

Isolation and characterization of purple non-sulfur anoxyphototropic bacteria from two microecosystems: tropical hypersaline microbial mats and bromeliads phytotelmata

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Purple non-sulfur anoxyphototropic bacteria (PNSAB) are a diverse and ubiquitous group of gram-negative anoxyphototroph microorganisms with members of α and β -Proteobacteria. This group is recognized by its capability of growing in diverse environments, and recognized by its physiological versatility, and ecological importance. This research presents the first report of PNSAB in two contrasting microenvironments: the Tropical Hypersaline Microbial Mats at Cabo Rojo Salterns and the phytotelmata of bromeliads from the subtropical forest of Puerto Rico. The colonies with the characteristic reddish pigments were isolated and characterized using microscopic, biochemical and molecular methods. A total of 49 PNSAB were characterized: 23 from microbial mats and 26 from bromeliad phytotelmata. While microbial members of the genera *Rhodospseudomonas* and *Rhodospirillum* were present in both microenvironments, members of the *Rhodobacter*, *Rhodothalassium* and *Rhodovulum* genus were only found in the microbial mats and *Rhodomicrobium* in bromeliads phytotelmata.

Keywords: purple non-sulfur anoxyphototropic bacteria, microbial mats, phytotelmata

1. Introduction

Purple non-sulfur anoxyphototropic bacteria (PNSAB) have a wide range of growth modes and are able to grow under photoautotrophic, photoheterotrophic and chemoheterotrophic conditions [1]. These groups of microbes are a cosmopolitan group located in water bodies below the layer of oxygenic photosynthetic organisms such as: algae, aquatic plants and cyanobacteria [2]. They can also be found in habitats such as: waste water ponds, sediments, moist soils, seawater pools, hypersaline environments [3]. In these habitats, the PNSAB are active participants in the carbon, nitrogen and sulfur cycles [4, 5]. The genes involved in photosynthesis are organized in clusters and have been identify and characterized [6]. These gene clusters are composed mainly of genes that encoded for bacteriochlorophyll, carotenoids, light harvesting complexes and reaction center. The reaction center of all PNSAB is composed by structure proteins called PUH and PUF (pigment binding protein) [7]. A molecular technique for identification of PNSAB were develop using *pufM* gene; based on the design and application of PCR primer sets to assess the biodiversity, metabolic activity and ecology of these phototrophs [8].

Purple non-sulfur bacteria have been studied and isolated from different ecosystems like aquatic and extreme environments. Examples of extreme microecosystem are the microbial mats. Mats are laminated organo-sedimentary structure stratified by a gradient of oxygen and sulfide [9]. The multilayer communities of prokaryotic microorganisms have been descended from the oldest and most widespread biological communities in the Earth; named stromatolites [10]. Mats built by photosynthetic organisms created the most studied stromatolites in fossil record. For that reason, microbial mats can be found in hostile environments such as hypersaline bodies of water [11], hot springs [12], dry temperate deserts and cold dry environments [13]. Typically, several discrete layers can be recognized, arranged vertically, where the organisms distribute themselves according to physiological requirements, such as amount of light, oxygen, nutrients and temperature. The upper layer is denominated green layer, which is dominated by cyanobacteria and organoheterotrophic bacteria [9]. Below this layer, a pink layer is found, which contains anoxyphototropic and chemolithotrophic surfur bacteria. The bottom layer of the microbial mats is often black due to iron sulfur precipitation by dissimilatory sulfate and sulfur reducing bacteria [14]. Some microbial mats are considered an aquatic environment; they are denominated ephemeral or benthic.

Another example of aquatic microenvironments is bromeliads phytotelmata. The bromeliads are plants of the family Bromeliaceae, adapted to a number of climates. All the bromeliads are composed of a spiral arrangement of leave sometimes called "rosette". The bases of the leaves in the rosette may overlap tightly to form an aquatic microhabitat that provides refuge to a wide variety of organisms [15]. This environment is called phytotelmata. Phytotelmatas are structures formed by non-aquatic plants that impound water, such as modified leaves, leaf axils, flowers, stem holes or depression [16]. The water accumulated on these plants may serve as substrate from associated fauna, and often the fauna associated with phytotelmata is unique. A variety of organisms live in these microenvironments such as: cyanobacteria, fungi, green algae, protozoan, ciliates, insects' larvae, aquatic insects, amphibians and fecal coliform bacteria [17]. In studies, realized in bromeliad phytotelmata from El Yunque Tropical Forest of Puerto Rico, it was shown the presence of cyanobacteria and fecal coliform bacteria [18].

