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Use of Probiotics in Aquaculture

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Abstract:
Probiotics, the live microorganisms which are extensively used now a days in aquaculture to improve growth, immune system, survival rate, reproduction, etc. The research on the use of probiotics in aquaculture is vast. There are many types of probiotics like yeast, Lactococcus, Bacillus and Pediococcus which have shown beneficial effects, however the important probiotic bacteria are Lactobacillus and Bifidobacterium.The possible modes of action are mainly competitive exclusion of pathogenic bacteria, production of inhibitory compounds and also enhancement of the immune system towards the pathogenic infections.In few studies, it has also shown to improve the water quality by controlling the growth of phytoplanktons. In this review, the history of probiotics, their mode of action, applications of probiotics in aquaculture, Interference with Quorum sensing and molecular approach has been presented.

Keywords: *Probiotics, Lactobacillus, mode of action, water quality, aquaculture, Quorum sensing*

1. Introduction

The name "probiotic" is derived from a Greek word *pro* and *bios* which means "prolife", which has several meanings with the changing time. Probiotic is a comparatively new term which means 'for life'.Such types of microbes are responsible to maintain the intestinal microbial balance, thus improving the health of the host. The probiotic microbes belong to mostly the strains of the genera of Bifidobacterium and Lactobacillus, but also some strains of Bacillus, yeast and Pediococcus have the characteristics of probiotics. These entire microorganisms together are responsible for protecting the host from the harmful pathogens and also claim to improve the immune system. Probiotics are mostly found in dairy and non-dairy products. (Bianchi Pedroni Medeiros, Caroline Tiemi Yamaguishi1, Juliano De Dea Lindner1, Ashok Pandey and VaneteThomaz-Soccol[5])

During the year 1905, Dr. Elie Metchnikoff discovered the first evidence on the positive impact of some bacteria in the farmers who had consumed milk containing pathogen; the bacteria's were responsible for changing the gut microflora by replacing harmful ones with the beneficial microorganisms. The name probiotic was introduced till 1965 by Lilly and Stillwell, which was a modification of the original word "probiotika." It was also called as a substance which plays the opposite function of antibiotics. Later, Spertiwith few modifications with the time called it as the "tissue extracts that stimulate microbial growth."

After sometimes, Parker in 1974 first used this term to explain the concept of food supplement/ microbial feed. He called it as "organisms and substances that contribute to intestinal microbial balance." Fuller extended the meaning to "live microbial food supplement that benefits the host (human or animal) by improving the microbial balance of the body" and also suggested its use in the conditions of extreme temperatures and variations in the salinity. Later on, it was suggested that probiotics are those "monocultures or mixed cultures of microbes supplemented to animals or humans that are useful to the host which improves the characteristics of the gutmicroflora". The first study was conducted in 1986, to study the effectiveness of probiotics on the growth of hydrobionts (organisms found in the water).

Guarner and Schaafsma in 1998, thought that probiotics are live microorganisms which, when administrated in adequate amounts, provide health benefits to the host.In 1999, Gatesoupe gave the definition as "microbial cells administered in a certain way, which reaches the gastrointestinal tract and remain alive with the aim of improving health". During the same year, research was carried out on the removal of pathogens using probiotics; this expanded the definition to "live microbial supplement which benefits the host by improving its microbial balance".(Patricia Martínez Cruz, Ana L. Ibáñez, Oscar A. Monroy Hermosillo, andHugo C. RamírezSaad[22])

Verschuere et al., improved the definition which helps us to understand the more broader use of the word probiotic and gives answers to the objections which were made before- "as a live microbial adjunct which has a beneficial effect on the host by modifying the host associated or ambient microbial community, by ensuring improved use of the feed or enhancing host response towards disease or by improving the quality of its ambient environment". The growth of the aqua culture industry has been increasing over the past decades; which has caused stress and low productivity of various crop species. The urgency for the improvement of health, the quality of diet and the requirement for reducing the disease in aquatic species have resulted in the increased use of probiotics in aquaculture.

