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Effect of two probiotics on bacterial community composition from biofloc system and their impact on survival and growth of tilapia (*Oreochromis niloticus*)

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Abstract
The main goal of this study was to evaluate the effect of two probiotics on bacterial community composition of Biofloc system using molasses as carbon source. 135 tilapia organisms with an initial mean weight of 7.08±0.17g and a total length of 7.47±0.16cm were used for the present study. The fish were cultivated in plastic containers of 100L testing three experimental diets: Biofloc as control, Biofloc enriched with *Bacillus subtilis* and Biofloc enriched with *Rhodococcus* sp. Every 15 days, weight and length, as well as water quality parameters were analyzed. For DNA extraction, 1L of water was extracted from each treatment (initial, middle and final phase). The samples were sent to IMR to make the massive sequencing of the region V4-V5 from 16S rRNA. The bioinformatic analysis was made with MOTHUR and STAMP programs. 20 phyla were obtained, from which the most abundant were: Proteobacteria, Bacteroidetes, Actinobacteria, Planctomycetes, Verrucomicrobia and Fusobacteria from each treatment. Bacterial community showed variations in different periods, at the beginning were similar in all the cases, while at the end *Rhodococcus* sp. was different because it showed variations in the composition. The probiotics did not show significant effects in growth but improved the tilapia organism's survival.

Keywords: biofloc, probiotics, massive sequencing, *Oreochromis niloticus*

1. Introduction
The Nile tilapia (*Oreochomis niloticus*) has become, in Mexico, an important economic species, as in 2016, 117,806 tons production was reported with an increase of 15% with respect to the last year [1]. It is a species with wide range tolerance to environmental variations, easy reproduction, and with possibility to culture in different aquaculture systems [2; 3]. However, this species is not exempted to show problems associated with diseases or nutritional deficiencies, which represents a limit factor to increase the tilapia production [4; 5]. At same time, the increase of their production has generated a concern to environmental impact, generated by this activity, because the effluent discharges derived from its production, rich in organic matter, chemicals, antibiotics, and hormones which were used in the culture system and impact on the ecosystem [6], which allow the development of new culture production systems with low environmental impact [7, 8]. This is the case of Biofloc technology, which is supported in generation of microbial floccules produced by external carbon source, where heterotrophic bacteria that develops have principal functions in carbon and nitrogen recycle (present in organic matter) in the nutrient availability, in better water quality, disease control, and cultured organism's nutrition [9; 10].
On the other hand, the use of probiotic additives in aquaculture have increased as an alternative to the use of chemicals and antibiotics for diseases control. Several studies have shown that it contributes to intestinal microbial balance, increase the immune response, and increase the nutrient assimilation, which leads to better survival and growth of cultured species [11]. Based on the above, the scientific community assures that microbial controlled manipulation can be beneficial in productive systems [12] and in recent years, diverse studies were focused in the use of Biofloc system and probiotic additives inclusion, trying to increase the obtained benefits when these technologies were applied separately. However, it is

unknown the microbial community's dynamic associated to floccules, to implicated taxonomic groups, the variation can be done when probiotic bacteria were applied to system, and their effect to cultured organisms.

With respect to probiotics, there is a biotechnology which in last 20 years has increased worldwide about fish and crustacean culture [13, 14], 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 [15].

The definition of probiotic [16] 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 [17, 18, 19]. 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 [17, 20].

So, the goal of this investigation is to make the comparison effect in tilapia organisms with two probiotic additives on bacteria community composition in Biofloc system, and their effect on *Oreochromis niloticus.*

2. Materials and Methods

This study was made in the Chemical Analysis of Live Food Laboratory and Microbiology and Molecular Biology Laboratory at Universidad Autónoma Metropolitana, Xochimilco.

2.1 Experimental design and culture conditions

The culture of tilapia in Biofloc system was made in nine plastic containers of 100L capacity, with an air diffuser to assure the particles movement. In each container 15 juvenile's tilapias with a mean weight of 7.08± 0.17 g and mean length of 7.47± 0.16 cm were placed. Dairy was applied commercial food (Alimentos del Pedregal®, Toluca, State of Mexico) with 45% of protein and particle size of 0.6-0.8 mm, considering 10% of total weight. Ratio was adjusted every 15 days. To assure the Biofloc development in culture system, the relation C/N=20:1 was maintained [9], by controlling the supply of molasses as external carbon source. Furthermore, were added two different probiotics: *Bacillus subtilis* and *Rhodococcus* sp., in bacterial concentration of 1 x 10^7 UFC mL^{-1}. The experiment was formed by one control treatment (Biofloc), one treatment with Biofloc enriched with *Bacillus subtilis*, and one treatment with Biofloc enriched with *Rhodococcus* sp. Each treatment has three replicates and the experiment was done in a period of 60 days.

