Biological activity screening of isolated freshwater and thermal water cyanobacteria from the Azores

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Abstract

The cyanobacteria, photosynthetic prokaryotes that are common inhabitants of inland waters, are increasingly considered an important and rich source of secondary metabolites with diverse biological activities, such as anticancer, antibacterial, antifungal, anti-inflammatory, etc., leading to an increase of bioprospecting in these organisms. In the Azores, several cyanobacteria species are present in lakes and hot springs but their biological activities were never tested. Due to the environmental conditions and isolation of the archipelago, the cyanobacteria present in the Azores might have unique proprieties and biological activities with great value.

In this work, water and biofilm samples from lakes and hot springs in the Azores where collect for the production of uni-algal cultures of cyanobacteria. The isolation work allowed the establishment of 44 strains of cyanobacteria from thermal, fresh and brackish water habitats. The identification of these strains allowed the enrichment of the basic knowledge of the Azorean cyanobacteria flora, contributing in total with 12 new species, in addition to many others still lacking identification and which are likely new for the Azores.

For the bioprospecting work 20 strains, isolated from thermal and fresh water habitats, were selected to test antioxidant, anti-acetylcholinesterase, antimicrobial, toxicity and cytotoxicity activity. All the strains tested showed activity in at least one of the selected tests, with exception for antimicrobial where none of the strains revealed any activity.

The most relevant result are the identified toxicity and cytotoxicity effects from T07 and L30 strains, that belong to cyanobacteria with no described toxic compounds and also the identified strains as potential sources of anti-acetylcholinesterasic and antioxidant compounds.

Although this work contributes significantly to the increase of knowledge about cyanobacteria in the Azores, our results show that cyanobacteria in the Azores are still understudied, with a lot of taxonomic diversity yet to be described. Also relevant are the important activities hereby identified that in future works might lead to the elucidation of compounds with pharmacological and/or biotechnological activities.

Resumo

As cianobactérias, procariotas fotossintéticos comuns em habitats lacustres, têm sido indicadas como uma importante fonte de metabolitos secundários com diversas atividades biológicas como anticancerígenas, antibacterianas, antifúngicas, anti-inflamatórias, etc., conduzindo a um aumento da bioprospeção nestes organismos. Nos Açores, várias espécies de cianobactérias estão presentes em lagos e fontes termais, mas nenhuma atividade biológica foi testada até agora. Devido às condições ambientais e isolamento do arquipélago, as cianobactérias presentes nos Açores poderão possuir propriedades únicas e atividades biológicas de valor acrescentado.

Neste trabalho foram recolhidas amostras de água e biofilmes de lagos e fontes termais nos Açores para a produção de culturas uni-algais de cianobactérias. O trabalho de isolamento permitiu a produção de 44 estirpes. A identificação destas estirpes permitiu enriquecer o conhecimento da flora de cianobactérias dos Açores, contribuindo no total com 12 novas espécies, para além de muitas por identificar e que provavelmente serão novas para a os Açores.

Para o trabalho de bioprospecção, 20 estirpes foram selecionadas, isoladas de habitats termais e lagos, para os testes de atividade antioxidante, anti-acetilcolinesterase, antimicrobiana, de toxicidade e citotoxicidade. Todas as estirpes selecionadas apresentaram atividade em pelo menos um dos testes selecionados, com exceção da atividade antimicrobiana em que nenhuma apresentou atividade.

Os resultados mais relevantes estão relacionados com os efeitos tóxicos e citotóxicos dos extratos das estirpes T07 e L30, pertencentes a cianobactérias nunca antes descritas com compostos tóxicos, mas também são de realçar as estirpes identificadas como potencial fonte de compostos com efeitos anti-acetilcolinesterásico e antioxidante.

Apesar do contributo significativo deste trabalho para o aumento do conhecimento sobre as cianobactérias nos Açores, os resultados mostram que as cianobactérias dos Açores continuam pouco estudadas, com uma grande diversidade taxonómica ainda por descrever. É também importante de referir as atividades aqui identificadas, que em trabalhos futuros poderão levar à elucidação estrutural de compostos com potenciais atividades na farmacologia e/ou biotecnologia.

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AChE - Acetylcholinesterase

AD - Alzheimer's Disease

DMSO - Dimethyl sulfoxide

DPPH - 2,2-diphenyl-1-picrylhydrazyl

MG - Myasthenia gravis

NRPS - Nonribosomal peptide synthetases

PKS - Polyketide synthases

1. Introduction

1.1. Work context and objectives

Several organisms are able to produce organic compounds that are not involved in the normal growth and development of the organism (Agostini-Costa *et al.*, 2012), but have high and diverse biological activities that can be used for human applications (Mandal & Rath, 2015). These compounds are usually known as secondary metabolites (Vining, 1990). From the various activities they might have some are more notorious as: antibacterial agents, antifungal agents, metal transporting agents, agents of symbiosis, sexual hormones and differentiation effectors (Demain & Fang, 2000).

Cyanobacteria have been revealed as an important source of compounds, recognized as one of the groups of organisms with the widest diversity and production of secondary metabolites with biological activity (Singh *et al.*, 2005; Welker & Von Dõhren, 2006; Arif *et al.*, 2012; Dixit & Suseela, 2013; Vijayakumar & Menakha, 2015; Mazard *et al.*, 2016; Swain *et al.*, 2017; Wang *et al.*, 2017) and/or with biotechnology applications (Abed *et al.*, 2008; Rastogi & Sinha, 2009; Wijffels *et al.*, 2013). For the most important and recognized activities we should emphasize anticancer, antibacterial, antiviral, antifungal and anti-inflammatory. The chemical structure of the most important cyanobacteria secondary metabolites are non-ribosomal peptides, polyketides, alkaloids and terpenes (Mandal & Rath 2015).

The most efficient method for the identification of new compounds with biological activities is by bioprospecting, based in the search of compounds with biological and commercial interests (Cox & King, 2013). Several bioprospecting studies have been realized using different approaches such as genetic methods (*e.g.* Prasanna *et al.* 2010; Brito *et al.*, 2015) or molecular methods (Montalvão *et al.*, 2016).

Cyanobacteria are gram-negative photosynthetic prokaryotes that are common inhabitants of terrestrial and aquatic ecosystems, such as lakes, thermal waters and brackish waters (Whitton & Potts, 2012). Due to their unique abilities they adapt very well to extreme environments of temperature, algae concentration, salinity, etc. (Waterbury, 2006). In the Azores islands, cyanobacteria are widely spread in lakes (Gonçalves, 2008; Cordeiro, 2015), streams (Johansson, 1977), wet soils and rocks (Bourrelly & Manguin, 1946; Johansson, 1977), and were also found in caves (Joahansson, 1977; Aguiar *et al.*, 2010) and thermal waters (Pereira *et al.*, 2010; Moreira *et al.*, 2011).

The great diversity and richness of aquatic ecosystems in the Azores, such as lakes (88, according to Porteiro, 2000) and thermal waters (19, referred by Cruz & França, 2006), creates habitats for the development of microalgae and cyanobacteria, resulting in a high potential to discover biologically active metabolites from the natural inhabitants of these ecosystems.

Therefore, the work proposed in this thesis should be a relevant contribution to (i) the isolation of new cyanobacteria with biological interest, (ii) their culture and identification, taking into advantage their possible unique qualities that might have developed by the influence of the insular isolation and special environmental conditions. With this in focus, the following objectives were set:

- Isolation, purification and maintenance of cultures of cyanobacteria from lakes and thermal waters from the Azores;
- Optimization of an extraction protocol of non-excreted compounds;
- Identification of species that produce compounds with potential biological activity.

1.2. Cyanobacteria

Cyanobacteria are a wide and diverse group of photosynthetic bacteria that are distinguished by their unique set of photosynthetic pigments: chlorophyll *a* and the phycobiliproteins phycocyanin, allophycocyanin and phycoerythrin (Waterbury, 2006). These prokaryotes are part of a phylogenetic group of phototropic bacteria, with significant different morphologies that have a very important impact in ecology, by the production of O_2 and fixation of CO_2 (Garcia-Pichel, 2009).

1.2.1. Morphology

Cyanobacteria are gram-negative prokaryotes with great morphological diversity (Whitton & Potts, 2002). They can go from unicellular forms to filamentous complexes that can have cellular differentiation, going from 0.5 μm to 50-100 μm (Whitton & Potts, 2002; Waterbury, 2006). Cells from unicellular cyanobacteria can present spherical form, bacillus or fusiform, but some species have pleomorphism. Filamentous cyanobacteria can present discoid or barrel shape cells and extend for several millimetres of length. Some species can produce an exopolysaccharide coating, forming a well-defined structure that influences the form of the cells (Garcia-Pichel, 2009).

Several species have heterocysts, specialized cells produced in response to lack of nitrogen in the environment (Garcia-Pichel, 2009). These cells are normally bigger than the others, have a double wall, are yellowish or colourless, and are connected to the adjacent cells by polar nodules. Depending on the species, these cells can acquire different forms, such as cylindrical or rectangular, being this characteristic important in the moment of identification (Sarma, 2013). The main function of the heterocysts is the fixation of atmospheric nitrogen (N_2) and delivery, in amino acids form, to the adjacent cells (Garcia-Pichel, 2009).

The akinetes are specialized cells without motility, differentiated from other cells by their augmented size, thick cellular wall and large nitrogen reservoirs in the form of cyanophycin granules. Due to their great resistance to adverse conditions, the akinetes are considered a latent state of the life cycle of the cyanobacteria and allows their re-growth when conditions become favourable (Garcia-Pichel, 2009).

1.2.2. Ecology

Cyanobacteria normally use water as electron donor during the photosynthesis producing oxygen. However, some strains have the ability to use hydrogen sulphide (H_2S) converting it to sulphur, with the advantage of resisting to low concentrations of O_2 and high concentrations of H_2S that would be toxic to the majority of algae (Vincent, 2009). This capacity can be fundamental to their resistance in eutrophic lakes or in the periphyton (Vincent, 2009).

Common in the periphyton, cyanobacteria may arise forming layers in sand, sediments, rocks, plants or other types of substrates. Depending on the environment, cyanobacteria may accumulate vertically to several centimetres of length. These layers can deposit in the bottom of water masses or arise and create a suspension layer composed mainly of cyanobacteria (Vincent, 2009).

In lakes where the nitrogen amount is high, in the form of nitrate or ammonium, cyanobacteria rarely are the dominant taxa, being competitively suppressed by better adapted algae (Assmy *et al.,* 2009). In the summer, the ratio between nitrogen and phosphorus decreases by the reduction of the amount of available nitrogen, reaching its minimum (Assmy *et al.,* 2009). This favours some species of cyanobacteria, as several of them hold the ability of atmospheric nitrogen fixation in the heterocysts (Whitton & Potts, 2012). Nonetheless, phosphorus is also indicated as a boundary to cyanobacteria growth in the natural environment, being the rise of its concentration, mainly by

anthropogenic influences, in the origin of proliferation of cyanobacteria in eutrophic lakes (Whitton & Potts, 2012).

Cyanobacteria can occur in a wide interval of temperature, but prefer warmer climates for optimum growth, normally above 15 ºC (Vincent, 2009). In *in vitro* culture conditions they show an optimum growth between 20 ºC and 35 ºC (Waterbury, 2006). Besides that, cyanobacteria prefer more alkaline water, growing in eutrophic lakes were the pH can reach a value of 9 (Vincent, 2009).

Cyanobacteria possess a high capacity of adaptability, surviving in zones with very low light (Waterbury, 2006). They also possess a great capacity to resist high light intensities, even when the UV radiation is high, capacity that could be an indicator of their resistance in superficial water blooms (Vincent, 2009).

1.2.1.1. Cyanobacteria in extreme environments

Extreme environments are, from an anthropocentric point of view, those inhospitable and abnormal places that some organisms may inhabit (Mandal & Rath, 2015). Cyanobacteria are often the main and/or sole autotrophic organisms inhabiting these environments (Komárek & Johansen, 2015a). Extremophile organisms are classified according to their type of "extreme" habitat, since these can present very low or high values of temperature, pH, salinity, heavy metal concentrations, etc. (Mandal & Rath, 2015).

Thermophilic cyanobacteria are those that grow well or resist temperatures above 45 ºC, with a maximum of 72-73 ºC (Castenholz, 1988). Different types of cyanobacteria have been identified in high temperature habitats, such as *Oscillatoria* and *Mastigocladus*, but the most common is *Synechococcus,* with a maximum upper temperature of 72-73 ºC (Mandal & Rath, 2015).

Marine and halophilic cyanobacteria are organisms that grown in salt obligatory environments (Oren, 2012), where cyanobacteria can tolerate salinity levels up to saturation (Mandal & Rath, 2015). The marine cyanobacteria *Coleofasciculus chthonoplastes* is the main mat building of cyanobacteria in littoral areas and can tolerate salinity as high as 200 g/L (Oren, 2012).

1.2.3. Taxonomy

Taxonomic classification is the main method used to evaluate biological diversity and has been in continuous modification since its creation. Although they are

considered bacteria, cyanobacteria classification has been traditionally done by algologists following the botanic taxonomic principles and their nomenclature code. The recent interest of bacteriologists in this group, together with the use of new techniques in taxonomy, profoundly changed the cyanobacteria classification. In addition, the great morphological diversity and the long evolutionary history of cyanobacteria caused higher difficulties, contributing to a continuous reconstruction of the classification of this group (Komárek *et al.*, 2014).

The older classification was based exclusively in their morphology, but nowadays new techniques such as electronic microscopy and phylogenetic data contribute to cyanobacteria classification (Richmond & Hu, 2013; Komárek, 2016).

After a phylogenetic revision in cyanobacteria, Komárek *et al.* (2014) suggested a new classification which recognizes eight orders: Gloeobacterales, Synechococcales, Spirulinales, Chroococcales, Pleurocapsales, Oscillatoriales, Chroococcidiopsidales and Nostocales (table 1).

Table 1 – Cyanobacteria orders, main characteristics and genera following Komárek *et al.* (2014).

Order	Characteristics	Exp. Genus
I. Gloeobacterales	Absent thylakoids	*Gloeobacter*
II. Synechococcales	Unicellular or filamentous; Parietal thylakoids	*Pseudanabaena*, *Eucapsis*
III. Spirulinales	Regular screw-like coiled trichomes without sheaths	*Spirulina*
IV. Chroococcales	Coccoids that have a more or less irregular thylakoid arrangement	*Microcystis*, *Aphanothece*
V. Pleurocapsales	Pseudo-filamentous which reproduce by baeocytes; division in multiple planes or irregular	*Xenococcus*, *Chroococcidium*
VI. Oscillatoriales	Radial, fasciculate or irregular thylakoid arrangement	*Oscillatoria*, *Lyngbya*
VII. Chroococcidiopsidales	Division in three or more planes; reproduction by baeocytes	*Chroococcidiopsis*
VIII. Nostocales	Filamentous with diversified thallus and specialized cells (heterocysts and akinetes)	*Anabaena*, *Calothrix*

1.3. Cyanobacteria secondary metabolism

The best characterized compounds of cyanobacteria are their toxins, due to their harmful effects to animals and humans when abundant in contaminated water masses (Dittmann *et al.*, 2012). Nonetheless, many other secondary metabolites are known, reaching 1100 compounds from 39 genera (Dittmann *et al.*, 2015). Most of these compounds, which comprise several different activities (table 2), are related to few

genera and confined to specific orders mainly because those species are the ones most studied due to their easier *in vitro* cultivation (Welker & Von Döhren, 2006).

The majority of biologically active metabolites that we can extract from cyanobacteria are either peptides, macrolides, or a combination of both (Welker & Von Döhren, 2006). Many of these metabolites have been characterized and are known to be produced by the Nonribosomal Peptide Synthetases (NRPS) and/or by the Polyketide Synthases (PKS) route (Kehr *et al.*, 2011), as illustrated in figure 1. Nonetheless, other synthetic pathways using cytochrome P450 are being studied and elucidated in the secondary metabolism of cyanobacteria (Mandal & Rath, 2015).

Table 2 – Cyanobacteria biological activity list (adapted from Singh *et al.* 2005).

Order	Biological activities
Chroococcales	Enzyme inhibitor, cytotoxic, tumor promoter, endotoxic, hepatotoxic
Pleurocapsales	Antifungal
Oscillatoriales	Anticancer, antifungal, anti-inflammatory, antimicrobial, antimitotic, antiproliferative, brine shrimp toxicity, cytotoxic, herbicidal, hepatotoxin, molluscidal, neurotoxic, tumor promoter, sunscreen pigment, toxin
Nostocales	Anticancer, antifungal, antimalarial, anti-HIV, hepatotoxic, antimicrobial, antimitotic, anti-inflammatory, cytotoxic, enzyme inhibitor, toxin, neurotoxin
Stigonematales	Antifungal, antibiotic, anticancer, antimitotic, cytotoxic, herbicidal

1.3.1. Biosynthetic pathways of peptides

Peptides in cyanobacteria may be produced by two types of biosynthetic pathways, (i) the NRPS or (ii) the ribosomal synthesis with post-translational modification and processing (Kehr *et al.*, 2011). The NRPS consist in domains that work in a progressive way to incorporate a single amino acid in the target molecule (Kehr *et al.*, 2011). The number of these domains can range from seven to 64 depending on the target molecule (Dittmann *et al.*, 2015). The ribosomal synthesis is limited but also contributes with direct gene products with only a few post-translational modifications (Dittmann *et al.*, 2015).

