



## Aquatic microbiota diversity in the culture of Nile tilapia (*Oreochromis niloticus*) using bioflocs or periphyton: virulence factors and biofilm formation

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**ABSTRACT.** The following research isolated and identified the main bacterial groups present in the culture of juvenile Nile tilapia in the presence of bioflocs and/or periphyton. The strains were also tested for the production of exoenzymes, indicative of potential virulence factors, and ability to form biofilm. The water samples were taken from tilapia cultured in the presence of bioflocs (T1), in the presence of bioflocs and periphyton (T2), from traditional culture (T3) and from culture in the presence of periphyton (T4). In the growth and selection of the bacterial groups, pour plate method was used, along with the following media: Plate Count Agar (PCA - DIFCO), Aero Pseudo Selective Agar (GSP - Himedia) and Nutrient Agar (AN - Merck). 46 strains were isolated in the following distribution: T1 (n = 12); T2 (n = 10); T3 (n = 14) and T4 (n = 10). Among the isolates, the most frequent genera were: *Pseudomonas* spp., *Aeromonas* spp., *Staphylococcus* spp., *Bacillus* spp., *Mycobacterium* spp., *Micrococcus* spp., and *Corynebacterium* spp. Bacterial isolates in treatments T1 and T3 tested positive for five virulence profiles each, while those isolated from T2 and T4 for two and three virulence profiles, respectively. Treatments in bioflocs and periphyton (T2) or only periphyton (T4) yielded bacteria of less pathogenic potentials. In relation to the fish growth, T1 and T4 resulted in a higher final weight.

**Keywords:** fish farming, microbial aggregates, pathogenicity.

### Diversidade da microbiota aquática em cultivo de tilápia do Nilo (*Oreochromis niloticus*) utilizando bioflocos ou perifiton: fatores de virulência e formação de biofilme

**RESUMO.** A presente pesquisa isolou e identificou os principais grupos bacterianos presentes no cultivo de juvenis de tilápia do Nilo na presença de bioflocos e/ou perifiton. Verificou-se a produção de exoenzimas como indicadores de potenciais fatores de virulência e a capacidade de formação de biofilme. As amostras de água foram retiradas do cultivo das tilápias na presença de bioflocos (T1), cultivo na presença de bioflocos e perifiton (T2), cultivo tradicional (T3) e cultivo na presença de perifiton (T4). Para o crescimento e seleção dos grupos bacterianos foi utilizada a técnica da semeadura em profundidade com os seguintes meios de cultura: Ágar para Contagem em Placas (PCA – DIFCO), Aero Pseudo Seletive Ágar (GSP – Himedia) e Ágar Nutriente (AN – Merck). Foram isoladas 46 cepas, distribuídas da seguinte forma: T1 (n = 12); T2 (n = 10); T3 (n = 14) e T4 (n = 10). Entre os isolados, os gêneros mais frequentes foram: *Pseudomonas* spp., *Aeromonas* spp., *Staphylococcus* spp., *Bacillus* spp., *Mycobacterium* spp., *Micrococcus* spp., e *Corynebacterium* spp. Os isolados bacterianos dos tratamentos T1 e T3 expressaram cinco perfis de virulência enquanto aqueles isolados dos tratamentos T2 e T4 apresentaram dois e três perfis de virulência, respectivamente. Os tratamentos com presença de bioflocos e perifiton (T2) ou somente perifiton (T4) apresentaram bactérias com menor potencial patogênico. Em relação ao crescimento dos peixes, o T1 e T4 possibilitaram maior ganho em peso final.