Egypt is one of the major contributors to the world aquaculture studies. The project has been done on the aquaculture as well as wild fishing and is of great importance in the fresh as well as marine continents. Egypt was facing problems in the aquaculture due to the pathogenic infections and the level of stress in the fish due to huge demand of the increasing population. Therefore, the use of probiotics was to be effective in this case. (Mai D. Ibrahem[16])

Afterwards, the applications of probiotics extended for the use of improvement of water quality and to cure the infections caused by the pathogens. Recently there is evidence which says that probiotics helps in increasing the stress tolerance, helps in the reproduction and helps in the better digestion of the essential nutrients.Nowadays, the probiotics consisting of the bacterial species like Enterococcus sp., yeast Saccharomyces cerevisiae, Lactobacillus sp., Bacillus sp., Carnobacterium sp. are available commercially, but are recommended with the careful management practices. (Patricia MartínezCruz,Ana L. Ibáñez, Oscar A. Monroy Hermosillo, andHugo C. RamírezSaad[22]) Empirical process can well define the process for selecting the appropriate probiotics which have limited scientific proofs. The disadvantage is mainly related to the selection of wrong probiotic species. (Bruno Gomez-Gil, Ana Roque[4]).

2. Mode of Action

There are varieties of microorganisms which are now useful to function as a natural bio filter, have a positive impact on the water quality and also used as a diet for aquatic organisms. However, the speed of production of the probiotics is very limited due to lack of knowledge in understanding its mechanism of action. In the recent research on the agricultural and medical probiotic there is an absolute proof of the accurate mechanism of action which has been reflected now.(Fuller, 1989).(Niall G. Vine, Winston D. Leukes, Horst Kaiser[21])

There are many studies which show the compounds secreted by bacteria play the role of immunostimulant in shrimp and fish cultures. Basically, the immune response might be positive by using probiotic in the following ways (Fuller, 1992):Increased activity of macrophage, seen by the improvement in ability to engulf the microorganisms or even carbon particles;

The increased production of specific antibodies, namely of interferon, immunoglobulin

Where, multiplication of the common antibodies on the surface of mucus membrane takes place near the gut wall. (Ali Farzanfar[3])

The use of probiotics proved to be an excellent source through several ways. The results can be seen in a single or in a combination of multiple factors. (Kesarcodi-Watson et al., 2008).

There is ample amount of studies showing the mechanism of action of the probiotics:

2.1. Competitive Exclusion of Pathogenic Bacteria

The competition for binding sites and nutrients by the process of adhesion and colonization to the mucosal membranes are possible protective mechanisms against pathogens (Westerdahl et al., 1991). Several species of *Lactobacilli* are seen to reduce the adhesion mechanism of A. *salmonicida,* C. *piscicola* and *Yersinia ruckeri* to the intestinal mucus in the rainbow trout (Balcázar et al., 2006).

2.2. Production of Inhibitory Compounds

Different species like *Enterococcus durans*, *Escherichia coli, Micrococcus luteus*and *Pseudomonas aeroginosa* produces antibacterial compounds towards the various pathogens of fish as understood by the recent study done on BIOMIN (2009).

2.3. Enhancement of the Immune Response against Pathogenic Microorganisms

Probiotics helps in stimulating non-specific immune system. The effect of Immunostimulants differs according to their use and the mode in which they are effective. The phagocytic activities are enhanced to produce macrophages by specific derivates, such as lipoproteins, ß-glucans, polysaccharides and nucleotides.

The study on tiger shrimp (*Penaeusmonodon*) showed that use of the species of *Bacillus* gave protection against disease after activating the humoral and cellular immune defenses. Rengpipat et al. (2000) (Wang et al., 2005) (Jose´ Luis Balcazar , Ignacio de Blas , Imanol Ruiz-Zarzuela , David Cunningham , Daniel Vendrell , Jose´ Luis Muzquiz[13])

3. Effect of Probiotics on the Water Quality

In order to improve water quality and the health of the aquatic species various vaccines, immunostimulants and even probiotics mainly consisting of the lactic acid bacteria are used in the aquaculture.(L. Villamil, C. Tafalla, A. Figueras, and B. Novoa[14]).There is wide use of probiotics by the shrimp farmers, where the live bacterial cells act as a factor which improves the water quality and can also help in curing the disease in the culture.The precisely controlled trails on the use of probiotics showed no beneficial impact on the water quality aspects when measured (Boyd and Gross 1998), although a small amount of experiments on the pathogenic disease resistance have been performed (Rengpipat et al., 1998; Rengpipat et al., 2003).(Timothy W. Flegel, Donald V. Lightner, Chu Fang Lo and Leigh Owens[28])