This study aimed to isolate purple non-sulfur anoxyphototrophic bacteria from two divergent microenvironments, tropical hypersaline microbial mats and bromeliads phytotelmata; and characterize them microscopically, biochemically and molecularly. The present study shows the first report of PNSAB in the Tropical Hypersaline Microbial Mats at Cabo Rojo Salterns, and in different bromeliads phytotelmata from the subtropical forest of Puerto Rico.

2. Methods

2.1 Enrichment and isolation of purple non-sulfur anoxyphototrophic bacteria

Two different methods were designed to isolate PNSB candidates from two microecosystems: microbial mats and bromeliads phytotelmata. Microbial mats samples from Cabo Rojo Salterns were collected and dissected in the three colored layers. Serial dilutions from 10^{-1} to 10^{-5} performed to the pink layer of the microbial mats filtered water (collected from sampling site). The diluted pink layer was filtered through a Nalgene[®] Analytical Filter attached to a nitrocellulose membrane with a pore size of 0.2 μ m. The nitrocellulose membranes were placed on Petri dishes with marine agar (Difco[™]), and incubated in an anaerobic jar of polycarbonated BBL[®] attached to a Gas Pack (following the manufacturer specifications) in the presence of light at 28-32°C; until reddish colonies were observed. The red colonies (putative PNSB candidates) were grown in marine broth anaerobic under photosynthetic conditions, the red colonies were re-streak for purification, then growth in liquid medium and storage in -80°C, followed by further characterization. The liquid present in the axial leaf of different bromeliads phytotelmata were collected using a sterile pipette. After the sample collection, they were placed in a 4mL bottle containing Sistrom 2X selective medium (Sistrom, 1960). The sample adding to the medium bottle diluted Sistrom 2X medium to 1X. The samples were incubated under photosynthetic conditions at 28-32°C, until medium turned red, this phenomenon is better known as “bloom effect”. The “bloomed” samples were streaked on Sistrom’s solid medium, and incubated in an anaerobic jar with a Gas Pack under anaerobic and photosynthetic conditions. The colonies that showed reddish pigmentation were purified and stored as described before.

2.2 Characterization of PNSAB candidates

2.2.1 Macroscopic and microscopic characterization

The PNSAB candidates were analyzed macroscopically considering the following features: colony pigmentation, margin, size and elevation of the colony [19]. The pigmentation of the PNSAB ranges from brown, red to red-purple (Munsell Color Chart[®]). The PNSAB size and shape were determined using light microscopy and Scanning Electron Microscopy (SEM). The morphology presence of structures, like external membrane, was determined by Gram staining [19]. The SEM analysis helped us to have a better idea of the shape of the bacteria. For the SEM, the bacteria were observed using a JEOL JSM-541 scanning electron microscope. To microscopically analyze the PNSAB, the isolated candidates were grown in their respective media for 48 hours at 30-32°C, under anaerobic and photosynthetic conditions. Then, approximately 1.5 mL of the culture was transferred to a microtube and centrifuged for 1 minute at 0.8xg (3,000 rpm) to obtain a cell pellet. A 4% solution of glutaraldehyde [$\text{CH}_2(\text{CH}_2\text{CHO})_2$] was added to fix the cell pellet and then was left resting for 24 hours at 4°C. The cell pellet was washed with 1X Phosphate Buffer Saline (PBS) three times. To dehydrate the pellet, different percentages of alcohol were used (10%-100%). Every ten minutes the pellet was transferred from one percentage of alcohol to another. To completely dry the sample, a critical point drier (Critical Point Drying Apparatus Polaron E3000) was used. A sputter coater was used to cover the samples with a palladium-gold coating to protect and to increase the conductivity of the candidate samples. The parameters used for the microscopic analysis were established by the Scanning Electron Microscopy Center at the University of Puerto Rico, Mayaguez Campus. Electron micrographs were taken at an accelerating voltage of 15 kV.

2.2.2 Photosynthetic pigments analysis of PNSAB

To determine the presence of photosynthetic pigments, like bacteriochlorophyll α and β , and carotenoids pigments, the bacteria culture was diluted in bovine serum albumin (BSA). Then, the whole cell spectra were recorded using UV-Vis spectroscopy within a range of 380-1100 nm [6]. The spectra was recorded in a Genesis 2 Thermospectronic[®] spectrophotometer.

