <i>Heteropneustes fossilis</i>	Proteolytic, Cellulolytic, and amylolytic enzyme activity	(Banerjee et al., 2013)
<i>Nitrosomonas Sp.</i> <i>Nitrobacters Sp.</i>	The gut of Catla (<i>Catla catla</i>), Rohu (<i>Labeo rohita</i>), and Grass carp (<i>Ctenopharyngodon idella</i>)	Block the pathogen entry in the culture pond and can raise the concentration of dissolved oxygen	Sunitha and Krishna, 2016)
<i>Pseudomonas sp.</i> (GP21) and <i>Psychrobacter sp.</i> (GP12)	The gastrointestinal tract of Atlantic cod	Elicit the immune response, particularly in mucosal surfaces such as the skin and intestine.	(Butt, et al., 2021)
<i>Clostridium butyricum</i>	Kuruma shrimp (<i>Marsupenaeus japonicas</i>)	Increased the content of intestinal short chain fatty acid along with propionic acid and butyric acid and increased the levels of body crude protein.	(Duan et al., 2018)
Yeast <i>Debaryomyces hansenii</i> HF1	Supplemented to Sea bass larvae	Promote growth by secreting amylase and trypsin, enzymes that help in digestion	Tovar et al., 2002)
<i>B. subtilis</i> , <i>B. licheniformis</i> , and <i>Enterococcus faecium</i>	Supplemented with a traditional meal of Rainbow trout	Enhance fish growth by increasing feed consumption efficiency	(Merrifield et al., 2010)
<i>Lactobacillus Plantarum</i> and <i>Pseudomonas fluorescent</i>	Added in pond water	Increase survival rate by 96.22% in <i>Clarias gariepinus</i>	(Omenwa et al., 2015)
<i>Lactobacillus acidophilus</i> (Fla) and <i>Saccharomyces boulardii</i> (FSB)	Supplemented with the diet of <i>Acipenser baerii</i>	Enhance growth, immunity, and survival rate	(Mocanu et al., 2022)
<i>Bacillus subtilis</i>	Gut of <i>Cirrhinus mrigala</i>	Increase the length and weight of guppies (<i>Poecilia reticulata</i> , <i>P. sphenops</i>), and swordtail (<i>Xiphophorus helleri</i> , <i>X. maculatus</i>), and increase the proteases and amylases activity in ornamental fish	Ghosh et al., 2008)

bifidum and yeast (*Saccharomyces cerevisiae*) on the growth performance and immune response of Nile tilapia (*Oreochromis niloticus*), reported a good growth performance was attributed to *S. cerevisiae* attached to the gut and the secretion of amylase enzymes which shared the

increased digestibility of the diet. Yassir, et al. (2002), identified that highest growth performance was recorded with *Micrococcus* as probiotics and the best conversion ratio was observed with the same organism, so the *Micrococcus luteus* may be considered as growth

promoters the added. The use of *Streptococcus* strain supplemented in the diet of Nile tilapia significantly increased the content of crude protein and lipid in the fish and the weight of the fish increased (Cruz, et al., 2012). Probiotics have been used in aquaculture to increase growth of cultured species through the mechanism, whether the probiotics increase appetite or improve digestibility is not very clear (Cruz et al. 2012), but evidence of increase growth from the use of probiotics have been demonstrated and reported with success (Sayed, et al., 2011, Simah, et al., 2011, Welker and Lim 2011). However, Shira and Sherwood (2014) and Drikso, et al. (2003) explained that this success are achieved through several mechanisms such as preventing colonization, cellular adhesion and invasion by pathogenic organisms, direct antimicrobial activity and modulating the host immunity.

8. Probiotics bacteria on immune-modulation of aquatic animals

Some probiotic bacteria are directly linked to the stimulation of the immune response, by increasing the production of antibodies, activation of macrophages, T-cell proliferation and production of interferon (Raphael and Luis, 2012). Cells such as lymphocytes and accessory cells like macrophages, antigen-presenting cells, and epithelial cells are the mediating cells (Badina, 2013). Immune-modulation refers to any process in which an immune response is altered to a desired level, while immune-modulator is a chemical agent that modifies the immune response or the functioning of the immune system (Bidhan, et al., 2014). The immune response involves recognition of the pathogen or foreign material and the mounting of a reaction to eliminate it. Immune-modulation can be beneficial or detrimental to the host (Snydman, 2008). Probiotics can modify the immune response of the host by interacting with epithelial cells and by modulating the secretion of anti-inflammatory

cytokines which could result in a reduction of inflammation (Stefan, et al. 2009). One target of modulation by microorganisms at several immune systems are small molecules known as cytokines which functions as messenger of the various immune response such as inflammation of the manufacturer of antibodies, others involved in down-regulating the immune responses. Variety of bacteria, viruses and parasite are also able to modulate the immune system by affecting the way antigens are exposed on their surface (Shira and Snydman, 2015)

