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INFLUENCE OF PROBIOTICS ON GROWTH AND DEVELOPMENT OF AQUACULTURE – A REVIEW

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ABSTRACT

Aquaculture has become an important economic activity in many countries. In large-scale production facilities, where aquatic animals are exposed to stressful conditions, problems related to diseases and deterioration of environmental conditions often occur and result in serious economic losses. Probiotic use in Aquaculture is increasing as producers attempt to improve pond soil and water quality, enhance survival and improve the growth of cultured species. The probiotics which are used as biological control agents in aquaculture belong to the Lactic acid bacteria, Bacillus and Vibrio are used as Probiotic bacteria. Probiotics, such as Bacillus spp. can be introduced into the culture environment to control and compete with pathogenic bacteria as well as to promote the growth of the cultured organisms. In addition, Bacillus spp. are non-pathogenic and non-toxic microorganisms without undesirable side-effects when administered to aquatic organisms. Probiotics can help to boost the immune system and ward off inflammatory responses in the gut. Application of *Bacillus subtilis* as probiotic has brought very promising results for shrimp aquaculture. This bacterium is a non-pathogenic Gram positive spore-forming which has been used to improve the growth performance and also shrimp health and disease management. The maintenance of good water quality is essential for optimum growth and survival of shrimps. The levels of physical, chemical and biological parameters control the quality of pond waters. The level of metabolites in pond water can have an adverse effect on the growth. Good water quality is characterized by adequate oxygen and limited level of metabolites. Recently, more studies on probiotics including *Bacillus subtilis* have concentrated its role in digestion, absorption, growth, nonspecific immunity, and pathogen resistance in aquatic animals.

KEYWORDS: Aquaculture, Probiotics, Growth, Development, Culture, Gram positive bacteria, ***Bacillus subtilis***.

INTRODUCTION

Today, Aquaculture is the fastest growing and food-producing sector in the world, with an average annual growth rate of 8.9% since 1970, compared to only 1.2% for capture fisheries and 2.8% for terrestrial farmed meat production systems over the same period. World aquaculture production has increased outstandingly in the last few years.[1] Among them, farming of shrimp is one of the fastest growing aquaculture sectors in many tropical countries; however, this development has been accompanied by adverse impacts due to the intensification, such as problems related to diseases and deterioration of environmental conditions.[2] The occurrence of infectious diseases in shrimp aquaculture is a serious problem due to the overuse or misuse of antibiotics and antibiotic resistance genes among opportunistic pathogens such as Vibrio species.[3,4,5] The occurrence of infectious diseases in shrimp aquaculture is a serious problem due to the overuse or misuse of antibiotics and antibiotic resistance genes among opportunistic pathogens.

During the last decades, antibiotics used as traditional strategy for fish diseases management and also for the improvement of growth and efficiency of feed conversion. However, the development and spread of antimicrobial resistant pathogens were well documented.[6,7] There is a risk associated with the transmission of resistant bacteria from aquaculture environments to humans, and risk associated with the introduction in the human environment of non-pathogenic bacteria, containing antimicrobial resistance genes, and the subsequent transfer of such genes to human pathogens.[8] Considering these factors, there has been heightened research in developing new dietary supplementation strategies in which various health and growth promoting compounds as probiotics, prebiotics, synbiotics, phytobiotics and other functional dietary supplements have been evaluated.[9] In this context, microbial intervention can play a vital role in aquaculture production, and effective probiotic treatments may provide broad spectrum and greater nonspecific disease protection.[10,11]

In addition, the invertebrate immune system is highly depends on the innate mechanisms and is incapable of responding to specific vaccines.[12] In fact, the development of vaccines for shrimp infectious diseases have had limited success and limited information has resulted from these studies.[13,14] Therefore, other alternatives such as probiotic bacteria have been examined in order to control the infectious diseases not only in shrimp aquaculture but also in other farmed aquatic species[15] recent study has demonstrated that dietary administration of an antagonistic bacterium, improved the feed utilization, enzyme activity and immune response.[16] Moreover, dietary administration of *Lactobacillus plantarum* resulted in an enhanced immune response in white shrimp, *Litopenaeus vannamei.*[16]

