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Original Article

Diversity of anoxygenic phototrophic bacteria in anaerobic lagoons and facultative stabilization pond used in treatment of sewage

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Abstract: The objective of this study was to identify the anoxygenic phototrophic bacteria in the anaerobic lagoons and facultative stabilization pond of the Vale do Ribeira, Cajati, São Paulo, Brazil, and their correlation with physical and chemical parameters of the ponds' water. The samples were collected seasonally (spring, summer, autumn and winter) in the sub-surface, intermediate layer and sediment-water interface. We used the PCR/DGGE with *puf*M 557FGC and *puf*M 750R primers specific to the reaction center of the photosynthetic phototrophic bacteria for their identification. The amplification products were separated by electrophoresis on denaturing gradient gel. From the bands cut out and sequenced from DGGE, the identified bacteria were *Rhodopseudomonas palustris* (99% similarity), *Chromatium* sp. (92%), *Thiocapsa* sp. (90%), *Rhodospirillum* sp. (95%), *Roseobacter* sp. (93%) and other uncultured bacteria.

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Introduction

The anoxygenic phototrophic bacteria are part of a metabolically diverse group of microorganisms, grouped according to the use of sulfate and specific pigmentation. They play an important role in anaerobic degradation of organic matter, as primary producers or consumers of the reduced organic compounds (Madigan et al., 2006). Stagnant water exposed to sunlight facilitates sulfate production by decomposing organic matter, allowing the growth of anoxygenic phototrophic bacteria. In such environments, these bacteria often occur in large numbers and are visible because they produce pink, red, brown and green blooms. In general, blooms of the anoxygenic phototrophic bacteria contain mostly purple and green sulfur bacteria (Hiraishi et al., 1995). Phototrophic bacteria species are generally found in concentrations of 10^5 cells.mL^{-1} in environments with high amounts of the organic matter. Moreover, they coexist with heterotrophic anaerobic microorganisms in hypolimnion, or in aquatic systems surface such as eutrophic lagoons or wastewater treatment plants. In wastewater treatment processes, anoxygenic phototrophic bacteria inhabit symbiosis with other heterotrophs and photoautotrophs (Ng et al., 1989).

Anoxygenic phototrophic bacteria often bloom in waste stabilisation ponds used to treat sanitary sewage and form a purple layer on their surface. These blooms can cause malfunctions and toxicity in these lagoons, because under anoxic and/or facultative conditions, color of the wastewater appears purple due to the predominance of purple anoxygenic phototrophic bacteria, and consequently the presence of sulfate. As a result of this massive growth, the physicochemical quality of pond effluents can be seriously affected, such as color, odor and concentration of suspended solids (Belila et al., 2013a, b). Therefore, study of the conditions that provide these blooms, the microbial diversity, potential for organic matter removal and establishment of relations between such knowledge, allow the comprehension of the system metabolism, and contributes to the efficiency maintenance of the

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stabilisation ponds, which are particularly sensitive to environmental changes such as temperature, nutrients and organic load instability (Belila et al., 2011).

In addition to visual verification of the phototrophic bacteria's blooms, it is possible to use molecular methods to characterize a microbial community and identify microorganisms. The analysis of nucleic substances extracted from the full community member of an environment allows the phylogenetic detection and identification of most organisms, including those are not cultivated. The denaturing gradient gel electrophoresis (DGGE) is widely used to describe a genetic diversity of microorganisms in different environments. Depending on the objective of the study, the total number of bands visualised in the DGGE gel indicates a spatial and temporal diversity of variations in the determined environment. Through this technique, it is possible to identify microorganisms by sequencing selected bands in the DGGE. The combination of these molecular methods with those related to microbiology allows the detailed analysis of the microorganisms' diversity in the system. Achenbach et al. (2001) reported the application of specific primers to evaluate green sulfur bacteria, non-sulfur green bacteria and *Heliobacteria*. In addition, specific photosynthetic primers, known as *puf*M, were used for anoxygenic phototrophic abnormalities detection, that encode a M subunit of the photosynthetic reaction center, which is universally distributed for purple photosynthetic anoxygenic bacteria. *Puf*M primers amplify, not only purple and non-sulfur anoxygenic phototrophic bacteria DNA, but also that of *Chloroflexus* sp. (non-sulfur green bacteria). Though the structure of the purple bacterium reaction center is similar to the photosystem II of green plants, *puf*M primers do not amplify cyanobacterial DNA, which indicates its specificity for anoxygenic phototrophic bacteria.

