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The role of probiotics in the pond management- a review

Naga Murali Chalamalasetti[1], Sreeya G. Nair[2], Kalidass Subramaniam[1], Patricio De los Ríos-Escalante[3,4], Eliana Ibáñez-Arancibia[3,5]

1- Manonmaniam Sundaranar University, Tirunelveli, India. *Corresponding Author Email: *chnmurali@gmail.com*

2- Assistant professor of Zoology, Sree Ayyappa College of Women, Tamil Nadu, India.

3- Departamento de Ciencias Biológicas y Químicas, Facultad de Recursos Naturales, Universidad Católica de Temuco, Casilla 15-D, Temuco, Chile.

4- Núcleo de Estudios Ambientales UC Temuco

5- Programa de Doctorado en Ciencias mención Biología Celular y Molecular, Universidad de la Frontera, Casilla 54-D, Temuco, Chile.

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Author for correspondence
chnmurali@gmail.com

Abstract

Aquaculture is one of the fastest emerging food manufacturing area which has become an economically important industry in recent years. Intensification of Aquaculture has also brought many challenges in which illness was a main difficulty. Since use of antibacterial mediators as a defensive preventive way is not an encouraging idea and due to potential health hazards linked with the usage of antibiotics in animal farming, the role of non-antibiotic substances became one of the primary factor in fisheries health management. Alternatively probiotics is a major technique which is developed in reaction to problems in infection control where these probiotic ingredients can diminish infection epidemics by increasing the immunity of shrimp and fish. The current study summarises the probiotics selection, procedures and application with its significance in aquaculture and also estimates additional uses of probiotics in Aquaculture.

1. Introduction

Aquaculture is one of the fastest emerging food manufacturing areas and during last years it has become an economically important Industry through various intensifying methods [1, 2]. Penaeid shrimps were the major significant species cultured in this growing period due to its high economic value and export [3, 4]. After introduction of *Litopenaeus vannamei* which is known as pacific white shrimp or American white shrimp in India, shrimp farming has taken a new dimension in terms of production from a unit area. Farmers who were stocking 5-8 shrimps per sq. meter started stocking 25-40 pcs per sq. meter. Ultimately the production capacity from a unit area got increased almost by five times in comparison to Penaeus monodon farming, also increase in feed consumption, leading to increase in feed wastage happened simultaneously. The feed wastage and faecal matter from shrimps started deteriorating pond

bottom faster than before which started bringing new complications in water quality. All these unfavourable environmental conditions lead the shrimps to stress and ultimately to new microbial diseases [5].

In general, intensification of Aquaculture has also brought many challenges like diseases, epizootics, brood stock development alongside domestication and improvement of suitable feed stuffs and mechanisms of feeding, hatchery as well as growing technology and quality of water management [6]. Among all the challenges, illness was a main difficulty in the aqua production [1]. Nevertheless, the use of antibacterial mediators as a defensive preventive way is not an encouraging idea based on the widespread assessment with antibacterial resistance amongst harmful microbes. Added to this, antibiotics (Abs) obstruct or impede useful microbes in the gastro-intestinal (GI) system and it also create antibiotic side chains accumulated in aqua-based yields like fish to be dangerous for human feed [3]. Due to its potential health hazards linked with the usage of antibiotics in animal farming, there is indeed an increasing understanding that antibiotics must be utilized with greater attention [7]. In consideration of the dangers involved with the usage of antibiotic, the role of non-antibiotic substances are one of the primary factor in fisheries health management. According to the literature, probiotics is a major technique which is developed in reaction to problems in infection control. Several studies have recommended that probiotic ingredients can diminish infection epidemics by increasing the immunity of shrimp and fish [8, 9, 10]. Use of probiotics may also reduce culture charges by enhancing the feeding and growth of fish [11, 12, 13, 14, 15].

The word probiotic describes life; which was originated from two Greek terms ‘Pro’ and ‘Bios’ [16]. In addition to improving the animal physiology like improving host intestinal microbial balance and growth performance, the use of probiotics may also results in the quality enhancement of water, improved feed efficiency resulting in low waste by fish [17, 18, 19, 20, 21, 22].