**Palavras-chave:** piscicultura, agregados microbianos, patogenicidade.

#### Introduction

Aquaculture is the food industry sector that presents the highest growth rate (Food and Agriculture Organization [FAO], 2014),

complementing current deficits in fishing. In Brazil, tilapia was the most produced freshwater fish (Brasil, 2014). As in any other activity, aquaculture brings about problems, such as fast accumulation of

organic matter from the feed and toxic nitrogen compounds (Avnimelech, 2006). Different culture systems create an artificial environment which are able to directly influence microorganisms, increasing the selection, adaptation and growth of specific bacterial communities in regular aquatic microbiota (Jiravanichpaisal, Miyazaki, & Limsuwan, 1994; Aguirre-Guzmán, Ruíz, & Ascencio, 2004). Moreover, a number of diseases might affect tilapia production, generating high mortality rates and, consequently, economic losses. Among the main pathogenic bacteria in the culture of tilapia are *Flavobacterium columnaris*, *Aeromonas* sp., *Vibrio* sp., *Streptococcus iniae*, *S. agalactiae*, *Edwardsiella tarda*, *Francisella* sp. (Kubtiza, 2008; Soto, Hawake, Fernandez, & Morales, 2009).

Aiming to minimize problems generated by aquaculture, new technologies have been employed to improve feeding, water quality and effluents, securing a healthy cultivation environment and helping in the development of an environmentally sustainable and correct activity (Crab, Avnimelech, Defoirdt, Bossier, & Verstraete, 2007).

The use of microbial communities is one of the alternatives that have been used in aquaculture. These communities can grow attached to substrates such as periphyton or in suspension as flakes (bioflocs). They are formed by diverse microorganisms, such as bacteria, fungi, microalgae and protozoa, aside from detritus and organic matter (Azim, Milstein, Wahab, & Verdegem, 2003; Azim, Beveridge, Van Dam, & Verdegem, 2005; Emerenciano et al., 2007). Many researches have shown the efficiency of these two systems in aquaculture (Zhang, Lin, Wang, & Xu, 2010; Sakr, Shalaby, Wassef, El-Sayed, & Moneim, 2015) and the positive results are attributed to the presence of microorganisms, specially bacteria, and the metabolites they produce (Avnimelech, 1999; Crab, Lambert, Defoirdt, Bossier, & Verstraete, 2010).

The microorganisms benefit aquaculture systems with numerous functions, such as nutrient cycling, reduction in the amount of toxic nitrogen compounds (Keshavanath et al., 2002; Asaduzzaman et al., 2009; Audelo-Naranjo, Martínez-Córdova, Voltolina, & Gómez-Jiménez, 2011) and extra source of organic foodstuff (Avnimelech, 2007; Uddin, Azim, Wahab, & Verdegem, 2009), compensating for a decrease in feeding levels.

Some of the negative factors that might be caused by the microorganisms are the development of diseases, toxin production, excessive consumption of dissolved oxygen and excretion of nitrogen metabolites (Martínez-Córdova, Emerenciano,

Miranda-Baeza, & Martínez-Porchas, 2015). For this reason, the main components of these microbial aggregates, as well as their diversity and dynamic, should be further studied. Aquatic microorganisms not only influence the environment, but are also known for bearing relations to the physiological state of fish and diseases (Al-Harbi & Uddin, 2005). The pathogenicity of microorganism is linked to virulence factors and the formation of biofilms, both regulated by the quorum sensing process (Zhao, Chen, Quan, & Fan, 2015). Bacterial enzymes expression is important and has many functions in the culture system of tilapia, such as organic matter processing, adaptation to different conditions and environments (Jose, Giridhar, Anas, Bharathi, & Nair, 2011) and is also a parameter for potential pathogenicity factors (Costa, Amorim, Araújo, & Vieira, 2013).

The comprehension of these biological systems, the main components of the microbial aggregates, their diversity, dynamics, and possible adaptation or mechanisms processes are fundamental steps in the mastery of technologies that favor beneficial microbial groups. The present research aimed to isolate, identify and subject the bacterial strains to tests for potential virulence factors and biofilm formation, in order to characterize the environmental strategies of the isolates present in the culture of juvenile Nile tilapia in four different treatments.

## Material and methods

### Experimental system

The culture of juvenile Nile tilapia, weighting  $0.99 \pm 0.04$  g, was conducted in the premises of an aquaculture laboratory in the Fishing Engineering Department of the Federal University of Ceará. The fish were kept in polyethylene tanks with 250 L of usable volume, in a density of 18 fish per tank ( $72 \text{ fish m}^{-3}$ ) and fed with commercial feed in pellets (40% BP- Brute Protein; 0.8 – 1.2 mm), four times a day (8, 11, 14 and 16 hour) for 10 weeks.

