Full Length Research Paper

Bacterial community in two subtropical fishponds in São Paulo, Brazil

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The knowledge concerning fishpond microorganisms is important considering the aquatic microbial ecology and water quality. In this study, we compared the bacterial communities present in freshwaters of two fish farm ponds from Brazil, one used as water reservoir (P1) and the other with conditions of high nutrient load (P4). The aim of this study was to identify and compare the bacterial population in two fishponds during dry and rainy seasons. The determination of the bacterial population was conducted through sequencing of the 16S rRNA gene. The results show that the bacterial population is different in the ponds and it changes according to the climatic period. Moreover, the diversity of microorganisms in P1 was greater than P4. Proteobacteria predominated in the rainy season in both ponds varied in proportion and classes composition. In the P4 during dry season, Cyanobacteria were predominant due to favorable conditions of environment. This study provides the first local investigation that demonstrates that the bacterial community is differ according to the trophic state of aquatic systems and between rainy and dry seasons, the latter of wich exerted more influence in bacterial communities. It can be suggested that the management in P4 promotes favorable environment for the high density of Cyanobacteria, demonstrating the need for improvements in management.

Key words: *16S rDNA*, biodiversity, freshwater, metagenome.

INTRODUCTION

Aquaculture is an important social and economic activity, currently accounting for 40% of world production of fish, which corresponds to approximately 150 million tonnes (FAO, 2012). This activity causes environmental impacts that affect society as a whole. In this respect, the deterioration of water is a major socio-environmental effect that occurs in aquaculture ponds in the absence of good management practices in aquaculture ponds

(Calijuri et al., 2008).

In aquaculture ponds, there is a constant inflow and outflow of water that has pronounced effect on the distribution of organisms, and the outflow effects on the residence time of water have become essential to the understanding of the internal processes of artificial systems (Pereira et al., 2004). Rainfall directly affects the dynamics in these environments by promoting the transport of nutrients and allochthonous materials, visual changes, and changes in the physical and chemical characteristics of the water (Sipaúba-Tavares et al., 2007). Furthermore, the expansion of aquaculture leads to increase in nutrients in the aquatic environment which can cause algae accelerated growth (Macedo and Sipaúba-Tavares 2010; Chislock et al., 2013) and alteration in diversity of microrganisms (Leff, 2009).

Microbial activity is important in the removal and transformation of organic matter in fish farms (Forster et al., 2003). In microbial ecology, one of the most critical determinants of an aquatic ecosystem it is trophic state which is heavily influenced by the food chain and biogeochemical cycles (Leff, 2009). The eutrophication of aquatic environments can be caused by man through disposal of effluent from aquaculture farm (Cao et al., 2007). The concentration increase of nitrogen and phosphorus are the principal cause of the eutrophication, which can affect the fast develop and excessive growth of algae and *Cyanobacteria* (Macedo and Sipaúba-Tavares, 2010).

The microrganisms in the water have an important interaction in food chain, they are directly involved with the process named microbial loop, first described by Azam et al. (1983). According to these authors, in summary, an important consequence of the microbial loop is the bacterial ability to soak up the nutrients in the water column, which leads to a fast recycle of these nutrients. Fenchel (2008) relates that the principal effect of microbial loop on nutrients cycle in the water column is the acceleration of the mineralization and the regeneration of production in systems limited of nutrients.

Despite the importance of the microbial dynamic of population in freshwater ecosystems (Zwart et al., 2002; Taipale et al., 2009) studies in this area are scarce and needed. In this sense, the aquatic bacteria are difficult to obtain in culture and consequently defining their biogeochemical role and understand the dynamics of populations in these systems becomes a challenge (Kemp and Aller, 2004).

The advents of molecular methods as metagenomic strategies, especially ribosomal RNA-based techniques, can be used to explore the microbial diversity in samples directly from the environment. Much of what is now known of the diversity of aquatic bacteria is based on distinguishing among different organisms, as represented by polymerase chain reaction (PCR) of 16S RNA gene, where it is unnecessary culturing them or having any direct knowledge of their morphology, physiology or ecology (Kemp and Aller, 2009; Petrosino et al., 2009).

The microorganisms in water present important interac-

tions in the food chain: they are directly involved with the process denoted microbial loop, which was first described by Azam et al. (1983).

According to these researchers, an important consequence of the microbial loop is the ability of bacteria to soak up the nutrients in the water column, which results in a rapid recycling of these nutrients. Fenchel (2008) reported that the principal effect of the microbial loop on the nutrient cycle in the water column is the acceleration of the mineralization and the regeneration of production in nutrient-limited systems.