Macrolides are compounds with a macrocyclic lactone ring of 12 or more elements (Mazzei *et al.*, 1993). In cyanobacteria they are produced by the PKS route, and contrary to NRPS this route performs the activation and assemblage of carboxylic acids (Kehr *et al.*, 2011).

1.3.2. Other compounds

Cyanobacteria also produce a vast array of other compounds such as retinoids, alkaloids, lactones and phospholipids (Elersek *et al.*, 2017). Among the most notorious there is saxitoxin, a neurotoxic alkaloid (Ballot *et al.*, 2017). Several natural compounds

found in plants such as terpenes can also be produced by cyanobacteria, e.g. geosmin (Dittmann *et al.*, 2015). Other compounds like hapalindole-type alkaloids are a group of hybrid isoprenoid-indole compounds found, according to Ditmann *et al.* (2015), only in *Stigonemataceae* species, though other species have been reported to produce it, extending the production families to *Hapalosiphonaceae* (Hillwig *et al.*, 2014).

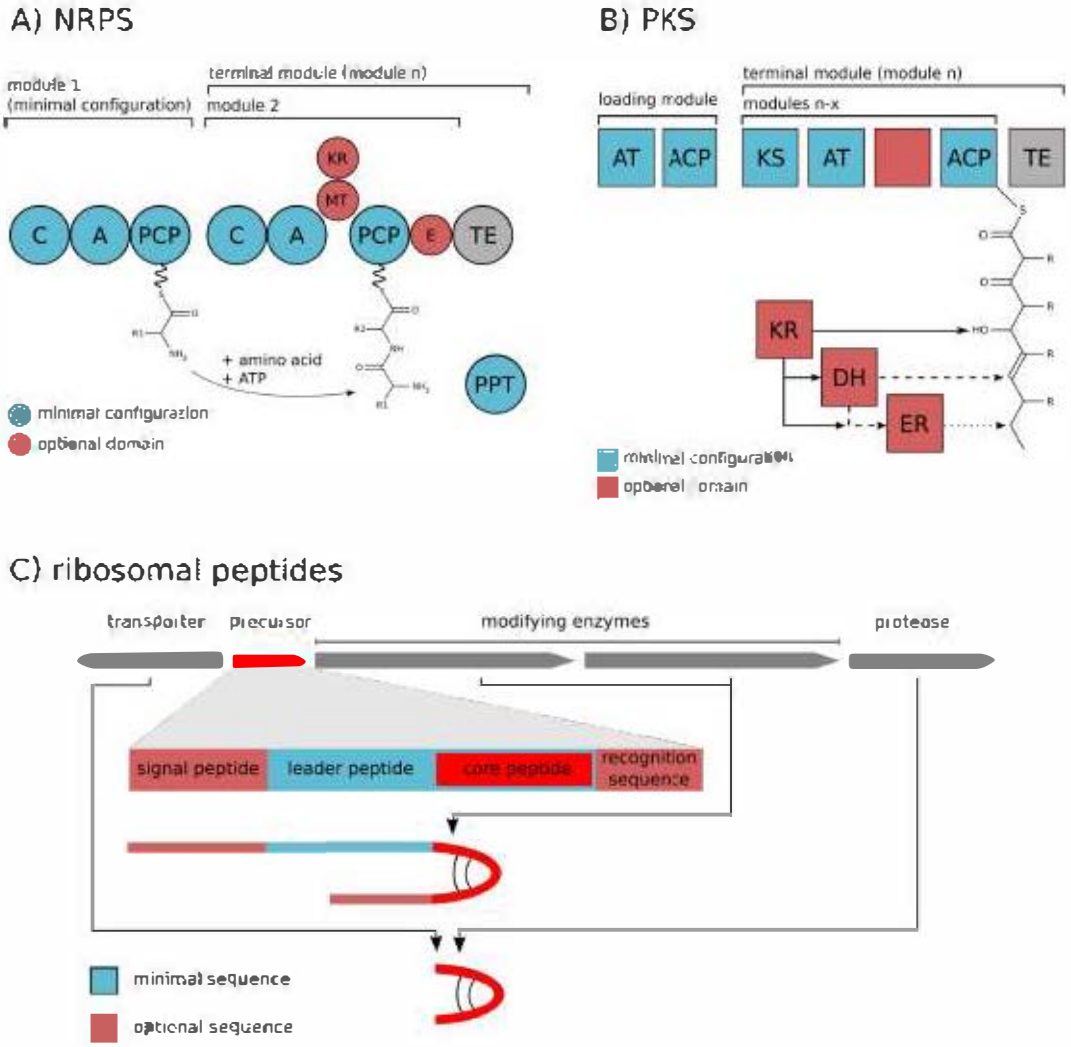

Figure 1 – NRPS, PKS and ribosomal pathways (from Kehr *et al.* 2011).

1.4. Pharmacological and biotechnological applications of secondary metabolites from cyanobacteria.

The wide diversity of secondary metabolites that has been identified in cyanobacteria render these organisms into excellent models for pharmacological and biotechnological studies, considering that a great part of cyanobacteria remains unstudied (Dittmann *et al.*, 2015). The search for applications for the metabolites of cyanobacteria is in focus with several reviews published in recent years with that topic (e.g. Dias *et al.*, 2015; Varshney *et al.*, 2015; Vijayakumar & Menakha, 2015; Mazard *et al.*, 2016; Raja *et al.*,

2016; Singh *et al.*, 2016; Haque *et al.*, 2017; Rajneesh *et al.*, 2017; Singh *et al.*, 2017; Swain *et al.*, 2017; Wang *et al.*, 2017).

Among the investigated activities the pharmaceutical field is the one most researched, with diverse activities such as antimicrobial, anticancer, antifungal, etc. Several works have focused on anticancer activity, as shown by Vijayakumar & Menakha (2015) and Wang *et al.* (2017) reviews, where a wide array of metabolites have been identified, many of which obtained from the marine cyanobacteria *Lyngbya majuscula*. Antimicrobial and antifungal activities have been widely detected in cyanobacteria from several identified and elucidated secondary metabolites, as reported by Swain *et al.* (2017) and Wang *et al.* (2017). Cyanobacteria also produce a wide array of toxins, many already identified and with also several identified activities in the pharmaceutical field (Dias *et al.*, 2015).

Several biotechnological applications have been proposed for cyanobacteria, such as biofertilizers, biofuels, bioremediation agents and food supplements. Among food supplements *Arthrospira platensis* (Spirulina) is already widely used due to its high protein and nutrient content (Raja *et al.*, 2016; Haque *et al.*, 2017). *Anabaena azollae* has been widely used in Asia as biofertilizer and is recognized as an important species due to its symbiotic relation with *Azolla*, an aquatic fern used to enrich Asian crops, allowing its use as a source of organic N_2 (Watanabe, 1982). Recently several other cyanobacteria have been studied to be used as biofertelizers, with their relation and interaction with the destination crop investigated (Singh *et al.*, 2016). Due to their nitrogen fixation ability and lipid content, cyanobacteria is also studied as possible sources of biofuel, which if used might minimize the cost and harmful effects of the presently used fuels (Mazard *et al.*, 2016; Haque *et al.*, 2017; Rajneesh *et al.*, 2017).

2. Material and methods

2.1. Sampling

2.1.1. Freshwater

Samples dated from 2014 until July 2015 were collected in the margin of 16 lakes of São Miguel island (Appendix I; see appendix II for location coordinates), by dragging the net from the inside out of the lake for at least 5 meters. Seasonally from autumn of 2015 to summer of 2016 the samples were collected in the deepest point of the lake from the bottom to the top of the water column in 22 lakes of 4 islands of the Azores (Appendix I; see appendix II for location coordinates). Each sampling was done with a phytoplankton net with 10 µm size pore. General characteristics like pH and water temperature were measured in the moment of sampling (Appendix III).

2.1.2. Brackish water

Sampling was done in August 2016 in Maia, Santa Maria (see appendix II for location coordinates) by collecting a biofilm in volcanic rocks near the ocean, where an old fountain used for water drinking had broken and continually ran. This was considered brackish water for its proximity to the ocean, approximately 5 meters, which may provide salinity influence to the small pond. Due to absence of technical support, characterization of the water was not possible.

2.1.3. Marine water

Sampling of marine water was performed in April 2017 in Fajã dos Cubres, São Jorge island (see appendix II for location coordinates). Biofilm from rocks that were under and above water were collected. A sample with a 10 µm size pore net from the inside out of the lake was also carried out, for planktonic species. Both samples were considered distinct and treated as such. General characteristics like pH, temperature and salinity were registered in the moment of sampling (Appendix III).

2.1.4. Thermal water

Sampling was done in June 2016 in Furnas, São Miguel island, by collecting biofilms present in or near thermal water running fountains or springs (see appendix II for location coordinates). General characteristics like pH and water temperature were measured in the moment of sampling (Appendix III).

2.2. Samples enrichment and maintenance

Each sample, on arrival to the lab, was diluted in three different media, BG-11 and BG-11_o (Appendix IV) and TFI+M (Appendix IV), in a 1:1 proportion for a total volume of 40 mL in 100 mL glass Erlenmeyers, at most 72 hours after sampling. The marine sample was enriched only with BG-11 and BG-11_o.

Fresh, brackish and marine water samples were maintained in controlled temperature chambers at 23(±2) ºC, in a 14:10 photoperiod and light intensity of 750-1000 LUX. Thermal water samples were maintained in controlled temperature chambers at 40(±2) ºC, with 14:10 photoperiod and light intensity of 3000-3500 LUX.

2.3. Isolation and maintenance of uni-algal cultures

2.3.1. Isolation

Isolation was done using serial dilution and streak plate method according to Stainer *et al.* (1971) and Rippka (1988). After 30 days of growth in lab after dilution, a drop of each enriched sample, without selection, was streaked in a plate with 0.7% of bacteriological agar. Growth of cyanobacteria were monitored and identified cyanobacteria colonies or filaments were transferred and put in sterile agar plates. If needed again, serial dilution was used.

Selective isolation was performed in glass plates by dilution under observation on a Euromex Holland, VT series inverted microscope and transferred directly from the original diluted sample to 10 mL tubes with 2 mL of medium and/or by transfer to agar plates, depending on the taxa. Taxa that had mobility or hormogonia production (*e.g. Calothrix*) were preferentially isolated in agar plates.

Isolation of cultures was always done in the same medium in which they were first diluted, always using sterile material. The verifications for uni-algal cultures were done by agar streak plating. The cultures used and characterized in this work were only the ones considered uni-algal.

Taxonomic identification was performed based on Komárek & Anagnostidis (1999), Komárek & Anagnostidis (2005), Komárek (2013), Komárek & Johansen (2015a) and Komárek & Johansen (2015b).

2.3.2. Nomenclature assignment/culture maintenance

Every isolated strain was catalogued and given an alphanumeric code related to the type of environment from where it was sampled and the order of isolation (table 3).

Table 3 – Nomenclature assignment.

Sampling type	Code (Example)
Freshwater (Lake)	L.XX
Brackish	B.XX
Thermal	T.XX

Maintenance of fresh, brackish and marine water strains of uni-algal cultures was done in a stable temperature of 20 (±0.5) ºC in 100 mL glass Erlenmeyer's with 50 mL in which the culture was isolated in a 14:10 photoperiod and a light intensity of 2500 LUX.

Thermal water strains of uni-algal cultures were maintain in 100 mL glass Erlenmeyer's with 50 mL in which the culture was isolated in a 14:10 photoperiod and a light intensity of 1000-1500 LUX.

2.4. Checklist of cyanobacteria from the Azores

From the available literature referring the presence of cyanobacteria in the Azores, a checklist of these organisms in the archipelago, including their distribution per island, was done. The classification and nomenclature of the taxa in the final list was updated to the currently taxonomy according to Komárek *et al.* (2014).

2.5. Extraction

For extraction of metabolites only freshwater and thermal water cultures were selected. Cultures for extraction where put to grow on January 3rd, 2017, and on January 6th, 2017, for thermal waters and freshwater species respectively, in 100 mL Erlenmeyers flasks with 50 mL of the medium in which they were isolated.

Dense cultures of 50 mL, grown for two months, were centrifuged during 20 minutes at 12000 rpm (18514 g) at 4 ºC. The supernatant was collected, frozen and lyophilized. The centrifugation pellet was also collected, weighed and freeze-dried for 3 days.

The extraction was performed with acetic acid for all analysed strains, adapted from Carvalho *et al.* (2013). The lyophilized biomass sample was transferred to a 15 mL Falkon tube with 10 mL 0.1M acetic acid; in a repetitive procedure the sample was submitted to 30 seconds of sonication at 100 watts with the sample in ice and 30 seconds of rest in ice, repeating this procedure for 5 times; in the end the extract was centrifuged at 12000 rpm (18514 g) for 20 minutes at 4 ºC. Each centrifugation pellet was separated by pipetting to a new clean Falkon tube and stored at -20 ºC until lyophilisation. For the assays, the lyophilized extracts were re-suspended in a known

concentration in DMSO (Dimethyl sulfoxide), sterile deionized water was added if needed to better solubilize, and transferred each to a glass flask. The supernatants that resulted from each extraction were also lyophilized, and stored at -20 ºC.

2.6. Biological activity tests

For strains T14 and L17 only the cytotoxic activity against the A-549 cell line was tested. All other strains chosen for bioactivity screening were tested for antioxidant activity by the DPPH (2,2-diphenyl-1-picrylhydrazyl) test, for AChE (Acetylcholinesterase) inhibition activity, for antibacterial activity against two gram-positive bacteria, *Bacillus subtilis* DSM10 and *Micrococcus luteus* DSM 20030, and a gram-negative bacteria *Escherichia coli* DSM 498 and for anticancer activity by the inhibition/cytotoxicity against A-549 cell line. These tests were chosen by the wide opportunity of possible applications that might be suggested by the analysis of their results, alone or together.

2.6.1. Antimicrobial assay

The antimicrobial activity was performed according to Barreto & Simões (2012) in 96 well microplates. For these tests, the models used were two gram positive bacteria, *Bacillus subtilis* DSM10 and *Micrococcus luteus* DSM 20030, and one gram negative bacteria, *Escherichia coli* DSM 498. The test was realized with an initial concentration of extract ranging from 50 to 200 µg/mL (for more information see appendix V). Penicillin and streptomycin were used as controls, with four replicates for each extract tested.

2.6.2. Anti-acetylcholinesterase assay

The anti-AChE assay was performed using acetylthiocholine as an analogue to acetylcholine, according to Barreto & Simões (2012). The test was carried out in 96 well microplate with an initial concentration of extract ranging from 40 to 150 µg/mL (for more information see appendix V). For control it was used galantamine, with four replicates for each tested extract.

2.6.3. Antioxidant assay

For the antioxidant assay it was used DPPH, a reagent in an oxidative state that changes colour in the presence of an antioxidant compound (Pyrzynska & Pękal, 2013). The assay was performed according to Barreto & Simões (2012) and the test performed

in 96 well microplate with an initial concentration of extract ranging from 65 to 250 μg/mL (for more information see appendix V). Gallic acid was used as control. Each experiment had four replicates.

2.6.4. Cytotoxicity assay

The cytotoxicity assay was performed according to Barreto & Simões (2012) using as target the A-549 pulmonary carcinoma cell lineage. The test was performed in a 96 well microplate with an extract concentration ranging from 50 to 200 μg/mL (for more information see appendix V), using taxol and colchicine as control. Each experiment had four replicates.

2.6.5. Toxicity assay

The toxicity assay was performed using *Artemia salina* as model organism. The assay was performed in triplicate in 96 well microplate according to Solis *et al.* (1993). The *nauplii* where submitted to the lyophilized supernatant from all strains tested at a concentration of 3.13 mg/mL.

3. Results

3.1. Checklist of Azorean cyanobacteria

A compilation of data was performed (Appendix VI) whose taxonomic analyse is descripted in table 4. Cyanobacteria in Azores has been cited 1618 times since 1874, the first description of cyanobacteria in Azores. In the archipelago, a total of 187 species have been identified prior to this work.

Table 4 – Azorean cyanobacteria distribution (1874–2017).