Four treatments were employed. In Treatment 1 (T1), the culture was conducted in the presence of bioflocs (the proportion of organic carbon to ammoniacal nitrogen was adjusted to 15:1). In Treatment 2 (T2), the culture presented both bioflocs and periphyton (adjustment of the C: N proportion in water and an artificial substrate in the system). In Treatment 3 (T3), neither bioflocs or periphyton were present and, in Treatment 4 (T4), the culture occurred in the presence of periphyton. Sanded PVC plates were used in the tanks as

substrates for fixation. The carbon source used to keep the C:N relation in 15:1 was molasses powder, according to Schryver and Verstraete (2009).

The dissolved oxygen was monitored weekly (following Apha, 2005), whereas pH was measured daily with a pH-meter. In the evaluation of growth and survival rate of the fish, biometric tests were conducted every two weeks.

#### Water samples processing

In the growth and selection of bacterial groups, pour-plate method was used along with the following media: Plate Count Agar (PCA - DIFCO), Aero Pseudo Selective Agar (GSP - Himedia) and Nutrient Agar (AN - Merck). Water samples of 1.0 L were collected from the tanks and serial dilutions were made in saline solution at 0.85% ( $10^{-1}$  to  $10^{-5}$ ). Parts of the diluted sample (1.0 mL) were distributed in Petri dishes and covered with 15.0 mL of the culture media specific to each bacterial group (pour plate technique) and left in an incubator for 48 hours at 35°C (Downes & Ito, 2001). After that, five colonies were isolated for each culture media used.

After purification, the bacterial cultures were subjected to Gram staining characterization (Tortora, Funke, & Case, 2005) and biochemical tests according to Bergey's (2014).

#### Tests for the detection of biofilm formation

Ability to form biofilms was determined through phenotypic test in plates containing Congo red agar (CRA) according to Freeman, Falkiner, and Keane (1989) and Abdallah, Chaieb, Zmantar, and Kallel (2009) with adaptations, and through glass adhesion test (GAT) tubes, with modifications (Christensen, Simpson, Bisno, & Beachey, 1982).

#### Detection of potential virulence factors

All bacterial isolates from the Nile tilapia culture water were subjected to the tests of virulence factor expression, with the help of the following exoenzymes: gelatinase (GEL), elastase (Elas), caseinase (CAS), lipase (LIP), phospholipase (Phos) and haemolytic activity ( $\beta$ -hemolysis in sheep blood) ( $\beta$ -hem), following the methodologies of Rust, Messing, and Iglewski (1994), Rodrigues, Ribeiro, Alves, and Hofer (1993) and Furniss and Donovan (1979).

### Results and discussion

#### Water quality variables and zootechnical performance

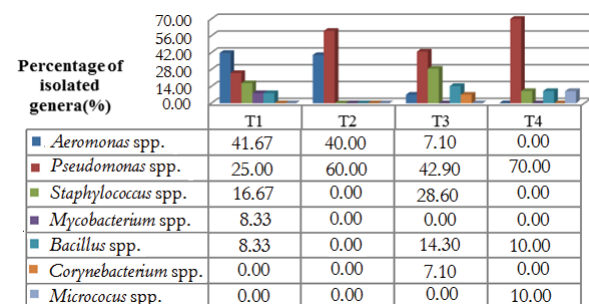
Concentration of dissolved oxygen has not varied among the treatments tested, in average they