2.2 DNA extraction

At initial, middle and final phase of the experiment (day one, 30 days, 60 days), water samples were taken from each treatment in sterile beakers of 1L capacity coded as: 1) IC Initial Control), IB (Initial *Bacillus subtilis*), IR (Initial *Rhodococcus* sp.); 2) MC (Middle Control), MB (middle *Bacillus subtilis*), MR (Middle *Rhodococcus* sp.); and 3) FC (Final Control), FB (Final *Bacillus subtilis*), FR (Final *Rhodococcus* sp.). After obtaining the sample, this was centrifuged to obtain the pellet and suddenly make the DNA extraction using Silica Extraction Kit (Gene Reach Biotecnology Corp) [21]. DNA concentration was measured with Bio-photometer Eppendorf. The quality of genomic DNA extraction was detected in 1% agarose gel [22].

2.3 Illumina sequencing

The samples were send to sequencing service Integrated Microbiome Resource (IMC) in Ontario, Canada to make the massive sequencing of 16S RNA gen V4 and V5 region with Illumina MiSeq with chemical of kit 300 + 300 V3. The amplification products for PCR of the regions V4-V5 variables of 16S rRNA gen were obtained using universal primers 515 F (Illumina adapters + 5'GTGYCAGCMGCCGCGGTAA3') and 926 R (Illumina adapters + 5'CCGYCAATTYMTTTRAGTTT3').

The multiplexing, and sequencing of amplicons were made by double label design of indexing using barcode of 8 pb with the kit Nextera XT Index v2 (Illumina, San Diego, CA, USA).

2.4 Bioinformatic analyses

Bioinformatics analyses were made using MOTHUR, version 1.39.5, following the MiSeq SOP technique [23]. Selected reads must meet the following criteria: no ambiguous bases, a minimum length of 400 bp, no homopolymers of 8 bp and above. Demultiplexing was done with a barcode mismatch tolerance of one base for the 8-base molecular identifier tags. Operational taxonomic units (OTUs) were assigned to qualified reads at 3% dissimilarity using average-neighbor algorithm, chimeric reads were identified and excluded using UCHIME [24], and singletons were excluded to avoid inflated OTUs numbers using recommended MOTHUR's commands. Species diversity and richness, as well as rarefaction curves were computed at 97% similarity, as part of MOTHUR's alpha diversity pipeline, with the database Silva bacterial reference alignment [25]. Five metrics were calculated to assess the bacterial communities including number of observed OTUs, Shannon diversity index, and the estimators Inv-Simpson and Chao1 for species richness. Calculation of these parameters was done by normalizing all the libraries. Principal coordinates analysis (PCoA) were performed with UniFrac [26] using clustering at 97 % sequence identity. The RDP taxonomy classifier function was used to align and assign identities to the Illumina sequence data [27]. Downstream statistical analysis was performed using STAMP [28].

2.5 Water and sedimentable solids quality

Once a week, water samples were taken from each experimental plastic container to evaluate their quality. For that, temperature (°C), pH, and dissolved oxygen (DO, %) were measured with a multiparametric equipment HANNA (HI 9829). At same time, were analyzed the nitrogen compounds, like nitrites (NO_{2-}, mg/L), nitrates (NO_{3-}mg/L), and ammonium (NH_{4+}, mg/L) with autoanalyzer HANNA Aquaculture Photometer (HI83203), according to standard methods of HANNA (HANNA Company, 2003) [29]. To

measure the sedimentable solids quantity produced by Biofloc system was use an Imhoff cone, introducing 1L of water from each plastic container and let it sedimented by 15 to 20 minutes to know the sedimentable solids volume in mL/L [9; 30].