This study aimed to identify the anoxygenic phototrophic bacteria in anaerobic lagoon and facultative stabilization ponds of the Vale do Ribeira (Cajati, São Paulo, Brazil) and their correlations with physical and chemical parameters of water from the ponds verified at different times of the year and different collection times. Identification is performed using a partial sequencing technique of *puf*M photosynthetic reaction center of the purple anoxygenic phototrophic bacteria.

Materials and Methods

The selected ecosystem for this study was the Cajati microbasin (São Paulo - Brasil), in the Baixo Ribeira de Iguape region, located at 23º30'-25º30' south latitude and 46º50'-50º00' west longitude, in an area of 24.980 km^2. Samples were taken from anaerobic and facultative ponds of Cajati, at subsurface (S) and intermediate layer (CI), and water-sediment interface (AS) twice a day (14:00 h and 02:00 h), as well as the affluent raw sewage and final effluent were sampled from the stabilisation ponds. In the anaerobic lagoon, the intermediate layer and water-sediment interface were collected at 1.50 and 3.00 m below the surface, respectively. In the facultative lagoon, the collections were made at 0.70 and 1.20 m from the surface. The specific collection point in the lagoons present circulation and mixing due to the wind at different times of year.

Microbial Diversity Analysis: Samples were collected using Van Dorn sampler, transferred to flasks and subjected to a temperature of 4°C. For measuring biomass concentration, 200 mL of the pond water was centrifuged at 6000 rpm for 10 min at 4°C. The created pellet was washed three times with 1X PBS phosphate (137.0 mM NaCl, 2.6 mM KCl, 1.7 mM KH_2PO_4, 10.0 mM Na_2HPO_4, pH=7.4).

DNA extraction was performed using glass beads, phenol and chloroform according to the Griffiths protocol et al. (2000). In polymerase chain reaction (PCR) for the amplification of the photosynthetic reaction center of the purple anoxygenic phototrophic bacteria, *puf*M.557F (5'-CGCACCTGGACTGGAC-3') with GC clamp and *puf*M.750R (5'-CCCATGGTC CAGCGCCAGAA-3') primers were used (Achenbach et al., 2001). PCR conditions were as follows: 1.5 μL $MgCl_2$ (50 mM), 5 μL 10X PCR buffer, 5 μL dNTPs (2 mM each), 0.5 μL each primer (100 μmol, Invitrogen), 0.5 U AmpliTaq DNA polymerase (5 $U.mL^{-1}$), containing 2 μL template DNA (50-100 ng)

completing at an ultrafurified rate up to 50 μL. Amplification cycles were as follows: denaturation at 94°C for 3 min, followed by 30 cycles of 1 min denaturation at 94°C, 1 min annealing at 55°C, and 1 min extension at 72°C; and final extension at 72°C for 10 min. The amplified product was analyzed on 1% agarose gel for use in DGGE (Muyzer et al., 1993).

The samples were transferred to 10% acrylamide/ bis-acrylamide gel prepared from 40% acrylamide/ bis-acrylamide (38.93 g acrylamide and 1.07 g bis-acrylamide dissolved in 100 mL ultrapure water) with 20 to 80% denaturants (urea and formamide) for the electrophoresis gel in DGGE. The electrophoretic run time of 16 hrs was used in TAE 1X buffer, with temperature of 65°C and constant voltage of 75 V. The DGGE gel was stained in ethidium bromide for 20 min and then visualised in UV transilluminator. The resulting predominant bands were cut out of the gel and eluted separately in 20 μL of ultrapure water and stored at 4°C overnight. The eluted DNA was re-amplified using the respective primer set (without GC clamp), purified and applied to GE Healthcare's Megabace 1000 sequencer.

The identification of organisms was made using a set of sequencing reagents based on fluorochrome-labeled dideoxynucleotides (dye terminators). The concentrations of DNA template and primers followed the manufacturer's instructions. Sequencing reactions were purified on Sephadex G50 column and applied to GE Healthcare's Megabace 1000 sequencer. The analysis was performed by the Sequence Analyzer 3.0 software with Cimarron 3.12 basecaller. The sequences were checked using DNA Star program (SeqMan) and then compared with other sequences previously in the GenBank using the Basic Local Aligment Search Tool (BLASTn). Aligned nucleotides were used to make the consensus phylogenetic tree using the Neighbor-joining algorithm (Kimura et al., 1980) in Mega 3.1 software (Kumar et al., 2004). The known sequences of *R. palustris* (CP000301), *R. rubrum* (J03731) and *Chromatium vinosum* (AJ544223) were retrieved from the GenBank to identify the sampled microorganisms.