remained within the range of  $5.9 \pm 0.8$  mg L<sup>-1</sup>. Values for pH were, in average,  $7.02 \pm 0.18$ ,  $7.03 \pm 0.17$ ,  $8.10 \pm 0.60$ ,  $8.13 \pm 0.61$  for T1, T2, T3 and T4, respectively. Adjustment of C: N relation had influence in the decrease of pH values, while the presence of periphyton bore no such influence. Values for oxygen and pH are considered acceptable for the culture of Nile tilapia. Survival rate of the fish was higher than 80% and their final weight was, in average,  $7.79 \pm 0.82$ ,  $28.14 \pm 2.7$ ,  $24.35 \pm 1.04$ ,  $26.04 \pm 2.07$  for T1, T2, T3 and T4, respectively. For further details on the culture system, variables in water quality and zootechnical performance, refer to Cavalcante, Lima, Rebouças, and Sá (2016). It is noticeable that the presence of bioflocs (T1 and T2) and of periphyton (T4) contributed to a higher final weight in comparison to T3. Their presence in the culture system yields extra natural feed for the cultured organisms, increasing their weight and productivity (Avnimelech, 2007; Uddin et al., 2009).

#### Isolation and identification

From the water samples collected in each treatment, 46 strains were isolated, distributed in the following way: T1 (n = 12); T2 (n = 10); T3 (n = 14) and T4 (n = 10).

In Figure 1, identification and percentage of isolated bacterial strains for each treatment is shown. In Treatment T1 (biofloc technology-BFT), genus *Aeromonas* spp. represented more than 41%, followed by 25% of *Pseudomonas* spp., 16.67% of *Staphylococcus* spp., and 8.33% of *Mycobacterium* spp. and *Bacillus* spp., respectively. Likewise, works of Monroy-Dosta et al. (2013) on the culture of tilapia with bioflocs during 14 weeks, found the genera *Aeromonas*, *Vibrio*, *Pseudomonas*, *Bacillus*, *Micrococcus* among others.



**Figure 1.** Identification and frequency of bacterial genera isolated in the four treatments employed in the culture of juvenile Nile tilapia (*O. niloticus*) with or without periphyton and bioflocs.

In Treatment T2, two genera were isolated, 60% of the bacteria were *Pseudomonas* and 40% *Aeromonas*. It was expected that, in T2 culture water, there

would be a higher diversity of bacterial genera, as the C: N relation was adjusted. Moreover, an artificial substrate was added to the tank, offering better environmental conditions for the development of microorganisms, as the bacteria do not live as isolated colonies in nature, but typically form biofilms, characterized by a structured and functional microbial community (Tortora, Funke, & Case, 2012).

In treatment T3, genus *Pseudomonas* spp. was the most prominent (42.9%), followed by *Staphylococcus* spp. (28.6%) and *Bacillus* spp. (14.3%), while the genera *Corynebacterium* spp. and *Aeromonas* spp. presented the lowest isolation percentage (7.1%). Differently from the present results, Newaj-Fyzul, Mutani, Ramsubhag, and Adesiyun (2008) found the following proportions of isolates, in decreasing order: *Bacillus* spp. (80%), *Staphylococcus* spp., *Alcaligenes* spp. and *Aeromonas* spp. (60%), and *Pseudomonas* spp. (53%). The prevalence of other organisms may be attributed to seasonal variations and different culture procedures, such as feeding rates, which contribute to the increase or decrease of substrates for the establishment of certain bacterial groups in spite of others.

In treatment T4, the most observed genus was *Pseudomonas* spp. (70%) followed by *Staphylococcus* spp., *Bacillus* spp. and *Micrococcus* spp., (10% each). Corroborating with the results, Shilta, Chadha, Pandey, and Sawant (2016), in a study on the culture of *Etroplus suratensis* (a type of cichlid) in the presence of periphytic biofilm, reported the dominance of the genera *Bacillus*, *Pseudomonas* and *Micrococcus* in the water. Heterotrophic bacteria such as *Pseudomonas* spp. and *Bacillus* spp., are known for their ability to degrade organic matter (Monroy-Dosta et al., 2013). Thus, the presence of an inductor substrate for periphyton had a purifying effect in the culture water, as it offered a larger surface for the adhesion of heterotrophic microbiota.

It is possible to suggest that the culture systems employed in the present research influenced the diversity and proportion of bacterial genera isolated, which are differently adapted to each environment tested.

Among all treatments, the most frequently isolated genera, in decreasing order, are *Pseudomonas* spp., *Aeromonas* spp., *Staphylococcus* spp., *Bacillus* spp., *Mycobacterium* spp., *Micrococcus* spp., and *Corynebacterium* spp..