Bacteria used as Probiotics in Aquaculture

In aquaculture practices, probiotics are used for a quite long time but in last few years probiotics became an integral part of the culture practices for improving growth and disease resistance. This strategy offers innumerable advantages to overcome the limitations and side effects of antibiotics and other drugs and also leads to high production through enhanced growth and disease prevention.[17,18]

The probiotics which are used as biological control agents in aquaculture belong to the Lactic acid bacteria (*Lactobacillus*, *Lactococcus* and *Carnobacterium*), such as *Lactococcus lactis, Lactobacillus plantarum, L. rhamnosus, L. sakei, L. delbrueckii, L. fermentum,L. Casei, Leuconostoc mesenteroides, Carnobacterium divergens* and *C. Inhubens.*[17,19-24]

Another important Probiotic group is Bacillus, such *as Bacillus subtilis, B. clausii, B.* coagulans, B. Pumilus, B. cereus, B. Firmus, B. Circulans, and B. Licheniformis, B. Claisey.[25-31]

Vibrio such as *Vibrio alginolyticus, Vibrio* fluvialis[32,33], Pseudomonas[34-38] and Aeromonas such as *Aeromonas hydrophila, A. Media* [39-41] are also used as probiotics.

These probiotics have been used in different aquatic organisms and have been shown to be successful, not only for their ability to prevent disease, but also for improving digestion and growth. Many of these applications have been targeted at the early stages of development of the aquatic organisms, such as the larval stages, because these stages are more susceptible to infections.[42-45]

[46]Hong et al.(2005) have reported that Bacillus strains are suitable as probiotics for aquaculture as they are commonly found as part of the microbiota in the gastrointestinal tract of animals. Some studies have reported that *Bacillus subtilis* enhances the growth of tilapia[47] the survival and net production of channel catfish[48] and the immune response of white shrimp.[49]

Application of *Bacillus subtilis* as probiotic has brought very promising results for shrimp aquaculture. This bacterium is a non-pathogenic Gram positive spore-forming which has been used to improve the growth performance and also shrimp health and disease management.[50-53] In addition it is well documented that Bacillus species are able to produce a wide range of extracellular substances and antimicrobial peptides against variety of microorganisms.[54-57]

Bacteria of the Bacillus genus are among the most widespread microorganisms in nature, they can be found in soil, water and air. Bacillus constitutes a diverse group of rod-shaped, Gram-positive bacteria, characterized by their ability to produce a robust spore.[58,20] The *Bacillus subtilis* genome is totally sequenced, leading to generation of a great amount of basic knowledge in this bacterium. Additionally, developments of molecular and genetic methodologies are well established in *B. subtilis.*[59,60] *B. subtilis* is not harmful to mammals, including humans, and is commercially important as producer of a high and diverse amount of secondary metabolites like antibiotics, fine chemicals and enzymes, as well as heterologous proteins, antigens and vaccines.[61-63] *B. subtilis* grow efficiently with low-cost carbon and nitrogen sources, because its enzymes are very efficient breaking down a great variety of proteins, carbohydrates and lipids from animal and vegetable origin, into their constituent units.[64-66] The enzymes also degrade organic accumulated debris from shrimp/fish cultures inducing ponds bioremediation and consequently the prevention of viral and bacterial diseases.[67-68] On the other hand, the antimicrobial activity of Bacillus is greatly determined by their ability to produce antibiotics, principally from peptide origin.[69] There were identified 795 antibiotics from Bacillus species. *B. subtilis* is the genus most productive species devoting 4-5% of its genome to their synthesis, producing 66 antibiotics.[62] Furthermore, *Bacillus subtilis* is Generally Recognized as Safe (GRAS) by the FDA, meaning this bacterium is not harmful to animals or humans (Figure - 1). Taking into account beneficial properties of *B. subtilis*, this bacterium is a potential probiotic candidate to be considered in “Functional Feeds” of crustaceans and fishes.[70]