Island	Nº of citations	Order	Family	Genus	Species
São Miguel	1080 **(30)**	4	23 **(1)**	48 **(1)**	126 **(6)**
Santa Maria	6 **(2)**	2	4 **(1)**	5 **(2)**	6 **(2)**
Terceira	44	4	14	20	38
Pico	182 **(9)**	4	14 **(2)**	24 **(4)**	40 **(5)**
São Jorge	35 **(7)**	5 **(1)**	14 **(3)**	15 **(3)**	30 **(3)**
Graciosa	1	1	1	1	1
Faial	18	5	8	11	18
Flores	219 **(2)**	4	17 **(1)**	28 **(1)**	50 **(2)**
Corvo	33	4	11	15	19
Azores	1618 **(50)**	6 **(1)**	28 **(2)**	64 **(4)**	198 **(11)**

In brackets the contribution by this work.

3.2. Isolated and cultured cyanobacteria

A total of 180 samples from four types of ecosystems (thermal, fresh, brackish and marine waters) were collected and enriched in at least one of the used media (BG-11, BG-11$_0$ and TFI+M). From those, 44 cyanobacteria isolates were obtained and are maintained in uni-algal cultures. The list of isolated and cultured species organized by origin of sampling (thermal, fresh and brackish water) are presented in tables 5 to 7. As mentioned in the methodology, a unique code was assigned to each strain and a database was build including all the available information related to the sampling site, taxon and culture conditions (*e.g.* taxon name, sampling location and characteristics, date of collection and the maintenance medium). The results of an identification work of cyanobacteria from a marine sample collected in São Jorge island are also presented. A comparison with previous known cyanobacteria from the Azores was preformed based on the compilation of data of all identified species in Azores (see checklist in appendix VI).

From thermal water samples 12 strains were isolated (table 5), of which eight unique genera and nine unique species where identified. Despite their previous record in

freshwater habitats, the genera *Coleospermum* and *Microchaete* were firstly reported in thermal habitats in this study. The species *Microchaete bulbosa*, *Leptolyngbya gelatinosa* and *Chlorogloeopsis fritschii* are reported in the Azores for the first time, and the later species corresponds to the first report of the genus *Chlorogloeopsis* in the Azorean islands. Figures 2 to 11 illustrates some of the cyanobacteria isolated from thermal habitats.

Table 5 – Strains of cyanobacteria isolated from thermal waters in São Miguel island.

Isolate Code	Taxa	Location Sampling	Date of collection	Medium	Figure
T02	*Chroccoccales*	Ribeira Grande - Lava Pés	Jun /16	TFI+M	-
T05	*Coleospermum* sp. 1	Furnas - Poça da Tia Silvina	Jun /16	BG-11$_0$	2
T06	*Microchaete bulbosa*	Furnas - Água da Prata	Jun /16	BG-11$_0$	3
T07	*Leptolyngbya gelatinosa*	Furnas - Água do Rego	Jun /16	BG-11	4
T08	*Leptolyngbya* sp. 1	Furnas - Água do Caldeirão	Jun/16	BG-11$_0$	5
T10	*Phormidium* sp. 1	Furnas - Dona Beja	Oct/16	BG-11$_0$	-
T12	*Phormidium* sp. 1	Furnas - Ribeira Amarela 1	Jun/16	BG-11$_0$	6
T13	*Mastigocladus laminosus*	Furnas - Ribeira Amarela 1	Jun /16	BG-11$_0$	7
T14	*Phormidium* sp. 2	Furnas - Água do Rego	Jun /16	TFI+M	8
T15	*Phormidium* sp. 3	Furnas - Ribeira Amarela 2	Jun /16	BG-11$_0$	9
T16	*Chlorogloeopsis fritschii*	Furnas - Poça da Tia Silvina	Jun /16	BG-11	10
T17	*Chlorogloeopsis fritschii*	Furnas - Poça da Tia Silvina	Jun /16	BG-11$_0$	11

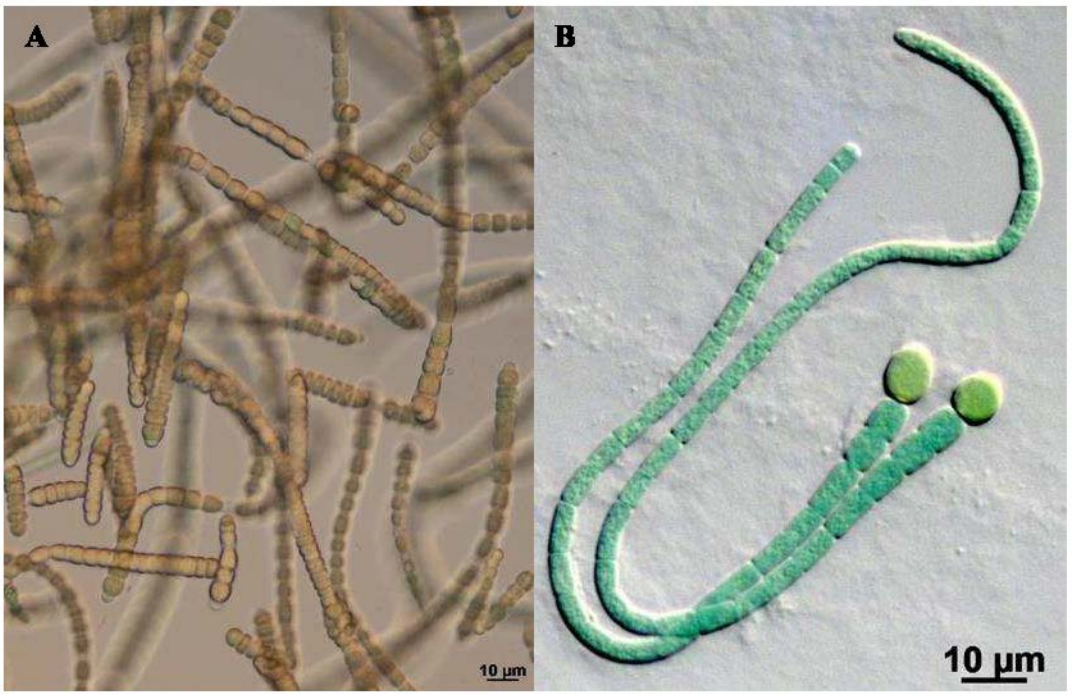

Figure 2 – *Coleospermum* sp. 1 (T05). A: Liquid medium; B: Agar solidified medium.

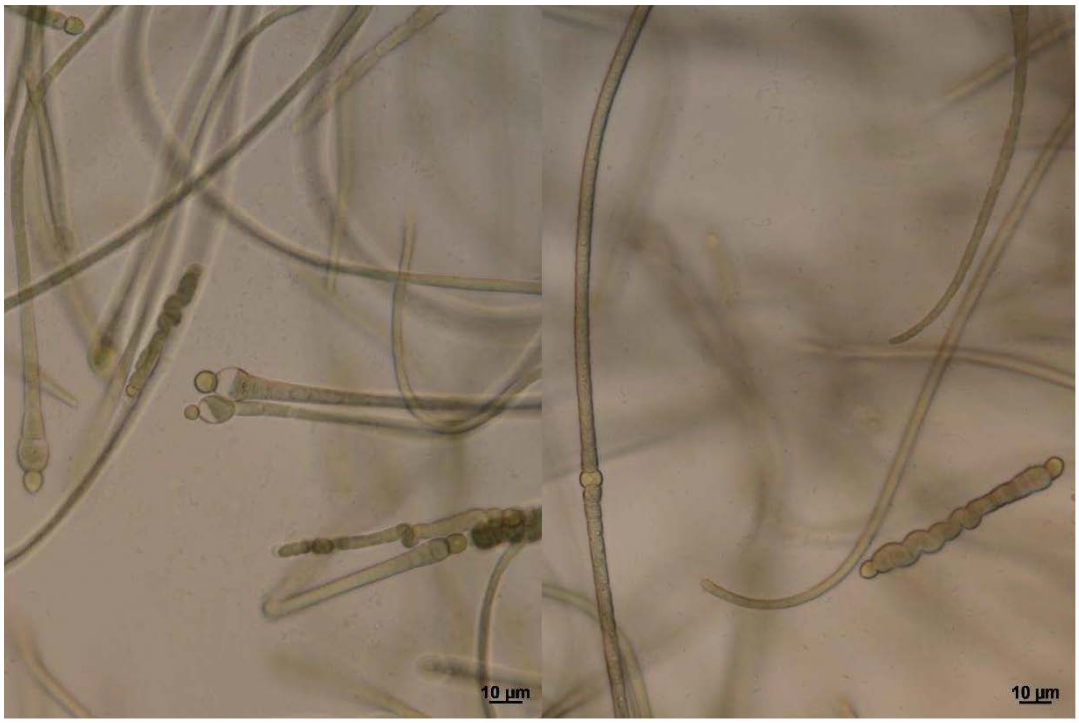

Figure 3 – *Microchaete bulbosa* (T06).

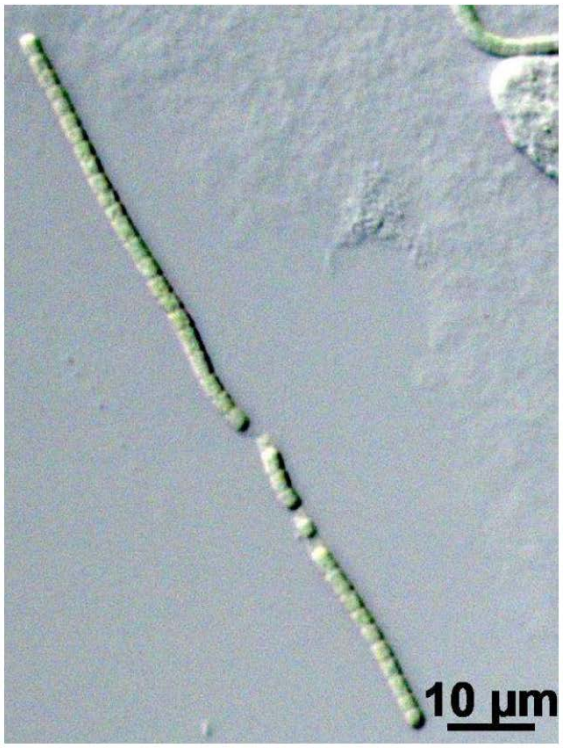

Figure 4 – *Leptolyngbya gelatinosa* (T07).

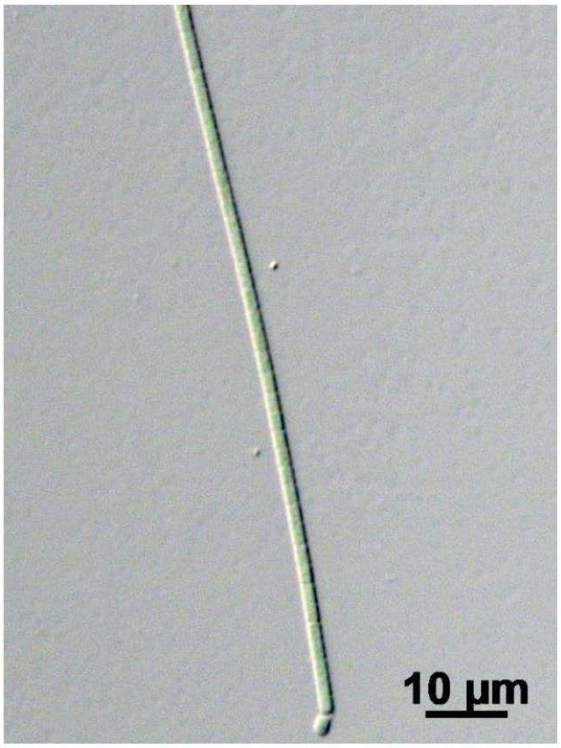

Figure 5 – *Leptolyngbya* sp. 1 (T08).

Figure 6 – *Phormidium* sp. 1 (T12).

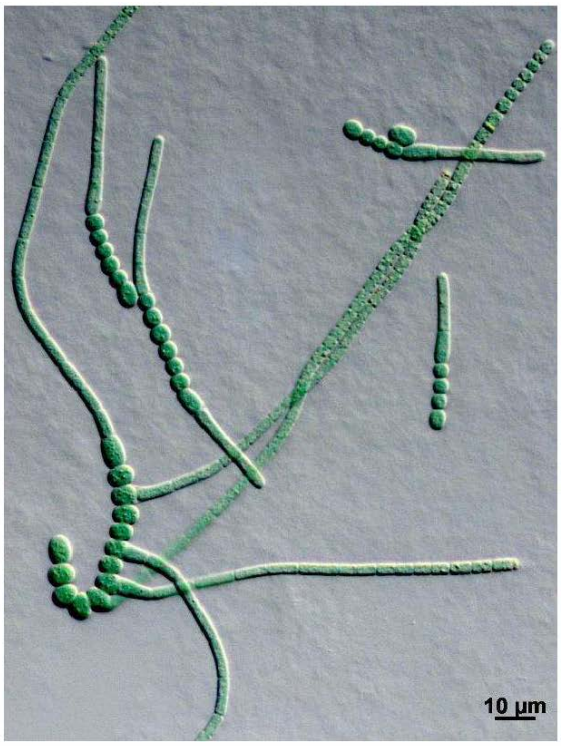

Figure 7 – *Mastigocladus laminosus* (T13).

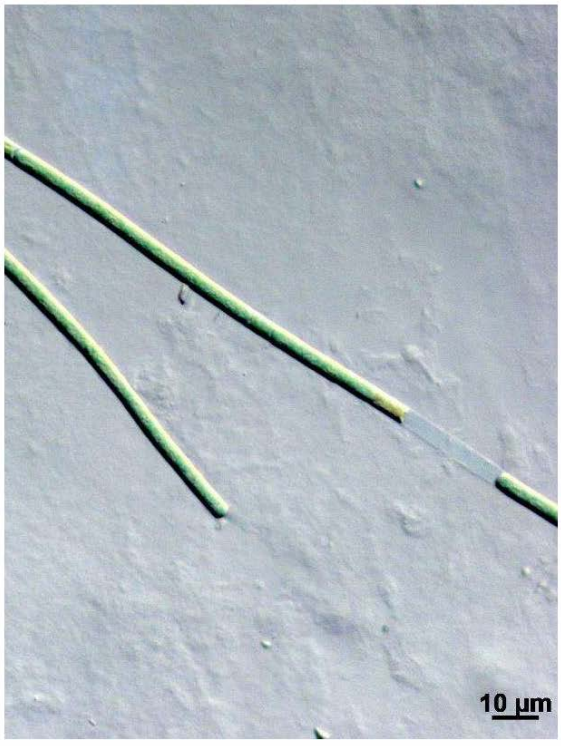

Figure 8 – *Phormidium* sp. 2 (T14).

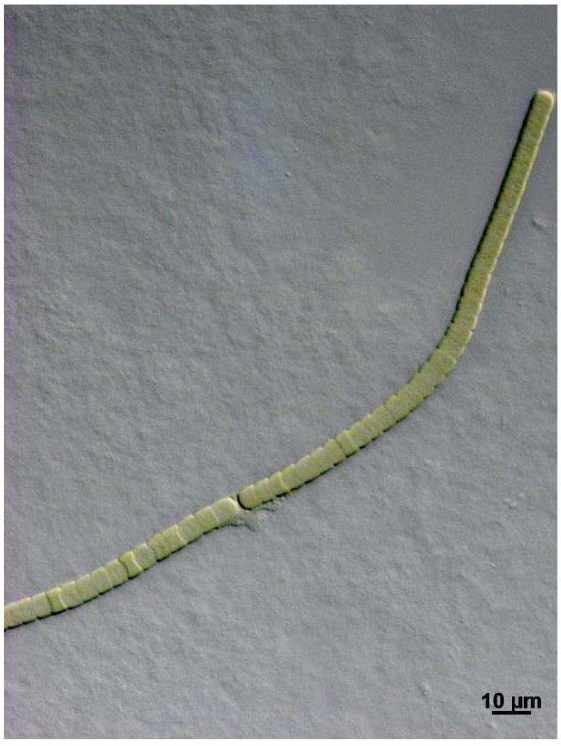

Figure 9 – *Phormidium* sp. 3 (T15).

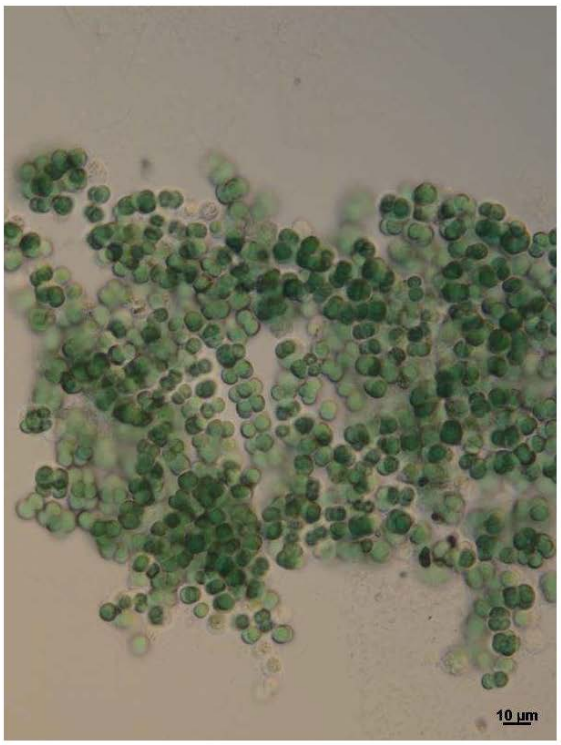

Figure 10 – *Chlorogloeopsis fritschii* (T16).