Members of the *Pseudomonas* spp. are frequently found in soil, continental and oceanic

waters (Raaijmakers, Weller, & Thomashow, 1997). They belong to the heterotrophic microbiota in water, helping on the mineralization of nutrients. In addition to that, they produce bioactive compounds that are used as pathogen inhibitors in shrimp farming (Vijayan et al., 2006; Rattanachuy, Kantachote, Tantirungkij, Nitoda, & Kanzaki, 2010).

The *Aeromonas* genus is widely known as the etiological agent in fish infections, causing septicemia and hemorrhagic ulcers, generating significant mortality rates in freshwater as well as in marine species, harming the economy of aquaculture (Hu, Wang, Pan, Lu, & Liu, 2012; Beaz-Hidalgo, & Figueras, 2013). Therefore, presence of these microorganisms in tilapia culture waters should be of concern, calling for the use of procedures which favor beneficial bacterial groups to grow in the culture environment, decreasing or even eliminating *Aeromonas* spp..

*Staphylococcus* spp., *Mycobacterium* spp., *Micrococcus* spp. and *Corynebacterium* spp. genera belong to the regular human microbiota (Kaushal, Gupta, & Van Hoek, 2016), or might be associated to it, hence it is believed that the presence of these groups are the consequence of human activity on the environment, which directly contribute to the dissemination of these microorganisms. Some bacterial species in these genera are opportunistic pathogens both to humans and fish (Austin & Austin, 2007; Santos et al., 2007).

Many species of the *Bacillus* genus are used as probiotics in aquaculture, in the strengthening of the immunological systems (Wang, Tian, Yao, & Li, 2008), stimulation of digestive enzymes, zootechnical improvement of the cultured animals (Standen et al., 2013; Jatobá & Mouriño, 2015), production of antibacterial compounds (Panigrahi & Azad, 2007), and degradation of organic matter. Therefore, it is considered a beneficial genus for the culture.

Once the main bacterial genera are identified in each treatment, adjustments in the water or feed can be made, in order to favor the main groups of interest in improving the environment, aside from preventing diseases from spreading.

#### Virulence factors and biofilm formation

Bacteria have a great potential to respond to stressing factors, be it competition between microorganisms or environmental fluctuations, such as sudden changes in temperature, low activity in

water, inanition, oxydative stress, etc (Stewart, 2002). Hence, bacteria possess adaptive mechanisms, biochemical or genetic (Meer Van Der, 2006), including new metabolic routes and pathogenic expression, for example. With that in mind, it can be noticed that the bacterial isolated from each treatment showed differences in the expression of potential virulence factors and biofilm formation, as can be observed in Table 1.

In treatments T1 (presence of biofloc) and T3 (control) the isolated genera displayed a higher pathogenic potential, as they produced up to five from the six virulence factors tested (Table 1). Aside that, it was possible to identify four and five virulence profiles, respectively, as can be seen on Table 1, and a positive result in at least one test for biofilm formation.

Potentially pathogenic bacteria were isolated and identified in the treatment with biofloc technology - BFT (T1). However, no case of disease was observed during the whole culture period. Some authors relate that the employment of biofloc may protect the cultivated organisms against the infection of pathogenic bacteria, because of the 'natural probiotic' effect acting against, for example, *Vibrio* spp. and ectoparasites. This effect is triggered by large groups of microorganism, mainly by bacteria that are considered to be on the first trophic level in

the system (Crab et al., 2010; Emerenciano, Gaxiola, & Cuzon, 2013).

