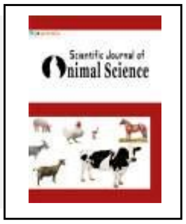
Sjournals
Scientific Journals
Scientific Journal of Animal Science (2015) 4(9) 103-110
ISSN 2322-1704
doi: 10.14196/sjas.v4i9.1941

Contents lists available at Sjournals
Scientific Journal of Animal Science
Journal homepage: www.Sjournals.com



Original article

Importance and function of microbial communities in aquaculture systems with no water exchange

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A R T I C L E I N F O

Article history,
Received 06 Oct 2015
Accepted 18 Sep 2015
Available online 25 Sep 2015

Keywords,
Bacteria
Biofloc
Aquatic system
Aquaculture

A B S T R A C T

The goal of this review is to analyze the importance of microbial communities in systems with zero or no water exchange or also called Bioflc, this because in recent decades has developed a strong interest in its use for the cultivation of various aquatic species such as shrimp and tilapia primarily though the function that these bacterial groups discharge in the system is unknown, making it relevant analysis mainly because the microorganisms are the foundation for the transfer of matter energy and enabling the production of small organisms such as ciliates, rotifers and protozoa, nematodes and others that can serve as natural food in situ for cultivated species, and positive impact on water quality due to microbial transformation of all performing waste generated in these systems where water exchange is limited. The knowledge of the behavior of microbial communities in the Biofloc systems will allow better management and therefore greater benefits obtained so far.

1. Introduction

Over recent years the environmental impact concern, generated by aquaculture activity, has led to development of more sustainable production systems (González–Ocampo et al., 2006. Primavera, 2006). Regarding this, it has been proposed new aquaculture methods, like Biofloc technology (BFT) which is based in microbial floccules generation, where bacterial communities are very important for system balance, because after adding a carbon source the bacterial communities help to minimize or avoid water exchange, and also produce microbial protein that can be used as primordial food source, with a significant saving of commercial food supply and a substantial improvement in discharge water quality, transforming organic matter, and also removal of contaminant compounds (Avnimelech, 2009. De Scryver et al., 2008).

There are several studies were its show the economic and environmental advantages of the use of BFT system for species like shrimp and tilapia, but there are many questions unanswered about the microbial communities that develop and ecological function they play (Ray et al., 2010). Another important aspect that recent studies point out is that this type of culture also can provoke the development of bacteria recognized for their probiotic potential, due to intestinal microflora that it is released in feces, which can proliferate if it integrates to a nutritive environment, therefore, it is used by cultured species (Crab et al., 2010). Nevertheless, there are not many studies about microbial groups that develop in BFT systems. So this review aims to present the groups of bacteria that develop in BFT systems, their function and variations that were obtained by using different species and carbon sources.

2. Microbial communities in aquatic systems

Microorganisms are a fundamental part of any organism life and ecosystem (López and Zaballos, 2005). Microbiota is an essential component of trophic networks of marine and freshwater environments both in activity and amount of biomass, contributing to nutrient regeneration and interacting with a wide range of organisms (Lyautey et al., 2005). In the same way, in aquaculture production systems many microbial groups develop, just like bacteria and unicellular fungus, which can act in a positive way in the organic matter transformation, removal of contaminant compounds and as source of microbial biomass available for larger organisms (De Scryver et al., 2008), but also they can act in a negative way when environment conditions are adverse and bacterial populations show virulent. Because of that, it is important to know the environmental and biological context where this microorganisms are found; this refers to environmental variations and surrounding species and in many cases to specie they depend on (host).

In a microbial system the cellular growth forms populations; metabolically related populations are called guilds or some authors call them functional groups and together, these groups interact forming microbial communities (De la Cruz-Leyva et al., 2015; Díaz and Wacher, 2013). However, to understand their function in the specific niches, it is essential to identify and quantify each one of the members that make these communities and establish their metabolic activities which defines its ecological function depending on the culture system where they develop (De la Cruz- Leyva et al., 2015). Due to the development in recent years of new aquaculture production systems using circulation systems for a better water quality control, with aim of biological filters or better yet the development of cultures with low or none water change called BFT, that promote the growth of heterotrophic bacteria for a better removal of contaminant residues, it is important to generate knowledge about microbial groups that are developing in these systems, but above all, to establish the ecological function that they are conducting, with the idea of make a more adequate use of it, being this information that has been slightly addressed at the moment.