2.6 Growth and survival of Oreochromis niloticus

To obtain the biometric values, every 15 days fish were weighted with Adventurer™ Pro OHAUS balance and the total length of tilapias were measured with digital Scala® Vernier. With these values were obtained the Intrinsic Growth Rate (IGR) and the Absolute Growth Rate (AGR). Likewise, the organisms were daily counted to determined survival rate.

2.7 Statistical analysis

A database was made in Excel 2013 program to obtain descriptive statistics of water quality and fish growth values, for later make a one-way ANOVA analysis of a random model. In case obtaining significant differences (P<0.05), a multiple mean comparison was made using Tukey test from SYSTAT 12.0 program.

3. Results

3.1 Bacterial communities

The metagenomic analysis results showed that microbial community associated to Biofloc is represented by 20 phyla. Those who showed highest relative abundance were: Proteobacteria, Bacteroidetes, Planctomycetes, Verrucromicrobia, and Actinobacteria (Fig. 1).

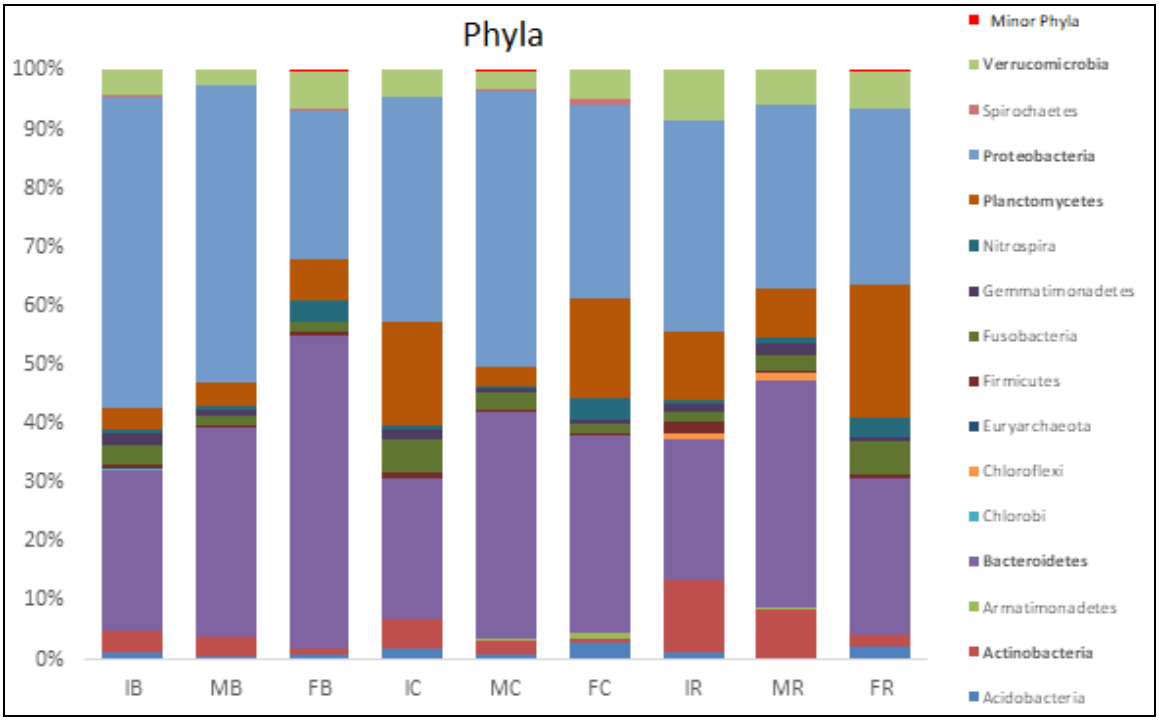

Fig 1: Relative abundance of bacteria phyla presents in Biofloc and Biofloc with probiotics

These phyla were present on all samples of three experimental phases. Nevertheless, in treatments with probiotic *B. subtilis* the dominant phylum was Proteobacteria, even though its relative abundance presented variations regarding time, at initial and middle phases of experiment shows 55 and 50% respectively, while at final phase show only 20%. Regarding to treatments with *Rhodococcus* sp. the relative abundance of Proteobacteria was the same (30%) in different analyzed phases. On the other hand, control treatment (Biofloc without probiotic) obtained a relative abundance of 35% at initial phase, 45% at middle phase and 30% at final phase. The phylum Firmicutes to which the genus *Bacillus* belongs and Actinobacteria, that includes *Rhodococcus* sp., are present in the study, nevertheless, were found with a relative abundance lower to 10%.