In different studies on the use of probiotics used for improving the water quality have been effective, when the gram-positive strains of probiotics like *Bacillus* was used as gram positive negative bacteria. Probiotics can also control the growth of phytoplankton and controls the level bacteria are more capable of converting organic matter into CO_2 as compared to the gram of dissolved as well as particulate organic carbon, when the fishes are in a growing phase. This experiment has a limited acceptance for enhancing the water quality as it has failed to show results in the cultivation of channel catfish and shrimps and also when the probiotic strains of *Nitrobacter,Enterobacter, Rhodopseudomonas, Bacillus* was used.

If the example of edible fishes was considered like trout production farms, it generated high concentration of nitrogen, which had a range of 0.05–3.3 mg L^{-1} of total Kjeldahl nitrogen 6.4 mg L^{-1} after 7 months, when the monitoring was carried out. Taking into consideration the example of total ammonia produced in the tilapia culture of a recirculating system. The rate of ammonia increased from a range of 4.73 to 14.87 mg L^{-1} in a 21-day-experiment and nitrite concentration from 3.75 to 9.77 mg L^{-1}.The reason for the increase in ammonia and nitrites was the increased production of compounds of nitrogen, which was generating high concentration of toxic compounds of ammonia. Therefore, probiotics is only referred when it is used for the water quality production.(Patricia Martínez Cruz, Ana L. Ibáñez, Oscar A. Monroy Hermosillo, and Hugo C. RamírezSaad[22])

4. Improvement in Nutrient Digestion

It is familiar that nutrition may change the fish health and immune responses. Nutritional components are part of the diet, which is important for development and usual growth of the fish. (EinarRingø, Rolf Erik Olsen, Jose L. Gonzalez Vecino, Simon Wadsworth and SeongKyu Song[8]).

It is many times not understood whether the probiotic impact is related to the deletion of pathogens or if it is directly or indirectly associated with the nutritional effects of probiotics.Verschuere et. al. selected nine types' bacterial strains that were found to improve the nutritional value of the dry food which was used for feed Artemia juveniles. (Laurent Verschuere, Geert Rombaut, Patrick Sorgeloos and Will-y Verstraete[15]).The impact of the indigenous microbiota is sustained to not only immune system, but also now to the structure, function, and metabolism process of the digestive tract of the fish. The gut microorganisms also known as probiotics have a major effect on priming immunophysiological regulation in the mucosal barrier of intestine, which has led to new approaches in the field of science of nutrition. (T Pérez, JL Balcázar, I Ruiz-Zarzuel, N Halaihel, D Vendrell , IdeBlas and JL Múzquiz[26])

After performing several experiments on the use of probiotics for the aquatic animals, it has shown a beneficial effect on the aquatic organisms. They have an ability to secrete growth factors such as fatty acids, amino acids and vitamins as well as some enzymes like lipases, amylases, and proteases. Hence, the nutrient absorption is easy if probiotics are mixed with the feed of the fish.

Probiotics are also used in the larvae of edible fishes, for example in European bass (*Dicentrarchuslabrax*). A probiotic species of yeast named *Debaryomyceshansenii* HF1 produces two types of polyamines i.e. spermidine and spermine which plays an important function in the maturation and differentiation in the gastrointestinal tract of mammals. It is also responsible for secreting enzymes like trypsin and amylase which helps in enhancing the digestion of sea bass larvae .When the diet of fish named rainbow trout was supplemented with *B. licheniformis, B.subtilis*, *Enterococcus faecium* for almost ten weeks also showed improvement in the health status of this fish.

Another experiment was performed with the shrimp *Litopenaeusvannamei* Boone and *Fenneropenaeusindicus* supplemented with strains of *Bacillus* used to increase the level of crude proteins, phosphorous and also helps in the digestion of the dry matter. Results improved in terms of weight when the diet was supplemented with 50 g of probiotic kg^{-1} of the food. Study on the ontogenetic stages in shrimp provided the importance of managing probiotics to produce a long term effect on the generation of digestive enzymes.