The use of probiotics in Aquaculture was considered as a means of infection control, improving immunity, provide enzymatic as well as nutritional inputs to the host's metabolism, and enhancing the quality of water [23]. The aquaculture production of probiotics will minimize the usage of drugs with prophyl active antimicrobes that have been over-reliant in latest days as danger to people who consuming it [16]. Probiotics were designed for the usage as medicinal substances and few cultivators were previously utilizing them favourably over antibiotics [24, 25]. Utilization of these probiotics, that prevent infections via a diverse mode of actions, viewed as a substitute to antibiotic treatment [26]. The current study summarises the probiotics selection, procedures and application with its significance in aquaculture and also estimates additional uses of probiotics in aquaculture.

The main reason to review the literature on the topic of probiotics in Aquaculture is to identify the best suitable strains of probiotics needed for a specific purpose of solution or application. Each and every probiotic strain will have its own properties of action like who take part in nitrogen cycle, or carbon cycle.

Various Probiotic Strains: The term probiotic was formerly utilized by Lilley and Stillwell [27] in order to illustrate the substances formed by protozoa which induces another microbes and in future utilized to illustrate animal food supplements that is beneficial for animal host [24, 25] and he even reviewed the probiotic definition as “living microbial feeding supplement which usefully impacts the animal host by enhancing the intestinal microbial balance”.

Aquaculture concept suggests that a probiotic is a live supplement of food to microbes that possesses beneficial effects on hosts [28]. A probiotics theory to aquaculture is fairly a recent one, and processes are needed to test probiotics effectiveness. The major aim of utilizing probiotics is to preserve or create a favourable interaction among pathogenic organism that constitutes the intestinal flora or

mucus of fish skin. Fuller [24, 25] suggested that a healthy probiotic must have attributes such as (i) formulation efficacy; (ii) non-toxic and non-pathogenic; (iii) use as cell viability; rather in massive quantities; (iv) sustaining and effectively participating in intestinal digestion and (v) stabilizing and keeping stable over longer storage period or underground conditions. Bioactive molecules with dual origins, the endogenous and exogenous microbes, were identified in marine animals. The prevalent endogenous microbiome of fish species are Gram -ve facultative anaerobic microbes, *Vibrio* and *Pseudomonas*. Certain big aquatic native microbes comprises of *Aeromonads, Plesiomonas*, group of entero-bacteriaceae family and obligatory anaerobic microbes of the classes Bacteroides, Fusobacterium and so on; nonetheless lactic acid microbes were commonly below the dominant in fishes [29, 30, 31, 32]. The maintenance and stability on microbial plants inside a marine animals is connected to exterior ecological aspects [33].

Concept for Usage of Probiotics: There are various types of Aquaculture systems like ponds, liner ponds, cages, RAS, tanks, pens etc. The ecosystem will be different in each of them. The scope for probiotics also will vary from one system to another. Here our study is mainly concentrated on the efficacy of probiotics in pond management. Here in ponds we have again earthier ponds and liner ponds. In lined ponds there will be no interface between water and soil. Usually soil acts as a substratum and dump for most of the wastes produced in the pond during the culture. Naturally available bacteria in the soil will decompose and disintegrate those wastes. Usually the wastes generated in the pond (both earthen and liner) will be feed wastage, faecal matter, dead plankton etc. The biological cycles involved in maintenance of pond ecosystem will be Nitrogen cycle and Carbon cycle. Carbon and Nitrogen ratio usually termed as C: N ratio plays a crucial role in the successful pond management (Figs. 1 and 2).

When we observe the Nitrogen cycle first, we can observe the role of bacteria in that cycle and when we observe the carbon cycle we can notice the importance of carbon as food source to the bacteria. When the ammonia, nitrates and nitrites increases in pond water that is an indication of waste accumulation of inefficient nitrogen cycle in pond. Here there will be no balance in C: N ratio. Nitrogen will be more than standard level of 6:1 to 12:1 [17], Microbes utilize organic material as an energy source in respiratory and CO2 is solubilized in the soil as organic material disintegrates. This decreases the quantity of organic material in the decaying residues whereas N2 is preserved only with residues in microbial activity. The effect is a reduction in the carbon to nitrogen ratio as the substance decomposition.