It is worth noting that in the control treatment (T3) no case of disease was observed, and it was necessary to conduct *in vivo* tests to ascertain the beneficial potential of bioflocs for fish. It is known that the presence of potentially pathogenic microorganisms only preferably causes damage when there is some unbalance in one or more of the following three elements: environment, host and pathogen. Thus, the culture is considered stable for the whole experimental period. In the culture of tilapia in the presence of both periphyton and bioflocs (T2), only two genera were isolated (*Pseudomonas* spp. and *Aeromonas* spp.). The *Pseudomonas* spp. group produced gelatinase, caseinase, elastase and hemolysin with  $\beta$ -hemolytic activity. *Aeromonas* spp., in the other hand, tested positive for the other enzymes, excepting caseinase, a total of five from six analyzed factors. Both groups were positive for biofilm formation. In this treatment only two virulence profiles were observed. In the case of environmental unbalance or the use of inadequate procedures in the culture, diseases might appear, causing high mortality rates and economic losses, since these genera are known for possessing various opportunistic pathogenic species (Peixoto, Sá, Gordiano, & Costa, 2012).

**Table 1.** Analysis of potential virulence factors and tests for the detection in the formation of biofilm of bacterial isolates for each treatment employed in the cultivation of juvenile Nile tilapia.

Isolates	Treatment	Virulence factors						Biofilm formation tests	
		GEL	CAS	ELAS	LIP	PHOS	$\beta$ -hem	CRA	GAT
<i>Pseudomonas</i> spp.	T1	+	+	+	-	-	+	+	-
	T2	+	+	+	-	-	+	+	+
	T3	+	+	+	-	-	+	+	+
	T4	+	+	+	-	-	+	+	+
<i>Aeromonas</i> spp.	T1	+	-	+	+	+	+	-	+
	T2	+	-	+	+	+	+	+	+
	T3	+	-	+	+	+	+	+	-
	T4	NI	NI	NI	NI	NI	NI	NI	NI
<i>Staphylococcus</i> spp.	T1	+	-	-	-	-	+	+	+
	T2	NI	NI	NI	NI	NI	NI	NI	NI
	T3	+	+	-	-	-	-	+	+
	T4	-	-	-	-	-	-	+	+
<i>Mycobacterium</i> spp.	T1	+	+	+	-	-	-	+	-
	T2	NI	NI	NI	NI	NI	NI	NI	NI
	T3	NI	NI	NI	NI	NI	NI	NI	NI
	T4	NI	NI	NI	NI	NI	NI	NI	NI
<i>Bacillus</i> spp.	T1	+	+	+	-	-	-	+	+
	T2	NI	NI	NI	NI	NI	NI	NI	NI
	T3	+	-	-	-	-	+	+	+
	T4	-	+	-	+	-	+	+	+
<i>Corynebacterium</i> spp.	T1	NI	NI	NI	NI	NI	NI	NI	NI
	T2	NI	NI	NI	NI	NI	NI	NI	NI
	T3	-	-	-	-	+	+	+	-
	T4	NI	NI	NI	NI	NI	NI	NI	NI
<i>Micrococcus</i> spp.	T1	NI	NI	NI	NI	NI	NI	NI	NI
	T2	NI	NI	NI	NI	NI	NI	NI	NI
	T3	NI	NI	NI	NI	NI	NI	NI	NI
	T4	+	+	-	-	-	-	+	+

NI: Not isolated; -: Negative reaction to virulence factor; +: Positive reaction to virulence factor.

The strains isolated from Treatment 4 presented up to four of the six virulence factors, while also being positive for biofilm formation (Table 1). Moreover, only three virulence profiles were attested (Table 2). This treatment contributed to a more balanced culture environment. Characterization of bacterial strains exoenzymes, plus biofilm formation were used as an expression of virulence potential factor in this study. With these parameters in mind, it can be argued that this treatment resulted in the isolated genera expressing less pathogenicity, seeing that they were positive with less exoenzymes. It is proved that the presence of periphytic biofilm in cultivation environments reduces the occurrence of pathogens (Thompson, Abreu, & Wasielesky, 2002) and helps in the fortification of the fish immune system, offering higher resistance to diseases (Zhang et al., 2010; Anand et al., 2014; Kumar et al., 2015).

Observing virulence profiles in Table 2, one can identify and establish the following order of potentially pathogenic strains: *Aeromonas* > *Pseudomonas* > *Bacillus* > *Staphylococcus* > *Mycobacterium*, *Corynebacterium* e *Micrococcus*.