Table 1 shown higher and lower relative frequency values in each treatment. Highest values were found in MB, MC, FC treatments (31.20, 31.10, 17.31% respectively) with Alphaprotobacteria group; IB, FB, MR treatments (11.78, 19.12, 16.94% respectively) with Flavobacteria group; Ir treatment (12.28%) with Actinobacteria group; and FR treatment (15.36%) with Planctomycetacia group. Lowest values were observed in IB, MB, IC, MC, IR, MR treatments (1.36, 0.63, 0.45, 0.50, 0.79, 0.33% respectively) with Gammaprotobacteria group; FB, FR treatments (0.22, 0.65% respectively) with Betaprotobacteria group; and FC treatment (0.21%) with Actinobacteria group.

Table 1: Relative frequency of bacteria families identified with most frequency from each treatment.

Taxonomic classification				Treatments								
Phyla	**Class**	**Order**	**Family**	**IB**	**MB**	**FB**	**IC**	**MC**	**FC**	**IR**	**MR**	**FR**
Proteobacteria	Gammaproteobacteria	Gammaproteobacteria	Pseudomonadaceae	1.36	0.63	3.22	0.45	0.50	2.35	0.79	0.33	2.54
	Betaproteobacteria	Burkholderials	Burkholderiales_incer	4.81	2.23	0.22	10.71	5.88	1.46	4.71	5.86	0.65
	Alphaproteobacteria	Caulobacterials	Caulobacteraceae	4.82	31.20	6.28	6.38	31.10	17.31	6.98	14.42	4.62
Bacteroidetes	Sphingobacteria	Sphingobacterials	Chitinophagaceae	3.96	4.58	18.49	2.58	4.17	5.95	6.86	5.78	2.61
			Sphingobacteriaceae	1.75	1.79	6.51	1.23	3.94	3.78	2.86	6.10	7.62

	Flavobacteria	Flavobacterials	Flavobacteriaceae	11.78	22.34	19.12	10.52	22.28	8.62	2.80	16.94	3.95
Actinobacteria	Actinobacteria	Actinomycetals	Mycobacteriaceae	3.75	3.03	0.57	5.05	2.32	0.21	12.28	6.99	1.12
Planctomycetes	Planctomycetacia	Planctomycetals	Planctomycetaceae	4.72	4.45	6.14	18.51	3.51	14.92	12.61	8.11	15.36
Verrucomicrobia	Opitutae	Opitutals	Opitutaceae	2.13	1.13	1.08	1.14	1.38	1.44	3.28	1.81	1.41
Fusobacteria	Fusobacteria	Fusobacterials	Fusobacteriaceae	3.92	1.79	1.54	5.91	3.32	1.30	1.89	2.61	3.96

The main component analysis for different treatments in experimental phases show that initial phase values of all treatments were near each other. All of them began with a similar microbial composition as shown in Figure 2. In middle phase at control and *B. subtilis* treatment were different regarding to *Rhodococcus* sp. which show that composition of bacterial community changed regarding to other treatments. In final phase, *Rhodococcus* sp. treatment was different from each other's, because have variation in their composition change.