MATERIALS AND METHODS

Study area

The study was carried out in two fishponds of the University of São Paulo State (UNESP at Jaboticabal (São Paulo, Brazil) 21°15'S and 48°18'W) during the rainy (January and February/2012) and dry (July and August/2012) seasons (Figure 1). The water samples were collected in the water outlet with two fishponds studied. The first fishpond (P1) is a water supply reservoir to the aquaculture farm with continuous water flow surface area of 3,472 m² and water depth ranging from 1 to 1.3 m. This shallow reservoir was in the past used as a fishpond, but since 1995, it is used only as a water reservoir for the aquaculture farm. The other fishpond studied is the fourth (P4) of six similary size at fishponds in a sequential line, and each one of them is directly or indirectly receiving water from the previous one (Figure 1C). This fishpond studied has an area of 4,268 m², a depth of 1-1.3 m, a continuous water flow with a daily exchange rate of 5% of its volume and an average fish density of 1 fish.m-2. A mix of fish species, such as *Cyprinus carpio, Piaractus mesopotamicus, Cichla ocellaris, Oreochromis niloticus and Brycon cephalus*, were found in this pond. These fishponds are affected by continuous water flow, wind, rainfall, and the transport of nutrients and allochthonous materials.

The aquaculture consist of 73 small fishponds (c.a. 140 m²) with a continuous water flow and six large fishponds, disposed in a sequence, ranging between 1.822 and 8,067 m². A semi intensive production is conducted for research.

The climate in the region is subtropical Cwc (Peel et al., 2007), relatively dry in the winter (June to August) and rainy in the summer (December to March), with mean yearly temperature of 22°C and mean yearly of rainfall 1,427 mm.

Water sampling

Physical and chemical parameters were sampled at 8:00 AM at 10 cm depth. Samples are collected with Van Dorn bottle and transported in refrigerated polyethethylene bottles to laboratory. Temperature, pH, conductivity and dissolved oxygen were measured *in situ* using a multi-probe Horiba U-10. Total phosphorus, orthophosphate, total ammonia nitrogen, nitrite and nitrate, were quantified spectrophotometrically according to Golterman et al. (1978) and Koroleff (1976). Analyses were performed immediately after sampling or samples were duly store under a refrigerator until analysis. All samples were carried out in triplicate.

Molecular analyses

Water samples for molecular analysis were stored in sterile 5 L bottles. The total DNA was extracted using UltraClean ™ Water DNA Isolation kit (0.22 µm) (MO BIO Laboratories, Inc., Carlsbad – CA), and then subjected to PCR amplification of the 16S rRNA
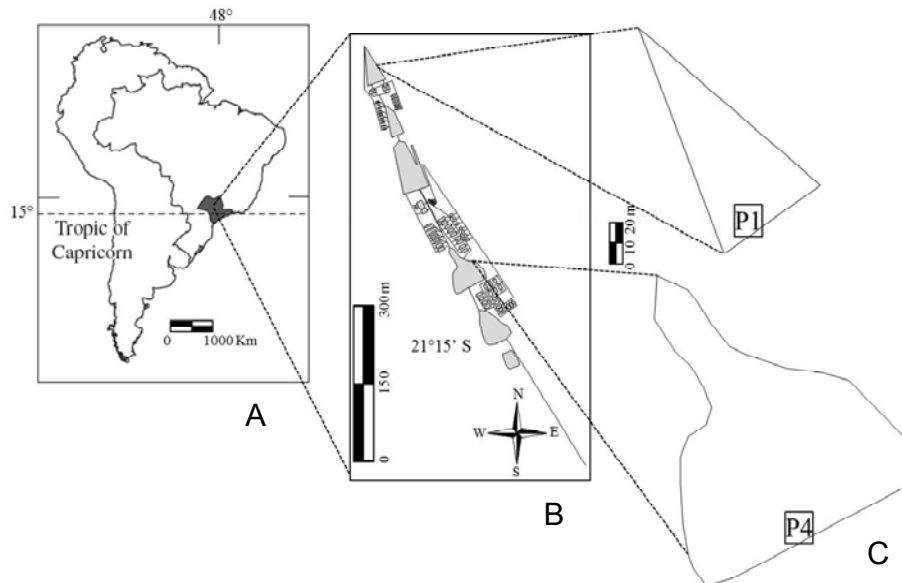
Figure 1. Cross section of aquaculture farm. A, shaded area indicates São Paulo state, in southeastern Brazil. B, Distribution of fishponds at UNESP farm. C, fishponds studied.

gene using Y1 and Y2 primers (Young et al., 1991). The PCR reaction were performed using 60 ng of DNA , 1X PCR buffer [20 mM Tris- HCl (pH 8.4), 50 mM KCl] , 1.5 mM MgCl2, 0.2 mM dNTP, 10 pmols of each primer and 0.8 U of Taq DNA polymerase. The reaction was subjected to the following the thermal profile: 5 min at 95°C, 35 cycles of 45 sat 95°C, 45 s at 65°C and 90 sat 72°C, followed by a final incubation at 72°C for 5 min. The amplicons were purified using the Wizard® SV Gel and PCR Clean-Up System (Promega). After purification, the amplicons were randomly cloned using the CloneJET™ PCR Cloning Kit (Fermentas) and chemically competent E. coli DH10B cells. Extraction of plasmid DNA of obtained clones was performed with the Wizard® kit MagneSil® Plasmid Purification System (Promega) in epMotion 5075 (Eppendorf) instrument.