2.2.3 Molecular analysis

Genomic DNA of isolated PNSAB candidates was extracted using the method described by Chen and Kuo (1993) [20]. Briefly, the bacteria cultures were grown in their respective media and incubated for 48 hours, under anaerobic and photosynthetic conditions. The cultures were transferred to a 1.5mL microtube and centrifuge at 15.7xg (13,000 rpm) to obtain a cell pellet. The cell lysis was performed using lysis buffer (40mM Tris-acetate pH 7.8, 20mM sodium acetate

pH 8.0, 1.0mM EDTA pH 8.0 and 1% SDS) and 5M sodium chloride, then treated with RNase (20 µg/µl) for 30 minutes at 37°C. The organic extraction was performed using one volume of chloroform and centrifuge at 15.7xg (13,000 rpm); this step was repeated twice. The samples were precipitated with absolute ethanol at -20°C. The isolated genomic DNA was resuspended in 50µL of 1X TE buffer (10mM Tris-Cl pH 8.0 and 1.0mM EDTA pH 8.0).

Once the all candidates' DNA was isolated, a Polymerase Chain Reaction (PCR) amplification of *16S rDNA* gene was performed. The amplification was done using the Green Taq Master Mix (Promega) and universal bacterial primer oligos 14-F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492-R (5'GGTTACCTTGTTACGACTT3'). The following parameters were used for the PCR reaction: initial denaturalization at 95°C for 3 minutes; then 30 cycles which include denaturalization at 95°C for 1 minute, annealing at 52°C for 30 seconds and extension at 72°C for 1 minute. A final extension was performed at 72°C for 10 minutes. Also, PNSAB candidates were analyzed by PCR amplification of *pufM* gene using specific primer sets: 557-F (5'-CGCACCTGGACTGGAC-3') and 750-R (5'-CCCATGGTCCAGCGCCAGAA-3') [8]. The amplifications were purified using a PCR purification kit (Qiagene). The PCR products were sequenced by MacroGen facilities (New York, US). *In silico* analysis was done by using online databases such as GenBank and BLAST from NCBI (<http://www.ncbi.nlm.nih.gov>)

The isolated PNSAB candidates' 16S rDNA sequences were edited using bioinformatics tools such as Chromas Lite 2.0 and then aligned and edited with BioEdit 7.0. The phylogenetic analysis was performed using MEGA 3.1 software. The distance model used was p-distance and the bootstrap test of phylogeny was calculated for 2000 replicates. The phylogenetic tree was constructed by the Neighbor Joining method.

3. Results

3.1 Isolation and characterization of PNSAB from two different microenvironments

The enrichment procedure used for microbial mats samples from Cabo Rojo Salterns resulted in the isolation of 23 PNSAB candidates and the other enrichment method used for phytotelmata resulted in the isolation of 26 candidates. Isolated PNSAB were grown in liquid and solid specific media, respectively, and showed the characteristic reddish bloom (Figure 1 and Figure 2). The colonies showed circular shape and convex elevation, entire margin and different intensities of red in the color scale (Figure 1 and Figure 2). The Gram staining suggest that all the isolated are gram negative rods of sizes that ranged from 1.5-3.0µm and 0.48-0.90µm for both microenvironments.