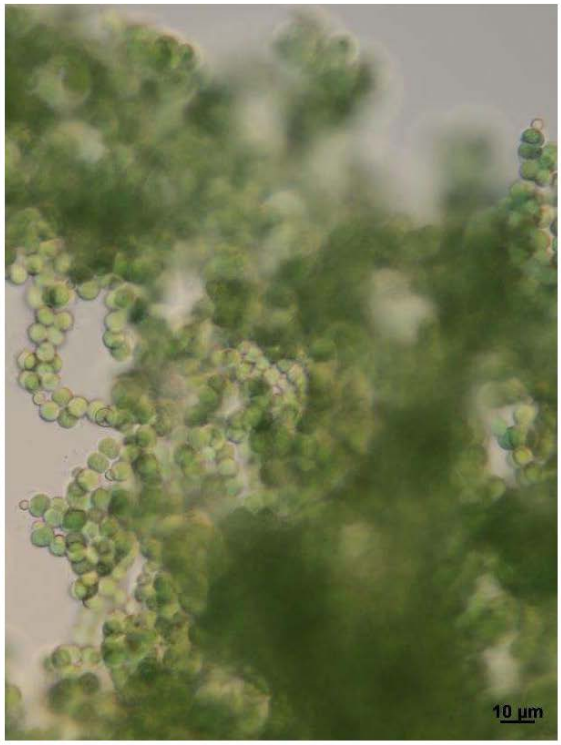

Figure 11 – *Chlorogloeopsis fritschii* (T17).

From the fresh water habitats 30 cyanobacteria strains were isolated, comprising 14 genera and 27 species (table 6). From the total of isolated strains 15 were identified as planktonic and 15 as benthic species. Among these, *Fortiea* is the only newly described genus for the Azores cyanobacteria flora, although there are some species that are first described in the present work (*Aphanizomenon manguinii*, *Calothrix breviarticulata*, *Calothrix marchica*, *Calothrix castellii*, *Pseudanabaena minima*). In addition, there might be a bigger contribution to the flora with this work in the future, since a large number of isolated strains (13) remained unidentified. Figures 12 to 35 illustrates some of the cyanobacteria isolated from freshwater habitats.

Table 6 – Strains of cyanobacteria isolated from freshwater ecosystems in São Miguel, Pico and Flores islands.

Isolate Code	Taxa	Island	Lake	Date of collection	Medium	Figure
L04	*Dolichospermum* sp.	Pico	Peixinho	Feb/16	BG-11$_0$	12
L05	*Anabaena* sp.	São Miguel	Furnas	Feb/16	BG-11$_0$	13
L06	*Aphanizomenon manguinii*	Pico	Capitão	Feb/16	BG-11$_0$	14
L07	*Calothrix marchica*	São Miguel	Azul	Feb/16	BG-11$_0$	15
L08	*Calothrix castellii*	Pico	Peixinho	Feb/16	BG-11$_0$	16
L09	*Calothrix castellii*	São Miguel	Empadadas Norte	Feb/16	BG-11$_0$	17
L10	*Phormidium* sp. 4	São Miguel	Empadadas Sul	Jul/15	BG-11	-
L11	*Phormidium* sp. 5	São Miguel	Pico D'el Rei	Jul/15	BG-11	18
L12	*Microcystis flos-aquae*	São Miguel	Furnas	Nov/14	TFI+M	-
L13	*Nostoc paludosum*	São Miguel	Azul	Feb/16	BG-11$_0$	19

Isolate Code	Taxa	Island	Lake	Date of collection	Medium	Figure
L14	*Nostoc punctiforme*	São Miguel	São Brás	Feb/16	BG-11$_0$	20
L15	*Oscillatoria tenuis*	São Miguel	Areeiro	Jul/15	BG-11	21
L16	*Planktolyngbya limnetica*	São Miguel	Furnas	Nov/14	BG-11	22
L17	*Pseudanabaena* sp. 1	São Miguel	Azul	Nov/14	BG-11	23
L18	*Pseudanabaena minima*	São Miguel	Rasa Serra Devassa	Jul/15	BG-11	24
L19	*Pseudanabaena* sp. 2	São Miguel	Areeiro	Jul/15	TFI+M	-
L20	*Pseudanabaena limnetica*	Pico	Caiado	Oct/15	BG-11	25
L21	*Microchaete tenera*	Pico	Rosada	Jul/16	BG-11$_0$	26
L22	*Coleospermum* sp. 2	Flores	Comprida	Jul/16	BG-11$_0$	27
L23	*Chroccoccales*	São Miguel	Verde	May/16	TFI+M	-
L24	*Calothrix breviarticulata*	Flores	Comprida	Jul/16	BG-11$_0$	28
L26	*Coleospermum* sp. 3	Pico	Peixinho	Oct/15	BG-11$_0$	29
L27	*Fortiea* sp.	Pico	Paul	Jul/16	BG-11$_0$	30
L28	*Nostoc commune*	Pico	Peixinho	Oct/15	BG-11$_0$	31
L29	*Planktolyngbya* sp. 2	São Miguel	Azul	Feb/16	BG-11	-
L30	*Sphaerospermopsis* sp.	Pico	Rosada	Jul/16	BG-11$_0$	32
L31	*Nostoc punctiforme*	São Miguel	São Brás	Feb/16	BG-11$_0$	33
L33	*Planktolyngbya* sp. 2	São Miguel	Furnas	Jul/16	BG-11	-
L34	*Tolypothrix* sp.	São Miguel	Rasa das Sete Cidades	May/16	BG-11$_0$	34
L37	*Aphanizomenon* sp.	São Miguel	Azul	May/16	BG-11$_0$	35

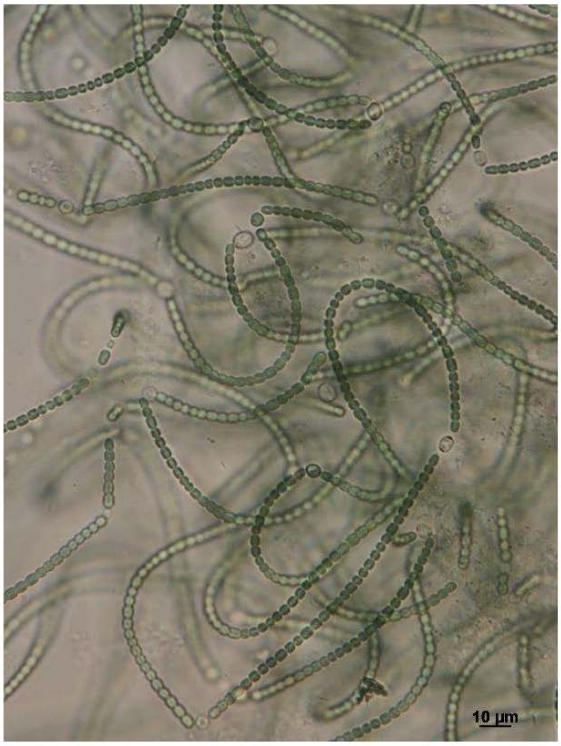

Figure 12 – *Dolichospermum* sp. (L04).

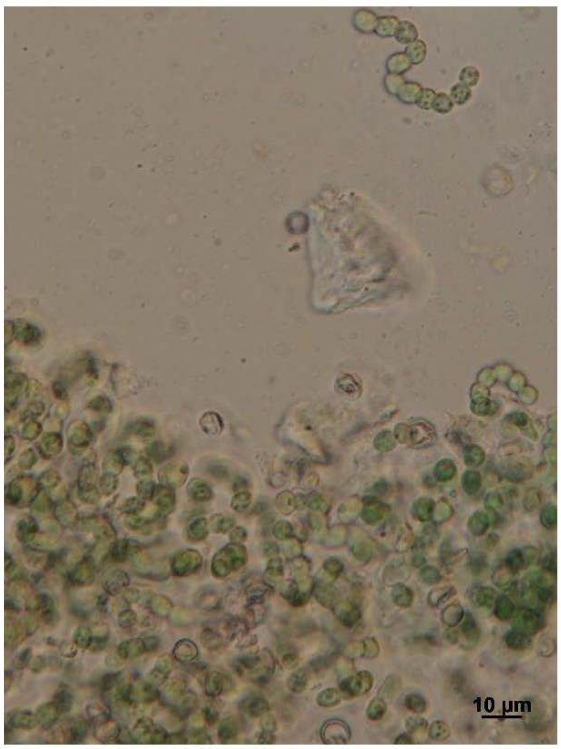

Figure 13 – *Anabaena* sp. (L05).

Figure 14 – *Aphanizomenon manguinii* (L06).

Figure 15 – *Calothrix marchica* (L07).

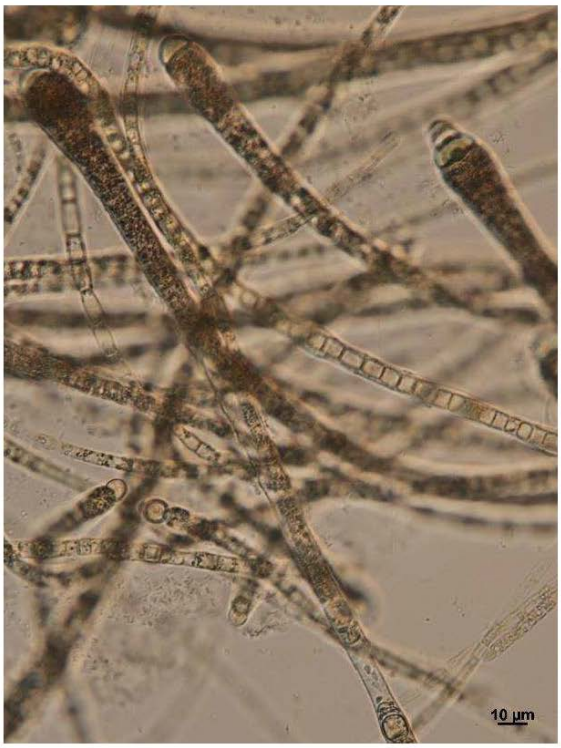

Figure 16 – *Calothrix castellii* (L08).

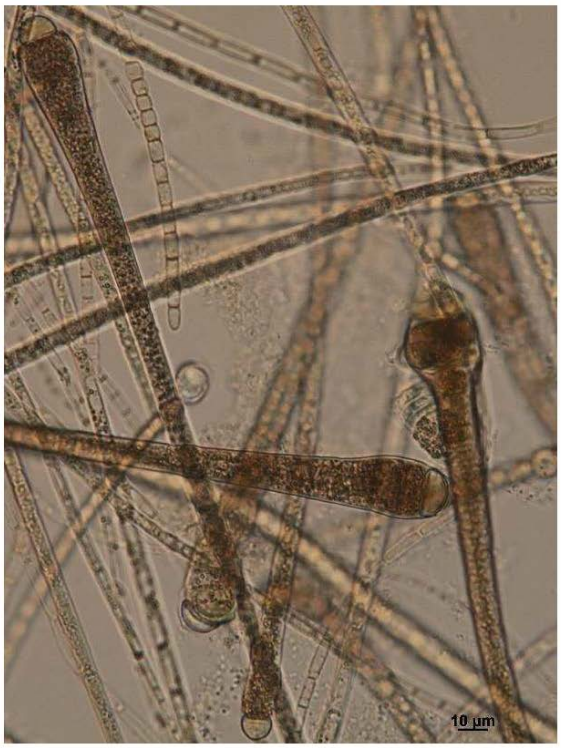

Figure 17 – *Calothrix castellii* (L09).

Figure 18 – *Phormidium* sp. 5 (L11).

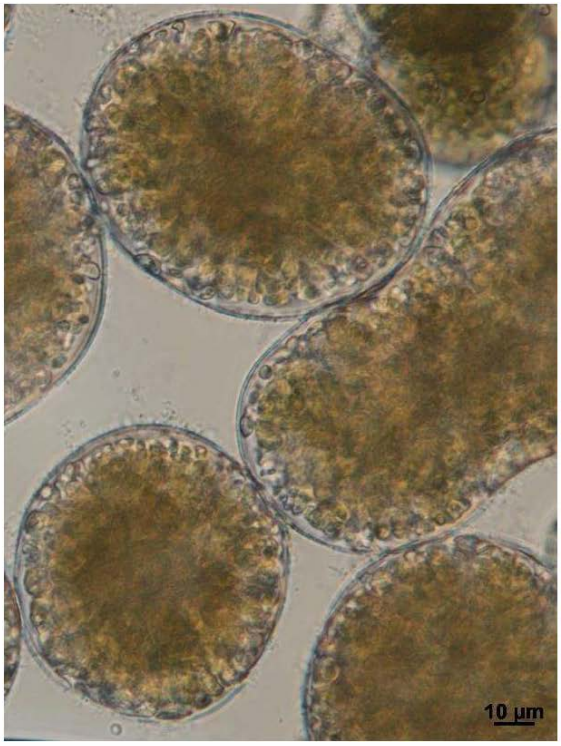

Figure 19 – *Nostoc paludosum* (L13).

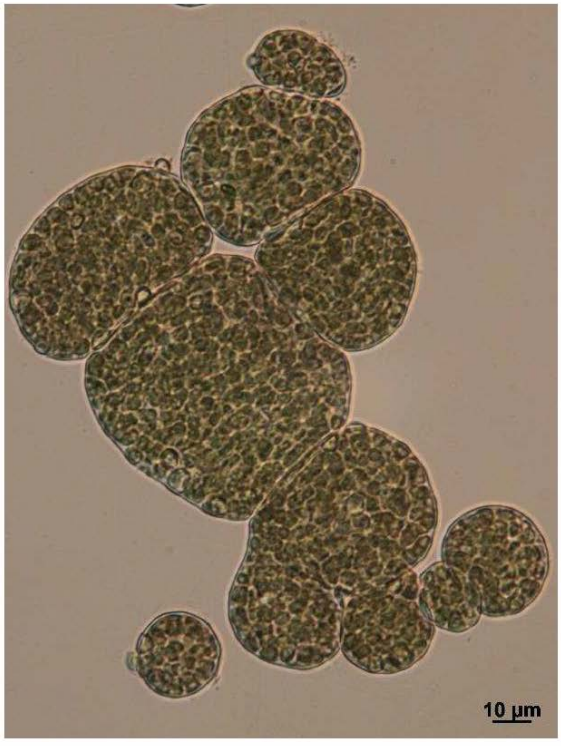

Figure 20 – *Nostoc punctiforme* (L14).

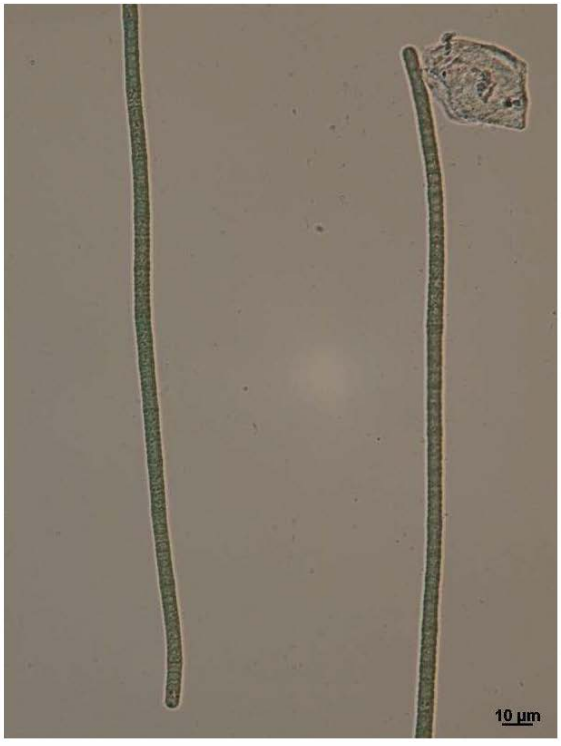

Figure 21 – *Oscillatoria tenuis* (L15).

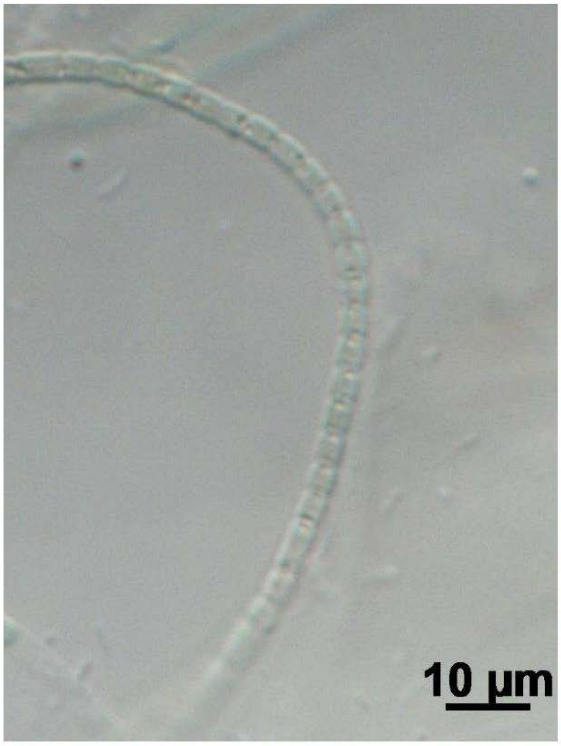

Figure 22 – *Planktolyngbya limnetica* (L16).