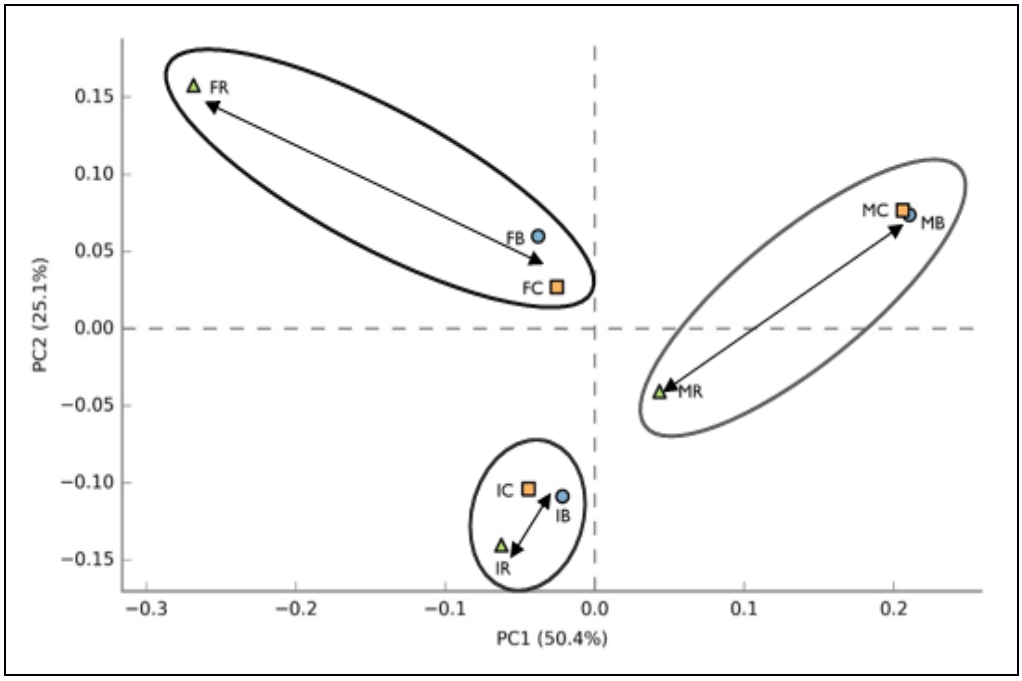

Fig 2: Microbial composition of Biofloc samples, Biofloc + *B. subtilis* and Biofloc + *Rhodococcus* sp. for each sequencing of 16s rRNA gen, V4 and V5 region

In general, diversity and richness index showed similar values between initial and middle phases, but specie richness was higher for *B. subtilis* treatment. In the initial phase, Sobs richness was 1497, Chao=3494.5 and in middle phase Sobs=1347, Chao=3052.02, while in final phase the highest richness was for *Rhodococcus* sp. treatment with values of Sobs=1716 and Chao=4066.62. Also, bacterial community diversity was higher at final phase of control treatment. Nevertheless, the number of coverage was high, because all samples obtained a value between 0.97 and 0.99. Regarding to sequencing number, the phase with lowest number was the middle with *Rhodococcus* sp. treatment (29250) and highest number was final phase with *B. subtilis* treatment (Table 2).

Table 2: Number of obtained sequences, sample coverture, richness and diversity of bacterial community of different treatments measured through Illumina MiSeq analysis of fragments of gen 16S rRNA amplified by PCR.

Samples	Sequences Number	Coverage	Community Richness		Community Diversity	
			Sobs (Observed richness)	Chao (Estimated richness)	InvSimpson (Richness)	Shannon (Diversity)
FB	34157	0.97	1548	3374.02	39.17	4.74
FC	32556	0.97	1561	3518.82	47.46	4.89
FR	31256	0.97	1716	4066.62	27.96	4.79
IB	32018	0.97	1497	3494.5	53.64	5.09
IC	30782	0.98	1259	2686.62	52.93	4.98
IR	24040	0.99	754	1281.65	41.37	4.68
MB	23782	0.97	1347	3052.02	33.21	4.69
MC	28447	0.97	1235	2965.94	35.87	4.55
MR	29250	0.98	1176	2265.66	64.19	4.90

On the other hand, the rarefaction curves of treatments in different experiment stages are shown in Fig. 3, where it is observed that number of Operational Taxonomic Units (OUT's) was high except in the initial phase sample of Biofloc + *Rhodococcus* sp. (IR). In general, the effort of sequencing was adequate with coverage values higher than 95% as shown in Table 2 and Figure 3.