Physicochemical analysis: The dissolved oxygen, pH and temperature were measured using membrane electrode (Yellow Springer, 556 MPS). Solar radiation was measured with a Quanta-Meter Ly-Cor radiometer with a sensitivity of 400-700 nm. Chemical oxygen demand (COD), and total and dissolved nutrients determinations were measure based on APHA (2012). Samples for analysis of volatile suspended solids were filtered on 0.45 μm pore diameter glass fiber membranes.

Results and Discussions

Physical and chemical characteristics of the stabilisation ponds: The raw tributary organic matter concentration was below the recommended values for strong sewage. According to Tchobanoglous (2016), domestic sewage is considered strong when it presents COD of 1,000 $mg.L^{-1}$. In the present study, the affluent COD ranged from 50 $mg.L^{-1}$ in spring to 290 $mg.L^{-1}$ in summer. Total phosphorus concentration ranged 0.6 $mg.L^{-1}$ in summer to 7.0 $mg.L^{-1}$ in spring; total nitrogen Kjeldahl (NTK) ranged 13.1 $mg.L^{-1}$ in spring to 39.9 $mg.L^{-1}$ in autumn; Ammonia nitrogen ranged 1.8 $mg.L^{-1}$ in summer to 36.8 $mg.L^{-1}$ in spring. All these values were below the values considered strong for sanitary sewage and equal to 15.0 $mg.L^{-1}$; 85.0 $mg.L^{-1}$ and 50.0 $mg.L^{-1}$, respectively. Nitrate remained above the recommended (0.0 $mg.L^{-1}$), as values ranged 1.3 $mg.L^{-1}$ in spring to 3.7 $mg.L^{-1}$ in summer. According to Andrade Neto (1997), in the stabilisation ponds without final effluents, COD range values should be between 200-300 $mg.L^{-1}$, NTK 28.0-45.0 $mg.L^{-1}$ and total phosphorus 4.5-6.9 $mg.L^{-1}$. In the present study, the organic matter was below this range, except in winter reaching 300.4 $mg.L^{-1}$. NTK values were below this range, except in spring with 28.7 $mg.L^{-1}$. Total phosphorus values were less than the recommended one by Andrade Neto (1997).

During four seasons, pH, dissolved oxygen and solar radiation fluctuated in the stabilisation lagoons. In the anaerobic lagoon, the solar radiation were similar in summer and autumn at 14:00 h, with 245.5 and 264.6 $\mu Em^{-2}s^{-1}$, respectively, which were possibly propitious for the development of anoxygenic

phototrophic bacteria, where a larger number of populations of these microorganisms were reported. In spring (545.8 $\mu Em^{-2}s^{-1}$) and winter (574.8 $\mu Em^{-2}s^{-1}$), the solar radiation was not favorable for the proper development of anoxygenic phototrophic bacteria. During summer, in the facultative lagoon, a higher light intensity was registered at 14:00 h (1344 $\mu Em^{-2}s^{-1}$) and a smaller number of populations of the anoxygenic phototrophic bacteria, compared to the anaerobic lagoons. The solar radiation during fall was 409.9 $\mu Em^{-2}s^{-1}$, which determined a lower number of anoxygenic phototrophic bacteria compared to those of the anaerobic lagoon at the same period. According to DGGE standard band profiles, anoxygenic phototrophic bacteria prefer a certain light intensity for their optimal development. In the this work, this range was 245.50-264.61 $\mu Em^{-2}s^{-1}$, for the 14:00 h. collection time, where 6, 10 and 10 bands were observed in the subsurface, intermediate layer and sediment water interface in summer, and 14, 17 and 18 bands in fall, respectively. According to Belila et al. (2013), the quality of the light spectrum stimulates different distribution of the photosynthetic bacteria, occupying different ecological niches along the visible spectrum, encouraging their coexistence.