We obtained and sequenced 1344 clones that were sequenced on a capillary device model 3100 DNA Analyzer ABI Prism (Pedrinho et al., 2012). After sequencing, the electropherograms were analyzed with the tool Phred/Phrap (Ewing et al., 1998; Ewing and Green, 1998) for detecting the reliability of each base sequenced and the ContGEN, for selection of only those sequences that presented more than 200 bases with Phred quality. Sequences with good quality were compared with the ribosomal genes database of Ribosomal Database Project (RDP) II (Cole et al., 2009), through Classifier (Wang et al., 2007) and also Library Compared (Cole et al., 2009), using confidence threshold the 70%. The data of Co-Occurrence were performed using MEGAN 5 (Huson et al., 2007).

The GenBank accession numbers of the clones from the four libraries deposited in NCBI are KJ609244-KJ609302, KJ663950-KJ664015, KJ664016-KJ664179 and KJ676390-KJ676449.

Statistical analyses

Principal components analysis (PCA) was performed using the average values measure in the fishponds in order to reduce the dimensionality of the environmental variables and to rank fishponds during the experiment (Legendre and Legendre, 1998).

Analysis of variance (ANOVA) was used to evaluate the differences among the seasons (dry and rainy) and fishponds and their interactions (Sokal and Rohlf, 1981).

RESULTS AND DISCUSSION

A comparison of physical and chemical parameters between the two ponds can be seen in Table 1. The temperature related results shows that there was an increase of 3-6°C in the rainy season in the two assessed ponds. At the same time, the pH was more acid. Conductivity, dissolved oxygen, nitrite, total ammonia nitrogen (TAN), orthophosphate and total phosphorus were higher in P4 regardless of the period. The concentration of TAN observed in P4 was even higher during the rainy period while the concentration of total phosphorus was higher in the dry period.

In Figure 2 shows the principal component analysis. This graph appointed four distinct groups according to the axis position. The first was positioned on the negative side of axis 1, which were grouped P4 in the dry period (P4-D) to the variables nitrate, nitrite, pH, dissolved oxygen (DO), orthophosphate and total phosphorus, indicating a higher degree of trophy. Positioned on the positive side of axis 1 and contrasting the group mentioned above, the group formed by the pond 1 in the rainy period (P1-R) was not associated with any variable, indicating a lower trophic level. The axis 2 shows the same contrast of group. On the negative side was positioned the point related to pond 4 during the rainy period (P4-R), associated to TAN and temperature. The positive side showed the point related to the pond 1 in the dry period (P1-D) not associated with any variable, which shows an increase in TAN load from P1 to P4.

Table 1. Means values and standard deviation for pH, conductivity, temperature, dissolved oxygen, nitrite, nitrate, ammonia, orthophosphate, total phosphorus in the fishponds studied, during the rain and dry seasons.

Parameter	Rainy		Dry	
	P1	P4	P1	P4
pH	4.7±0.2[d]	5.7±0.2[c]	6.5±0.5[b]	7.2±0.4[a]
Conductivity (μS.cm^{-1})	40.0±0.1[b]	103.5±6.7[a]	36±0.5[b]	103.8±3.9[a]
Temperature (°C)	23.4±0.3[b]	26.6±0.8[a]	19.0±0.3[d]	20.9±0.6[c]
Dissolved Oxigen (μg.L^{-1})	1.4±0.2[b]	2.7±0.5[b]	2.4±1.2[b]	6.6±1.5[a]
Nitrite (μg.L^{-1})	5.0±2.7[c]	36.9±9.4[b]	8.2±4.7[c]	78.9±15.3[a]
Nitrate (μg.L^{-1})	322.2±56.8[a]	308.3±60.4[a]	190.1±73.4[b]	379.9±63.2[a]
TAN (μg.L^{-1})	61.2±34.2[c]	439.3±95.6[a]	25.4±18.6[c]	156.5±29.3[b]
Orthophosphate (μg.L^{-1})	ND	11.2±10.5[b]	7.8±3.5[b]	56.0±12.2[a]
Total phosphorus (μg.L^{-1})	11.6±21[b]	96.7±26.1[b]	42.1±35.6[c]	214.8±18.0[a]