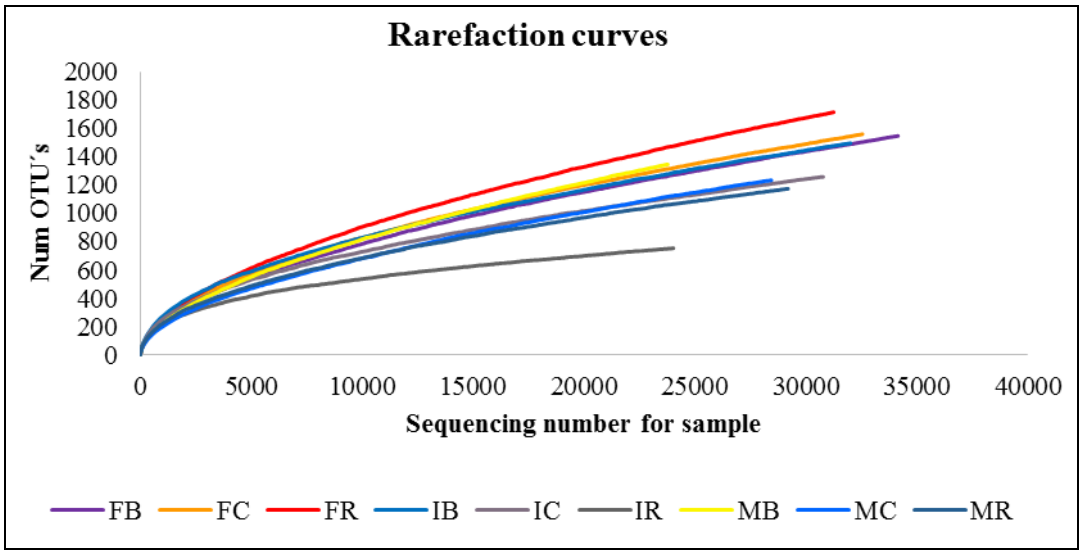

Fig 3: Rarefaction curves of the samples of Biofloc with corresponding probiotics in the different stages of experiment

3.2 Water quality and sedimentable solids parameters

Regarding water quality parameters as temperature, dissolved oxygen, pH and nitrogenous compounds did not show significant variations during the whole experiment ($P<0.05$) in all experimental treatments (Table 3).

Table 3: Average values and standard deviation of the water quality parameters of the treatments.

Parameters	Biofloc + *Rhodococcus sp.* Biofloc + *Bacillus subtilis* Biofloc									
	Mean SD	Min	Max		Mean SD	Min	Max		Mean SD	Min	Max
pH	7.67 (±0.21)a	7.20	7.85		7.57 (±0.18)a	7.36	8.01		7.67 (±0.16)a	7.45	7.95
Temperature (ºC)	21.58 (±0.72)a	20.69	22.77		21.86 (±0.78)a	20.29	22.44		21.69 (±0.66)a	20.63	22.40
Dissolved Oxygen mg/L	89.89 (±6.67)a	74.83	94		87.39 (±7.25)a	77.67	96.37		89.85 (±5.73)a	80.10	95.1
NO2- mg/L	0.59 (±0.28)a	0.28	1.15		0.52 (±0.26)a	0.22	1.15		0.58 (±0.31)a	0.19	1.15
NO3- mg/L	24.17 (±5.40)a	18.73	30.0		24.70 (±4.13)a	13.6	30.0		22.96 (±4.26)a	13.43	30.0
NH4+ mg/L	0.80 (±0.16)a	0.39	0.80		0.63 (±0.12)b	0.51	1.55		0.61 (±0.15)b	0.41	0.87
Sedimentable solids	5	0	5		5	5	5		5	5	10

The data are mean values (± standard deviation). Different letters in a row show significant differences ($P<0.05$) between treatments.

3.3 Growth of tilapia

The intrinsic growth rate (IGR) and absolute growth rate (AGR) in length and weight of fish, are shown in Table 4. Both variables did not show significant differences between treatments with $P=0.755$ and $P=0.472$ respectively.

The survival was different for *Rhodococcus* sp. treatment (91%) and *B. subtilis* treatment (89%) regarding to control group (71%).

Table 4. Growth parameters of *O. niloticus* in the different treatments

Parameter	Control	BFT + *Bacillus subtilis*	BFT + *Rhodococcus* sp.
Initial weight (g)	7.15 ± 1.03^a	6.88 ± 0.98^a	7.21 ± 1.01^a
Final weight (g)	8.97 ± 2.61^a	9.21 ± 2.38^a	9.54 ± 1.97^a
AGR (g day^{-1})	0.030	0.039	0.039
IGR (%/day)	0.37	0.48	0.46
Initial length	7.61 ± 0.59^a	7.28 ± 0.46^a	7.52 ± 0.49^b
Final length	8.26 ± 1.25^a	8.11 ± 1.08^a	8.24 ± 0.25^a
AGR (g day^{-1})	0.011	0.014	0.012
IGR (%/day)	0.14	0.18	0.15
Survival (%)	71	89	91

The data are mean values (± standard deviation). Different letters in a row show significant differences ($P<0.05$) between treatments.