The minimum and maximum pH in the anaerobic lagoon in both collection times were 6.81 and 10.91, respectively. During fall, the pH were lower i.e. 6.81 and 9.08, respectively. In this pH range, a greater diversity of the anoxygenic phototrophic bacteria was found. In the facultative lagoon, the minimum pH value was 6.98 recorded in fall, and maximum of 12.05 in summer. Also, in the facultative lagoon, the lowest pH along with greater diversity of these microorganisms were observed in autumn. This range of pH in both lagoon system possibly allow a better development of the anoxygenic phototrophic bacteria.

Temperature ranged 19.6 to 35.1°C during the seasons at both collection times. The highest temperature was recorded in autumn, when a greater diversity of anoxygenic phototrophic bacteria was verified. Above-mentioned temperature range is probably favored for development of these microorganisms. According to Von Sperling et al. (2003), optimum removal efficiency ranges may serve as a reference for most Brazilian regions, mentioned as 20-25ºC, in the coldest month of the year. The minimum temperature of 18.4°C was found in winter and a maximum of 34.3°C in autumn. Greater temperature values were recorded in the facultative lagoon in fall collection, where the lower number of the anoxygenic phototrophic bacteria was detected in relation to the anaerobic lagoon at the same time.

The lowest dissolved oxygen (DO) was close to zero, while the maximum one was 8.22 $mg.L^{-1}$. DO on the subsurface during collections at different times ranged 0.14 to 8.22 $mg.L^{-1}$. In fall, in the intermediate layer and water-sediment interface, no dissolved oxygen was detected. During this period, a greater diversity of anoxygenic phototrophic bacteria was verified at the sampling points, possibly favored for this facultative environment. Minimum DO of 0.02 $mg.L^{-1}$ and maximum one of 9.54 $mg.L^{-1}$ were recorded in summer. DO was variable during four seasons, different sampling points and collection times. Its values in autumn were higher than those of the anaerobic lagoon, where a greater diversity of anoxygenic phototrophic bacteria was observed. Possibly, the lower DO in the anaerobic lagoon favored the development of these microorganisms.

A higher removal of organic material (COD) was verified in the facultative lagoon, with 25.6%. The removal of 62.4% of volatile suspended solids was verified, in spring, with a temperature of 22.9˚C for affluent and 24.3˚C for effluent. Removal of 60.2% of NTK was obtained in fall (affluent temperature of 27.2˚C and effluent temperature of 32.4˚C) and 58.8% for total phosphorus in fall. Higher nitrate removal was observed in spring, with 14.6%, and 73.0% of ammonia nitrogen in fall. Higher removal of total dissolved phosphorus and 95.8% for orthophosphate were verified in spring.

In this work, the nutritional conditions were verified in different collections and sample locations probably favored the development of anaerobic, facultative anaerobic, microaerophilic and aerobic micro-organisms. When considering the temperature, development of the psychrophilic microorganisms in

winter and mesophilic during other times were favored. The variation of pH noted in the ponds suggests presence of the neutrophils and alkaliphiles microorganisms. Evaluating the nutrients i.e. ammonia nitrogen, nitrite and nitrate, the highest temperature was verified in fall, which favored a major metabolic activity, as well as a faster ammonia nitrogen oxidation. Almost every ammonia nitrogen was oxidized to nitrate in the facultative pond. DO was not limited by nitrifying activity associated to the highest temperatures registered during summer and fall.

In the anaerobic pond, the ammonia nitrogen concentration was higher than the facultative pond during the study, associated to the greatest organic charge and ammonification process in anaerobic environment. On the other side, there was a major nitrifying activity in the facultative pond, mainly during summer and fall, possibly due to the temperature and algal biomass (primary production) increase, consequently, a greater oxygenation of the environment. During this period, it was noticed that almost all ammonia nitrogen was oxidized to nitrate in the facultative pond, contrary to the anaerobic pond. Hence, there was a bacteria population raise during summer and fall, both autotrophs (including nitrifying) and heterotrophs confirmed by the major diversity Bacteria Domain in the DGGE standard band profile.

Anoxygenic phototrophic bacteria identification: Sampling from the stabilisation ponds were done two times (14:00 h and 02:00 h) to verify presence of different anoxygenic phototropic bacteria in three sampling locations (subsurface: S; intermediate layer: CI; and water-sediment interface: AS), as well as different times of year (spring: p; summer: v; fall: o, and winter: i). Table 1 shows the cut-off DGGE bands and phylogenetic approximation with the sequences of anoxygenic phototrophic bacteria deposited in GeneBank, samples from anaerobic and facultative lagoons at different times of year and different collection points. Approximately 229 base pairs (bp) were sequenced.