3. BFT systems

BFT is gaining worldwide interest in aquatic organisms production (Azim and Litlle, 2008), it consist in the development of microbial floccules, formed from a high carbon : nitrogen (C/N) relation, with low or none change in the water and a high oxygenation in the water column, also the use of diets with low raw protein content and addition of external carbon sources such as molasses (sugar cane), rice bran, wheat bran, between other, that function as organic matter substrate that allows microbial aerobic decomposition that is used as substrate source for growth of various organisms (Avnimelech, 1999. Hargreaves, 2013). These includes photoautotrophic organisms like algae and heterotrophic microorganisms (bacteria, ciliates, protozoa, rotifers and nematodes among others) (De Schryver et al., 2008). These microorganisms are very important in the nutrition of animals in culture because they are a rich natural source of protein-lipid "in situ" available all day. Nevertheless, the microbial density and diversity to be developed depends on the carbon source used in the development of floccules, because their chemical constitution determines the capacity of bacterial species than can use and the superior groups that develop later.

4. Heterotrophic bacteria in culture systems

Heterotrophic bacteria are abundant in ecosystems, particularly in aquatic environments. Adapting to diverse environments, they are more efficient in the way of substrate utilization, resulting in an extensive physiological bacterial diversity (Miravet, 2003). Through their metabolism, this bacteria liberate many inorganic compounds to environment that can be used by other living organisms, also they produce exoenzymes that decompose diverse compounds like cellulose, lignin, keratin and other natural molecules that are hard to transform. Processes of denitrification and decomposition does not perform in a more efficient way without functions made by heterotrophic microorganisms (Acinas, 2001).

5. Heterotrophic bacterial transformation of nitrogenous compounds

In aquaculture production systems, water quality is affected by unconsumed food, toxic wastes as ammonia nitrogen that is liberated by ionic diffusion and exchange through gills, urine and feces of organisms in culture (Crab et al., 2102); and by fertilization process in production units, nevertheless, there are diverse microorganisms in charge of minimize the impact through the removal of ammonia nitrogen by nitrification process that consist in successive ammonia oxidation, first to nitrite and finally to nitrate (Ebeling, et al., 2006). Also a anaerobic reduction of nitrate process to gaseous molecular nitrogen denominated denitrification, is made (Borja, 2002). Unionized ammonia is transformed by bacteria of the genus Nitrosomonas, Nitrosococcus, Nitrosospira, Nitrosolobus and Nitrosovibrio. The oxidation bacteria of nitrite to nitrate are mainly: Nitrobacter, Nitrococcus and Nitrospira (Hagopiany Riley, 1998) (Fig 1).

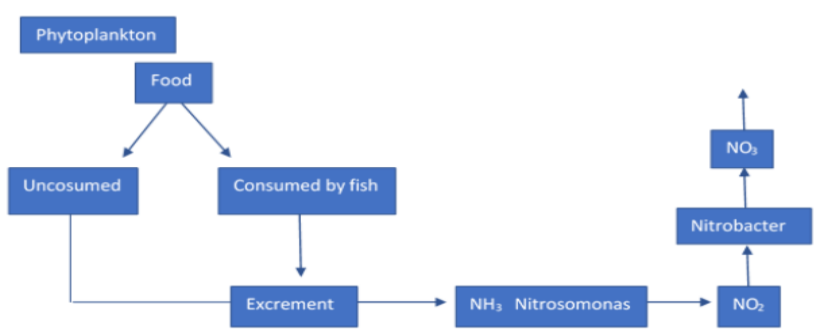

Fig 1. Nitrogen cycle in a conventional culture system.

In BFT systems the transformation of toxic nitrogenous compounds is more efficient, because this process is made by facultative heterotrophic bacteria that mainly correspond to genus Bacillus and Pseudomonas (Daims et al, 2001). Aerobic and facultative aerobic bacteria with high enzymatic activity that oxidize organic matter and also nitrites and nitrates without confronting the problems that autotrophic nitrifying bacteria present, because these bacteria seems affected when biological demand of oxygen is above 2 mg L-1 (Amilcar, 2012). Besides, the presence of organic matter as carbon, inhibits the denitrification by autotrophic organisms, that's why they grow more slowly than heterotrophic which increase their population quicker in water with high organic matter content and high oxygenation, so they can transform ammonia nitrogen in compounds with low toxicity; even some of them transform this compound to produce microbial biomass and allows the generation of diverse protozoa in a short time (days) compared to conventional systems (Figure 2) (Paniagua-Michel and García, 2003; Ebeling et al., 2006).