Bacillus subtilis Probiotic Capacities

B. subtilis is between the oldest species on earth, reason why animals and humans since the beginning of their existence have been in contact with this bacterium. In this sense, recognition of *B. subtilis* for animal and human immune system is well established and a symbiotic relationship had been developed for a long period of time.[20,71-73] On the other hand, million years of evolution has created an amazing quorum sensing communicationrecognition mechanism between beneficial and pathogenic bacteria.[70,74] However, animals and humans only sense pathogens when the disease had been developed, due equilibrium between beneficial and pathogenic bacteria had been broken because; environmental, nutritional and/or metabolic changes has occurred favouring pathogens proliferation. Synthetic antibiotics was the first option to control pathogens overgrowth in humans and animals, however the unregulated use of these compounds induced a multi-resistant mechanisms preferentially developed by the pathogenic bacteria.[75] Today, antibiotics utilization is well regulated in humans and also in animals including aquaculture. In this sense, the utilization of beneficial bacteria (probiotics) has emerge as an alternative due good results are been obtained in both; animals and humans.[76,77]

Probiotics are Gram-positive, lactic acid-producing bacteria that cause an increase in enzymatic activity, leading to a better feed digestibility. The criteria for lactic acid bacteria strains to be used as "Probiotics" include[78]: (i) exert a beneficial effect on the host; (ii) endure into a food product at high cell counts; (iii) survive during passage through the gastrointestinal tract; (iv) adhere to the intestinal epithelium; (v) produce antimicrobial substances; (vi) have antagonistic activity against pathogenic bacteria; (vii) stabilize the intestinal microflora; (viii) stable against bile, acid, enzyme and oxygen; and (ix) safety assessment (non-pathogenic, non-toxic and non-allergic).

A probiotic organism must possess certain properties, such as[79]: (i) not be harmful to the host; (ii) be accepted by the host; (iii) reach the location where the effect is required to take place; (iv) work in vivo as opposed to in vitro findings; and (v) not contain virulence resistance genes.

Probiotics are used in: (i) human nutrition, as dietary supplements; (ii) animal feed, as growth promoters and competitive exclusion agents; and (iii) aquaculture systems, for enhancing the growth and disease-resistance.[80] Methods to select probiotic bacteria for use in the aquaculture industry might include the following steps[18,81]: (i) collection of background information; (ii) acquisition of potential probiotics; (iii) evaluation of the ability of potential probiotics to out-compete pathogenic strains (in vitro evaluation); (iv) evaluation of pathogenicity and survival test; (v) evaluation of the effect of the potential probiotics in the host (in vivo evaluation); and (vi) economic cost/benefit analysis.

Probiotics, such as Bacillus spp. (a Gram-positive endospore-forming bacteria), can be introduced into the culture environment to control and compete with pathogenic bacteria as well as to promote the growth of the cultured organisms. In addition, Bacillus spp. are non-pathogenic and non-toxic microorganisms without undesirable side-effects when administered to aquatic organisms.[82,83] Their levels of incorporation range between 10^{7} and 10^{8} CFU/g feed, and the application period between 2–4 weeks for enhanced immune response and improved host survival.[84]

Spore probiotics Bacillus spp. (such as *Bacillus subtilis, Bacillus clausii, Bacillus cereus, Bacillus coagulans* and *Bacillus licheniformis*) have a number of advantages over non-spore forming bacteria (such as Lactobacillus spp.), as follows[73]: (i) spores can be stored at room temperature in a desiccated form without any effect on viability; and (ii) spores are capable of surviving the low pH of the gastric barrier.

The benefits of probiotics in aquaculture farming include several effects, such as[85,86]: (i) modulation of immune system; (ii) immunity stimulation under in vitro and in vivo conditions; (iii) feed conversion improvement on the organism through microbial colonization of the digestive tract; and (iv) disease control.

Effects of Bacillus spp. in shrimp culture

According to[87] Zokaeifar et al. (2014), the administration of *Bacillus subtilis* at a dose of 10^{8} CFU/mL in the rearing water of shrimp (*Litopenaeus vannamei*) for 8 weeks confers beneficial effects for shrimp culture, such as: (i) water quality; (ii) growth performance; (iii) digestive enzyme activity; (iv) immune response; and (v) disease resistance.