According to Moriarty, *Bacillus* secretes a wide range of exoenzymes which helps in the nutrient digestion of the fish. And the dissection of some of the species showed the presence of cellulases, trypsin, proteases, lipases and chitinases in its body. (Patricia martínezcruz, ana l. Ibáñez, oscar a. Monroyhermosillo, and hugo c. Ramírezsaad[22])

In 1993, Douillet and Langdon performed trial on the Pacific oyster larvae which was bacteria-free (*Crassostreagigas*) to check the impact of various bacterial strains, which were isolated from the gut of mainly non-axenic adult oysters, used to better oyster survival and enhance growth. But only *Alteromonas*sp.strain CA2 was able to improve the larval survival and growth, if compared with the bacteria-free control, the reason may be due to the nutritional benefit provided by this bacterium. (A. Marques, F. Ollevier, W. Verstraete, P. Sorgeloos, P. Bossier[1])

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5. Impact on the Fish

It is also seen that *Enterobacter cloacae* and *B. mojavensi*also helps in the prevention and control from yersiniosis disease. (E. Capkin ,I. Altinok[7])The probiotics has more of a positive impact than negative impact. For example, a Fin rot was injected into fish by intradermal injection with 0·1 ml volume containing 10^5 cells per ml.

Fish fed with probiotic GC2 had 100% survival after the experiment if compared with Ich with 2% for probiotic BA211 and 0% for controls. Study of innate immune responses showed that probiotic GC2 helps in promoting higher phagocytic activity, whereas probiotic BA211 led to increased respiratory burst activity. This was the first demonstration that probiotics can protect fish from surface infections. Also, this was the first time a probiotic had shown protection against a eucaryotic pathogen, named *I. multifiliis*. (N. Pieters,J. Brunt, B. Austin and A.R. Lyndon[20])

In 2003, Kavitha performed experiments to study the impact of Lactobacillus on two fresh water fishes Oreochromismossambicus and Labeorohita which resulted in the improved growth, nutritional value and digestive enzyme activities. A study was done to check the effect of two probiotic *Bacillus subtilis* strains on the growth performance, digestive enzyme activity in the juvenile white shrimp

(Litopenaeusvannamei). The mixture of two probiotic strains was taken, i.e. L10 and G1 in the equal proportions, given at two different doses105 (BM5) and 108 (BM8) CFU g_1 feed to the shrimp culture for eight weeks.
The final weight, weight gain and digestive enzyme activity was checked of treated and untreated group was measured. Hence, the use of probiotics strains of B. subtilis strains, L10 and G1,helps in improving the growth rate and disease prevention in the shrimp. (HadiZokaeifar , José Luis Balcázar , CheRoosSaad , MohdSallehKamarudin , KamaruzamanSijam , Aziz Arshad , Naghmeh Nejat[12]).
On the one hand, the bacterial strain helps the host with the process of nutrients. On the other hand, the bacterial strains can also be a source for opportunistic pathogens. (Erwin G. Zoetendal, Elaine E. Vaughan, Willem M. De Vos[9])
The probiotics named *Streptococcus* strain was mixed in the diet of *Oreochromisniloticus*(Nile tilapia), which resulted in the increased weight of crude lipid and protein in the fish. The increased weight was from 0.154 g to 6.164 g when used for 9 weeks culture. Therefore, it proves that probiotics can be used for edible fishes.