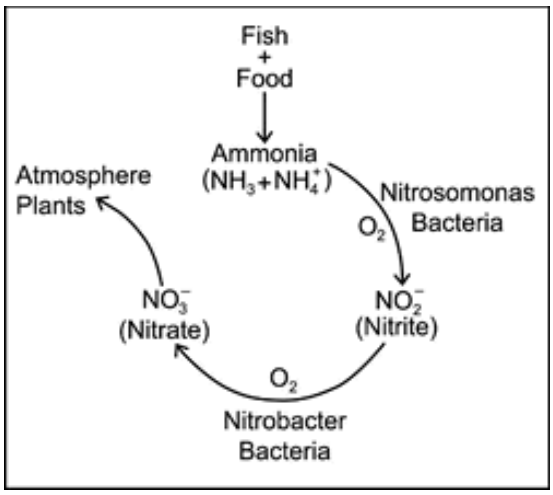

Fig. 1. Nitrogen Cycle in Fish Pond Ecosystem

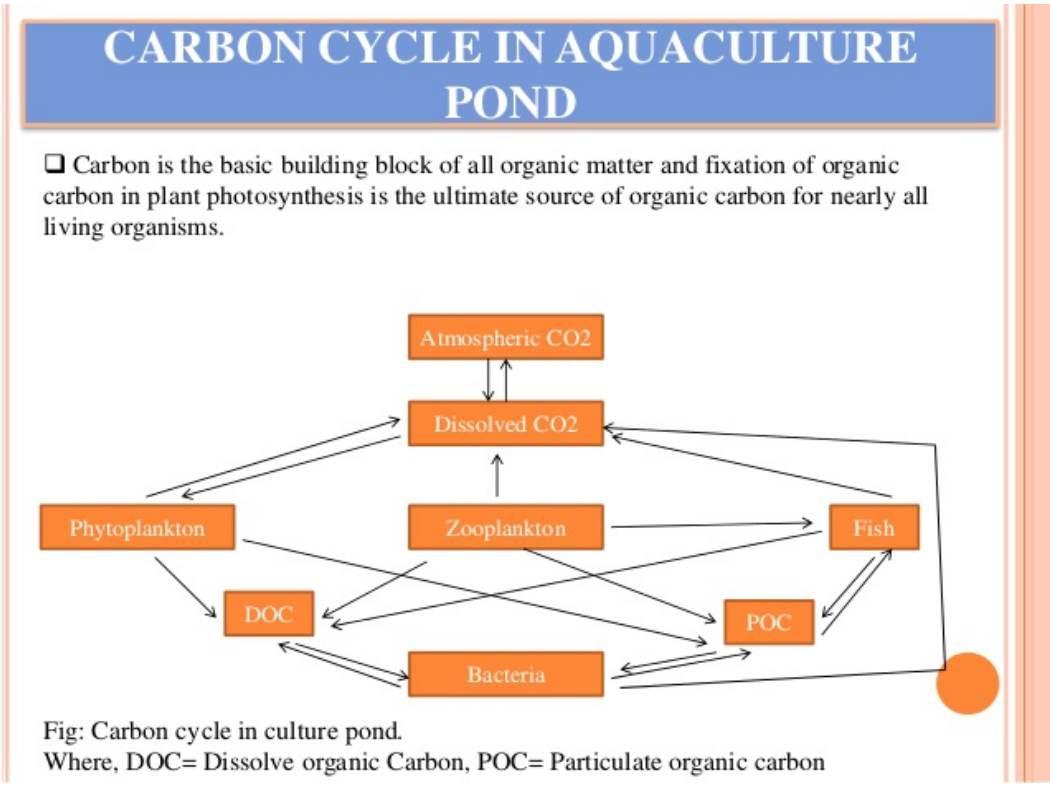

Fig 2. Carbon cycle in culture ponds.

Decomposition microbes have a higher N2 content (10 percent in bacteria and 5 percent in fungi). Since microbes need a great deal of N2 to generate newer cells, biological residues usually disintegrate more rapidly with N2 than they disintegrate with much lesser nitrogen. Particulates are typically higher in intensity of carbon (30-45 per cent), but viruses and bacteria are Fifty per cent. Because carbon is destroyed by microbial metabolism, a relatively consistent carbon to nitrogen ratio is achieved in stabilized organic material wherein microbes have slow respiration in fresh organic material [17].