In addition, administration of *Bacillus subtilis* strains increased total protein, protease and amylase activity of treated shrimp after the colonization in the gastro-intestinal tract. A study of[88] Li et al. (2007) revealed that *Bacillus licheniformis* administered to white shrimp *Litopenaeus vannamei* water culture at 10^{5} CFU/mL inhibited Vibrio species by competitive exclusion, and improved shrimp immunity by increasing their total haemocyte count activity, superoxide dismutase activity and phenoloxidase activity.[67] Guo et al. (2009) investigated the effect of three probiotics (*Bacillus foraminis, Bacillus cereus biovar toyoi* and *Bacillus fusiformis*), isolated from hydrogen-producing fermented solution, against pathogenic bacteria in shrimp larviculture. Results showed that a daily addition of Bacillus fusiformis at an optimum concentration of 10^{5} CFU/mL can improve the survival and accelerate the metamorphosis of the larval shrimp *Penaeus monodon* and *Litopenaeus vannamei* by competitive exclusion effect against pathogens.

The continuing goal of new world aquaculture[89] is to maximise the efficacy and optimise the profitability of fish production. As a result, global aquaculture is becoming more intensified. This may lead to increased fish yields and per capita fish production; however, it is also directly leading to deterioration in water quality resulting in outbreaks of fish diseases.[90] Farmers usually control fish diseases by using antibiotics as feed supplements. The excessive use of antibiotics results in the development of antimicrobial-resistant pathogens, inhibits or kills the beneficial microbiota in the gastrointestinal (GI) system, and produces antibiotic residues in the fish body that are accumulated in fish products and may be harmful for human consumption[8] The European Union banned the import of fish fed with antibiotic supplements in 2006. Subsequently, aquaculture scientists began to explore new strategies to replace the antibiotics used in the feeding and health management of fish in aquaculture[3] These researchers have evaluated new dietary supplements[91] such as dietary prebiotics, probiotics, symbiotics, phytobiotics, and other functional dietary supplements.[92]

The growth of the shrimp Aquaculture industry also increased the need to intensify farming practices to maximize the profits. Problems of diseases often accompanied this intensification as environmental conditions deteriorated and brought the decline of the industry. Pressure to ensure production led to reliance on Antibiotics and Chemotherapeutic agents. The regular and indiscriminate use of Antibiotics and Chemotherapeutic agents in Aquaculture has led to problems of drug resistance.[93,26] The use of preventive and environmentally friendly approaches, namely Antibacterial peptides, probiotics and prebiotics are becoming increasingly important on Aquaculture, particularly in light of new trends toward organic production systems.[95,96]

The word ‘probiotic’ comes from Greek language ‘pro bios’ which means ‘for life’ opposed to ‘antibiotics’ which means ‘against life’. The term ‘probiotic’ firstly used in 1965 by Lilly and Stillwell to describe substances which stimulate the growth of other microorganisms.

The original definition of probiotics is organisms and substances contributing to intestinal microbial balance.[97] The probiotics are a live microbial feed supplement, which beneficially affects the host by improving its intestinal balance.[98] Moriarty et al. (2005) proposed to extend the definition of probiotics in Aquaculture to microbial “water additives”. Probiotics can also be defined as microbial cells administered through the gastrointestinal tract with the aim of improving health of the hosts.[76]

The term "Probiotics" was derived from the Greek word, meaning "for life".[99] According to the currently adopted definition by FAO/WHO, probiotics are: "Live microorganisms which when administered in adequate amounts confer a health benefit on the host.[100] Since 50 year ago exists a scientific and commercial interest in the use of beneficial bacteria for the prevention and treatment of diseases.[101] Probiotics are live microorganisms that provide colonization resistance to the pathogenic microbes and thus are effective in prevention and treatment of some diseases.

Nowadays, the term refers to viable, non-pathogenic microorganisms (bacteria or yeasts) that, when ingested, are able to reach the intestine in sufficient numbers to confer health benefits to the host.[102] A probiotic microorganism that will be used as a microbial supplement in animal feeding should survive and grow under rumen-like conditions, resist to gastric acidity and the presence of lysozyme, bile salts and pancreatic enzymes. These characteristics can be evaluated in vitro and can be used for strain selection.[103] Duodenal acidities and the high concentration of bile in the intestine are the first factors to consider in probiotic selection. Several culture media have been developed and evaluated for the selective enumeration of probiotic Lactic Acid Bacteria in yoghurts.[104] A large number of studies have been carried out for the identification of Lactic Acid Bacteria, which include; gram staining, catalase test, long term preservation of isolates, resistance to low pH, tolerance against bile salt, antimicrobial test, antibiotic test, and carbohydrate fermentation. It is well known that, the major selection criteria (antibiotic resistance, resistance to low pH, tolerance against bile salt, bile salt hydrolysis and antimicrobial activity) used for the determination of probiotic properties of lactic acid bacteria isolates.[105]