P1, Pond 1; P4, Pond 4; Simple ANOVA, with mean and standard deviation. Tukey´s test.
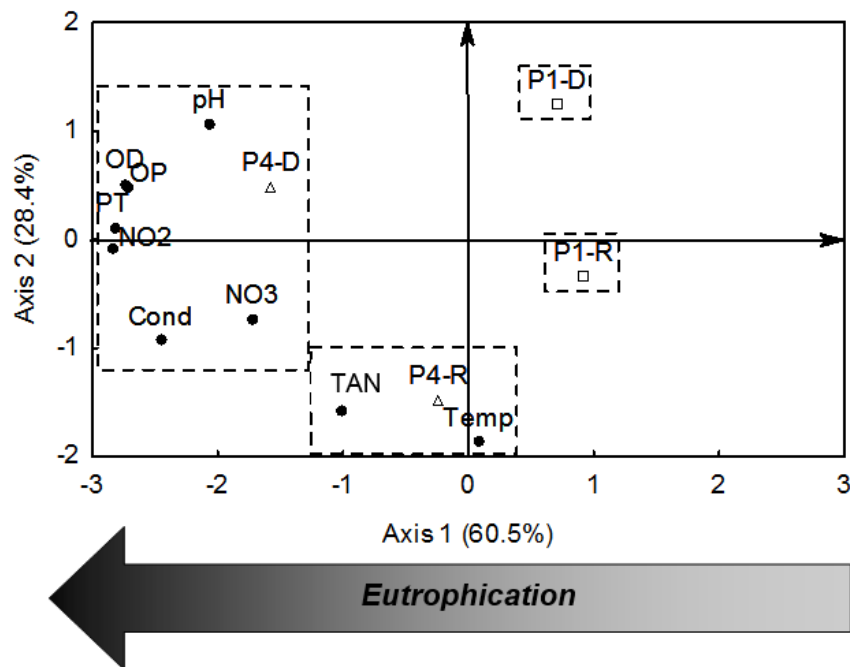

Figure 2. Interpolation of eigenvalues from the matrix of water variables. First two axis the principal component analysis (PCA); filled circles= water variable, open triangles= fishponds, R= rain season, D= dry season, DO= dissolved oxygen; OP= orthophosphate; PT= total phosphorus; Cond= conductivity; pH= hydrogen potential; Temp= temperature; NO_2= nitrite; NH4= total ammonia nitrogen; NO_3= nitrate.

Sequencing of 16S rRNA gene from DNA samples revealed variation in bacterial community according to the nutrient load of the ponds and the climatic seasons.

The sequences were classified into distinct phyla (Figure 3). The composition of the bacterial population was different between the ponds and some phyla showed greater variation between the dry and rainy periods.

The lowest concentration of nutrients found in P1 may be associated with the great diversity and quantity of aquatic macrophytes present in this pond, which may act as a biofilter capturing the nutrients from the environment, mainly phosphate. There was no farming at P1; the aquatic organisms that live there survive from the aquatic biota, and therefore metabolites of animals are low and there is not availability of nutrients resulting from feed. In P4, a more eutrophic pond (Sipaúba-Tavares et al., 2010), the concentration of nutrients was higher due to the discharge of effluents from the raniculture and
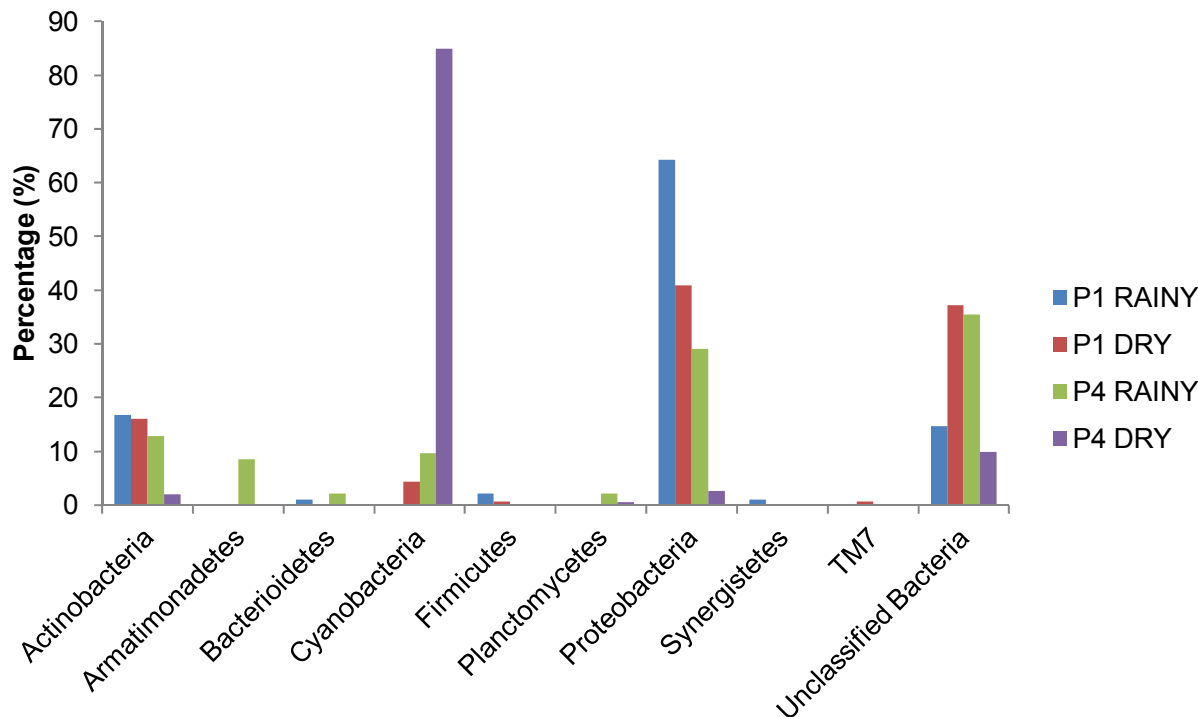
Figure 3. Percentage of different phyla and unclassified bacteria found in water samples from the studied ponds (P1 and P4) during the different climatic seasons (dry and rainy).