Today we have probiotics available not only to maintain nitrogen cycle but also bacteria which can decompose Hydrogen sulphide etc. toxic gases too. Probiotics are also used in eradication of pathogenic bacteria on the concept of completion for food and space. If we inoculate the pond TPC/TVC (total plate count or total viable count) with good bacteria then the scope for proliferation of harmful bacteria which can cause diseases will be low, in the same way the good bacteria or beneficial bacteria will be using away the food needed for pathogenic bacteria to proliferate and in that way also probiotics gives good results. *Bacillus subtilis* is the common bacteria used for this principle since that is the easiest and fastest growing bacteria.

2-Methods of Application

Probiotics are been directly applied to shrimp and fish ponds to enhance the quality of water environment [17] and ability to survive in culture species [34]. Probiotics efficacy could be clarified through processes of biological control or biological augmentation through which the sediment and water microbial ecosystem is enhanced [29]. The process of inserting probiotic substances into marine organisms was utilized to enhance the fish immune reaction to infection of pathogenic bacteria [35, 36]. In freshwater aquaculture, many biological processes like pathogenic microorganisms inoculate, enzymes formulations, and crop surface compounds were utilized as ways of enhancing soil and water quality

conditions [17]. Freeze-dried probiotics could also be utilized in shrimp immunizations [37], as the probiotics stimulate the immunity of host animal via improving antibodies action.
Purposes of Probiotics: Various research have shown the merits of probiotics for marine animals, like stimulating growth or improving feed digestive system, immunity and controlling quality of water.

3- Development and Digestive Method Promoter

Adding probiotics to fish nutrition will decrease the usage of Abs and chemical compounds in the feed [24, 25]. Subsequently, the introduction of supplements to the fish diet on fish farming is becoming common. The use of probiotics leads to decreased feed prices, which shows a major role in deciding aquaculture. Interestingly, prior studies has reported that the potential benefits of probiotic strains can be seen as increased feed of marine animals by supplementing gut bacteria, improving feed quality and increased growth, preventing gastrointestinal diseases and pro-nutrition features exist in the complex feed [30, 31, 38]. The role on probiotics particularly in improving growth and feed in fish was regarded with improving the digestibility of nutrient [13, 14]. Although, many probiotics repopulate the host and significantly impact gastrointestinal tract by growing in numbers and producing microorganisms, helping to improve the microbial composition of the intestines and subsequently the digestibility and uptake of feed [15, 39]. The microorganisms colonize in the digestive system after transformation via the abdomen, and use a huge proportion of carbohydrates to develop and lead to a variety of proteolytic enzymes [39]. Nonetheless, it is critical to analyse the feeding process in order to prevent deactivation or degradation of useful probiotic organisms in the culture [15]. The microbiome can act as an alternative protein source and biological growth in the intestinal system, and can be an origin of amino acids, nutrients and micronutrients [30, 31]. In practice, a range of chemicals and substances are being documented to provide stimulating action as a probiotic in defined rate of growth, food palatability, and productivity improvements of consumption, and marine species sustainability. The digestive organs are actually quite responsive to fortified foods and cause major modification in gastrointestinal enzymatic activity [15]. Nevertheless, the use of higher probiotic quantities may not necessarily contribute to increased growth efficiency [40]. Distinct probiotics provide distinct factors in supporting development and utilization of nutrients in fishing species [15]. Consequently, probiotic strains efficacy on fish culture was based on species of hydrobiont, temperature, level of enzymes and hereditary tolerance and quality of water [41, 42]. In addition, the phase of hosting existence often plays a significant role in determining the efficacy of probiotic application. This was apparent to create good microbes in bivalve larvae, because transition period of microbes in bivalve larvae became too limited and microbial appeared to be complicated to create colonies [32, 43, 44, 45]. Furthermore, prebiotics were classified as an un-digestable dietary supplements, which were helpful in accelerating health by enhancing microbes in a digestive toward improving host microbial colonies [46, 47, 48, 49]. Nonetheless, several authors were observed with prebiotics consumption which has no positive impact on aquaculture and feed digestibility [41, 42]. To a certain point, an increasing quantity of substances in abdomen holds a possibility and perhaps infections may be able to digest the intestinal compounds [46, 47, 49]. Therefore, further research on the prebiotics efficacy on aquaculture are required before applying prebiotics to farming and fish hatchery or shrimp.