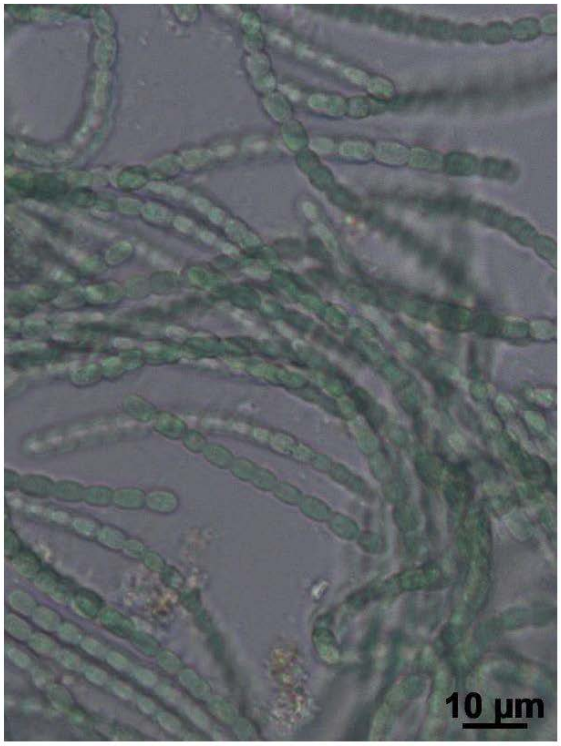

Figure 23 – *Pseudanabaena* sp. 1 (L17).

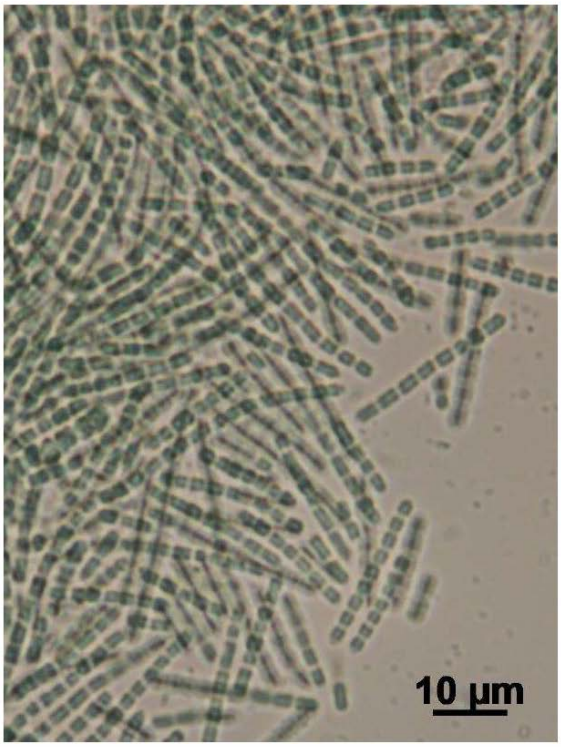

Figure 24 – *Pseudanabaena minima* (L18

Figure 25 – *Pseudanabaena* sp. 2 (L20).

Figure 26 – *Microchaete tenera* (L21).

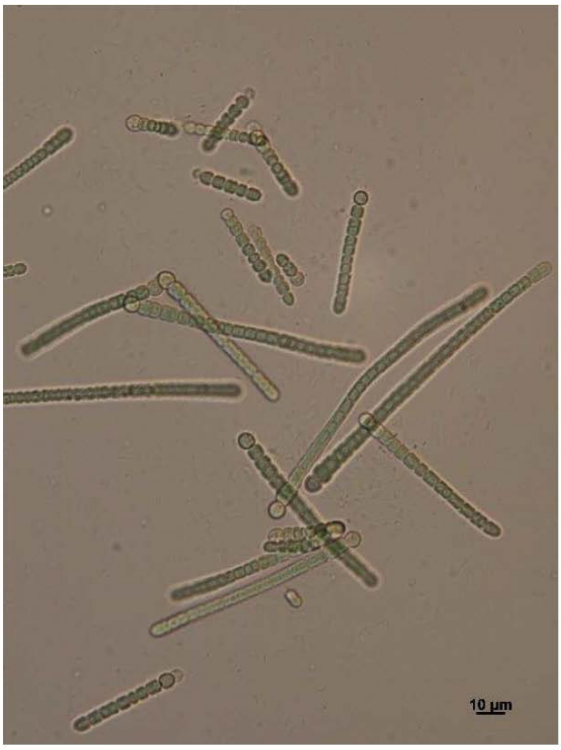

Figure 27 – *Coleospermum* sp. 2 (L22).

Figure 28 – *Calothrix breviarticulata* (L24).

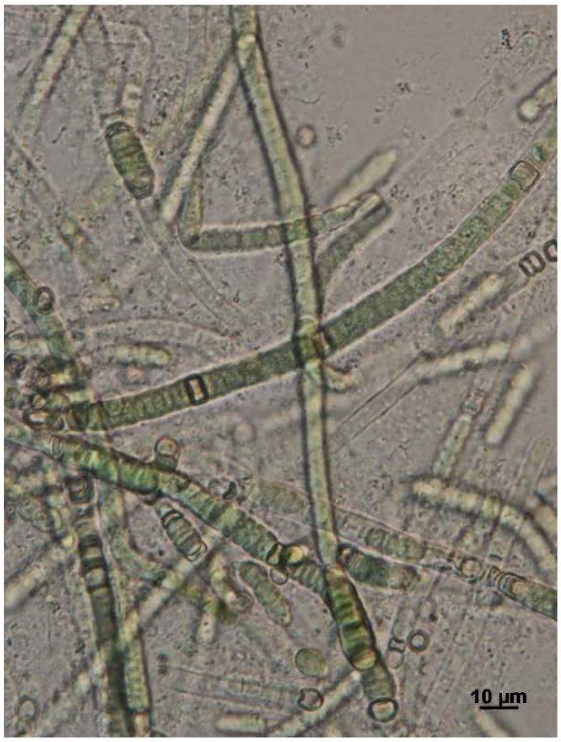

Figure 29 – *Coleospermum* sp. 3 (L26).

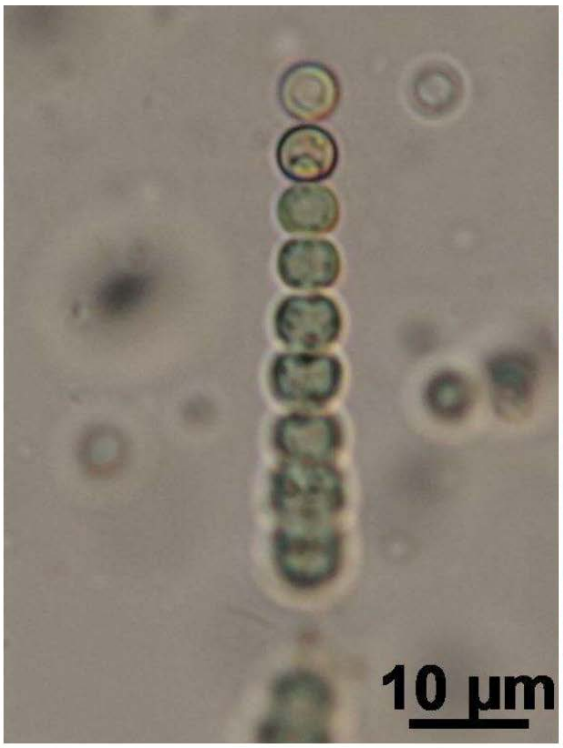

Figure 30 – *Fortiea* sp. (L27).

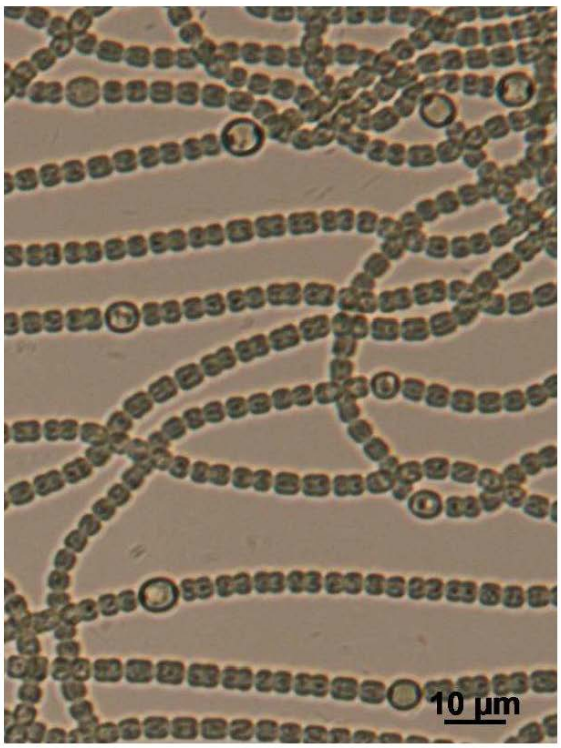

Figure 31 – *Nostoc commune* (L28).

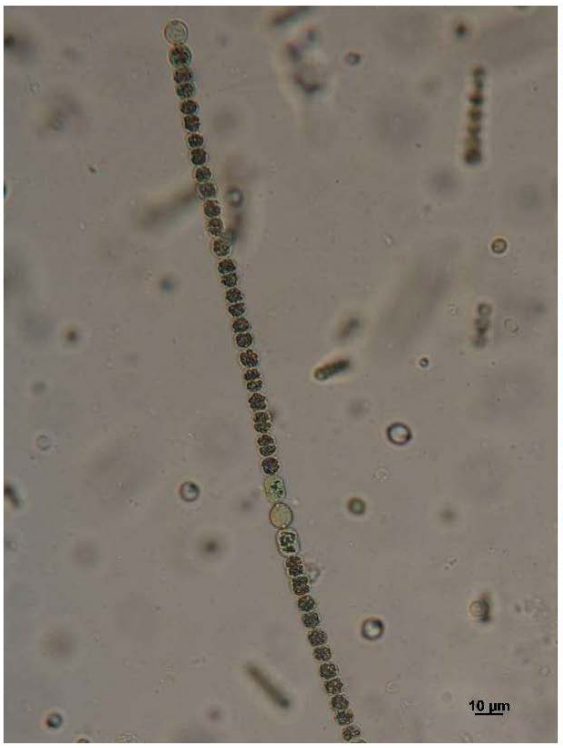

Figure 32 – *Sphaerospermopsis* sp. (L30).

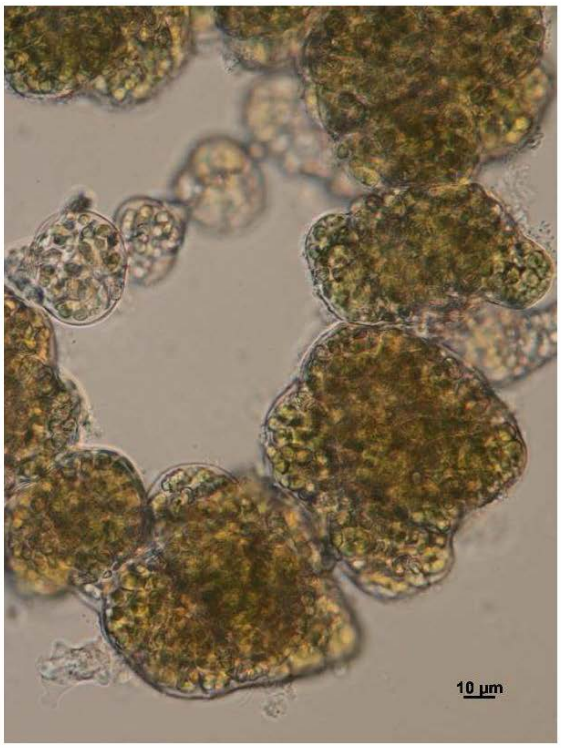

Figure 33 – *Nostoc paludosum* (L31).

Figure 34 – *Tolypothrix* sp. (L34).

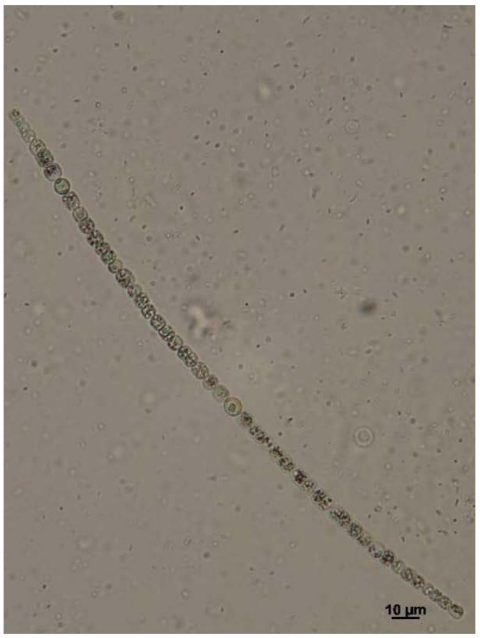

Figure 35 – *Aphanizomenon* sp. (L37).

From the brackish water samples, collected in Santa Maria island, two cyanobacteria strains were isolated (table 7, figures 36 and 37), one of which, *Pseudophormidium pauciramosum*, is described in the Azores islands for the first time.

Table 7 – Strains of cyanobacteria isolated from brackish water sites in Santa Maria island.

Isolate Code	Taxa	Island	Location	Date of collection	Medium	Figure
B01	*Pseudophormidium pauciramosum*	Santa Maria	Maia	Jul/16	BG-11	36
B02	*Hapalosiphon pumilus*	Santa Maria	Maia	Jul/16	BG-11$_0$	37

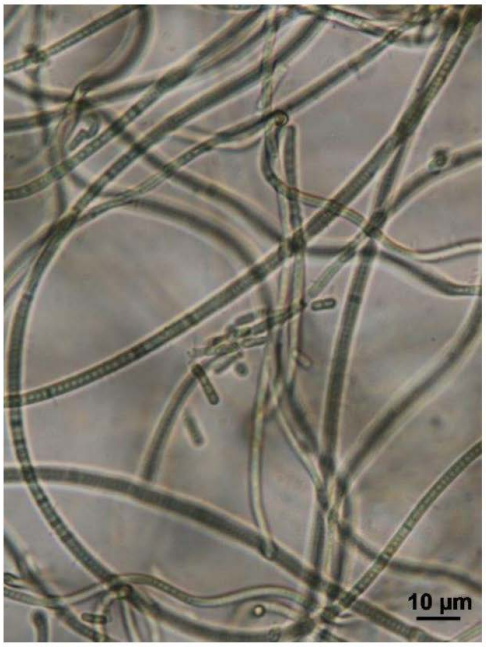

Figure 36 – *Pseudophormidium pauciramosum* (B01).

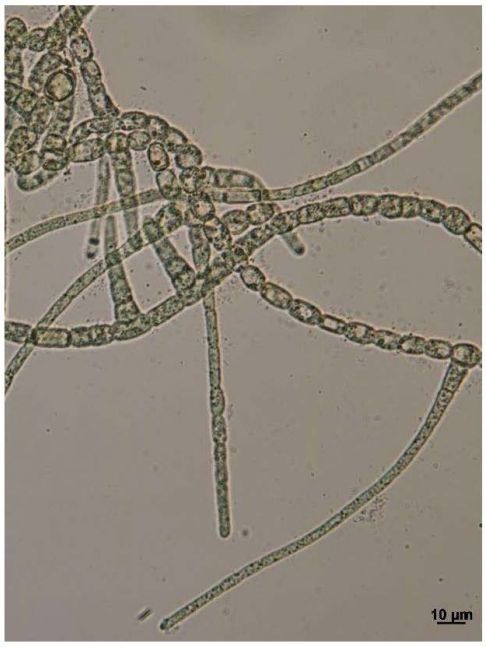

Figure 37 – *Hapalosiphon pumilus* (B02).

The sample collected in the coastal saline lake located in Fajã dos Cubres (São Jorge island) was put in culture but no cyanobacteria was isolated. Observation of the original and enriched sample allowed the identification of seven cyanobacteria taxa (table 8, figures 38 to 44).

Table 8 – Cyanobacteria observed in the sample collected from the coastal lake in Fajã dos Cubres, São Jorge island.