previous fishponds. Consequently, it promoted increased amount of nutrients in the water column, especially in the forms of nitrogen and phosphorus.

The increase of nutrients in the aquatic environment was an important factor to local bacterial population changes. When in P4, during dry period, nitrogen levels were relatively low com-pared to the rainy period, the nitrogen fixing *Cyanobacteria* have proliferated due to the availability of phosphorus, winning the competition with other bacterial species.

The ability of organic matter degradation, assimilation and cycling of nutrients in water were related to the bacterial community which forms the basis of the food web as the microbial loop model proposed by Azam et al. (1983) and supplemented by Fenchel (2008).

In this study, the *Proteobacteria* were found in abundance in the bacterial communities identified mainly in P1 and in the rainy period for P4. According to Newton et al. (2011), *Proteobacteria* residing in the freshwater generally were competitive under conditions of low nutrient/substrate. This group is also able to degrade complex organic compounds as well as participate in the nitrogen cycle.

High conductivity values were found in P1 during the rainy period, indicating that the site had a high degree of organic matter decomposition. During this period there was predominantly *β-Proteobacteria*, which is related to the degradation of complex organic matter. In the dry period, there was a balance between the *β-Proteobacteria* and *α-Proteobacteria*, which in turn are related to biological nitrogen fixation. According to Nishimura and Nagata (2007), *α-Proteobacteria* is abundant in freshwater. The source of complex organic matter in P1 was the macrophytes. This pond had different macrophytes species with different content of organic matter such as *Eichhornia azurea* (Sw.) Kunth and *Salvinia auriculata* Aublet. These species compete with each other and have alternating life cycle in the pond (Dias and Sipaúba-Tavares, 2012).

Table 2 shows the significant difference between each library, according to the tool Library Compare from RDP II (Cole et al., 2009). It can be observed that only *Proteobacteria* demonstrated significant difference bet-ween fishpond 1 and 4 in dry and rainy seasons. In contrast, the fishpond 4, for the same seasons showed significant difference too between *Armatimonadetes*, *Cyanobacteria* and *Actinobacteria*.

For each pond, the percentage of *Proteobacteria* was higher in the rainy period. Large percentage of *Cyanobacteria* (almost 80%) was observed in P4-D. The percentage of *Actinobacteria* remained similar in the two periods for P1, but demonstrated a remarkable reduction in P4, especially during dry period.

Some phyla were identified as unique to certain samples, such as: *Synergistetes* and *Firmicutes* in P1-R; TM7 in P1-D; *Armatimonadetes* and *Planctomycetes* in P4-R. In all samples, the presence of unclassified bacteria was noted.

The significant difference involving the *Proteobacteria*

Table 2. Values obtained with Library Compare tool from RDP comparing the phyla of the libraries of fishpond 1 (P1) and fishpond 4 (P4) in the rainy and dry seasons. The symbol (*) demonstrates a significant difference at 0.01 for P1 and the symbol (**) demonstrates a significant difference at 0.01 for P4.

Phylum	% P1 Rainy	% P1 Dry	% P4 Rainy	%P4 Dry
TM7	0.0	0.7	0.0	0.0
Cyanobacteria**	0.0	4.4	9.7	84.9
Bacteroidetes	1.1	0.0	2.2	0.0
Synergistetes	1.1	0.0	0.0	0.0
Firmicutes	2.1	0.7	0.0	0.0
Actinobacteria**	16.8	16.1	12.9	2.1
Proteobacteria*/**	65.3	40.9	29.0	2.6
Armatimonadetes**	0.0	0.0	8.6	0.0
Planctomycetes	0.0	0.0	2.2	0.5
Unclassified Bacteria	13.7	37.2	35.5	9.9

Table 3. A significant difference in the classes of Proteobacteria found was detected.