4- Immune system promoters.

The usage of beneficial microorganisms to dislodge infections by competition was utilized as a favoured approach for the administration of antibiotics in the livestock industry, and is still gradually gaining for infections control in aquaculture [34, 49, 50]. Amongst these defensive microflorae found to be quite

effective in barriers to bacterial agents [51, 52, 53, 54, 55, 56] as well as in regulatory factor production, like single-chain fatty acids and bacteriocins, in the digestive system of fish [57]. Larval fish, shrimps as well as other invertebrate immune systems are quite well grown than in adult stages. Subsequently, larvaeis usually most reliant on unspecific response of immunity to the disease. Nevertheless, latest researches have shown that the unspecific immune defence of species mentioned in the table above might be enhanced by supplementing the dietary probiotics to the culture water. The colonization level of bacteria in the gastro intestine was stated based on the range of microbes in the feed [58]. Another report has explicated the various mode of actions by which probiotics moderate the fishing immune response, namely inducing outcome of better seditious cytokines on an action of immunogen based tissues, Abs and antibacterial peptides etc. in reaction to intrusive infections [28, 59]. In general, probiotics effectively prevent a proliferation of possible viruses throughout a digestive system via antibiosis/food and space rivalry, and also altering the cellular diversity and enhancing immune system [39]. Nonetheless, an efficacy on various probiotics towards infections varies to specific organisms and pathogenic mode of actions of the pathogen based on the aquatic species defence mechanism [40].

Impacts on these probiotics especially in the immunity and microbial processing are well established in fish animals and in environment. Oral dosing of *Clostridium butyricum* microbes to rainbow trout was shown to improve the tolerance to Vibriosis by enhancing the leucocyte phagocytic action [60]. Probiotic administering by food source and crop groundwater significantly reduced microbial growth in certain teleost like *Sparus aurata* and *Salmo salar* [38]. A beneficial rod type microorganisms, *Lactobacillus* (strains MMI and MM4) have been documented together with the production of hydrogen peroxide and bacitracin-like compounds that have significant inhibitory activity towards gram-negative *Vibrio* organisms and gram-positive *S. aureus* that causes orange spotted infection, the phagocytic field of activity and serum [56]; moreover the phagocytic action, serum compliment C3 and IgM concentrations of *E. coioides* are elevated in *Bacillus* cured in groups of fish. Nikoskelainen et al., [50] study demonstrated the presence of *Lactobacillus rhamnosus*, lactic acid pathogen on the stage of around 10 colony forming unit per gram feed induced a pulmonary eruption in rainbow trout.

By comparison to greater vertebrate's animals with inherited immune system, shrimps get an inborn immunized reaction [10, 12, 51, 52, 61]. Probiotics were supposed to play an essential part in stimulating the immune reaction in shrimps because of the simple immune system [62]. Rengpipat et al. [51] study developed its usage of *Bacillus* sp. supplied disease defence in tiger shrimps by stimulating both humoral and cellular immune defences. Administering a combination of strains of bacteria favourably impacted the production and development of juvenile white shrimps and offered a defensive impact towards *V. harveyi* infections and white spot syndrome virus. Through growing a defence, the activation of the immune response was linked to improving phagocytosis and anti-bacterial activity [30, 31]. Through growing a defence, the activation of the immune response by improving the phagocytosis and anti-microbial movement [30, 31]. Bacillus species were employed to enhance and prevent a *Vibrio* sp. contagion to penned crabs [34, 63]. Additionally, *Vibrio* sp., peptidoglycan, β-1-3 and yeast glucan has evaluated in laboratory in a minor scale and the outcomes implies that it may be employed as significant substance in controlling the infection in shrimp via immune stimulus [28]. Furthermore, a probiotic microbes like *L. planetarium* and *B. subtilis* have stated to stimulate immune reaction and genetic factor expression in white shimps once provided in the diet. L. planetarium enhanced phenol and pro phenol oxidase, lung bursts and clearance effectiveness of *Vibrio* species micro RNA transcript and existence proportion after trial with *Vibrio* species for 168 hours when provided to shrimp at 1010 colony forming unit probiotics per Kg diet [64]