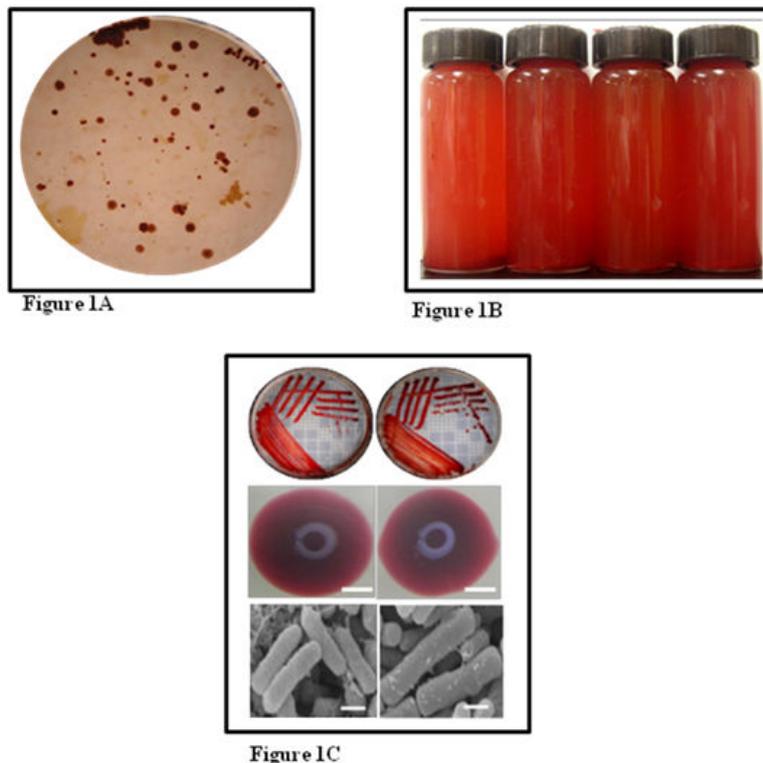


Fig 1. Isolated PNSAB from tropical hypersaline microbial mats. After filtering the diluted microbial mat the red pigmented colonies on the nitrocellulose filter (A). Were pick and inoculated in liquid media until showing the characteristic reddish bloom (B). The bloomed samples were streaked, and the colonies analyzed macroscopically and microscopically (C). The bars in the colonies represent 1mm, while in the SEM images represent 1µm.

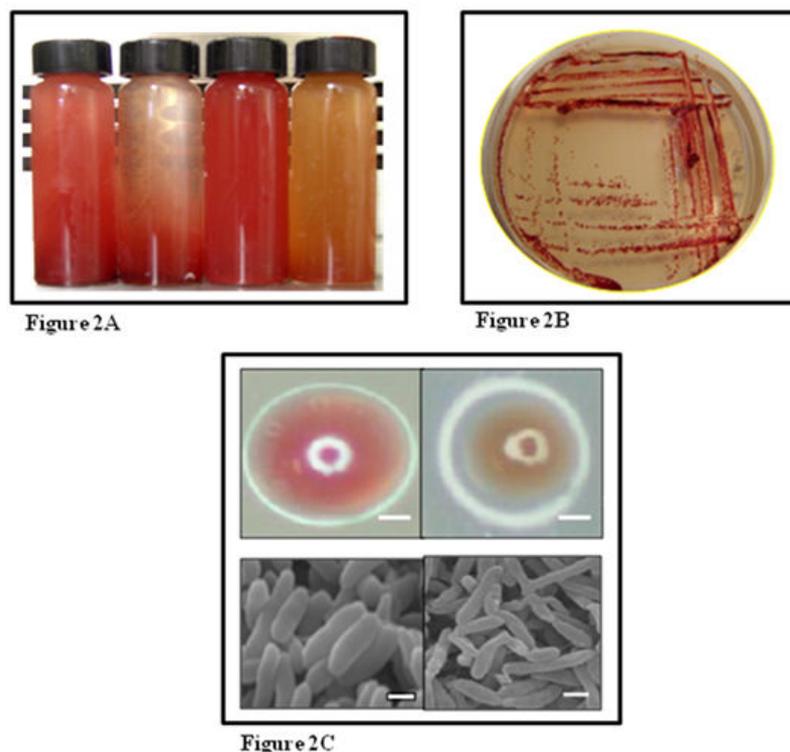


Fig 2. Isolated PNSAB from bromeliads phytotelmata from subtropical forest of Puerto Rico. After adding the liquid collected from the bromeliads phytotelmata to the Sistrom selective media, and incubated under photosynthetic conditions (A), the bloomed samples were streaked in solid medium (B) and the colonies analyzed macroscopically and microscopically (C). The bars in colonies represent 1mm, while in the SEM images represent 1µm.

3.2 Photosynthetic pigment analysis

The photosynthetic pigment analysis using UV-Vis spectroscopy showed whole cell absorption peaks from 300 nm to 1100 nm. Characteristics bacteriochlorophyll signals were found on peaks to 805 nm and 865 nm. Carotenoids pigments of the spirilloxantin series were found due to absorbance peaks at 492, 515 and 549 (characteristics of purple non-sulfur bacteria).