Class	Order	Genus	Sample
β-Proteobacteria	Rhodocyclales	*Dechloromonas*	P1-D
		Unclassified	P1-D/P1-R
	Burkholderiales	*Polynucleobacter*	P1-D/P1-R/P4-R
		Pseudorhodoferax	P1-D/P1-R
		Macromonas	P1-R
		Limnohabitans	P1-D/P1-R
		Inhella	P1-D
		Ideonella	P1-D
		Piscinibacter	P1-D
		Curvibacter	P1-D
		Pelomonas	P1-D
α-Proteobacteria	Rhizobiales	*Labrys*	P1-D
		Bradyrhizobium	P1-D

was in the classes found. In Table 3, we showed the distribution of classes of the phylum *Proteobacteria* found in the libraries studied.

Among bacteria in order Rhodocyclales the genus *Dechloromonas* was found only in P1-D. Although the high frequency was in rainy seasons (P1-R); it was not classified in any genus.

Most of these bacteria genus, are classified as chemoorganotrophic, aerobic and anaerobic facultative, and are frequently found in freshwater habitats (Hahn et al., 2010a, 2010b; Kasalicky et al., 2010).

The presence of *β-Proteobacteria* in P4 was probably associated with the discharge of effluents from other ponds used for farming of aquatic organisms and also the algae decomposition present in the study site. In P4-R was also observed the presence of *γ-Proteobacteria*. The organisms found in this phylum are mostly of enteric origin and they indicate anthropogenic or zoonotic conta-

mination and can be pathogenic to several species (Newton et al., 2011). This is a factor to be considered when determining the management of aquaculture farm concerning their role in human nutrition and its economic importance.

The significant difference between phyla Proteobacteria in P4, was in classes *β-Proteobacteria* and *γ-Proteobacteria*. Both this classes were in rainy season (P4-R) and was predominant. The genus *Polynucleobacter*, order Burkholderiales, class *β-Proteobacteria*, was the only one found in P4-R. This genus is cosmopolitan and has high ability of adaption in distinct environments (Hahn et al, 2010a; Newton et al, 2011). When the same phylum in P4-D, was observed *α-Proteobacteria* was predominant.

In general, in P4-R, the classes *β-Proteobacteria* and *γ-Proteobacteria* were predominant, and consequently different according to Library Compare. No order of *γ-*
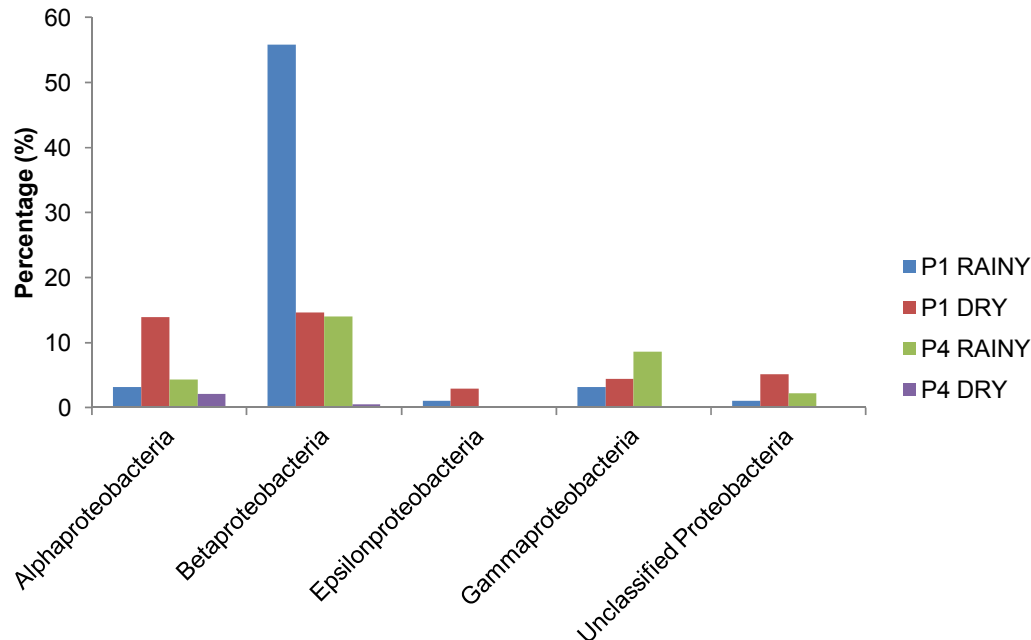
Figure 4. Percentage of different classes of Proteobacteria found in water samples from studied (P1 and P4) during the different climatic seasons (dry and rainy).