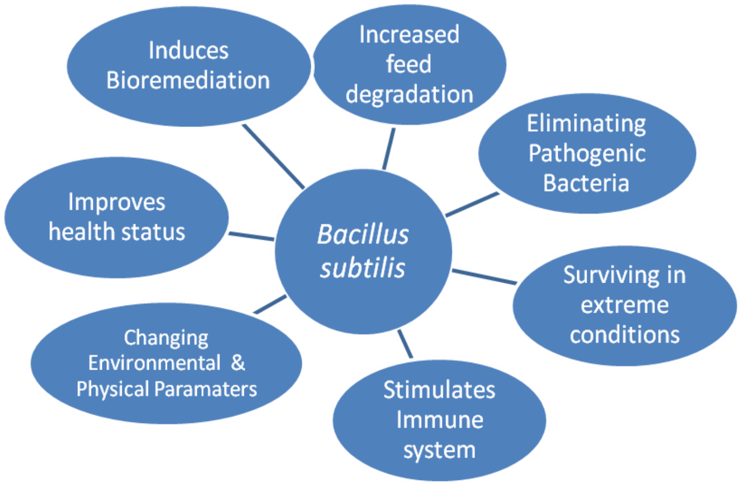

Figure 1: *Bacillus subtilis* probiotic capacities in humans and animals.

In this sense, probiotics as a living microbial supplement that:

(a) Positively affects host health status by modifying the host-associated microbial community and its immune system;

(b) Secrete a variety of enzymes to increases feed degradation-assimilation enhancing its nutritional values;

(c) Improves quality of environmental parameters by the bioremediation of waste products and

(d) Support extreme changing environmental and physical parameters (Figure- 1).

However, to accomplish all these objectives the utilization of "*Bacillus subtilis* spore producing multifunctional probiotics bacterium" it is recommended, to support environmental, nutritional and metabolic changing conditions where probiotic will be involve and most importantly, to take advantage of them.[27,106]

***B. subtilis* has**

a) Versatility of growth nutrients utilization,

b) High level of enzymes production,

c) Secretion of antimicrobial compounds,

d) Spore producer,

e) Develops in aerobic and anaerobic conditions, and

f) *B. subtilis* is Generally Recognized As Safe (GRAS) by the Food and Drug Administration (FDA) (**Figure 2**). In this sense, *B. subtilis* in "theory" could be considered as a perfect multifunctional probiotic bacterium for humans and animals.[107,108]

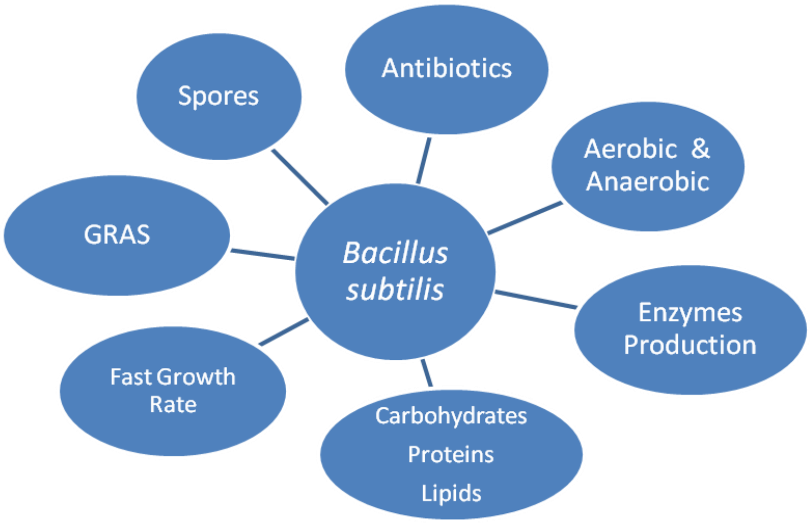

Figure 2: *Bacillus subtilis* Intrinsic Properties.