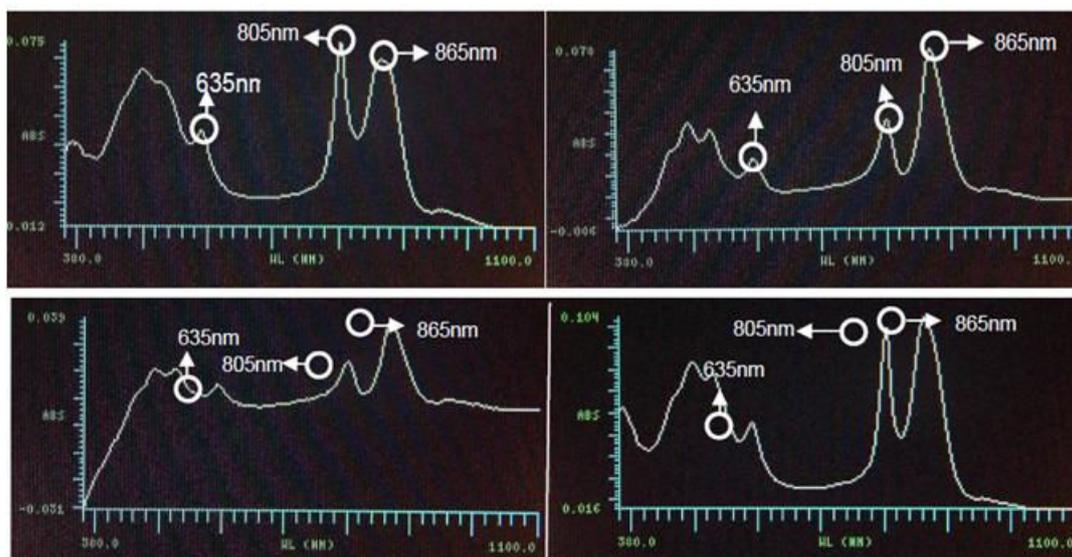


Fig 3. Whole absorption spectra of isolated purple non-sulfur anoxyphototrophic bacteria samples from the microenvironments tested. The whole cell absorption spectra was done from 300nm to 1100nm. The four isolated bacteria showed absorption peaks characteristic of photosynthetic pigments in 492, 515 and 549nm (carotenoids pigments) and at 805 and 865nm for bacteriochlorophyll *a*.

3.3 Molecular analysis

The presences of the *pufM* gene were detected by PCR in all the isolated samples in both microenvironments. The *in silico* analysis performed to the *16S rDNA* suggest the presence of purple non sulfur bacteria from the genus *Rhodopseudomonas* and *Rhodospirillum* for both environments. In contrast, the genus *Rhodobacter*, *Rhodothalassium* and *Rhodovulum* were only presence in the microbial mats and genera *Rhodomicrobium* were only present in the bromeliad's phytotelmata.

A phylogenetic tree constructed with the *16S rDNA* sequences (715pb) showed the evolutionary relationship between the isolated candidates from microbial mats and phytotelmata to other bacterial groups. The generated phylogenetic tree showed that the majority of the isolated are based on microenvironment. In the case of microbial mats, twelve isolated were similar to *Rhodothalassium* (MM19, MM14, MM21, MM13, MM4, MM11, MM16, MM22, MM10 and MM20), six to *Rhodobacter* (MM15, MM2 and MM5) and one to *Rhodovulum*. For bromeliads phytotelmata eight isolated are similar to *Rhodopseudomonas* (BP2, BP9, BP6, BP1, BP3, BP11, BP4 and BP7). A total of eight samples do not allow relation to known PNSAB, suggested the possibility of being new genus.