*Pseudomonas* spp. and *Aeromonas* spp. did not alter the virulence profile, remaining the same in all treatments: Gel-Cas-Elas- $\beta$ -hem and Gel-Elas-Lip-Fosf- $\beta$ -hem, respectively (Table 2).

Sung and Hong (1997) isolated *Aeromonas* and *Pseudomonas* from freshwater shrimp *Macrobrachium rosenbergii* and identified that these bacteria were able to produce four and five extracellular products, respectively. The virulence of *Aeromonas* is considered multifactorial, as it is practically impossible to establish a hierarchy in the classification of its virulence factors according to their part in the disease process, and the following factors have been identified as virulence indicators: hemolysins, proteases, lipases, enterotoxins, gelatinases, elastases, among others (Rabaan, Gryllos, Tomas, & Shaw, 2001; Nam & John, 2007; Peixoto et al., 2012; Yadav, Verma, Pradhan, Dobriyal, & Sood, 2014).

*Bacillus* spp. and *Staphylococcus* spp. group exhibited three different virulence profiles, and were also positive for biofilm formation, being the third

group in pathogenic potential. Many studies in literature have used *Bacillus* species as probiotics (Cruz, Ibáñez, Hermsillo, & Saad, 2012; Albuquerque, Marengoni, & Boscolo, 2013), however, in this study, the isolated strains showed an enzymatic profile that probably conditioned them to the non-expression of their beneficial effects on the cultured organisms. *Staphylococcus* spp. possess various virulence mechanisms (Otto, 2012), and some of its species cause diseases in fish (Carvalho, Belém-Costa, & Porto, 2015). Although the genera *Mycobacterium* spp., *Corynebacterium* spp. and *Micrococcus* spp. isolated in this research have presented only one virulence profile, they are considered important pathogens of fish (Austin & Austin, 2007).

All genera identified were positive to at least one of the biofilm formation tests, independent of the treatment from which they were isolated. This biofilm formation factor, associated with the remaining exoenzyme production tests, presents a risk for cultivation, as biofilm formation is also considered an important factor in the pathogenicity of microorganisms, and can be used as an advantage in resisting environmental factors, aside from avoiding cellular and chemical defenses of the hosts immune system (Wakimoto et al., 2004; Dong, Fan, Wang, Shi, & Zhang, 2013).

## Conclusion

Treatments T1 and T3 presented a higher total of isolates, which expressed five virulence profiles. While genera from treatments T2 and T4 had two and three virulence profiles, respectively. The genus *Aeromonas*, considered one of the groups most pathogenic to fish, was not isolated in T4. The fish cultivated with bioflocs (T1) and periphyton (T4) presented a higher final weight.

The employment of growth inductor substrate for the periphyton and the manipulation of the C: N relation is a good alternative to aquaculture, since it is possible to increase the diversity of less pathogenic bacteria.

**Table 2.** Profile of potential virulence factors in bacterial isolates for each treatment tested in the culture of juvenile Nile tilapia (*O. niloticus*).

Genera	Treatments			
	T1	T2	T3	T4
	Virulence profile			
<i>Pseudomonas</i> spp.	Gel-Cas-Elas- $\beta$ -hem	Gel-Cas-Elas- $\beta$ -hem	Gel-Cas-Elas- $\beta$ -hem	Gel-Cas-Elas- $\beta$ -hem
<i>Aeromonas</i> spp.	Gel-Elas-Lip-Phos- $\beta$ -hem	Gel-Elas-Lip-Phos- $\beta$ -hem	Gel-Elas-Lip-Phos- $\beta$ -hem	NI
<i>Staphylococcus</i> spp.	Gel- $\beta$ -hem	NI	Gel-Cas	-
<i>Mycobacterium</i> spp.	Gel-Cas-Elas	NI	NI	NI
<i>Bacillus</i> spp.	Gel-Cas-Elas	NI	Gel- $\beta$ -hem	Cas-Lip- $\beta$ -hem
<i>Corynebacterium</i> spp.	NI	NI	Phos- $\beta$ -hem	NI
<i>Micrococcus</i> spp.	NI	NI	NI	Gel-Cas

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