In the anaerobic pond, the bands 3, 4, 6, 7 and 9 (uncultivated bacteria), 8 (*Rhodospirillum* sp. AY390602), and 11 (*Rhodopseudomonas palustris*, AB015977) were verified. The sequence for band 8 was similar to *Rhodospirillum* sp., it is a non-sulphurous, Gram negative, anoxygenic phototrophic purple bacterium with flagella. Such bacteria grow preferentially under anaerobic photoheterotrophic conditions, in the presence of various organic compounds, or under dark microaerobic conditions (Beja et al., 2002). *Rhodospirillum* and *Rhodoferax* are known for their key roles in carbon and nitrogen metabolism, and often found in sewage, activated sludge and wastewater treatment plants (Wan et al., 2011; Belila et al., 2013; Hulsen et al., 2014). The sequence of band 11 was similar to *R. palustris*, a purple non-sulfur phototrophic, Gram negative bacterium. Its photoautotrophic growth is possible using hydrogen, sulfite and thiosulfate as electron donors in the presence of yeast extract and carbon dioxide. Photoheterotrophic growth is occurred in the presence of organic substrates such as benzoate, acetate, pyruvate, malate, fumarate, lactate and succinate. Such bacteria may grow under dark microaerobic and aerobic conditions, or under anaerobic conditions in the presence of some organic substrates. Sulphate can be used as a source of sulfur, while ammonia, dinitrogen, and some amino acids are used as nitrogen sources (Madigan et al., 2006). These microorganisms represented part of the autochthonous bacterial community of the anaerobic lagoon, probably responsible for the blooms in the stabilisation lagoons, causing purple color in the surface. In general, purple non-sulphurous phototrophic bacteria are characterised by their metabolic versatility, including photoautotrophic, chemoheterotrophic, and photoheterotrophic organisms, using sugars and a variety of organic acids as electron donors and carbon sources (Kim et al., 2004; Hulsen et al., 2014).

In the facultative pond, the bacteria similar to *Thiocapsa* sp. (AJ544223, band 5) were found. *Thiocapsa* sp. is a purple phototrophic sulfur, and Gram negative bacteria. Under anaerobic conditions, it has phototrophic growth and under aerobic or

Table 1. Sequence information obtained from DGGE cropped bands with *puf*M primers from anaerobic and facultative lagoons.

Band	Microorganism	Anaerobic pond		Facultative pond		#access	Similarity %	Reference
		14:00 h	02:00 h	14:00 h	02:00 h			
Band 1	*Chromatium* sp.	Sp, CIp, ASp, Sv, CIv, ASv, So, CIo, ASo, Si, CIi, ASi	Sp, CIp, ASp, Sv, CIv, ASv, So, CIo, ASo, Si, CIi, ASi	Sp, CIp, ASp, Sv, CIv, ASv, So, CIo, ASo	Sp, CIp, ASp, Sv, CIv, ASv, So, CIo, ASo	ABO11811	92	Nagashima (1998)
Band 2	*Roseobacter* sp.	Sp, CIp, ASp, Sv, CIv, ASv, So, CIo, ASo, Si, CIi, ASi	Sp, CIp, ASp, Sv, CIv, ASv, So, CIo, ASo, Si, CIi, ASi	Sp, CIp, ASp, Sv, CIv, ASv, So, CIo, ASo	Sp, CIp, ASp, Sv, CIv, ASv, So, CIo, ASo	AY675565	91	Oz et al. (2005)
Band 3	Uncultivated bacteria	So, CIo, ASo				AM162697	99	Ranchou-Peyruse et al. (2006)
Band 4	Uncultivated bacteria	So, CIo, ASo				AY044247	89	Beja *et al*. (2002)
Band 5	*Thiocapsa* sp.			So, CIo, ASo, Si, CIi, ASi		AJ544223	90	Wieland et al. (2003)
Band 6	Uncultivated bacteria	CIv, ASv, ASo				AM162737	88	Ranchou-Peyruse *et al*. (2005)
Band 7	Uncultivated bacteria	CIv, ASv				DQ080988	89	Yutin et al. (2000). Not published (GenBanK)
Band 8	*Rhodospirillum* sp.	So, CIo	So, CIo			AY390602	95	Lee et al. (2003)
Band 9	Uncultivated bacteria	ASo				AY044246	90	Beja et al. (2002)
Band 10	*Chromatium* sp.	Sp, Sv, So, CIo, Si, CIi, ASi	Sv, So, Cio, Si	So, CIo, ASo	Sp, CIp, ASp, So, CIo, ASo	D50647	91	Nagashima et al. (1997)
Band 11	*Rhodopseudomonas palustris*	CIv, ASv, CIo, ASo,	CIv, ASv, CIo, ASo,			AB015977	99	Beja et al. (2002)