Fig 2. Use of microbial chain in BFT (modified from Azam et al., 1995).

Laloo et al. (2007) checked the capacity of three isolates of genus Bacillus to decrease nitrites, nitrates and ammonia concentration in water of ornamental fish culture. This was also observed by Kim et al. (2005) in B. subtilis, B. cereus and B. licheniformis, which contributes to a reduction in nitrogenized compounds to bioaccumulation, bio-assimilation and bacterial nitrification.

6. Degrading bacteria

In addition to bacteria that oxidize nitrogenous compounds, there are a variety of heterotrophic microorganisms in charge of starch, cellulose, chitin and phenols degradation, among other compounds that are produced by the waste of organisms cultured with scales, skeletons, tissues and shells (Alvarado- Castro, 2012).

7. Chitinolytic bacteria

Chitinolytic bacteria are a fundamental part of marine and freshwater cultures, because they enable the restoration of carbon and nitrogen levels through the degradation of chitin in the water column, and are highly efficient at using waste shells of diverse crustaceans or other organisms that possess chitin in their structure and that are in the system (Cardoso, et al., 2012). In this group there is a large variety in microorganisms as genus Pseudomonas, Bacillus, Alteromonas and Micrococcus. Studies like Pelczar et al. (2002), point out that these genus are important for solubilization of elements like calcium phosphate, iron, and aluminum, making them available in the environment for production of diverse protozoa, rotifers, nematodes and a number of organisms that can be used as natural food in situ by cultured species. That's why diverse investigators have suggested that heterotrophic microorganisms induction, combined with adequate aeration protocols can provides benefits to aquaculture production; not only in profitability but also in sustainability (Avnimelech, 2009; Martínez-Córdova et al., 2011).

8. Amylolytic and cellulolytic bacteria

In the group of degrading bacteria, amylolytic bacteria has high relevance in culture systems where an external carbon source is added, because generally vegetable meals with a high starch content are used, like rice flour that have high quantities of starch in form of amylose (which give cohesion to grains) and amylopectin (Arellano-Carbajal and Olmos Soto, 1999). Starch and cellulose, are the two carbon sources more distributed in nature, these polymers are made by subunits of glucose, presenting different chemical conformations, so diverse enzymatic systems are required to degrade them; in culture systems this process is made by diverse bacteria that through exoenzyme production, they hydrolyze the starch with different specificity. Until today it has been identified seven classes of amylolytic enzymes of microbial origin, within the most effective microorganisms in degradation of starch it can be found Bacillus subtilis,Bacillus macerans, Bacillus circulans, Klebsiella pneumoneae and Candida utilis yeast(Barrera, 2008).

On other hand, due to extensive distribution of cellulolytic and lignocellulosic compounds in nature, there is a large variety of microorganisms that possess enzymes capable of hydrolyze, such as cellulase, xylanase and lignolytic enzymes. Microorganisms of aquatic environments with a high cellulite activity belong to genus: Cellulomonas, Pseudomonas, Thermomonospora, Microbispora Streptomyces, and Clostridium (González et al., 2005). Filamentous bacteria of Streptomyces genus, are the more efficient lignolytic bacteria because they mineralize >59% of this compound.

9. Carbon sources and microbial communities in BFT systems

According with what was mentioned before, micrbial communities developed in BFT systems, will depend on the used carbon source and the cultured organisms. Molasses has been used, nevertheless, in many cases sub products derived from human and/or animal food industry of easy acquisition and handling, have been used (Asaduzzaman et al., 2008; Samocha et al., 2007). Chemical composition of the carbon source in a matter of starch, cellulose, fructose, sucrose and other compounds will determine the bacterial load that integrates to the floccules and therefore in their nutritional content. In table 1, resume some of carbon sources that are more used in aquaculture.

Table 1
Carbon sources that are more used in BFT systems for fish and crustaceans.

