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Probiotics used in Biofloc system for fish and crustacean culture: A review

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Abstract
The review was made with results obtained in fish and crustacean culture with probiotics and Biofloc system culture. The importance of bacteria in aquaculture production systems was discussed and showed the advances in probiotics and Biofloc system application, separately and in combination. But it is necessary to increase studies on implicit associated bacterial communities, also the variations of this community throughout production process and especially, probiotic addition to displace microbiota. Microbiome studies could show a better general view of abundance, diversity and bacterial ecological function in fish and crustacean culture.

Keywords: aquaculture, microbial communities, Biofloc, probiotics.

1. Introduction

Microbial communities in aquatic habitat respond quickly to changes in their immediate environment. These changes can be subtle and manifest as activation or inactivation of certain metabolic pathways of bacterial community or change in their composition and functionality [1]. The same happens in aquaculture production systems where microorganisms that develop, are diverse and can act in a positive way in transformation of organic matter and compounds generated in production systems. At the same time, they can be used as microbial biomass source available for higher size organisms, but also, they can act in a negative way to develop virulence factors in response to environmental variations [2].

Recent studies suggest that proper handling of microbial communities can help to get better water quality, on one side reducing nutrient levels (nitrogen compounds and bacteria biomass) and for other side, reducing pathogen bacteria abundance, consequently increasing survival of cultured species, without use toxic chemical substances or antimicrobials [1; 3]. Considering this point of view, one of the most used technology in aquaculture to modified microbiota is the application of probiotics [4], which have shown health benefits and water quality of aquatic cultured species, through different action mechanisms like: stimulation of immune response, substances segregation which inhibited the microorganisms pathogen growth, enzyme production which induce absorption and improve fish and crustacean nutrition and, nitrogen compound reduction which were transformed by microorganism in culture systems [5; 6].

Otherwise, in last years the searching of new sustainable aquaculture technologies allows the development of culture method called Biofloc [7], which uses food waste, organic matter and, compounds produced during the production, through proliferation of heterotrophic microorganisms which develop in water using an external carbon source and high oxygen levels [8]. This technology has demonstrated positive results in fish and crustacean production, with significantly saving of commercial food, also in medicines and chemical supply for disease control and, reduction of water exchange which reduce significantly the environmental impact on water and limit pollution reducing aquaculture effluent discharges [9; 10]. Recently, diverse studies were implementing both technologies, through endogenous addition of probiotic bacteria in Biofloc system, considering that they improve the obtained results, with respect to their application separately [11]. So, this review described the impacts of probiotics in fish and crustaceans culture systems using these two technologies: probiotics and Biofloc.

2. Importance of microbial communities in aquaculture

Microorganisms constitute an essential part of trophic ecosystems chains, because their capacity to mineralize nitrogen compounds and clean water ecosystems, making available to primary producer's nutritive substances; also contribute with biomass production, which are consumed efficiently by eukaryotes predators and finally, were obligatory intermediates between detrital organic matter and organisms which were found in higher trophic levels [12]. In some cases, and in determined conditions, microorganisms can be found in densities higher than 10 million per milliliter (10^6 mL^{-1}) and acting in a complex way, mainly through substances that they produce and excrete [13]. Despite the importance of microbial communities in culture systems, most studies focuses in pathogenic microorganisms detection, within which they stand out the genders: *Aeromonas, Vibrio, Fotobacterium, Enterobacter,* among others [14; 15] and only few studies about benefit of heterotrophic microbial diversity which show the ability to adapt to different environments and the efficiency in the way they use the substratum [16], given rise to broad physiological diversity in cellulosic, amylolytic, chitinolytic, and nitrogen-transforming bacteria and other waste compounds generated in culture process and fish and crustacean production. The genders with higher abundance are: *Bacillus, Alteromonas, Micrococcus, Rhodococcus, Pseudomonas* and some yeast [17; 18]. Previous studies [19], demonstrated the capacity of three isolated gender of *Bacillus* sp. to decrease nitrites, nitrates and, ammonia concentration in fish and crustacean water culture medium. Same phenomenon was observed by other authors [20] with *B. subtilus* and *B. licheniformis,* which attribute these effects to mechanism such as bacterial bioaccumulation, bio assimilation and nitrification.