Taxa	O/E	Island	Location	Date of collection	Sample enrichment	Figure
Chroococcales	E	São Jorge	Fajã dos Cubres lake	Apr/17	BG-11$_0$	38
Synechococcales	E	São Jorge	Fajã dos Cubres lake	Apr/17	BG-11$_0$	39
Calothrix sp.	O/E	São Jorge	Fajã dos Cubres lake	Apr/17	BG-11$_0$	40
Leptolyngbya sp. 3	O/E	São Jorge	Fajã dos Cubres lake	Apr/17	BG-11	41
Phormidium sp. 6	O/E	São Jorge	Fajã dos Cubres lake	Apr/17	BG-11	42
Phormidium sp. 7	O/E	São Jorge	Fajã dos Cubres lake	Apr/17	BG-11	43
Spirulina subsalsa	O/E	São Jorge	Fajã dos Cubres lake	Apr/17	BG-11	44

O: Original sample; E: Enriched sample.

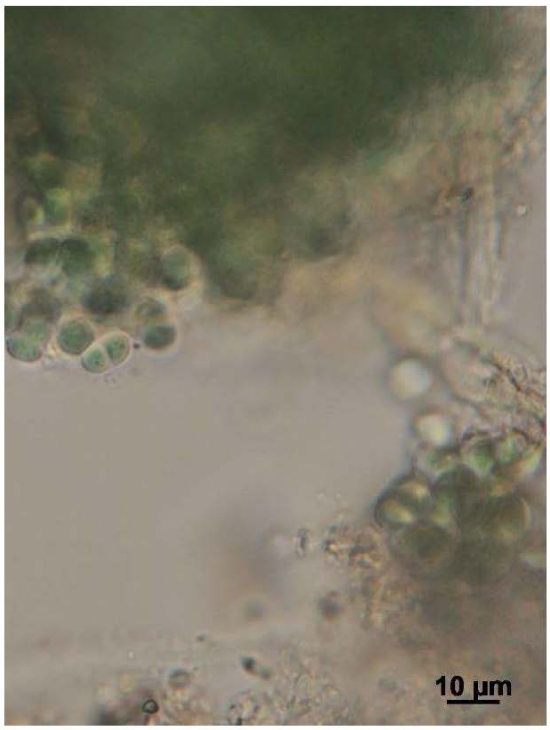

Figure 38 – *Chroococcales.*

Figure 39 – *Synechococcales.*

Figure 40 – *Calothrix* sp..

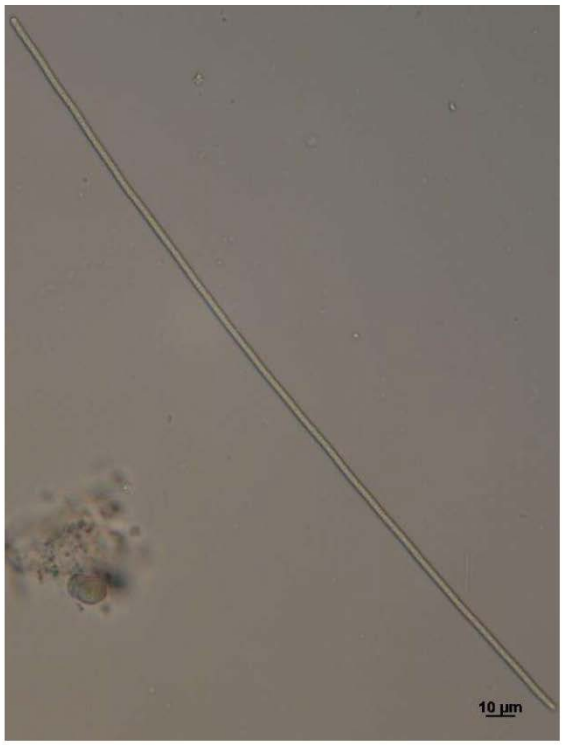

Figure 41 – *Leptolyngbya* sp. 3.

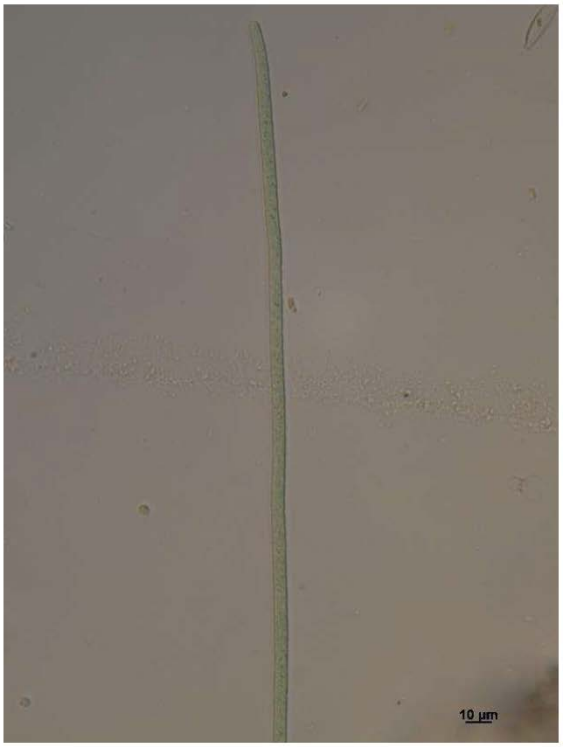

Figure 42 – *Phormidium* sp. 6.

Figure 43 – *Phormidium* sp. 7.

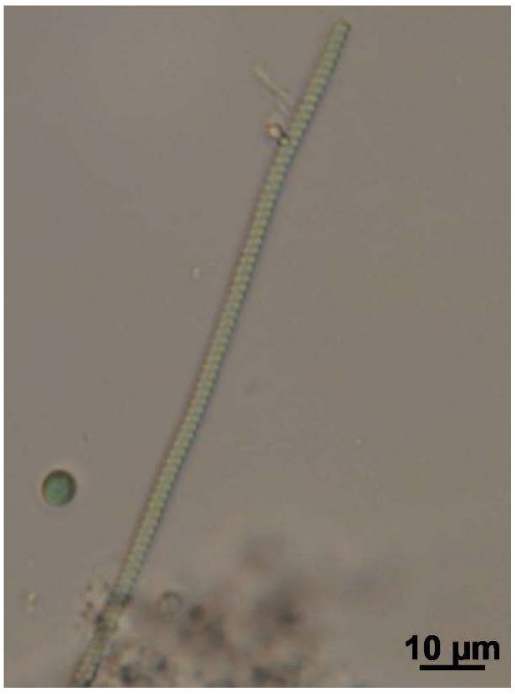

Figure 44 – *Spirulina subsalsa.*

3.3. Biological activity of the extracts

Lyophilized centrifugation fractions from each sample after extraction were weighed and stored. In table 9 the results of this extraction work in milligrams of dry weight are presented.

Table 9 – Quantities of lyophilized sample obtained for each strain tested.

Strain	Lyophilized Supernatant	Lyophilized Biomass	Lyophilized Biomass extract	
	Dry weight (mg)	Dry weight (mg)	Dry weight (mg)	Yield (%)*
T5	9.2	60	5.1	8.5
T6	4.5	90	17.1	19.0
T7	19.9	40	3.9	9.7
T13	3.8	70	6.3	9.0
T14	3.6	20	0.1	0.5
T16	17.5	120	16.0	13.3
T17	9.0	30	1.7	5.7
L4	2.5	40	9.3	23.2
L7	12.8	60	5.3	8.8
L9	10.6	70	6.9	9.9
L14	3.9	190	1.8	0.9
L15	29.3	10	1.0	10.0
L16	-	60	2.7	4.5
L17	5.2	20	0.1	0.5
L18	5.4	50	2.1	4.2
L20	6.2	50	1.1	2.2
L21	15.2	50	3.5	7.0
L28	27.3	40	2.0	5.0
L30	12.0	20	1.3	6.5
L34	7.3	30	7.3	24.3

*Yield of biomass extract is presented as % of lyophilized biomass.

The assessment of the bioactivity was carried out by five tests (antioxidant, anti-acetylcholinesterase, toxicity, anticancer and antimicrobial). These tests allowed the identification of possible applications, specific for each tested strain. Despite the low biomass yield due to the method of production (100 mL Erlenmeyer's flasks with 50 mL of fresh medium), it was important to analyse the extracts in the higher number of tests possible. Therefore, taking in account the obtained dry mass of each extract, the highest concentrations in the tests were varied when necessary. These concentrations are described in appendix V.

Bioactivities are presented in table 10, where all results above 10% of activity and with a reduced standard deviation were considered positive.

Anti-microbial activity was tested against two gram-positive (*Bacillus subtilis* DSM10 and *Micrococcus luteus* DSM 20030) and one gram-negative bacteria (*Escherichia coli* DSM 498). No activity was recorded against these bacteria.

Table 10 – Bioactivity of selected cyanobacteria strains.

Strain	Species	DPPH	AChE	Toxicity (*Artemia salina*)	Cytotoxicity (A-549)
T05	*Coleospermum* sp. 1	-	-	31.75% ± 2.24	-
T06	*Microchaete bulbosa*	-	-	12.90% ± 2.93	-
T07	*Leptolyngbya gelatinosa*	-	-	51.80% ± 4.78	39.98% ± 8.28 [b]
T13	*Mastigocladus laminosus*	15.33% ± 0.72 [a]	16.74% ± 4.40 [c]	-	-
T14	*Phormidium* sp. 2	/	/	37.05% ± 3.70	-
T16	*Chlorogloeopsis fritschii*	-	12.80% ± 0.99 [c]	26.98% ± 8.98	24.17% ± 3.12 [b]
T17	*Chlorogloeopsis fritschii*	13.27% ± 2.24 [c]	/	25.26% ± 2.60	37.22% ± 0.20 [c]
L04	*Dolichospermum* sp.	-	13.30% ± 3.42 [c]	18.55% ± 6.63	27.51% ± 2.65 [b]
L07	*Calothrix marchica*	-	14.70% ± 2.08 [c]	-	-
L09	*Calothrix castelli*	17.84% ± 5.35 [a]	14.33% ± 0.09 [c]	24.30% ± 4.76	-
L14	*Nostoc punctiforme*	11.80% ± 0.36 [c]	-	-	-
L15	*Oscillatoria tenuis*	-	11.43% ± 1.38 [f]	23.38% ± 6.45	26.85% ± 2.46 [e]
L16	*Planktolyngbya limnetica*	-	-	-	-
L17	*Pseudanabaena* sp. 1	/	/	26.09% ± 8.80	/
L18	*Pseudanabaena minima*	-	18.43% ± 2.82 [d]	-	-
L20	*Pseudanabaena limnetica*	-	-	17.35% ± 1.95	-
L21	*Microchaete tenera*	-	11.00% ± 1.79 [c]	41.67% ± 5.89	-
L28	*Nostoc commune*	-	-	40.28% ± 7.08	-
L30	*Sphaeropermospsis* sp.	-	-	53.33% ± 7.50	23.99% ± 0.97 [e]
L34	*Tolypothrix* sp.	-	-	18.10% ± 2.69	-

(/): not analysed; (-) results below 10%; (a): 250 µg/mL; (b): 200 µg/mL; (c) 150 µg/mL; (d) 65 µg/mL; (e) 50 µg/mL; (f) 40 µg/mL. DPPH control (Gallic acid): IC_{50} = 2.08 ± 0.09 µg/mL; AChE control (Galantamine): IC_{50} = 1.35 ± 0.36 µg/mL; Cytotoxicity control, Taxol: IC_{50} = 5.96 ± 0.48 µg/mL and colchicine: IC_{50} = 2.78 ± 0.71 µg/mL.

4. Discussion

4.1. Distribution of cyanobacteria in the Azores islands

Studies that focus only in cyanobacteria of the Azores islands are rare, but many authors have contributed to the knowledge of Azorean cyanobacteria flora during more broad studies, such as Archer (1874), Moseley (1874), Trelease (1897), Bohlin (1901), Krieger (1931), Cedercreutz (1941), Bourrelly & Manguim (1946) and Johansson (1977). These authors have contributed to the general knowledge of benthic and planktonic freshwater, thermal and marine cyanobacteria. Other studies were dedicated to planktonic freshwater cyanobacteria (*e.g.* Vasconcelos *et al.*, 1994; INOVA, 1999; Santos *et al.*, 2005; Gonçalves *et al.*, 2012b; Cordeiro, 2015; Gonçalves *et al.*, 2016e) due to the growing importance and awareness of toxic planktonic cyanobacteria present in the Azorean lakes.

Prior to this study 187 cyanobacteria species have been identified in the Azores (see appendix VI). This is probably far below the real number of existing cyanobacteria species in the Azores islands, since many habitats commonly inhabited by cyanobacteria have not been studied or monitored for a long time. Recent works by the University of the Azores, as part of the monitoring of lake's ecological status, have studied the cyanobacteria in the plankton of 24 lakes of the 88 described in the Azores (Porteiro *et al.*, 2000). No recent work has been done in creeks, tributaries of lakes, thermal springs or pools and saline lakes, leaving unstudied several habitats with great potential for cyanobacteria study.

Taking in account the compilation of data for taxonomic analysis, several observations were possible. São Miguel is the island with the higher amount of identified cyanobacteria but it is also the most studied island, with 41 studies. Flores is the second island with more identified cyanobacteria (50 species) and only has 22 studies, approximately half of São Miguel. For other islands this numbers are much lower but a direct relation between the number of published works and the number of identified cyanobacteria was found. Nonetheless, a more important relation between island area and the number of identified species can also be established (Figure 45). Higher area corresponds to a larger amount of identified cyanobacteria species, with two exceptions, Flores and Corvo, a pattern also observed in macroinvertebrate distribution analysis (Raposeiro *et al.*, 2013). A possibly explanation for this exceptions is the higher percentage that water bodies represents on total island area (Gonçalves,

2008). When compared, for instance, to Pico island, the percentage of land cover with water is the double in Corvo and almost six times higher in Flores. No relations were identified between islands age (Forjaz *et al.*, 2000) and number of species.

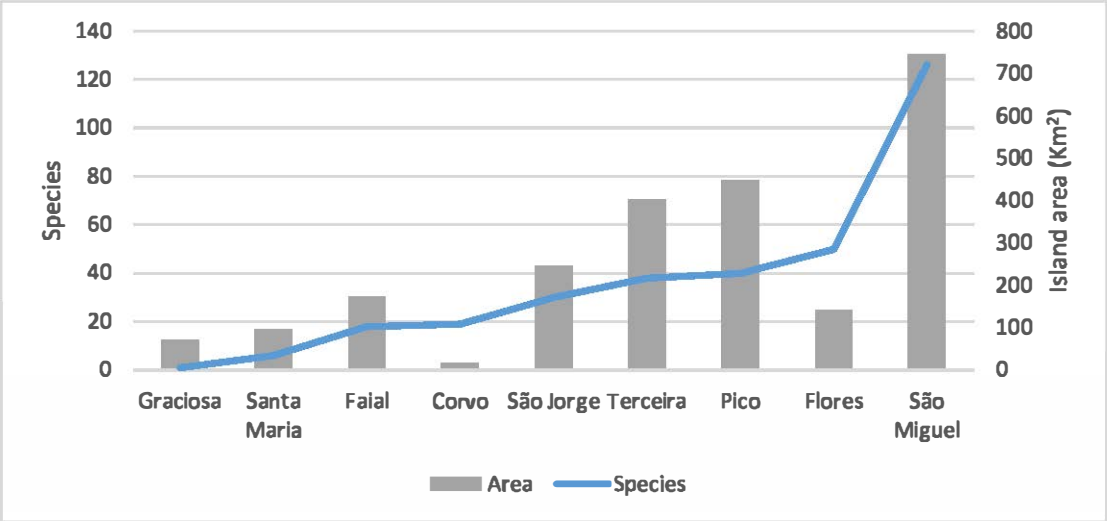

Figure 45 – Number of species compared with Azores islands area.

4.2. Cyanobacteria flora in the Azores islands with reported and isolated species

With the use of enrichment methods, the identification of both dominant and uncommon/rare cyanobacteria from inland waters was facilitated. On the total of freshwater habitats sampled, it was possible to isolate 44 strains, 30 from lake samples, 12 from thermal samplings and two from a brackish water sample. From those it was only possible the identification of 23 strains to species level, 21 strains remaining to be identified the species and in two it was only possible to identify to the order level. In the marine samples seven taxa were identified, but only one to the species level. Nonetheless, the impossibility of species identification, due to the nature of this work based on the growth of cyanobacteria in controlled conditions, is a common issue caused by the different growth conditions (Ripka *et al.*, 1979; Rudi *et al.*, 1997; Lyra *et al.*, 2001). This identification problem can be solved in the future by the sequencing of 16S rDNA and comparing with already identified and sequenced species.