Immune modulation of blood leukocyte in humans by lactic acid bacteria had been observed (Bidhan, et al., 2014). *Lactobacillus casei* in particular was found to be active in the stimulation of phagocytic activity when administered to mice (Rocha-Romirez, et al., 2017). Probiotics such as *Lactobacillus acidophilus* and *Bifidobacterium bifidum* have been shown to influence some immune functions, which involve one or several components of an immune response including humoral, cellular or nonspecific immunity (Drisko, et al., 2003). For example, *Lactobacillus casei*, *L. acidophilus* and yogurt enhanced the number of IgA-producing plasma cells in a dose-dependent manner (Mikulic, et al., 2017). For bacteria to be effective in the process of immune-stimulation, it may be necessary for them to migrate from the gut to the system of circulation (Oyetayo and Oyetayo, 2005). Mixture of bacterial strains comprises of *Bacillus* and *Vibrio* spp were administered to juvenile of white shrimps presented a protective effect against pathogen *Vibrio haveyi* and white spot syndrome (Balcazar, et al. 2006) and the protection was attributed to a stimulation of the immune system by increasing phagocytosis and anti-bacterial. While Nikoskelainen et al. (2003) has reported the success of *Lactobacillus rhamnosus* in stimulating the respiratory burst in rainbow trout (*Oncorhynchus mykiss*). Stefan, et al. (2009) summarized various probiotic bacteria

that are effective in immune-modulation as seen in the table 4.

9. Probiotics in the improvement of water quality of earthen ponds

In intensive and semi-intensive aquaculture practices, high stocking densities of fish along with intense feeding and fertilization, the quality of water during the culture period will deteriorate faster due to the accumulation of metabolic wastes, decomposition of unutilized feed, decay of biotic materials and proliferation of pathogens (Padmavathi et al., 2012, Sunitha and Padmavathi, 2013). It has been reported that probiotic bacteria directly uptake or decompose the organic matter or toxic material in the water, thus improving the water quality (Verschuere et al., 2000). Probiotic bacterial excretions such as exo-enzymes as a natural by-product of their metabolic activity make the pond medium less inhabitable for bad bacteria (Addo, 2013). These enzyme excretions infuse and spread throughout the pond medium, changing its chemistry and destroying bacteria (Sunitha and Padmavathi, 2013). Lactic acid bacteria are among one of the most used probiotics in aquaculture, due to their rapid reproduction, production of antimicrobial compounds (organic acids, lactic acid, bacteriocins, and hydrogen peroxide), and stimulation of non-specific immune responses in their hosts (Pandivan, et al. 2013).

In a study elsewhere, probiotics played a major role in maintaining optimum water quality parameters especially dissolved oxygen, ammonia, nitrate, nitrite and phosphates throughout the culture period (Sunitha and Padmavathi, 2013). It was clear, that *Nitrosomonas* and *Nitrobacter* species were dominated among the bacterial load and suppressed *Pseudomonas* species in probiotic treated ponds when compared to the control pond. According to Addo, (2013), the application of a probiotic to manipulate the composition and load of pathogenic bacterial

community on the skin, gills, gut and the water environment of a fish may be used in preventing or reducing the severity diseases caused by pathogenic bacteria. Hence by using probiotics, it can be possible to improve water quality and prevent the occurrence of bacterial diseases in fish ponds.

10. Probiotics in concrete ponds and plastic tanks

The benefits of probiotic bacteria either in earthen and concrete ponds or plastic tanks are not different from one another, since same is found in any receptacles. The probiotic bacteria such as *Bacillus* species are associated with development of water quality, reduction of pathogenic population in culture environment, enhancement of survival and growth rate and better health condition of the aquatic organism. Ngwu, et al. (2011) outlined the general benefit of probiotics in in aquatic environment which include: enhancing decomposition of organic matter, reduction in nitrogen and phosphorus concentration, better alga growth, greater availability of dissolve oxygen, lessening cyanobacteria, control of ammonia, nitrite, and hydrogen sulfide lower incidence of disease and greater survival of the aquatic animal. However, Wang and Wang (2008) have reported that there is strong tendency to combine the photosynthetic bacteria, *Bacillus*, nitrifiers and denitrifiers as probiotic compounds often referred to as multifunctional probiotic that can be applied to different culture species in various culture conditions to produce good success.

11. Probiotics on Aquatic Animal Disease Control and Prevention

One major impediment restricting great expansion of aquaculture development, domestication and expansion are diseases which occur in all stages of production and species of fish. Several bacteria have been reported as pathogenic to fish and shellfish. Welker, et al. (2005)

Table 4: Activity of Specific Probiotics bacteria in Health Maintenance and Disease Prevention