3. Probiotics used in aquaculture

Aquaculture system based on microbiota represents a viable strategy to reach sustainability [21]. With respect to probiotics, there is a biotechnology which in last 20 years has increased worldwide about fish and crustacean culture [4; 22], not only because their application was associated with gut health in cultured species, but also with environmental bioremediation of soil and water in aquaculture systems. The effects of strains of *Bacillus subtilis*, *Paracoccus* sp., *Bacillus pumillus*, among others, added to water directly, involve the modulation of microbiological profile in ponds, degradation of undesirable residues (ammonic, nitrite, and hydrogen sulfide), higher mineralization of organic matter and, decrease of anaerobic conditions in lower surface of ponds, avoiding noxious anoxic areas [23].

The definition of probiotic [24] is: "Live microorganisms which added in adequate quantities provide benefit actions on host and environment wellness that surrounds them". With respect to the advantages that probiotics grant it can be mentioned the nutrient assimilation increase, stimulation of immune system, potential pathogen exclusion, and nitrogen compound degradation in culture water. That's why they were used to prevent infections, growth promoters, and improve water quality [25; 26; 27]. Between probiotics most used were lactic bacteria, bifidobacteria, and yeast. In recent years, bacteria strains which are used have been expanded, principally *Bacillus* sp. genus, which were commercialized in preparation form, with one or more live microorganisms, which allow the production of diverse aquatic organisms in aquaculture [23; 28].

4. Biofloc culture system

In last decades, inside aquaculture sector, it has been designed different production systems to diverse aquatic organisms, aimed to reduce water use and culture space, considerably increasing culture densities. Likewise, those systems that limit infection outbreaks and minimize operating costs have taken relevance [29; 17]. An interesting example of this system type was so-called Biofloc, which consist of developing microbial aggregates formed from carbon:nitrogen ratio (C:N) in water, with low or zero exchange and high oxygenation, diets with low crude protein content and external carbon source like molasse (sugar cane), rice bran, wheat bran, among others. This C:N ratio allows microbial community growth, especially of heterotrophic bacteria, which metabolize carbohydrates and consume inorganic nitrogen (principally ammonia NH_4^+), reducing their concentration and improve water quality [10; 30; 31; 32].

Biofloc technology (BFT) is one of the innovative methodologies for waste management and nutrient retention that offers a solution to solve environmental problems in aquaculture [33]; because it doesn't use water exchange to solve nitrogen compound elimination, but it utilizes microbial assimilation, stimulated for addition of material rich in carbon, to transform those compounds. Also, microbial protein can be used as food for culture species [9; 34]. This is very important because in any fish and crustacean culture system, water quality management is the principal factor that impacts in production sector, particularly in intensive and hypertensive culture systems [35].

In conventional fish and crustacean culture systems, the nitrogen compounds transformation is made by autotrophic microorganisms of *Nitrospira, Nitrobacter* and *Nitrosomonas* genders. However, an excess of organic matter in form of carbon, inhibit their efficiency by limiting their growth [34]. Contrary, in Biofloc systems, nitrogen compounds transformation is more efficient, because this process is made by facultative heterotrophic bacteria that correspond principally to *Bacillus* and *Pseudomonas* gender. These two genders are more efficiently in organic matter presence, which allow increasing their population abundances quickly and oxide-reduction process [16]. Some authors have shown different benefits using Biofloc systems to fish and crustacean culture, as it is observed in Table 1.

Table 1: Obtained advantages to culture fish and crustacean in Biofloc systems.

Author(s) and year	Studied specie	Results
Gaona et al. (2017) [36]	*Litopenaeus vannamei*	Increase in 30% growth and survival of shrimp in Biofloc treatment.
Mansour and Esteban (2017) [37]	*Oreochromis niloticus*	Better growth and immune response in Biofloc treatment with respect to control treatment (without Biofloc).
Liu *et al.* (2017) [38]	*Litopenaeus vannamei*	Better growth was observed in shrimps with low culture density with respect middle or high-density treatment with Biofloc.
Suita *et al.* (2015) [39]	*Litopenaeus vannamei*	Dextrose and molasses have used to produce flocs. The results shown that dextrose has better effect on growth and culture water quality.
Anand *et al.* (2017) [40]	*Penaeus monodon*	Different dry Biofloc percentages were added (4, 8 y 12%). Concentration of 4%, shown