microaerophilic conditions, it has chemoautotrophic or chemoorganotrophic growth in the darkness. According to Wieland et al. (2003), the purple sulfur bacteria release sulfate, which can be used as an electron acceptor in anaerobic respiration by sulfate reducing bacteria, producing sulfide. The latter compound may have been used by phototrophic bacteria as an electron donor in anoxygenic photosynthesis.

Belila et al. (2013) evaluated red-coloured blooms in stabilisation ponds for domestic sewage treatment (anaerobic pond, facultative pond and two serial maturation ponds), and identified anoxygenic phototrophic bacteria using the PCR/DGGE technique (with pufM 557FGC and *puf*M 750R) from the selected and sequenced DGGE bands. Molecular analysis revealed that 59.3% of *puf*M sequences corresponded to non-sulfur purple bacteria (*Rhodospirillum, Rhodoferax, Rhodobaca, Rhodobacter, Rhodoplanes* and *Phaeospirillum*); 27.8% to purple sulfur bacteria (*Thiocapsa, Roseopersicin* and *Chromatium weissei*), and 12.9% to purple anoxygenic phototrophic bacteria (*Roseobacter* sp. and *Erythrobacter* sp.).

In both ponds, band 1 (*Chromatium* sp., ABO11811), 2 (*Roseobacter* sp., AY675565), and 10 (*Chromatium* sp., D50647) were verified. Band 1 was similar to *Chromatium* sp., Gram-negative purple