6. Effect of Probiotics on fish Metabolism

Lactic acid bacteria arecapable of producing different of antagonistic factors consisting of end products which are obtained metabolically, bactericidal proteins as well as substances like antibiotic termed bacteriocins. It has a capability of survival, proliferation and undergoes metabolic activity at the target surface. (SwatiChauhan[25]). In practice, prebiotic compounds must be in refraction to the host digestive process and the combined catabolic activities of bacteria increase in the gastrointestinal tract, so that prebiotic compounds are usually oligosaccharides towards which the probiotic microbes produce valid hydrolases. (Paul W. O'Toole1 and Jakki C. Cooney[23])
It has also been found that when bacteria was isolated in the study performed to check the increased survival of the fish when it was exposed to *Y. ruckeri*, probiotics helped in the increasing the growth and had no harmful effect on the fish.
Thus, the strains of *E. cloacae* and *B. mojavensis* isolated in this study had no harmful effect on rainbow trout and they also showed to enhance the immune system of fish. Hence, they can be safely used as a probiotic as they stop the colonization of *Y. ruckeri* in the digestive tract competition for nutrients andalterthe microbial metabolism (El-Haroun*et al.* 2006). (E. Capkin ,I. Altinok[7])

7. Effect on digestive enzymes

Nair et al. proved that a large proportion of marine bacteria were responsible for producing bacteriolytic enzymes against V.parahaemolyticus. (Laurent Verschuere, Geert Rombaut, Patrick Sorgeloos and Willy Verstraete[15]).Probiotics has an ability to tolerate digestive stresses also has a capability sustainoxygen stresses, tolerate heat, and osmotic pressure are few useful characteristics for the supplementation of probiotic bacteria into essential food products. (R.P. Ross, C. Desmond, G.F. Fitzgerald, and C. Stanton[24]). The probiotics requires following modes to reach their location for showing their effects those are :production of inhibitory compounds; competition for adhesion sites; competition for chemicals; improvement of water quality; enhancement of the immune response ;interaction with phytoplankton; source of macro- and micronutrients; and one of the important mode is its contribution enzymatic activity useful for digestion. (Laurent Verschuere, Geert Rombaut, Patrick Sorgeloos and Willy Verstraete[15])
In conclusion, continuous use of *Ent. cloacae* and *B. mojavensis* as a supplement for food is effective to rainbow trout. Use of these microorganisms can prevent fish from yersiniosis and improve digestibility and utility of feed.Probiotics may be useful in enhancing digestive processes by multiplying the amount of beneficial microbes population, improves microbial enzyme activity; helps in improving the intestinal microbial balance, and subsequently helps in increasing the digestibility and absorption of feed and its utilization (Bomba*et al.* 2002; El-Haroun*et al.* 2006). The intestinal bacteria present in the digestive tract may prevent fish against infections produced by pathogenic bacteria. (E. Capkin ,I. Altinok[7])

8. Interference with Quorum Sensing

Quorum sensing,is beneficial when used for the antimicrobial therapy. The continuous requirement for drug resistant strains of microbes has allowed us to find the effective method to be used for treating the pathogenic infections. (Teresa R. deKievit and Barbara H. Iglewski[27]).The discovery which shows that almost every bacteria can show the property of quorum sensing which bacteria uses to control the virulence is to be an effective method for the antimicrobial therapy. Bacterial quorum-sensing induces the bacteria to produce biofilms. Almost, 10–12 bacteria in bio-films are resistant to antibiotics, disinfectants, and the action of host immune defence. Biofilm formation plays an essential role in the pathogenicity at the time of chronic infections. (H. Wu1, Z. Song1, M. Hentzer, J. B. Andersen, S. Molin, M. Givskov and N. Høiby)
V. harveyi quorum sensing system in vitro controlled the bioluminescence (Bassler et al., 1993) and also produced various virulence factors such as a type III secretion system (Henke and Bassler, 2004a),extracellular toxin (Manefield et al., 2000) ,metallo- protease (Mok et al., 2003), a siderophore (Lilley and Bassler, 2000). Recently, the use of quorum sensing mutants, the discovery that the AI-2-mediated channel of the V. harveyi quorum sensing system stimulates virulence of the bacterium towards the brine shrimp Artemiafranciscana in vivo (Defoirdt et al., 2005). (Tom Defoirdt1, Nico Boon, Patrick Sorgeloos, Willy Verstraete and Peter Bossier[29]). Examples like V. fischerican delay the onset of bioluminescence, before by competing with 3-oxo-C6-HSL for LuxR binding. Also, the seaweed Deliseapulchrahas ability to produce furanone compounds, they are structurally similar to AHLs, they have an ability to interfere with the quorum-sensing systems of Vibrio harveyi ,Serratialiquefaciens and V. fischeri, .(Teresa R. de Kievit and Barbara H. Iglewski[27])