		low mortality and better immune response.
Widanarn and Mayram (2012) [41]	*Oreochromis* sp	Water quality was better in Biofloc treatment. Could maintain in culture water a 7.5 pH values and ammonia concentration was under 1.1 mg L^{-1}.
Emerenciano *et al.* (2012) [32]	*Farfantepenaeus brasiliensis*	Biofloc system improves growth shrimp. Likewise, authors shown protozoa, rotifers, cyanobacteria and diatoms, contribution to improve growth and survival of cultured organisms. Also, commercial food reduction.
Maya *et al.* (2016) [42]	*Oreochromis niloticus*	Bacteria with probiotic potential were identified in Biofloc system, like *Bacillus, Lactobacillus*, *Lactococcus* and S*accharomices,* genders to mention some.
Najdegerami *et al.* (2016) [43]	*Cyprinus carpio*	Growth, digestive enzyme activity (protease and pepsin) and hepatic condition, increase in fishes when 75% of food was replace with Biofloc.
Valle *et al.* (2015) [44]	*Litopenaeus vannamei*	Gradual substitution of fish flour with Biofloc flour was evaluated. After 42 days, shrimp survival was 99%. Authors prove that fish flour can be substituted with Biofloc flour.
Azim and Little (2008) [10]	*Oreochromis niloticus*	BFT system was evaluated in Nile tilapia ponds (*Oreochromis niloticus*). They found that net production was 45% higher with respect conventional system.
Avnimelech (20017) [9]	*Oreochromis niloticus*	Flocs assimilation was evaluated in tilapia. Author stablishes that microbial biomass produced in this system can be used as natural food source which contributes with 50% of protein requirement for tilapia.

5. Bacteria identified in Biofloc

In BFT system, several studies have been made related to culture species in Biofloc, but only few scientific studies were related to know microbial taxonomic groups that develop in this system. However, there's a study [45], where authors show different type of bacteria in *Litopenaeus vannamei* culture, where dominant groups were: *Vibrio* sp., *Vibrio rotiferianus, Photobacterium* sp., *Proteus mirabilis* and *Marinobacter goseongensis*, the most abundant was *Vibrio rotiferianus.*

Other study [46], show the presence of different Phyla in Biofloc system like: Protobacteria, Bacteroidetes, Cyanobacteria, Actinobacteria, Planctomycetes and Verrumibrobia in *Litopenaeus stylirostris* culture system, being Protobacteria Phyla most abundant (with 60%), because it can be widely distributed in the marine environment.

In a study with *Puntius conchonius* fish [42], bacteria associated to Biofloc were analyzed and it was observed that in first weeks it predominated *Aeromonas* and *Vibrio* bacteria, common in aquatic environment. At week 12, these two bacteria showed very low or null density in culture medium; the last two weeks, *Bacillus subtilis* and yeast *Rhodotorulla* sp were identified in culture medium and showed probiotic characteristic. Authors mentioned that this BFT system, can be used as microbial ecological succession model [42].

In Tilapia culture study with Biofloc [47], it was also observed that 3 to 7 culture weeks *Aeromonas* and *Vibrio* genders were identified (with high virulence factor and capable of causing diseases). It is from weeks 4 to 5 it was observed the increasing of heterotrophic bacterial diversity represented by: *Sphingomonas paucimobilis*, *Pseudomonas luteola*, *Pseudomonas mendocina*, *Bacillus* sp. *Micrococcus* sp. and *Rhodotorula* sp. yeast. Authors remark that there could be variations between develop microbial groups with respect culture specie, food and specially carbohydrate source applied.

In 2017, other researchers [48], isolated *Bacillus* sp. from microbial flocs in white shrimp (*Litopennaeus vannamei*) culture system, demonstrating that this type of culture system can act as natural probiotic source or like biocontrol of water from super intensive aquaculture systems.

According to above, Biofloc system could be considered like new strategy for pathogen control, without chemical substances, antibiotics and antifungals, because the feces eliminated by fish and crustaceans are associated with beneficial microbiota, which released to enriched system culture with a carbohydrate source allows their proliferation, resulting a competitive exclusion effect over pathogen bacteria versus natural probiotic bacteria [49; 16].