Microorganisms in Probiotic Products

Probiotics are used for long times in food ingredients for human and also to feed the animals without any side effects. Also probiotics are acceptable because of being naturally in intestinal tract of healthy human and in foods .The probiotics which are use to feed both man and animals are shown in the Table -1.

Mechanism of Probiotics

Lactobacillus and Bifidobacterium species are the most commonly used probiotics in foods for human consumption given the significant health benefits associated with ingestion of these micro-organisms.[109] The ability to resist gastric acidity, adhere to intestinal cells, reduce pathogen adherence, be safe and non-pathogenic and to live and grow as part of a normal, healthy microbiota are characteristics that are considered to make lactobacilli successful probiotics.[110,111]

1. The mechanisms by which probiotics exert their effects are largely unknown, but may involve modifying gut pH, antagonizing pathogens through production of antimicrobial compounds, competing for pathogen binding and receptor sites as well as for available nutrients and growth factors, stimulating immunomodulatory cells, and producing lactase.

2. These bacteria are also able to synthesize vitamins, help in digestion and absorption, inhibit growth of exogenous organisms and stimulate the immune system.[112]

3. The therapeutic potential of probiotics appears through a number of mechanisms of action, including modulation of the immune response, competitive inhibition of invading microbiota in the gut, modification of pathogenic toxins and host products, and enhancement of epithelial barrier function.[113]
4. It was postulated that Gram-positive Bacteriocins produced by lactic acid bacteria (LAB) have received great attention in the past decade owing to their potential applications as food preservatives. Production of bacteriocins and bacteriocin-like inhibitory substances (BLIS) from the Bacillus.[114]
5. Many bacteriocins and BLIS kill sensitive cells by a common mechanism of action through the formation of a transient pore in the cytoplasmic membrane resulting in the leakage of small intracellular compounds and dissipation of proton motive force (PMF) component(s). Antimicrobial proteins may deplete either or both components of PMF (proton motive force) and cause intracellular ATP depletion by either efflux or intracellular ATP hydrolysis.
6. Lactic acid bacteria play a key role in maintaining the balance of normal gastrointestinal microflora[17] One of the key properties of probiotic lactic acid bacteria is the adhesion of cells to epithelial cells or intestinal mucus. This requires strong interaction between receptor molecules on epithelial cells and bacterial surfaces.[110] Adhesion of probiotic cells prevent the adhesion of pathogens[111] and stimulate the immune system.[115]
7. The antibacterial activities of the lactobacilli involve numerous mechanisms of action, including the production of H_2O_2, metabolites, and antimicrobial substances, including bacteriocins and nonbacteriocin molecules.[116]
8. Bifidobacteria, obligate anaerobic rods play an important role in maintaining the healthy function of the colon in a number of ways, including production of short chain fatty acids, pro-motion of the host immunological activity and production of digestive enzymes and vitamins.[117] It is generally accepted that successful delivery and colonization of viable probiotic cells in the host large intestine is essential for probiotics to be efficacious, although a few studies have indicated that non-viable probiotics have similar effects.[118]

Table 1: Microorganisms applied in Probiotic products.

Lactobacillusspecies	Bifidobacterium species	Others
L. acidophilus	B. bifidum	Enterococcus faecalis
L. rhamnosus	B. animalis	Enterococcus faecium
L. gasseri	B. breve	Streptococcus salivarus subsp. thermophilus
L. casei	B. infantis	Lactococcus lactis subsp. lactis
L. reuteri	B. longum	Lactococcus lactis subsp. cremoris
L. delbrueckii subsp. bulgaricus	B. lactis	Propionibacterium freudenreichii
L. crispatus	B. adolascentis	Pediococcus acidilactici
L. plantarum		Saccharomyces boulardii
L. salivarus		Leuoconostoc mesenteroides
L. johnsonii		
L. gallinarum		
L.plantarum		
L. fermentum		
L. helveticus		

Selection Criteria for all Probiotics

In order to be able to exert its beneficial effects, a successful potential probiotic strain is expected to have a number of desirable properties. The desired properties of probiotics strains.[119]

- Acid and bile stability
- Adherence to human intestinal cells
- Ability to reduce the adhesion of pathogens to surfaces
- Colonization of human GI tract
- Antagonism against carcinogenic and pathogenic bacteria
- Production of anti-microbial substances
- Survive the various technological processes of production
- Safety evaluation: non-pathogenic, nontoxic, non allergic, non mutagenic
- Desirable metabolic activity and antibiotic resistance/sensitivity
- Clinically validated and documented health effects

The selection criteria can be categorized in four basic groups

- Appropriateness
- Technological suitability
- Competitiveness
- Performance and functionality[120]

Strains which have these criteria should be used in order to get effective on health and Functional probiotic strains. Probiotics are choosed by using the criteria in Table - 1.