The thermal water cyanobacteria strains produced allowed the identification of new cyanobacteria for the Azores and the confirmation of some previously identified species. The presence of the cosmopolitan species *Mastigocladus laminosus*, also widely present in our samples, has been already reported in the literature (Bohlin, 1901). Other genera like *Leptolyngbya* and *Phormidium* have also been reported

(Trelease, 1897; Bohlin, 1901; Cedercreutz, 1941). The *Coleospermum* and *Microchaete* genera, already described in soils and lakes (Bohlin, 1901; Krieger, 1931), are reported here for the first time in thermal habitats. This is perhaps because their low abundance did not allow their identification on the environmental samples collected in previous works, but with the enrichment that was carried out in the present work it was possible to grow them to a detectable level. Two new records to the Azores flora were isolated, *Leptolyngbya gelatinosa* and *Chlorogloeopsis fritschii*, and it should be pointed out that this is the first description of the genus *Chlorogloeopsis* in the Azores. The identification of this cyanobacteria can be important due to the diversity of morphologies that it presents, that may cause misidentification in field samples (Hindák, 2008).

The present identification work by isolation allowed the description of new genera and species in thermal habitats and surely will allow a better perception of what may be found in future thermal samples that may be studied from these sites, contributing to a better knowledge of the thermal cyanobacteria that inhabit thermal habitats of the Azores.

The lake sampling was performed using a phytoplankton net with a 10 μm pore, in the deepest point of the lake or in the margin of the lake. Despite this methodology, half of the isolates were benthic species, according to Komárek *et al.* (2014), which may be due either to the breaking of filaments that become suspended and are collected by the net at the margins, or at the deepest point of the lake by the attachment and growth of these species on the rope and ball that commonly mark this position, and therefore are collect when the sample is carried out.

The isolation of 30 strains was possible from these samples, of which 14 genera, making it a good diversity of genera when compared to the ones already described in the lakes. None of those are common planktonic habitants of the lakes where the samples were retrieved (Gonçalves *et al.*, 2016a; Gonçalves *et al.*, 2016b; Gonçalves *et al.*, 2016c; Gonçalves *et al.*, 2016d), which means that the isolation was mainly of species that are rare or at least uncommon. This is proved by the description of a new genus for the Azores, *Fortiea*, and of several new species such as *Aphanizomenon manguinii*, *Calothrix breviarticulata*, *Calothrix castellii, Calothrix marchica* and *Pseudanabaena minima* and also by the isolation of species for which the identification was not possible, but whose description does not meet the ones already identified in the Azores (*e.g.* *Anabaena* sp., *Coleospermum* sp. 2, *Pseudanabaena* sp. 1 and

Sphaerospermopsis sp.). This confirms once again that the Azorean cyanobacteria flora is understudied, and that the less common and distributed species are still far from being identified.

It was possible to identify new species for each island (with the exception of Corvo), almost in equal number. For genera, which may contribute to a bigger diversity, the most important was Pico, as it was possible to identify four new genera (*Calothrix*, *Coleospermum*, *Fortiea* and *Microchaete*), followed by Flores with one genus (*Coleospermum*). An interesting point is that no new planktonic genus was identified in any island, but all new genera found in Pico and Flores were benthic. This may be due to lake morphology and geographic location. Lakes from Pico island are all shallow lakes located at high altitudes and Lomba is a deep lake at high altitude in Flores (Gonçalves, 2008), where the wind have a great influence in lake turbulence, giving a higher chance of collecting benthic cyanobacteria in the plankton.

The biggest contribution to the cyanobacterial flora was to the genus *Calothrix*, where it was possible to isolate 4 strains of which 3 species, *Calothrix breviarticulata* from Flores, *Calothrix marchica* from São Miguel and *Calothrix castellii* from São Miguel and Pico. Besides that, the isolation and identification of at least two distinct *Coleospermum* species was possible, none of which matched the description of *Coleospermum goeppertianum*, the only species of this genus that had been previously identified in the Azores. In agreement with this, the isolation of the already mentioned *Fortiea* genus enhances the lack of knowledge of the benthic flora of the Azores islands in general, and the contribution that this work gives to that knowledge. In addition, the present study also gives a great contribution concerning planktonic species. Besides the isolation and identification of some common planktonic species such as *Oscillatoria tenuis*, *Planktolynbya limnetica* and *Pseudanabaena limnetica*, it was also possible to isolate species never identified before such as *Aphanizomenon manguinii*, and some other species like *Pseudanabaena* sp. 1 or *Sphaerospermopsis* sp., which were not identified to the species level but that differ greatly from the already identified species of the respective genus.

The brackish water isolates were performed from a unique sample collected from a little stream in rocks near the ocean. Two species were able to grown in the enrichment medium, a Nostocales, *Hapalosiphon pumilus*, and an Oscillatoriales, *Pseudophormidium pauciramosum*, and were isolated. Both species are new to Santa Maria and *Pseudophormidium pauciramosum* is here first reported in the Azores.

The marine sample collected from Fajã dos Cubres in São Jorge island allowed the identification of 7 species. No cyanobacteria were observed in the unenriched plankton sample (72 hours max. after sampling) nor after one-month of grow in enriched medium. In the unenriched sample from the benthos four cyanobacteria were identified: *Spirulina subsalsa*, *Calotrix*, *Leptolyngbya*, and *Phormidium*. All these taxa maintained viability in the enriched medium and two new cyanobacteria were observed, a *Chroococcales* and a *Synechococcales,* after one month of grow. In the enriched sample with BG-11 the *Leptolyngbya* sp. 3 became the dominant.

For the production of all isolated strains standard techniques described in the literature, as serial dilution and streak plate, were used, and also well know media as BG-11, BG-11_o and, in addition, TFI+M a commercial medium used for microalga production. The results obtained with the methods used was as expected, where cyanobacteria morphology and differentiation characteristics was take in account to improve isolation efficiency. Streak plate was very effective in the isolation of motile cyanobacteria or cyanobacteria that produced motile hormogonia, the majority of strains being produced this way in preference to serial dilution, although serial dilution was always used as an intermediate technique of purification or elimination of contaminants between streaks.

The medium that allowed the highest number of isolates was BG-11_o even within non nitrogen-fixing cyanobacteria. This result can be explained by the higher resistance of cyanobacteria to the lack of dissolved nitrogen when compared to microalgae (Scott & Marcarelli, 2012), giving them a competitive advantage in nature and in enriched samples. Despite their isolation in BG-11_o, all the isolated strains that did not possess or produce heterocysts were later transferred to BG-11 for maintenance purposes in better growth conditions. Besides the isolation of both non-fixing and fixing nitrogen species in BG-11_o, a higher percentage of nitrogen fixing cyanobacteria was isolated, mainly because of their ability to dominate in the enriched sample. Although the enrichment with the algal medium TFI+M allowed the isolation of some species that did not grow in BG-11 or BG-11_o, it was much less effective than the media BG-11 in the grow phase in enriched samples. For that reason, TFI+M was abandoned as an isolation and enrichment medium and used only for the maintenance of established uni-algal cultures.

The isolates produced proved that there is still much to discover in the Azorean flora from all the habitats here studied, especially in the benthic habitat. In the present

work it was possible to identify eleven new freshwater species and one new marine species to the Azores. The amount of unidentified strains suggests that many more cyanobacteria species will be identified for the Azores in a near future, contributing to better understand the diversity of the Azorean biota.

4.3. Bioactive extracts of cyanobacteria isolated from lakes and thermal waters

By the bioprospecting work, this study contributes with valuable information about these species and strains, but it is also a possible start to the discovery of novel compounds by the use of new and unique strains of cyanobacteria. Twenty uni-algal cultures of cyanobacteria isolated from the Azorean islands from thermal waters and lakes were studied, taking into advantage the possible evolution and unique development of these species by the island isolation and great diversity of compounds that cyanobacteria are able to provide (Dittmann *et al.*, 2015).

As this is a bioprospecting work that intends to evaluate the potential of the selected strains, the tests were chosen in a manner that in the end, when the results were analysed, specific applications might be proposed for each strain (*e.g.* pesticides, anticancer, etc.). Also, taking in account the taxonomic diversity of the isolates obtained in this work, the choice of strains for bioprospecting represented the higher taxonomic diversity possible, in order to give a better understating if there might be a correlation between genus and any kind of activities. No evident relation was identified.

In this work the main purpose was the extraction and study of peptides produced by the cyanobacteria and present in the biomass. The lyophilized growth medium was also analysed for its toxicity. The extraction yield was not equal to all strains, varying between 5 and 20%, nonetheless all planned tests were performed for 18 of the 20 strains.

The antioxidant analyses by the DPPH assay, a procedure where the capacity of reduction of the 2,2-diphenyl-1-picrylhydrazyl compound is analysed, is a very sensitive test. Antioxidant activity is highly searched because of its contribution to the cosmetic, pharmaceutic and food industry (Pisoschi & Negulescu, 2011). From the 18 tested strains four present viable results, where it was obtained around 15% of reduction activity. Since we used crude extracts this represents a potential activity, as no characterization of the active compounds was performed. Therefore, there is no way to know the quantity that each active compound represents. From the strains with some

activity this may come from the extracted pigments (*e.g.* phycocyanin), which are widely recognized as good antioxidants (Romay *et al.*, 1998; Romay *et al.*, 2003; Wu *et al.*, 2016). However, since all strains possess pigments and not all showed antioxidant activity, we may conclude that this activity is due to other type of compounds but we need to consider that the concentration of pigments in the strains could be different. From the four strains where positive results were obtained, T13 (*Mastigocladus laminosus*) has been already described with some antioxidant activity (Singh *et al.*, 2017) as also L14, *Nostoc punctiforme* (Assunção *et al.*, 2017). The T17 and L09 strains that represents the *Chlorogloeopsis fritschii* and *Calothrix castelli* have not been thoroughly study for their possible bioactivities and there are no records of their scavenging activities. Nonetheless, all these species have in common the description of being scytonemin producers, a pigment that gives a characteristic yellow-brown colour (Garcia-Pichel & Castenholz, 1991) that has been described with radical scavenging activity (Matsui *et al.*, 2012). Despite the positive results and in set with the literature, these cyanobacteria extracts do not appear to have great antioxidant activity, although a progressive work to fmd UV-absorbing and antioxidant activity in some compounds of cyanobacteria could really be beneficial to the cosmetically and pharmaceutically field of creams and solar protectors. However, it should be pointed out that acetic acid only extracts a group of compounds, and that extraction with solvents with lower polarities, such as ethanol, methanol, acetone, and chloroform, would result in other types of compounds, *e.g.*, pigments which would most likely have antioxidant properties, as described by Garcia-Pichel & Castenholz, (1991) that with 100% acetone and 100% ethyl acetate extracted scytonemin with great efficiency.

Anticholinesterasic activity may be used for several types of application, therefore the results of an anti-AChE assay may be the base in the search for neurotoxins, drugs against Alzheimer's Disease (AD) or Myasthenia gravis (MG), pesticides, biosensors, among others (Pundir & Chauhan, 2012; Čolović *et al.*, 2013; Kais *et al.*, 2015; Peng *et al.*, 2017). Eight of the 18 tested strains gave positive results in this activity. As it happens with the DPPH assay the results didn't differ much from each other reaching maximum inhibition of 18.43% by L18 strain. This species hasn't been thoroughly studied for anti-AChE activity neither have compounds with anti-AChE activity been described for it. Despite that, taking in account the genetic diversity of cyanobacteria, and the wide range of secondary metabolites that they can produce it is possible that the compounds causing this effect are anatoxin analogues. Anatoxin is

described as a neurotoxin capable of irreversibly biding to acetylcholine receptors, and is not degraded by acetylcholinesterase (Mahmood & Carmichael, 1987). Some species of *Oscillatoria* and *Dolichospermum* have already been described as anatoxin producers (Pearson *et al.*, 2016; Bernard *et al.*, 2017), and these isolated strains might be candidates for analogues studies, and depending of the structure, those might be useful compounds for use as drugs or pesticides.

The compounds excreted were only analysed for toxicity, using as test model the aquatic crustacean *Artemia salina*. This is a quick and cheap test with high sensibility (Rajabi *et al.*, 2015) that allows the identification of possible toxic compounds that are produced and mainly excreted to the exterior of the cell. From the 19 tested strains only five had no activity. From the 14 with positive results two reached above 50% of mortality of the brine shrimp, respectively T07 and L30, neither species with identified cyanotoxins production. The most studied cyanotoxins are recognized to be genus or species specific, but less ones exist and are believed to be more widely produced, such as BMAA and lipopolysaccharides (Monteiro *et al.*, 2017; Ploux *et al.*, 2017). Lipopolysaccharides are generally produced by almost all cyanobacteria and are proved to cause some kind of toxicity, mainly irritation and weak inflammation (Monteiro *et al.*, 2017). BMAA is a neurotoxin understudied but believed to be more widely produced (Cox *et al.*, 2005; Ploux *et al.*, 2017). These type of toxins, common to several species, are probably the cause for some mortality observed in the test. Nonetheless more detailed tests are necessary to the identification of the compounds with higher activity in T07 and L30, where valuable compounds might be produced.

The anticancer activity was tested against the pulmonary cancer cell line known as A-549. Six strains showed activity against this cell line. With activity between 23.99% and 39.98% for the highest concentration tested, these are mortality percentages to be considered, as each extract make part of complex matrix of compounds where the active compound is unknown. From the tested species there is no information about species specific compounds with anticancer activity. Nonetheless common compounds of cyanobacteria have been reported with anticancer activity. C-Phycocyanin (phycocyanin harvest from cyanobacteria) is described with anticancer activity (Liu *et al.*, 2016) with maximum activity of 31% obtained with an 80 µg/mL concentration of pure compound against HeLa lineage (Wang *et al.*, 2000). From the tested species, *Oscillatoria tenuis* already has been described as good source of this pigment with a good anticancer activity (Thangam *et al.*, 2013). The results obtained for anticancer

activity by T16 and T17 strains are very interesting. Although belonging to the same species with equal origins but cultivated in different media, these strains showed different activity that may be related the different morphology stages they were in the moment of extraction. This results highlight the influenced of culture stage in the production of the active compound(s).

A resumed proposal of possible applications that the tested strains may have is presented in table 11. Taking in account the results of all performed tests, applications for some of the strains are suggested, in a time and cost saving manner, therefore suggested six applications groups: toxins, antioxidant, pesticides, specific drug applications (AD/MG), anticancer and biofertilizers. A search on toxins is proposed in two strains, possible antioxidant compounds in four strains, pesticides in six strains, AD/MG drugs in three strains, anticancer drugs in four strains and use as biofertilizer in three strains.

Table 11 – Possible applications of strains in the cyanobacteria culture collection from the Azores.

Strain	Species	Toxins	Antioxidant	Pesticides	AD/MG	Anticancer	Biofertilizer
T07	*Leptolyngbya gelatinosa*	●	-	-	-	-	-
T13	*Mastigocladus laminosus*	-	●	●	●	-	-
T16	*Chlorogloeopsis fritschii*	-	-	-	-	●	-
T17	*Chlorogloeopsis fritschii*	-	●	-	-	●	-
L04	*Dolichospermum sp.*	-	-	-	-	●	-
L07	*Calothrix marchica*	-	-	●	●	-	●
L09	*Calothrix castelli*	-	●	●	-	-	-
L14	*Nostoc punctiforme*	-	●	-	-	-	●
L15	*Oscillatoria tenuis*	-	-	●	-	●	-
L18	*Pseudanabaena minima*	-	-	●	●	-	-
L21	*Microchaete tenera*	-	-	●	-	-	-
L30	*Sphaeropermospsis sp.*	●	-	-	-	-	●

For toxins search, based on the results from toxicity and cytotoxic test, a more profound research work in T07 and T30 strains is proposed, as there are no toxins described for both species and the results sustain some produced toxin. The antioxidant application takes in account the DPPH activity result with no toxic or cytotoxic activity. Therefore, T13, T17, L09 and L14 strains are good candidates for more studies. The pesticide applications take into account two tests, the anti-AChE and toxicity activities. Strains that produced good anti-AChE activity with low toxic effect, *i.e.,* T13, L07, L09, L15, L18 and L21, were chosen ensuring the exclusion of those that may have compounds with undesired toxic effects. The AD/MG drug applications take in account

three tests, the anti-AChE, toxicity and cytotoxic activities. The strains which produced good anti-AChE activity with low toxic and cytotoxic effect, *i.e.,* T13, L07 and L18, were chosen excluding therefore compounds with undesired toxic and cytotoxic effects. The anticancer applications take in account two results, the toxicity against *A. salina* and the cytotoxic tests. The strains with lower toxicity and higher cytotoxicity against the A-549 lineage were selected for this application, and so, future works should focus on T16, T17, L04 and L15 strains. The use as a biofertilizer had in account the toxicity, cytotoxicity and anti-AChE activity, where the choice of species was based on their null or reduced effects and the ability to produce nitrogen fixing cells (heterocysts). As a result, we suggest further research on strains L07, L14 and L34 for this application. Although several thermal cyanobacteria strains have similar potential for biofertilizers as the three selected strains (all freshwater cyanobacteria with heterocysts production), their production would be too expensive since their optimum grown requires high temperatures (above 40 °C).