9. Molecular Approach in Probiotic Use

From the past few years, it has created a revolution in the development of very rapid, sensitive, automated, molecular detection methods for a different species of lactic acid bacteria (LAB) dealing with the food industry.(DheerajMohania, RavinderNagpal,Manoj Kumar, AartiBhardwaj,MukeshYadav, Shalini Jain, Francesco Marotta, Vinod Singh, Om Parkash,HariomYadav[6]). Molecular techniques, specific polymerase chain reaction (PCR)-dependent methods, which includes rep-PCR fingerprinting and restriction fragment length polymorphism (RFLP) and also the pulse-field gel electrophoresis (PFGE) are considered as essential for the specific characterization and detection of LAB strains.The PCR technique uses species-specific primers, which have been developed for some species such as *J. lividum* (Harris *et al.* 2009a)

Molecular Ribotypingis a technique which uses nucleic acid probes to recognize ribosomal genes. Nowadays, the bacterial chromosomal DNA is separated and patterns such as restriction are created by hybridization with a 16S rRNA and 23S gene probe.It is well experimented that some probiotics can stop inflammation by preventing the proinflammatory cytokine production, the modes and molecules have recently been studied in pathogens. (Paul W. O'Toole1 and Jakki C. Cooney[23]). Transfer of the probiotic to the host can be studied using the culture-based or molecular methods (Becker *et al.* 2009; Muletz*et al.* 2012). (Molly C. Bletz, Andrew H. Loudon,Matthew H. Becker, Sara C. Bell, Douglas C. Woodhams, Kevin P. C. Minbiole and Reid N. Harris[19])

10. Conclusion and Future Prospective

Probiotic agents are live microorganisms belonging to the normal flora, with less or no pathogenicity and has a positive impact on the health of the host. Therefore, probiotics are very effective live microorganisms having several benefits to the fish cultures. Probiotic therapy uses bacterial interference as well as immunomodulation for the control of various infectitious, inflammation conditions. It is accepted that probiotic has a health benefit on host, but there are limitations to its selection and its use. Proper selection of agents and dose standardization of bacterial strains mainly which is used for the commercial and scientific purpose is required.

The essential role of the gut microbes is in the maintenance of health and the prevention of disease is well known (Holzapfel and Schillinger, 2002). Use of probiotics is likely to be the most natural and safe means for improving gut flora balance to prevent pathogens by using the competing for important nutrients or attachment to the site (Chukeatirote, 2003). According to Kesarcodi-Watson et al. (2008), a probiotic in aquaculture must possess specific properties which are as follow: (1) the probiotic should not harm to the host, (2) it should be acceptable by the host, (3) it should be reachable to the target location where the effect is required to take place, (4) it should be working in the in vivo as opposed to in vitro findings, and (5) it should not have virulence resistance genes or antibiotic resistance genes. (XuxiaZhou1 and Yanbo Wang[31])

Probiotics may also be used in the treatment of the STDs, transmission of HIV- 1, due to its ability to cure various infections in the host. Due to its immunostimulant activity, it may also be used in the production of vaccines in future. (Martha I. Alvarez-Olmos and Richard A. Oberhelma[18]). The present studies say that the risk of getting infections from the lacto- bacilli or bifidobacteria is similar to that commensal strains, and that intake of such products has very small or a negligible risk to consumers/ host, which includes immune compromised hosts. (S. P. Borriello) (W. P Hammes, W. Holzapfel, P. Marteau, J. Schrezenmeir, M. Vaaraand V. Valtonen[30])

Discoveries in the biomedical engineering will be important in the field of molecular biology in the developing systems that transfer the bacteria or nutritional benefits to the host.

It includes encapsulating probiotics, so that they rehydrate at specific sites, and encasing prebiotics in nano- aggregates that prevents against stomach acid and transfer their payload when the pH reaches to 7.4 (Gregor Reid[10])Studies show evidences on the potential medical benefits on the use of probiotics for the treatment and prevention of a various types of infection which includes mucosal surfaces, as well as pediatric gastroenteritis and vaginitis.

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