Proteobacteria was possible to be classified according to the tool used, because they showed threshold under 30%. But Newton et al. (2011) demonstrated that this class is more abundant in ocean, however when found in freshwater many of these organisms were enterics, for example, *Escherichia coli*, indicated anthropogenic contamination or zoonotic sources.

Glöckner et al. (2000) in a study cluster the α-Proteobacteria in six phylogenetic groups: *αI, αII, αIII, αIV, αV* e *αVI*, which according to his results, the groups αII, αIV, αV really are from freshwater, but the others showed different origins. According Newton et al. (2011) αI included *Rhizobiales, αII* the *Caulobacter* and *Brevundimonas*, genus generally found in libraries clones of freshwater.

The importance of the α-Proteobacteria and β-Proteobacteria found in the fishponds are in the fact that the microorganisms belongs to this classes; it is fundamental in biomass degrading (Newton et al., 2011) and there are bacteria nitrogen fixing (Souza et al., 2012; Noar and Buckley, 2009). These are characteristics necessary in fishponds to regulate the environment production and equilibria.

Alterations were observed in the prevalence of *Proteobacteria* classes depending on the hydrological period. In the rainy period there was a preponderance of *β-Proteobacteria*, while in the dry period there was a more diverse distribution of present classes, being the α-Proteobacteria present in the highest proportion. In the P4-D, the presence of *Proteobacteria* was negligible (Figure 4), probably because of the higher presence of *Cyanobacteria*.

Specifically for the P4, it can be observed that phyla *Armatimonadetes*, *Actinobacteria* and *Cyanobacteria*, also had significant difference in the climatic seasons explored.

Dunfield et al. (2012) clustering the sequences of the phylum *Armatimonadetes* and in the tree constructed found 12 groups, the group 1 belongs to class Armatimonadia, the same was found in this work and were frequently found in soil and water. This phylum are also frequently found in areas where photosynthetic bacterias are predominant which includes *Cyanobacteria* bloom in freshwater.

The *Actinobacteria* explored also is different according to Library Compare, which showed high frequency in P4-R. The bacteria in these phyla can grow up in environments with lower pH and high heavy metals concentration, nitrate and organic matter (Bollmann et al., 2010). The abundance of these group in freshwater is connected with physical and chemical properties, as pH (Newton et al., 2011).

It can be observed, that there was abundance of *Cyanobacteria* in P4, especially during dry period. According to the tool Classifier from RDP, all of them belongs to the same family II, and the genus GpIIa. The *Cyanobacteria* are atmospheric nitrogen-fixing; these organisms can rapidly proliferate when the availability of phosphorus is high (Conley et al., 2009; Yuan et al., 2009), which was observed in P4. The domain of *Cyanobacteria* in this pond during dry period suggests that the bloom of these bacteria caused inhibition of proliferation of other bacterial groups. The bloom of *Cyanobacteria* are common in eutrophic freshwaters, and
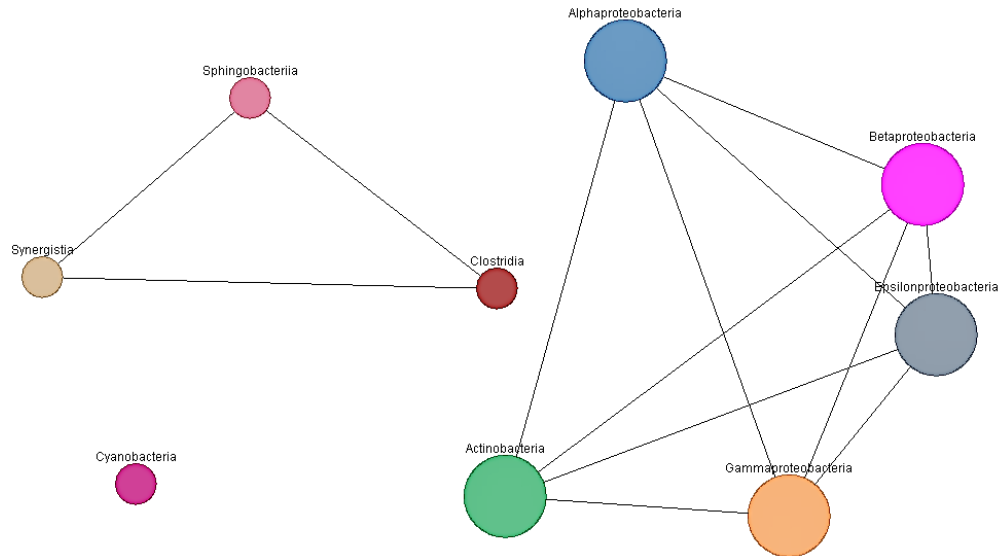
Figure 5. Taxonomic profile for Co-occurrence of classes from 2 libraries (P1 dry and P1 rainy) clustered for MEGAN5.
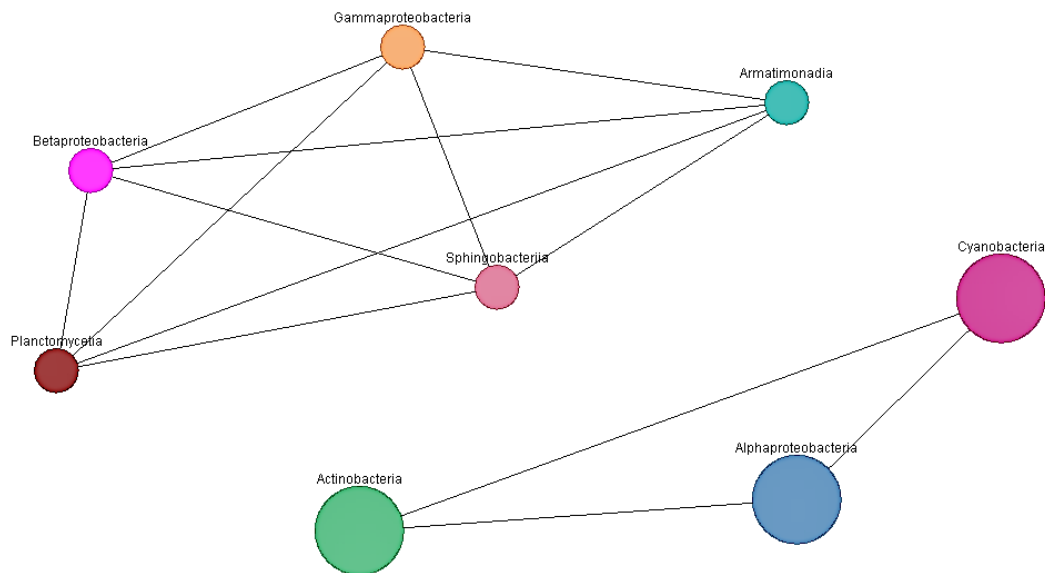