Immune System and Probiotics

Probiotics can help to boost the immune system and ward off inflammatory responses in the gut. However, when inflammation is present but not needed, illnesses such as allergies, flu and chronic infections can occur. Probiotics offer a beneficial defense against the effects caused by this unwarranted inflammation. The effects of immune system are promising. However, the mechanism is not well understood. Human studies have shown that probiotic bacteria can have positive effects on the immune system of their hosts.[121]

Probiotics affect the immune system in different ways such as

- Producing cytokines
- Stimulating macrophages
- Increasing secretory IgA concentrations.[122]

In the last decade, the consumption of Aquatic products has been increased substantially, but the world fishery production was decreased and hence the productions of Aquatic products through controlled conditions have been come into lime light. The probiotics are known to play an important role in carrying out a wide variety of functions including modulation of mucosal and systemic immunity, improving microbial balance by preventing colonization of undesirable bacteria in the intestinal tract.[123,124]

The common probiotics used in pond management are live Bacterial inocula (Non-Pathogenic organisms) rich in extracellular enzymes claims about the potential benefits of probiotics in Aquaculture ponds enhances decomposition of organic matter, reduction of Nitrogen and Phosphorous concentrations, enhances the availability of Oxygen, reducing the Blue-Green Algae, controls Ammonia, controls Nitrate and Hydrogen sulfide, enhances the production rates.

The sustainability and the success of Aquaculture depend on the quality of soil, water, seed selected and feed used. A good quality of soil, water and seed and feed plays an important role in the successful yield under skilful management practices. The ponds often accumulate with uneaten feed materials excreta, moulted shells, dead algae and surface run of organic matter carried by wind and water. When all the above mentioned materials remain undegraded or partially degraded in reduced oxygen condition and toxic gases such as H_2S and NH_3 will be produced. These gases give rise to stress to the cultured organisms resulting in the loss of appetite sluggishness, gulping for oxygen etc and ultimately results in the reduction of growth patterns.[125]

The maintenance of good water quality is essential for optimum growth and survival of shrimps. The levels of physical, chemical and biological parameters control the quality of pond waters. The level of metabolites in pond water can have an adverse effect on the growth. Good water quality is characterized by adequate oxygen and limited level of metabolites. Excess feed, faecal matter and metabolites will exert tremendous influence on the water quality of the shrimp ponds. Hence critical water quality parameters are to be monitored carefully as adverse conditions may be disastrous effect on the growing shrimps.[126,127] The optimum range of temperature for the black tiger shrimp is between 28 to 30ºC. The temperature in the present study was 26 to 30ºC and the low temperature 25ºC was observed due to cloudy weather. The optimum range of temperature of *P. monodon* was between 26 to 33ºC[126,128,149,150] and temperature range of 28 to 33ºC supports normal growth as observed in the present study. Salinity is important parameters to control growth and survival of shrimps.

Recently, more studies on probiotics including *Bacillus subtilis* have concentrated its role in digestion, absorption, growth, nonspecific immunity, and pathogen resistance in aquatic animals. However, few reports have studied a relationship between probiotics[129] and enhanced intestinal mucosal barrier function in aquatic animals. Probiotics improve mucosal barrier function against pathogens, but the specific mechanisms are not well understood. Several possible mechanisms in mammals are presented here:

1. Probiotics can enhance immune function by stimulating mucus and antimicrobial peptide production.
2. Probiotics can improve mechanical barrier function by enhancing tight junction proteins expression and/or localization.[130,131]
3. Probiotics can prevent epithelial cell apoptosis through inhibiting pro-apoptotic p38/MAPK activation[132] and
4. Probiotics can inhibit adhesion of intestinal pathogens by competing for binding sites on the intestinal mucosa surface and inducing mucus secretion.[133]

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