Carbon source	Specie	Reference
Acetate	Macrobranchium rosenbergii	(Crab et al., 2010)
Cornmeal	Tilapia hybrid	(Asaduzzaman et al., 2010)
Molasses	Litopenaeus vannamei and Penaeus monodon	(Samocha et al., 2007)
Tapioca	Litopenaeus vannamei and Macrobrachium rosenbergii	(Hari et al., 2004)
Wheat flour	Oreochromis niloticus	(Azim and Little, 2008)
Wheat and molasses bran	Farfantepenaeus brasiensis	(Emerenciano et al., 2012)
Starch	Oreochromis niloticus	(Crab et al., 2009)
Glycerol and glucose	Macrobranchium rosenbergii	(Crab et al., 2010; Ekasari et al., 2010)
Saccharose	Litopenaeus vannamei	(Khun et al., 2009)

10. Pathogens control

One of the biggest worldwide challenge that aquaculture sector confronts is infectious diseases, which are caused by virus, fungus and bacteria (Del Mar et al., 2004; López et al., 2007). Bacterial infections are more frequent because most of the cases, microorganisms that are part of fish and culture water normal microbiota, can be virulent as a result of environmental variations and handling and culture conditions (Negrete et al., 2004). Although for many years it has been used various chemicals and antibiotics for the control of diseases, the inadequate application of these, have led to a development of antibiotic resistance and an environmental impact. As a consequence, there is an urgent need of alternative and more sustainable control techniques (Defoirdt et al., 2007). BFT can be a new strategy for pathogens control in contrast with conventional approaches. First the addition of a carbon source to productive systems as molasses, enrich the medium with sugars that are used by heterotrophic bacteria, removing space to pathogenic bacteria that do not have the physiological capacity to neutralize them. On the other hand it has been observed that in culture systems with low or none change of water develop bacteria recognized by their probiotic potential, this may be due to the liberation of some intestinal microbiota in feces, that by being in a nutritive environment it allows their proliferation and with that, cultured species use the benefits of this microorganisms (Crab et al., 2010).

Among the obtained benefits, it can be noted the increase in nutrients assimilation leading to a higher survival and increase in cultured species, also the immune response against infectious processes increase. Kim et al. (2010), point out that BFT reduce the introduction of pathogens in culture of Litopenaeus vannameil shrimp, because Biofloc is constituted by microbiota rich in lipopolysaccharides, peptidoglycans and 3-glucans ß-1; components that activate a chain reactions that lead to prophenoloxidase production, increasing immune response from the shrimp. Ekasari and collaborators (2014), mention that phenoloxidase activity increase in response to the organic carbon load of different external sources that are added to the system (molasses, tapioca sub products and rice bran).

It has been also mentioned that there is a competitive exclusion effect from the probiotic bacteria against other microbial groups, because they secrete a large variety of exoenzymes and polymers that generate a hostile environment for bacteria and pathogenic bacteria (Hong et al., 2005; Ziaei-Nejad et al., 2006), this was also reported by an study made by Monroy et al. (2013), were it was compared the microbial composition in a BFT system for tilapia during twelve weeks of experimentation, where they observed an important variation between the developed microbial groups in the system and pointed out that as the culture matures, pathogenic bacteria are reduced and degrading heterotrophic increase but specially probiotic bacteria represented by genus Bacillus and Rhodotorula sp. yeast, reinforcing what was mentioned by Wu et al. (2012), which assure that one of the benefits of using Biofloc is the competitive exclusion capacity that some heterotrophic bacterial population have against pathogenic bacteria. Emerenciano et al. (2013), claim that the "natural probiotic effect" in BFT systems help the reduction of Vibrios and ectoparasites in culture systems.

Even though the evaluation of probiotics in aquaculture have been probed in different scientific articles where it has been reported the benefits of its application, selection processes, growth, scaling and administration, they remain deficient and expensive for fish and crustaceans production, that's why the implementation of culture systems where probiotic bacteria develop in situ in a natural way, will be of great importance in aquaculture sector.

It can be resume that BFT gives diverse advantages above conventional culture systems, where an improved removal of nitrogen compounds pollutants, increased feed conversion, increased water and land use efficiency and control of potential pathogens stand out; benefits where heterotrophic bacterial communities have an important role. Therefore there are still challenges for future investigations related to microorganisms identifications produced in Biofloc, the benefits they provide and variation related to the used carbon source, environmental parameters, cultures species and the handling in productive systems, to be use as with the idea of obtaining more benefits.

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