Figure 6. Taxonomic profile for Co-occurrence of classes from 2 libraries (P4 dry and P4 rainy) clustered for MEGAN5.

this proliferation can lead to high production of cyanotoxins and other toxic compounds that impair water quality (Eiler and Bertilsson, 2004).

Observation of the distribution of bacteria in the four fishponds, the analysis of Co-occurrence (Figures 5 and 6), becomes important because there are the clustering of the classes found in the libraries which demonstrated how the population is correlated.

Figure 5, shows the fishpond 1 in dry and rainy seasons. The fact that draws attention is that only

Cyanobacteria was not cluster with any other phyla; this probably occurs because this phyla was showed only in the dry season. All classes of *Proteobacteria* found is related with *Actinobacteria*, and according to literature data, describes above, this occurs frequently.

Figure 6 shows the data about the fishpond 4 in dry and rainy seasons. All classes of bacteria are clustered. The important fact was that the data showed was similar to that we found in literature; *Cyanobacteria*, *Actinobacteria* and *α-Proteobacteria* were related, as described above.

Generally, *Cyanobacteria* and *Proteobacteria* were asso-ciated with nitrogen cycle. When we observed the graphic of co-occurrence this phyla were correlated, it indicates that this population have a possible interaction in these nutrients recycle.

Although, to understand better what really happens between them in these specifically sys-tem, there are necessary other kind of analysis. The importance of the co-occurrence data was the detection of the organization of the microbial population in these systems.

The simultaneous view of microbial composition in aquaculture farms has not often been held. Most studies found in the literature are restricted to analysis of sites already impacted by the production process. In turn, knowledge of microbial systems for fish farming has great ecological and economic importance considering that microorganisms play a key role in biogeochemical cycles, in bioremediation processes and also as indicators of water quality.

The study of bacterial diversity developed in this work provides an assessment of the variations in bacterial communities residing in freshwater fishponds belonging to a sequential system with a continuous water flow. The results of this work allow the identification and compa-rison of the bacterial populations from two freshwater fishponds. In addition to these differences, ponds 1 and 4 presented a significance difference, suggesting that the inadequate management of artificial shallow systems and high concentrations of phosphorus and nitrogenous (P4) promote the appearance of bacteria originating from anthropogenic contamination (*γ-Proteobacteria* and *Cyanobacteria*). These findings indicate the need for improvements in the flow of effluents into this pond (P4). With respect to P1, the presence of macrophytes allows the removal of nutrients and consequently the presence of bacteria that do not affect water quality.

A microbiological study may be crucial to the definition of management tools that aim to prevent, or at least minimize, the environmental impact of improper manage-ment of fish farming.

Conflict of interests

The authors have not declared any conflict of interests.

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