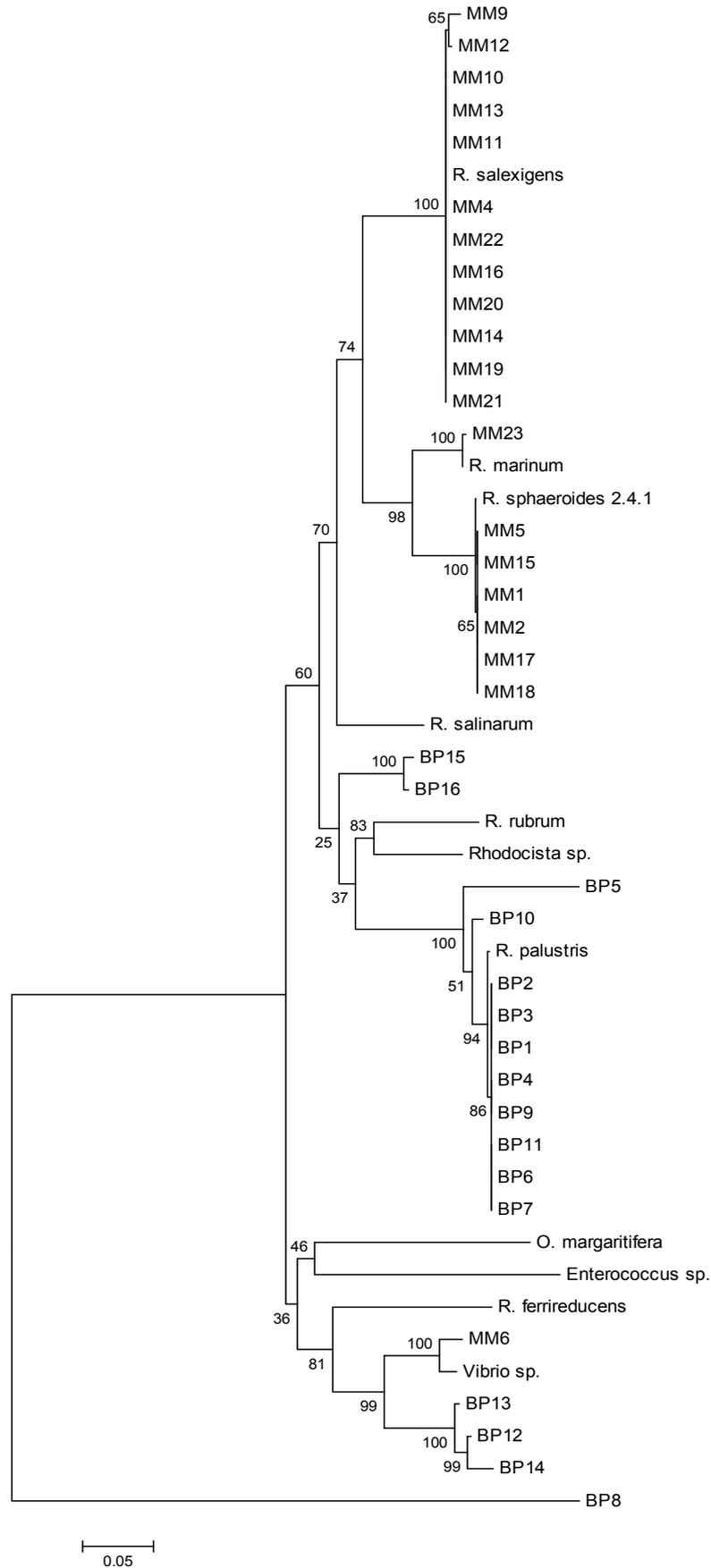


Fig 4. Phylogenetic analysis of the purple non-sulfur anoxygenic phototrophic bacteria. The generation of the consensus tree was made by the Neighbor-Joining method and the final tree was constructed with the use of MEGA 3 Tree Explorer. The tree based on 715 nucleotide position of the *16S rDNA* gene. Numbers in the nodes are the bootstrap values. The scale bar indicates the number of substitutions per nucleotide position (0.05). All sequence beginning with MM refers to microbial mats and BP to bromeliads phytotelmata.

4. Discussion

The main purpose of this research was to isolate and characterize purple non-sulfur anoxyphototrophic bacteria from two different microenvironments. Two different enrichment techniques were used to isolate the bacterial group. These techniques were similar to the isolation techniques used by Imhoff and Caumette in 2004 [21]. Both techniques were efficient, because they provided the non fermentative carbon sources such CO₂ (photoautotroph) malate and succinate (photoheterotroph), important for the physiology of the bacteria and the electron donor that this group of bacteria needs to undergo anoxygenic photosynthesis. A total of 23 and 26 PNSAB candidates were isolated from microbial mats and phytotelmata, respectively. When compared at the level of diversity, MM shows four phylotypes and BP are at least six phylotypes. These differences are due to the number of different bromeliads genera and the number of forests sampled.

Morphologically, one important characteristic of the PNSAB is the reddish pigmentation which indicated the presence of the photosynthetic pigments (carotenoids and bacteriochlorophyll) [22]. These pigments can be detected spectrophotometrically (Figure 4) due to its distinctive chemical structures. The photosynthetic pigment present in PNSAB could be bacteriochlorophyll *a*, *b* or both. The isolated bacteria in this study showed the characteristic reddish bloom under anoxy-photosynthetic conditions and the presence of only bacteriochlorophyll *a* (805-1040nm) and carotenoids pigments (435-590nm), specifically spirilloxanthin series [23, 24]. The photosynthesis apparatus in these organisms is controlled through a genes cluster that are composed basically of genes that encoded for bacteriochlorophyll, carotenoids pigments, light harvesting complex and a reaction center (*puh*, *puf* and *puc*) [25].

Molecularly, a technique frequently used to determine if the bacteria have an anoxygenic photosynthetic apparatus is the presence of a pigment binding protein encoded gene, known as *pufM* [7]. The presence of *pufM* gene was determined by PCR using *pufM* primer specific design by Achenbach [8]. In both microenvironments, the presence of the *pufM* gene was detected indicated that this component of the anoxygenic photosynthetic machinery is present.