Microflora	Associated Actions	Reference
<i>Bacteroides</i> species	Chronic colitis, gastritis, arthritis	Vanderhoof (2001)
<i>Bifidobacterium animalis</i>	Decreases <i>Candida albicans</i> systemic dissemination in euthymic or athymic beige mice	Gionchetti et al. (2000)
<i>Bifidobacteria</i> species	Reduced incidence of neonatal necrotizing enterocolitis	Caplan and Jilling (2000)
<i>Enterococcus faecium</i> SF 68	Decreased duration of acute diarrhea from gastroenteritis	Marteau et al. (2001); Wunderlich et al. (1989)
<i>Lactobacillus</i> strains	Lactobacillus strains shown to be effective in ameliorating pouchitis Improved mucosal immune function, mucin secretion, and prevention of disease	Vanderhoof (2001), Mack, et al. (1999) Wagner, et al. (1997)
<i>Lactobacillus acidophilus</i>	b. Decreased <i>Candida albicans</i> systemic dissemination in euthymic or athymic beige mice d. Prevented urogenital infection with subsequent exposure to three uropathogens <i>E. coli</i> , <i>K. pneumoniae</i> , <i>P. aeruginosae</i> . Lowered serum cholesterol levels	Sanders and Klaenhammer, (2001) Percival (1997) Gorbach (2000)
<i>Lactobacillus</i> GG	Reduced duration and/or risk of rotavirus diarrhea Reduced risk of traveler's diarrhea Reduced severity of pneumonia in children with cystic fibrosis Increased IgA-specific antibody secreting cells to rotavirus and reduced the duration of diarrhea Increased IgA secretion in Crohn's disease	Caplan and Jilling (2000) Gorbach (2000) Caplan and Jilling (2000) Marteau et al. (2001) Marteau et al. (2001)
<i>Lactobacillus plantarum</i>	Produced and preserves key nutrients, vitamins, and antioxidants; eliminated toxic components from food'	Bengmark, (2000)
<i>Lactobacillus plantarum</i> (299v and DSM 9843)	a. Reduced incidence of diarrhea in daycare centers when administered to only half of the children b. Positive effect on immunity in HIV+ children	Caplan and Jilling (2000) Schultz and Sartor (2000)
<i>Lactobacillus reuteri</i>	a. Shortened the duration of acute gastroenteritis b. Decreased <i>Candida albicans</i> systemic dissemination in euthymic or athymic beige mice c. d. Shortened acute diarrhea	Marteau et al. (2001); (2) Shornikova et al (1997)
<i>Lactobacillus rhamnosus</i> (HN001)	Enhanced cellular immunity in healthy adults in controlled trial	Sheih et al. (2001)
<i>Lactobacillus salivarius</i>	Suppressed and eradicated <i>Helicobacter pylori</i> in tissue cultures and animal models by lactic acid secretion	Aiba et al. (1998)
<i>Saccharomyces boulardii</i> (yeast)	a. Reduced recurrence of <i>Clostridium difficile</i> diarrhea c. Shortened the duration of acute gastroenteritis g. May reduce HIV-related chronic diarrhea	(Pochapin 2000; Marteau et al. (2001)) Guslandi et al (2000)
<i>Saccharomyces cerevisiae</i> (a yeast containing sucrase)	Enhanced digestion of sucrose load was shown in infants with sucrase deficiency	Marteau et al. (2001)

reported six Gram negative rods bacteria (*Aeromonas*, *Proteus*, *Citrobacter*, *Pseudomonas*, *Flavobacterium* and *Chromobacterium*) and 3 Gram positive cocci (*Micrococci*, *Streptococcus*, and *Staphylococcus*) genera as potential pathogens and serve as serious threat when fish are under environmental and physiological stress. Shrimps and freshwater prawn are not exception commonly affected by *Vibrio anguillarum*, *V. alginolyticus*, *A. hydrophila* and *Enterobacter aerogens* (Sihang and Sharma, 2012)

Use of probiotics for disease prevention and control has been highlighted by many workers (Anukam and Koyama, 2007, Sihag, 2010, Eslamifar, 2011; Yesillik et al., 2011). Improved resistance against infectious diseases can be achieved by the use of probiotics. 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 (Sihag and Sharma, 2012). 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 (Vine, et al., 2004). The organisms that offer promise to improve health and/or survival of farmed aquatic species are more diverse and greater in number than those considered for human nutrition (i.e. mostly the lactic acid bacteria) and terrestrial veterinary medicine. Among the Gram-positive bacteria, *Bacillus* species (such as *B. subtilis*) is the best evaluated genus for use in fish and shellfish farming, followed by various species of *Lactobacillus* (*L. plantarum*, *L. rhamnosus*).

Regular uses of probiotics in feed of fish have been found to have several health benefits (Rehana, 2003) with less implication than the use of antimicrobial agents. An experimental infection with *Lactococcus garvieae* and

Streptococcus iniae in rainbow trout was controlled with *Aeromonas sobria* GC2 which was isolated from the digestive tract of same species of fish, and incorporated into feed and fed to rainbow trout for 14 days at a dose equivalent to 5×10^7 cells/g of feed recorded only 0-6% mortality in the group treated with the probiotic and remained healthier compared to 75-100% mortality in the untreated group according to the work of Brunt and Austin (2005). In addition, there was stimulation of innate immunity, as evidenced by an increased number of leucocytes and the enhancement of the phagocytic and respiratory burst activity from the same experiment. So probiotics in recent years have been used as alternative measures to control the aquatic diseases since, because of its availability, efficacy and safety to both environment and public health hazard.

12. Statements and Declarations

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