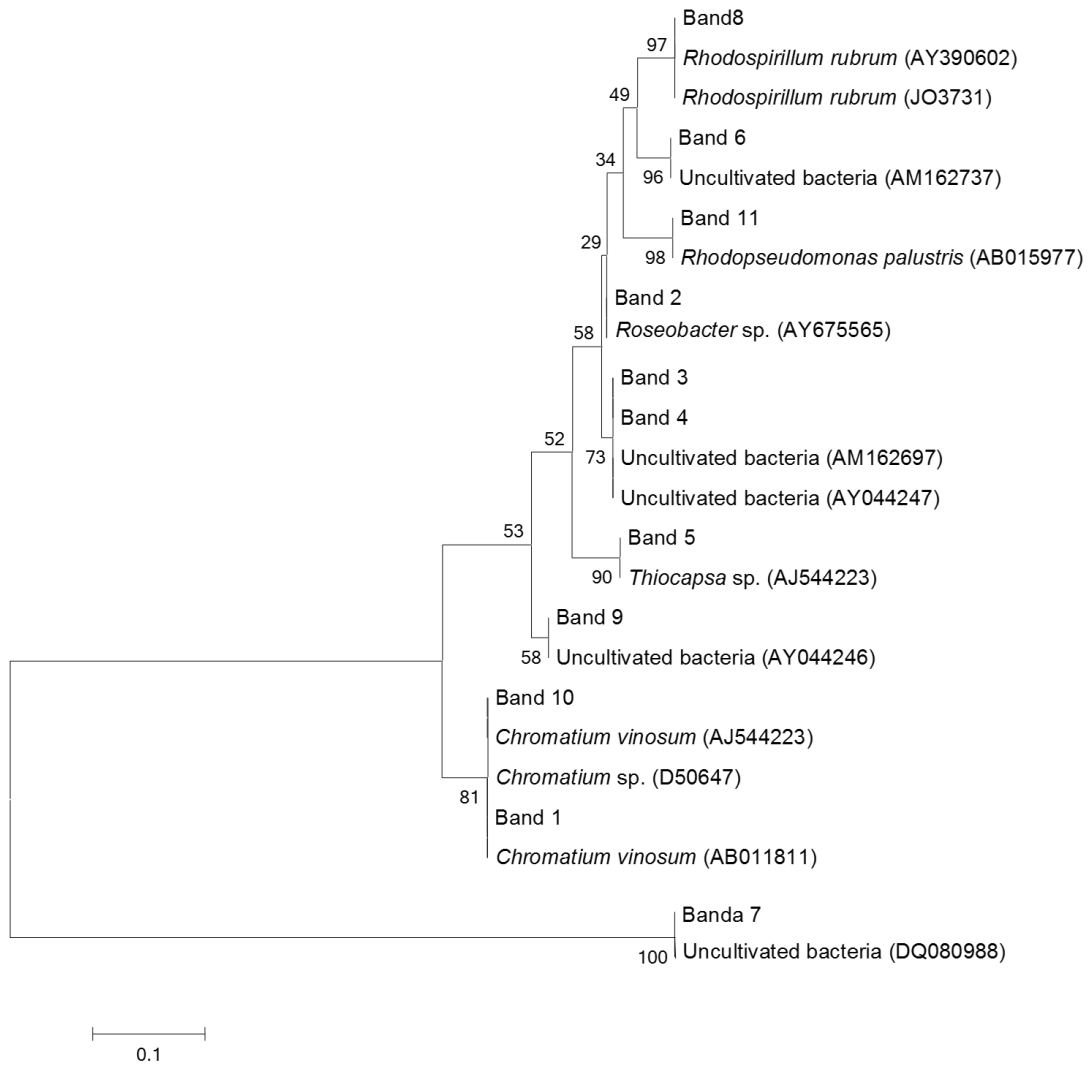

Figure 1. Consensus phylogenetic tree based on cut-off DGGE sequences with primers from the *puf*M photosynthetic center, obtained from anaerobic and facultative lagoons. Values present in tree nodes indicate percentages that branch repeated (500 bootstrap resamples).

sulfur phototrophic bacteria. *Chromatium* species present flagella with no gas vacuoles and intracellular sulfur granules in photoautotrophic growth in the presence of hydrogen sulfide. Under microaerophilic conditions, they grow under chemoautotrophic and mixotrophic conditions. In addition, they may grow under photoheterotrophic conditions in the presence of ammonium acetate (Madigan, 2006). This microorganism, which survives in microaerophilic conditions, possibly uses sulfur compounds and organic acids as electron donors in anoxygenic photosynthesis. When using sulfide in photo-autotrophic growth, intracellular sulfur accumulates, which will be oxidized culminating with increased sulfate. In general, such bacteria grow close to sulfate-reducing bacteria and both actively participate through syntrophic relations in the establishment of the sulfur cycle.

The sequence for band 2 was similar to *Roseobacter* sp., Gram-negative anoxygenic phototrophic bacterium with one or two flagella, of the α-proteobacterium group. However, it performs aerobic anoxygenic photosynthesis, i.e. requiring oxygen for growth and bacteriochlorophylla (Bchla) syntheses. These microorganisms are not able to grow under strict photoautotrophic conditions as they require organic carbon supplementation (OZ et al., 2005).

At 14:00 h, in both lagoons, bands 3, 4, 6, 7 and 9 (uncultivated bacteria) and 5 (*Thiocapsa* sp.) were

verified. The other sequences at both times indicate the presence of anoxygenic phototrophic bacteria day and night, contributing in the degradation of the organic matter in the stabilisation ponds. The primer sets of *puf*M gene not only detected sulphurous and non-sulphurous purple bacteria, but also aerobic anoxygenic phototrophic bacteria (*Roseobacter* sp.), as verified by Ranchou-Peyruse et al. (2006). However, such microorganisms are also capable to grow in an anaerobic environment. Figure 1 shows the result of phylogenetic tree based on the sequence of the organisms from the stabilisation lagoons. Similarity ranges 88-99%, indicating phylogenetic diversity of microorganisms, possibly due to the vertical transfer of genes from the photosynthetic center, which occurs between photosynthetic bacteria (Karr et al., 2003).

Conclusions

Although bacteria blooms were not found during this study, it was possible to verify their presence in anaerobic and facultative stabilising ponds. In both lagoons, it was detected a major number of bacteria during the fall. In Spring, it was ascertained a lower number of bands in DGGE profile. A greater number of DGGE bands of bacteria was found in the anaerobic lagoon compared to the facultative lagoon, this was probably facilitated by the lower concentrations of DO, observed in the first lagoon. It is very important to keep track of these communities to maintain the operation and guarantee the efficiency of stabilising lagoons. Researches on evaluations of seasonal and temporal diversity of bacteria community in stabilizing ponds are rare. Thereby, there is not much knowledge concerning the variability of water column and its connections to the system nutritional conditions.

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