The molecular analysis was completed with an *in silico* and phylogenetic analysis using the *16S rDNA* sequences of the isolates. The *in silico* analysis suggests the presence of members of the genera *Rhodopseudomonas* and *Rhodospirillum* for both microenvironments; but the genera *Rhodobacter*, *Rhodothalassium* and *Rhodovulum* are only present in MM. In the case of *Rhodomicrobium* this genus was found only in BP. The phylogenetic tree shows an evolutionary relationship between the isolated PNSAB and other purple non sulfur bacteria that have been already described. As showed in the phylogenetic analysis there are three principal branches; the first branch dominated by PNSAB isolated from MM, the second by isolates from BP and the third one a combination of both. While most of the bacteria members present in the first branch have been already described for hypersaline environments [1, 26]. The members of the second branch are more ubiquitous microorganisms, also found in aquatic environments [27].

The phylogenetic tree have been generated using almost all of the sequences of the entire *16S rDNA* (715bp), the 23% of the isolated from BP could represent novel genus. Most of the research performed to the bromeliads phytotelmata are ecological and at macroscopic level. In contrast, there microbiological studies (bacterial) are scarce.

During this research, purple non sulfur anoxyphototrophic bacteria have been isolated and identified from two contrasting microenvironments: tropical hypersaline microbial mats and bromeliads phytotelmata. Common as well as distinctive PNSAB to each microenvironments were also found. While this is the first report of PNSAB in mats in Puerto Rico, to our knowledge there are not previous taxonomic report in the literature of PNSAB in bromeliads phytotelmata.

Acknowledgments The support by NSF: RUI Cabo Rojo Salterns Microbial Observatory grant #0455620 is gratefully acknowledged.

References

- [1] Imhoff, J.F and Trüper, H.G. *Bergey's Manual of Determinative Bacteriology*. Vol. 2. 2nd ed. Michigan State University; 2005.
- [2] Oda Y., Wanders W., Huisman L.A., Meijer W. G., Gottschal J.C., Forney, L.J. Genotypic and Phenotypic Diversity within Species of Purple Nonsulfur Bacteria Isolated from Aquatic Sediments. *Applied and Environmental Microbiology*. 2002; 68: 3467-3477.
- [3] Okubo Y., Futamata H., Hiraishi A. Characterization of phototrophic nonsulfur bacteria forming colored microbial mats in a swine wastewater ditch. *Applied and Environmental Microbiology*. 2006; 72: 6225-6233.
- [4] Wang X., Falcone D.L, Tabita F.R. Reductive pentose phosphate-independent CO₂ fixation in *Rhodobacter sphaeroides* and evidence that ribulose biphosphate carboxylase/oxygenase activity serves to maintain the redox balance of the cell. *Journal of Bacteriology*. 1993; 175: 3372-3379.
- [5] Madigan M.T. Martinko, J.M. and Parker, J. *Brock Biology of Microorganism*. 10th ed. New Jersey: Pearson Education; 2007.
- [6] Kiley P.J., Kaplan S. Molecular genetics of photosynthetic membrane biosynthesis in *Rhodobacter sphaeroides*. *Microbiological Reviews*. 1988; 52: 50-69.
- [7] Karr E. A., Matthew W., Jung D., Madigan M., Achenbach, L. Remarkable diversity of phototrophic purple bacteria in a permanently frozen Antarctic lake. *Applied and Environmental Microbiology*. 2003; 69: 4910-4914.
- [8] Achenbach L.A., Carey J. and Madigan M. Photosynthetic and phylogenetic primers for detection of anoxygenic phototrophs in natural environments. *Applied and Environmental Microbiology*. 2001; 67: 2922-2926.
- [9] Gemerden V.H. Microbial mats: A joint venture. *Marine Geology*. 1993; 113:3-25.