4. Discussion

Microorganisms are a fundamental part of trophic networks in aquatic environments, by contributing to nutrient recirculation and interacting with a broad range of organisms [31]. To understand their specific niche function, it is essential to identify and quantify each integrant of this community. That's why metagenomics techniques are very important as an emergent tool for studying non-cultures microorganism's communities [32]. The results of metagenomics analysis in this study show that microbial community associated to Biofloc was represented by 20 phyla, which highest relative abundance were: Proteobacteria, Bacteroidetes, Planctomycetes, Verrucromicrobia, and Actinobacteria, but Protobacteria and Bacteroidetes phyla were the ones with higher relative abundances in all treatments. These results agree with respect those reported in an investigation [33], which made an experiment to evaluate the effect of addition of tapioca starch, cellulose, and combination of both sources on Biofloc microbial diversity when herbivorous carp was cultured, using massive sequencing. These authors report that

independent that carbon source were added to culture system, Proteobacteria and Bacteroidetes phyla show the highest relative abundance. It should be noted that bacteria of these phyla were reported in different studies as ubiquitous in aquatic environments and aquaculture production systems [34; 25, 36].

On other hand, it has been proved that microbial Proteobacteria group is important to nutrient recycling process and mineralization of organic components in aquatic systems [37], and this group include several phototrophic and heterotrophic genders with high degradative capacity of compounds like methane and methanol in aquatic environments [38]. Other study [39], mentioned that Betaproteobacteria, also identified in this investigation, was aerobic or facultative bacteria group, which can transform nitrogen compounds in aquatic environments. One of principally genus is *Nitrosomonas* that oxidize ammonia.

Bacteria families with highest relative abundance in this study, have also been reported in aquaculture systems and Biofloc systems like Pseudomonadaceae [40], Caulobacteraceae [34], Chitinophagaceae [41], Sphingobacteriaceae [42], which were efficient bacteria to transform several compounds in water column like cellulose, chitin, collagen, and nitrogen compounds produced during culture process.

One of the most important objectives of this investigation was to evaluate the effect of probiotic addition on Biofloc microbial community in tilapia culture. The analysis results showed that probiotic addition does not modify the present microbial community and neither dominates within the system because relative abundance of *B. subtilis* was very low specially in the final phase of the experiment (lower than 10%), opposite to what was expected, because it is one the genus most used as probiotic in aquaculture with positive effects in growth, survival, immune response, and disease control of fishes and crustaceans, which allow their capacity of competitive exclusion [15, 43, 44]. Nevertheless, in Biofloc culture system, must take into account that production conditions of aquatic organisms differ significantly regarding to conventional systems, because promote the growth of complex diversity of heterotrophic bacteria using an external carbon source and high aeration supply, which act not only cultivated species, but also in bacterial groups that can adapted to such conditions [45, 46], but unlike some authors [47] which mentioned that they do not obtain any positive effects when commercial probiotic like *Bacillus* spp. and *Lactobacillus* sp. in Biofloc system for shrimp culture were supplied. Authors mentioned that this is possible when processes of anaerobiosis happens at pond bottom or by probiotic concentration which probiotic was added.

Regarding to treatments where *Rhodococcus* sp. was added, it was not observed an effect on Biofloc bacterial community, also their relative abundance at three experimental phases were lower than 10%, even though it is known that this microorganism easily adapts to aquaculture systems, even in adverse conditions, for other genders, because their high metabolic diversity with which it can biodegrade and use diverse carbon sources, metals, hydrocarbons, among others [48].

It is important to mention that in Biofloc systems it has been documented the presence of probiotic bacteria that develops *in situ* in natural way, because in this systems with low or non-water exchange, fishes feces which were liberate to water, there are bacteria that are part of intestinal microbiota and when they have contact with water, rich in carbohydrates due to the input of external carbon source, proliferate significantly without need of probiotic addition of external sources [49, 50].

The analyzed diversity index in this work indicates that bacterial richness (Chao) and diversity (Shannon) in Biofloc is high (4066.62 and 5.08 respectively) considering the mentioned in an investigation [33, 37], which obtained bacterial richness and diversity values of 677 and 4.82 respectively in Biofloc cultures, indicating that are high values for aquatic environments. Also, in other study [51] which reported Shannon index value of >5.0 in tilapia culture with Biofloc, showed high diversity. The Chao index was also high with a value of 7656.