5- Water Quality Improvement

A vulnerability of cultivated marine organisms with high levels of nitrogen substances like NH3, NO2- and NO_3^- are usually non-specific but higher concentrations of these substances impact in aquaculture organisms and are likely to induce higher death rate. Gram +ve uses of *Bacillus* species is usually more affordable than applying gram -ve microbes to transform organic compounds back to carbon dioxide, resulting in a higher quantity of natural carbon being converted into microbial activity [24]. An efficacy of +ve endospore producing microbes, like *Bacillus* sp. for enhancing quality of water by affecting the structure and productivity of water-borne disease communities correlated with harvested organisms were assessed [54]. *Bacillus* sp. are linked by enhancing quality of water, decrease of harmful *Vibrio* species in the surroundings, improvement of shelf-life rate and enhanced state of health of Juvenile *Penaeus monodon* [19, 65]. Additionally, another intestinal organisms in which belonging to the genus *Nitrobacteria, Pseudomonas*, and plant-derived probiotics, namely yucca extract, tannic and citric acid, etc. are also evaluated and it has utilized in the culture systems pointed to have a significant enhancement in quality of water [17, 24]. A prerequisite on the usage of prototype probiotics in ponds of aquaculture were increased a decay on natural substances, diminished quantities of N_2 and P, enhanced algal blooms, increased accessibility of O_2 in the water, depressed cyanobacteria blooms, regulated levels of NH_3, NO_2^- and H_2 sulphide, reduced illness incidences, higher existence and better production [17].

6- Future Prospective and Challenges

The usage of probiotics in aquaculture has becoming most common. As stated here, the usage of probiotics gives several benefits like higher production, feed performance, increased reaction to the immune response, and also enhanced the quality of the water. More experiments are necessary to know the probiotics pathways in detail. When we begin utilizing them from initial phases of the community, probiotics become more successful. Because marine lives are in direct communication with their surroundings, supplementing the waters with probiotics may be beneficial. For instance, exposed probiotics in food at the bacterial stage, can contribute to the creation of a healthy transitional gastrointestinal tract that may be formed later [48, 49]. The routine use of probiotics by feeding animals raised in captivity could be utilized to sustain the bacterial community in the intestinal system at a degree that can convey adequate versatility.

In theory, the bacterial species were specifically extracted from the intestinal system and then introduced towards this host species, and many industrial probiotics has lately been found to have utilized. Probiotics get the ability to affect that both animals and the surroundings in a positive or negative effect. The identification of the species and hosts of the microbiota is quite significant, and typically defines the interaction features. Hence, the variety and origin of probiotics in general shows a significant role in optimizing the probiotic uses to avoid unnecessary cost. In addition, mutations that happen in the habitat environment and therefore predominant communities of provided probiotics may become infective and may be dangerous to distressed or damaged organisms in the host [48, 49]. A variety of probiotic strains were tested, as shown by their effectiveness in aquaculture. The aquaculture sector is becoming commonly accessible for intestinal microbes inoculum which are organisms-specific probiotics. These formulations were optimized to provide more efficient purpose as the probiotics is added. In addition, the consistency of the bioactive components must be analysed in detail. The implementation of novel techniques of study, like molecular strategies for analysing probiotic products and for in vivo testing, is anticipated to vastly enhance both probiotics consistency and functional attributes.

Conclusively, the precise purposes of probiotics in aquaculture cannot be repudiated. It has proven their profits of improved infection resistance, enhanced food digestion and growth enhancement in the species

and moreover improved the water and soil quality during the culture period. There were plenty studies and numerous reports which has established the effectiveness of probiotics, but major research was showed and appraised underneath lab-based circumstances, henceforth the utilization of probiotics under cultured condition is indispensable in order to estimate their usage accurately. Particularly, the attention of source, species superiority and application techniques would be required to estimate the usage of probiotics.

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