- [10] Des Marais D. J. Biogeochemistry of hypersaline microbial mats illustrates the dynamics of modern microbial ecosystems and the early evolution of the biosphere. *Biology Bulletin*. 2001; 204: 160–167.
- [11] Casillas-Martínez L., González L. M., Fuentes-Figueroa Z., Castro C.M., Nieves D., Hernández C., Ramírez W., Sytsma R.E, Pérez-Jiménez J., Visscher, P. Community structure, geochemical characteristics and mineralogy of a hypersaline microbial mat, Cabo Rojo, PR. *Geomicrobiology Journal*. 2005; 22(6): 269-288.
- [12] Hiraishi A., Kato K. Quinone profiles in lake sediments: implications for microbial diversity and community structures. *The Journal of General and Applied Microbiology*. 1999; 45: 221-227.
- [13] Clocksin K. M., Jung D.O., Madigan, M.T. Cold-active chemoorganotrophic bacteria from permanently ice-covered lake hoare, McMurdo Dry Valleys, Antarctica. *Applied and Environmental Microbiology*. 2007; 73: 3077-3083.
- [14] Urmeneta J., Navarrete A., Huete J., Guerrero, R. Isolation and characterization of cyanobacteria from microbial mats of the Ebro Delta, Spain. *Current Microbiology*. 2003; 46: 199-204.
- [15] Richardson B.A., Richardson M.J., Scatena F.N., McDowells W.H. Effects of nutrients availability and other elevational changes on bromeliads populations and their invertebrate communities in a humid tropical forest in Puerto Rico. *Journal of Tropical Ecology*. 2000; 16:167-188.
- [16] Fish D. Phytotelmata: flora and fauna. En Frank J.H., Lounibos L.P. (eds). *Phytotelmata: terrestrial plants as hosts for aquatic insect communities*. New Jersey, Plexus Publishing; 1983: 1-25.
- [17] Frank J.H. Bromeliad phytotelmata and their biota, especially mosquitoes. Frank J.H., Lounibos L.P. *Phytotelmata: terrestrial plants as host for aquatic insect communities*. New Jersey, Plexus Publishing; 1983: 101-128.
- [18] Rivera S.C., Hazen T.C., Toranzo G.A. Isolation of fecal coliforms from pristine sites in a tropical rain forest. *Applied and Environmental Microbiology*. 1988; 54: 513-517.
- [19] Harley J.P., Prescott L.M. *Laboratory Exercises in Microbiology*. 7th Edition. Mc Graw- Hill; 2007.
- [20] Chen W.P., Kuo T.T. A simple and rapid method for the preparation of gram-negative bacterial genomic DNA. *Nucleic Acids Research Journal*. 1993; 21: 2260.
- [21] Imhoff J., Caumette P. Recommended standards for the description of the new species of anoxygenic phototrophic bacteria. *International Journal of Systematic and Evolutionary Microbiology*. 2004; 54: 1415- 1421.
- [22] Melendez-Martínez A., Vicario I., Heredia F. Pigmentos carotenoides: consideraciones estructurales y fisicoquímicas. *Archivos latinoamericanos de nutrición*. 2007; 57: 2
- [23] Braun P., Scherz A. Polypeptides and bacteriochlorophyll organization in the light-harvesting complex B850 of *Rhodobacter sphaeroides* R-26.1. *Biochemistry*. 1990; 30: 5177-5184.
- [24] Glaeser J., Overmann J. Selective enrichment and characterization of *Roseospirillum parvum*, gen. nov. and sp. Nov., a new purple nonsulfur bacterium with unusual light absorption properties. *Archives of Microbiology*. 1999; 171: 405-416.
- [25] Zeilstra J.H., Gabbert K., Mouncey H.J., Kaplan S., Kranz R.G. Analysis of the *fnrL* gene and its function in *Rhodobacter capsulatus*. *Journal of Bacteriology*. 1997; 179: 7264-7273.
- [26] Imhoff J.F., Petri R., Suling J. Reclassification of species of the spiral-shaped phototrophic purple non-sulfur bacteria of the α -*Proteobacteria*: description of the new genera *Phaeospirillum* gen. nov., *Rhodovibrio* gen. nov., *Rhodothalassium* gen. nov. and *Roseospira* gen. nov. as well as transfer of *Rhodospirillum fulvum* to *Phaeospirillum fulvum* comb. nov., of *Rhodospirillum molischianum* to *Phaeospirillum molischianum* comb. nov., of *Rhodospirillum salinarum* to *Rhodovibrio salinarum* comb. nov., of *Rhodospirillum sodomense* to *Rhodovibrio sodomensis* comb. nov., of *Rhodospirillum salexigens* to *Rhodothalassium salexigens* comb. nov. and of *Rhodospirillum mediosalinum* to *Roseospira mediosalina* comb. nov. *International Journal of Systematic Bacteriology*. 1998; 48: 793-798.
- [27] Smiejan A., Wilkinson K.J., Rossier C. Cd Bioaccumulation by a freshwater bacterium, *Rhodospirillum rubrum*. *Environmental Science and Technology*. 2003; 37: 701-706