Rarefaction curves of treatment at different experimental stages showed a high number of OUT's (1716), higher than reported by other studies with values between 623 and 277.59 [33, 37]. According to some authors [51], coverage value was high when was close to 1.

Regarding to physicochemical parameters there were not any significant variation between treatments in all experimental period, mainly in nitrogenous compounds which are one of the more stressful factors for fish, because nitrites values were between 0.52 and 0.59 mg/L, nitrates between 22.96 and 24.17 mg/L and ammonia between 0.61 and 0.80 mg/L being within optimal ranges for tilapia culture [3, 52, 53]. Some authors [54], mentioned that one of Biofloc benefits used to growth aquatic organisms, was the reduction and stabilization of nitrogenous compounds, because organic wastes accumulated in ponds was degraded, and produced ammonia by fishes was nitrified or assimilated by microbial community, without the need of water exchange on conventional culture systems. It has also been reported that cyanobacteria transform ammoniac nitrogen in compounds that are not harmful for fish in culture and produce microbial biomass available for following trophic levels [55].

Regarding the weight and length organisms increase, there were not significant differences between treatments, but with respect IGR values that show significant differences (P=0.030 and P=0.039 respectively), was agree with a study [56], which made an 84 days' experiment to saw tilapia growth rate, comparing recirculation system with respect Biofloc system, and observed too, significant difference in IGR values compared to control treatment, but different with AGR, which did not show significant differences (P=0.37 in weight; P=0.48 in length). In other study [57], which evaluated the effect that Biofloc system produced on tilapia growth for eight experimental weeks, they observed that IGR was significantly higher in Biofloc system than in conventional system. On other hand, other study [58], which applied diets with 32% of protein, different percentages of tryptophan and 20% of Biofloc flake, observed that tilapia weight increase significantly because they began with a weight under 5g and in 60 days' treatment, organisms obtained highest mean weight value of 41.84g. The authors mentioned that these results were provoked because Biofloc positively affect the digestive enzymatic activity of cultured organisms, improving the nutrient assimilation. Some authors evaluated the *O. niloticus* growth in Biofloc [59], adding rice flour and molasses as external carbon source. The weight gain was 40g in Biofloc system in an experimental period of 12 weeks, because tilapia organisms can use food particles produced *in situ*, included suspended bacteria.

Survival was higher in treatments with Biofloc enriched with

probiotics with respect control treatment, even when metagenomics analysis did not showed any important effects on Biofloc microbiota, *Rhodococcus* sp and *B. subtilis* improved the wealth being health of fish, because they improve, not only the immune system but also water quality, which allowed to reach a higher survival values as mentioned in a study [60], which indicates that by adding probiotics to diet, immune response increments and therefore this variable.

5. Conclusions

The present work shows great relevance because the use of massive sequencing technique, as important tool to obtain better information about bacterial community, which were developed in Biofloc systems, although there are some works that made isolation and identification with conventional microbiological tests and cultured organisms, these doesn't give all the information that is obtained with this metagenomics analyses, which not only allow to identify the diversity and abundance of species, but also the ecological function that those bacteria have in their habitat. This study expands the knowledge about Phyla that develop in Biofloc systems, because in most cases, it was only considered the dominant Phyla Firmucutes, Proteobacteria, and Actinobacteria, but with this metagenomics tool were identified 20 phyla among those that were considered with greater abundance Proteobacteria, Bacteroidetes, Actinobacteria, Planctomycetes, Verrucomicrobia and Fusobacteria.

The addition of probiotics *B. subtilis* and *Rhodococcus* sp. Did not affect in bacterial composition of Biofloc, because these bacteria isolated from tilapia intestinal, did not support environmental conditions of culture system or were displaced by Biofloc microbiota. Also, it must be considered the dose and time of administration. Therefore, it is required to make more investigations to evaluate relevance of probiotic addition in Biofloc systems.

Although no significant differences were observed in length, weight and survival of organisms, improved substantially with probiotic use. Also, water quality parameters, especially nitrogenous compounds, were maintained optimum to those levels was required for tilapia culture, which was important, because there is a lower environmental impact using water resource for this aquaculture activity.

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