Some authors [5; 48] consider improving the handling of microbial community with probiotic addition with specific characteristics, which allow to obtain not only benefits to microbial community, also enhance water quality of culture medium (quickly nitrogen compounds transformation and organic matter) and increasing, in cultured organisms, nutrient assimilation and immune response.

6. Probiotics addition to Biofloc systems

In recent years, scientific studies were made with different goals to evaluate probiotic application in fish and crustacean production culture systems with Biofloc, with the idea to increase the result obtained for these technologies separately. About this [51], it was made the application of *Bacillus amyloliquefaciens* probiotic to improve the immune system of white shrimp *Litopenaeus vannamei* with Biofloc.

An experimental study was made with juveniles of *Clarias gariepinus* [52], in Biofloc system added with *Bacillus* sp. molasses and fish pellets. The results shown better growth with Biofloc and probiotics (30%) with respect experimental treatment without Biofloc.

An experimental study was made with commercial probiotics added in Biofloc system with *Litopenaeus vannamei* [50], infected with *Vibrio parahaemolyticus*. Vibriosis was controlled, but with respect to growth rates, it did not show significant differences. Survival increase with probiotics (83%), with respect to control treatment (52%). However, in other study [53], significant differences were not observed when commercial probiotic was added, composed by *Bacillus* spp. and *Lactobacillus* sp. in Biofloc system, in shrimp culture. These results could be assigned to anaerobiosis and deplections that can exist at pond bottom, could be influenced in action reduction of probiotic. Another factor that could affect was commercial probiotic concentration (2.2 $x10^8$ UFC g^{-1}), with respect to recommended probiotic concentration in other studies [54] (1.0 x 10^9 UFC g^{-1}).

Other studies with positive results adding probiotics to fish and crustacean culture systems in Biofloc, are shown in table 2.

Table 2: Scientific studies review which describe the effect of probiotic addition in Biofloc system, with various times of application in fishes and crustaceans culture system.

Author(s) and year	Studied species	Probiotic used	Results
Hu *et al.* (2017) [55]	*Litopenaeus vannamei*	*Bacillus*	Probiotic use with molasses can inhibit pathogens promoting benefit bacteria.
Miaou *et al.* (2017) [56]	*Macrobrachium rosenbergii*	*Bacillus subtilis* y *Lactobacillus*	Can improve humoral immune of shrimp, using a probiotic source in Biofloc.
Kim *et al.* (2015) [57]	*Fennerpenaeus chinensis*	*Bacillus* sp., *Lactobacillus* sp. y *Rhodobacter* sp.	Shrimp growth and immune response increase in different concentrations with Biofloc and furthermore decreases oxidative stress.
Ferreira *et al*. (2017) [48]	*Litopenaeus vannamei*	*Bacillus subtilis* y *B. licheniformis*	Bacteria from Biofloc system controlled *Vibrio* proliferation and not the probiotic bacteria because did not show significant differences between experimental treatments with respect control treatment.
Aly *et al.* (2008) [58]	*Oreochromis niloticus*	*Bacillus subtilis* y *Lactobacillus acidophilus*	Survival percentage and weight of fishes were significant higher in probiotics with Biofloc treatments.

7. Final considerations
According with this review, we can conclude that in 80% of scientific studies reported some benefit with probiotic use, either in growth, immune response or also water quality from cultured species.

Likewise, culture with Biofloc have shown better productive yields in fish and crustaceans like shrimp at intensive system, in addition to reduce commercial food up to 30%, decrease the exchange of water of ponds almost in 100% and enhance water quality by stabilization of nitrogen compounds for bacterial action.

Unfortunately, the effects of adding of probiotics in Biofloc are not clear yet, because results depend according to their concentration and used type, but above all, their impact in microbiota community developed in Biofloc system. This is because microbiota community handling can be exploited when producers use strains with different specific characteristics to increase benefits in cultured aquatic organisms. That's why microbiota community dynamic studies are important to increase the knowledge at this respect.

Studies with Biofloc microbiome and Biofloc with probiotics, can expand the view of variations in abundance, diversity and ecological bacterial functions using these biotechnologies in aquaculture.

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