5. Final remarks

The isolation and culture of cyanobacteria from the Azores allowed the creation of a cyanobacteria bank, bringing up opportunities for the bioprospection of bioactive compounds. The preservation of these strains and the implementation of quality preservation methods is of the utmost importance, as many of this compounds might be strain and/or species specific. To achieve this goal, the recent published guidelines for cyanobacteria cultures preservation by Gaget *et al.* (2017) and the improvement of the *in vitro* culture maintenance in sub-optimum conditions should be implemented in a near future.

This was a preliminary study and its outputs were constrained by the low biomass yield due to the choice of culture containers (100 mL flasks). These limitations influenced the choice for only one type of extraction and the extract concentrations tested. Despite this, the bioactivity found highly recommends a deeper research with the selected strains, involving a higher production of biomass, different types of extracts and the identification of the bioactive compounds. The increase in biomass production is also required for IC_{50} quantification.

Nonetheless, it was proved in this work that cyanobacteria have the potential to provide useful compounds for several applications, but more thoughtful works in the extracts are required for the real assessment of the potential. Interestingly, besides all negative results for anti-microbial activity, there exist records of an Azorean strain with remarkably good results, tested by Fish & Codd (1994), which indicates that there is also a great potential in this area, although not identified in this work.

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Appendix I – Lake sampling campaigns

Table 1 – Lakes sampled by campaign

Island	Lakes	2014-July/2015	Oct/2015-July/2016
São Miguel	Lake Verde	✓	✓
	Lake Azul	✓	✓
	Lake Areeiro	✓	-
	Lake Canário	✓	✓
	Lake Congro	✓	✓
	Lake Empadadas Norte	✓	✓
	Lake Empadadas Sul	✓	✓
	Lake Fogo	✓	✓
	Lake Pico D'el Rei	✓	-
	Lake Furnas	✓	✓
	Lake Rasa das Sete Cidades	✓	✓
	Lake Rasa da Serra Devassa	✓	✓
	Lake Santiago	✓	✓
	Lake São Brás	✓	✓
	Lake Verde	✓	✓
Pico	Lake Caiado	-	✓
	Lake Capitão	-	✓
	Lake Caveiro	-	✓
	Lake Paúl	-	✓
	Lake Peixinho	-	✓
	Lake Rosada	-	✓
Flores	Lake Comprida	-	✓
	Lake Funda	-	✓
	Lake Lomba	-	✓
	Lake Negra	-	✓
	Lake Rasa	-	✓
Corvo	Lake Caldeirão	-	✓

Appendix II – Sampled locations coordinates

Table 1 – Decimal degrees coordinates of sampled locations

Type	Sample place	Island	Municipality	GPS
LAKES				
	Areeiro	São Miguel	Vila Franca	-25.426916; 37.763062
	Azul	São Miguel	Ponta Delgada	-25.781702; 37.869683
	Canário	São Miguel	Ponta Delgada	-25.759733; 37.836100
	Congro	São Miguel	Vila Franca	-25.407290; 37.756384
	Empadadas Norte	São Miguel	Ponta Delgada	-25.748405; 37.826337
	Empadadas Sul	São Miguel	Ponta Delgada	-25.747020; 37.824472
	Fogo	São Miguel	Vila Franca – R.Grande	-25.473272; 37.763450
	Furnas	São Miguel	Povoação	-25.333590; 37.757686
	Pico d'el Rei	São Miguel	Vila Franca	-25.392607; 37.772971
	Rasa da Serra de Devassa	São Miguel	Ponta Delgada	-25.751296; 37.824858
	Rasa das Sete Cidades	São Miguel	Ponta Delgada	-25.779602; 37.843046
	Santiago	São Miguel	Ponta Delgada	-25.774190; 37.848729
	São Brás	São Miguel	Ribeira Grande	-25.409733; 37.793151
	Verde	São Miguel	Ponta Delgada	-25.788701; 37.847284
	Caiado	Pico	São Roque	-28.250094; 38.456807
	Capitão	Pico	São Roque	-28.318834; 38.487672
	Caveiro	Pico	Lajes	-28.196778; 38.435133
	Paúl	Pico	Lajes	-28.232291; 38.427808
	Peixinho	Pico	São Roque	-28.173872; 38.434597
	Rosada	Pico	Lajes	-28.185787; 38.432691
	Comprida	Flores	Lajes	-31.221303; 39.441260
	Funda	Flores	Lajes	-31.217641; 39.405818
	Lomba	Flores	Lajes	-31.188759; 39.425255
	Negra	Flores	Lajes	-31.225905; 39.442389
	Rasa	Flores	Lajes	-31.224723; 39.409555
	Caldeirão	Corvo	Vila do Corvo	-31.110746; 39.711949
THERMAL				
	Água do Caldeirão	São Miguel	Povoação	-25.303511; 37.773424
	Água da Prata	São Miguel	Povoação	-25.303914; 37.773357
	Água do Rego	São Miguel	Povoação	-25.302973; 37.773379
	Caldeira da Ribeira Grande	São Miguel	Ribeira Grande	-25.487078; 37.797879
	Poça da Dona Beja	São Miguel	Povoação	-25.320250; 37.769621
	Nascente do Morangueiro	São Miguel	Povoação	-25.306499; 37.770393
	Poça da Tia Silvina	São Miguel	Povoação	-25.307006; 37.770762
	Ribeira Amarela 1	São Miguel	Povoação	-25.319013; 37.769501
	Ribeira Amarela 2	São Miguel	Povoação	-25.309627; 37.771214
	Ribeira da Caldeira	São Miguel	Povoação	-25.304182; 37.772261
BRACKISH				
	Maia	Santa Maria	Vila do Porto	-25.016676; 36.946329
MARINE				
	Fajã dos Cubres lake	São Jorge	Calheta	-27.966591; 38.641770

Appendix III – Chemical characterization of sampled places

Table 1 – Characterization values of sampled lakes.

Island	Sampling lake	Sampling date	Temperature (°C)	O_2 (mg/L)	Conductivity (µS/cm)	pH
São Miguel						
	Lake Azul	nov/14	-	-	-	-
		jul/15	26	-	102	8.1
		out/15	20	8.7	99	7.3
		fev/16	14	10.4	100	7.4
		mai/16	20	9.8	100	7.6
		set/16	24	8.8	110	7.6
	Mean	-	21	9.4	102	7.6
	Lake Areeiro	nov/14	-	-	-	-
		jul/15	23	-	97	8.3
	Mean	-	23	-	97	8.3
	Lake Canário	nov/14	-	-	-	-
		jul/15	23	-	37	7.4
		out/15	16	8.6	31	7.2
		fev/16	12	10.3	38	8.0
		mai/16	18	9.2	39	7.3
		set/16	24	8.2	39	6.1
	Mean	-	19	9.1	37	7.2
	Lake Congro	nov/14	-	-	-	-
		jul/15	23	-	97	8.3
		out/15	19	8.5	92	7.4
		fev/16	13	10.7	100	7.3
		mai/16	18	11.4	100	9.4
		set/16	24	9.0	100	9.2
	Mean	-	19	9.9	98	8.3
	Lake Empadadas Norte	nov/14	-	-	-	-
		jul/15	21	-	37	7.4
		out/15	15	8.9	32	7.6
		fev/16	12	10.2	42	7.6
		mai/16	18	9.2	40	7.3
		set/16	25	8.6	38	6.5
	Mean	-	18	9.2	38	7.3
	Lake Empadadas Sul	nov/14	-	-	-	-
		jul/15	23	-	47	8.4
		out/15	15	8.7	43	7.8
		fev/16	12	10.1	51	8.0
		mai/16	18	9.1	49	7.5
		set/16	20	8.2	53	7.1
	Mean	-	18	9.0	49	7.8
	Lake Fogo	nov/14	-	-	-	-
		jul/15	21	-	51	6.9

	out/15	17	8.8	49	7.6
	fev/16	12	10.6	45	7.2
	mai/16	15	9.6	49	8.1
	set/16	20	9.2	51	7.2
Mean	-	17	9.6	49	7.4
Lake Furnas	nov/14	-	-	-	-
	jul/15	26	-	142	7.7
	out/15	20	9.2	140	7.7
	fev/16	13	10.5	130	7.4
	mai/16	18	9.8	150	7.6
	set/16	23	8.9	160	8.1
Mean	-	20	9.6	144	7.7
Lake Pico D'el Rei	nov/14	-	-	-	-
	jul/15	22	-	84	9.3
Mean	-	22	-	84	9.3
Lake Rasa das Sete Cidades	nov/14	-	-	-	-
	jul/15	25	-	46	5.8
	out/15	16	9.2	45	5.8
	fev/16	12	10.2	48	6.6
	mai/16	18	9.4	45	6.8
	ago/16	23	8.5	47	5.5
Mean	-	19	9.3	46	6.1
Lake Rasa da Serra Devassa	nov/14	-	-	-	-
	jul/15	22	-	35	7.5
	out/15	14	9.5	33	7.4
	fev/16	11	9.7	38	7.6
	mai/16	20	8.6	43	7.1
	set/16	19	8.5	39	6.2
Mean	-	17	9.1	38	7.2
Lake Santiago	nov/14	-	-	-	-
	jul/15	25	-	126	9.4
	out/15	18	8.5	120	7.0
	-	-	-	-	-
	-	-	-	-	-
	ago/16	23	8.4	130	8.0
Mean	-	22	8.5	125	8.1
Lake São Brás	nov/14	-	-	-	-
	jul/15	23	-	41	7.1
	out/15	16	9.6	33	6.8
	fev/16	12	10.3	37	6.9
	mai/16	18	9.8	38	7.2
	set/16	21	8.8	39	6.2
Mean	-	18	9.6	38	6.8
Lake Verde	nov/14	-	-	-	-
	jul/15	26	-	128	9.4
	out/15	19	8.6	120	7.5
	fev/16	13	10.5	120	7.4

		mai/16	19	11.7	130	9.6
		set/16	23	9.0	130	9.0
	Mean	-	20	10.0	126	8.6
Pico						
	Lake Caiado	out/15	13	9.8	27	6.5
		mar/16	11	10.7	34	6.8
		abr/16	13	9.6	30	7.0
		jul/16	19	8.6	29	6.4
	Mean	-	14	9.7	30	6.7
	Lake Capitão	out/15	13	10.1	30	6.8
		mar/16	12	11.2	30	6.6
		abr/16	13	9.5	46	4.7
		jul/16	19	9.5	36	6.2
	Mean	-	14	10.1	36	6.1
	Lake Paúl	out/15	14	8.9	34	6.6
		mar/16	13	10.1	32	6.3
		abr/16	13	9.6	35	5.4
		jul/16	19	8.2	23	6.4
	Mean	-	15	9.2	31	6.2
	Lake Peixinho	out/15	13	10.2	29	7.3
		mar/16	10	11.2	32	6.6
		abr/16	12	9.9	33	6.0
		jul/16	20	8.1	30	6.7
	Mean	-	14	9.9	31	6.7
	Lake Rosada	out/15	13	9.6	52	7.1
		fev/16	10	10.8	30	6.5
		abr/16	12	10.0	30	5.9
		jul/16	20	8.6	28	6.6
	Mean	-	14	9.8	35	6.5
Flores						
	Lake Comprida	out/15	15	9.5	66	7.4
		mar/16	12	10.5	76	7.3
		mai/16	15	9.6	90	7.1
		jul/16	21	8.7	83	7.6
	Mean	-	16	9.6	79	7.4
	Lake Funda	out/15	17	9.5	120	7.5
		mar/16	13	12.4	120	8.8
		mai/16	17	10.6	120	7.5
		jul/16	21	8.4	120	7.4
	Mean	-	17	10.5	120	7.9
	Lake Lomba	out/15	14	9.4	47	6.9
		mar/16	12	10.0	54	7.4
		mai/16	15	9.4	66	6.9
		jul/16	23	8.5	54	7.2
	Mean	-	16	9.3	55	7.1
	Lake Negra	out/15	15	8.8	130	7.6
		mar/16	13	13.6	130	8.6

		mai/16	16	10.5	140	7.6
		jul/16	20	9.2	130	9.6
	Mean	-	16	10.5	133	8.4
	Lake Rasa	out/15	15	9.5	58	6.8
		mar/16	12	10.2	63	6.8
		mai/16	16	9.7	62	6.6
		jul/16	20	8.5	62	6.1
	Mean	-	16	9.5	61	6.6
Corvo						
	Lake Caldeirão	nov/15	13	10.0	53	6.8
		mar/16	13	10.5	94	6.5
		mai/16	15	9.3	82	6.3
		jul/16	19	6.7	73	7.0
		-	15	9.1	76	6.7

Table 2 – Characterization values of sampled thermal waters.

Sampling	Sampling date	Temperature (ºC)	pH	Conductivity (µS/cm)	O_2 (mg/L)
Água do Caldeirão	26/06/2016	>55	6.78	0.88	3.26
Água da Prata	26/06/2016	29.43	5.99	0.61	5.99
Água do Rego	26/06/2016	34.80	6.52	0.62	3.94
Caldeira da Ribeira Grande	26/06/2016	-	-	-	-
Poça da Dona Beja	01/10/2016	38.41	5.48	0.78	5.48
Nascente do Morangueiro	26/06/2016	31.34	5.97	2.44	2.82
Poça da Tia Silvina (interior)	26/06/2016	43.50	6.05	2.15	1.99
Poça da Tia Silvina (exterior)	26/06/2016	39.54	6.08	1.88	4.79
Ribeira Amarela 1	26/06/2016	-	-	-	-
Ribeira Amarela 2	26/06/2016	-	-	-	-
Ribeira da Caldeira	26/06/2016	-	-	-	-

Table 3 – Characterization values of sampled marine lake.

Sampling	Sampling date	Temperature (ºC)	pH	Conductivity (µS/cm)	O_2 (mg/L)	Salinity (‰)
Fajã dos Cubres	06/04/2017	17.0	8.6	31333	12.85	26.22

Appendix IV – Media

Table 1 – BG-11 and BG-11$_0$ medium.

	BG-11	BG-11$_0$
	Quantity	
$NaNO_3$	1.5 g	-
K_2HPO_4	0.04 g	0.04 g
$MgSO_4 \cdot 7H_2O$	0.075 g	0.075 g
$CaCl_2 \cdot 2H_2O$	0.036 g	0.036 g
Citric acid	0.006 g	0.006 g
Ferric ammonium citrate	0.006 g	0.006 g
EDTA (disodium salt)	0.001 g	0.001 g
$NaCO_3$	0.02 g	0.02 g
Trace metal mix A5	1.0 mL	
Agar (if needed)	7.0 g	
Distilled water	1.0 L	

Table 2 – A5 trace metal elements medium.

	A5
	Quantity
H_3BO_3	1.5 g
$MnCl_2 \cdot 4H_2O$	0.04 g
$ZnSO_4 \cdot 7H_2O$	0.075 g
$NaMoO_4 \cdot 2H_2O$	0.036 g
$CuSO_4 \cdot 5H_2O$	0.006 g
$Co(NO_3)_2 \cdot 6H_2O$	0.006 g
Distilled water	1.0 L

Table 3 – TFI+M medium composition.

Stock		**Quantity**
TFI	20%	10% Amide nitrogen
		5% Nitric nitrogen
		5% Ammoniacal nitrogen
		5% P_2O_5
		4% K_2O
M		4.5% MgO
		6% S
		0.08% B
		0.016% Cm
		0.016% Fe
		0.08% Mn
		0.0016% Mo
		0.05% Zn

For one litter of medium it's used 0.2 mL of each stock with one litter of distilled water.

Appendix V – Applied concentration in assays

Table 1 – Applied concentration in first column of the 96 well plate.

Group	AChE (µg/mL)	DPPH (µg/mL)	BACTER (µg/mL)	CANCER (µg/mL)	Strains
1	150	250	200	200	T05;T06;T07:T13;T16;L04; L07;L09;L21;L34
2	85	250	150	150	L16
3	65	150	75	100	T17;L14;L18;L28
4	40	65	50	50	L15;L30
5	-	-	-	50	T14;L17

Appendix VI – Reported and isolated cyanobacteria

Table 1 – Reported cyanobacteria in the literature with the isolated cyanobacteria in this work.

		SMG	STM	TER	PIC	SJO	FAI	GRA	FLO	COR
II. Synechococcales	L.Hoffmann, J.Komárek & J.Kastovsky	●▲	-	●	●▲	●▲	●	-	●	●
Coelosphaeriaceae	Elenkin	●	-	-	●	-	-	-	●	●
Coelosphaerium	Nägeli	●	-	-	-	-	-	-	-	-
Coelosphaerium kuetzingianum	Nägeli	●	-	-	-	-	-	-	-	-
Coelosphaerium sp.	Nägeli	●	-	-	-	-	-	-	-	-
Snowella	A.A.Elenkin	●	-	-	-	-	-	-	-	-
Snowella lacustris	(Chodat) Komárek & Hindák	●	-	-	-	-	-	-	-	-
Snowella sp.	A.A.Elenkin	●	-	-	-	-	-	-	-	-
Woronichinia	A.A.Elenkin	●	-	-	●	-	-	-	●	●
Woronichinia naegeliana	(Unger) Elenkin	●	-	-	●	-	-	-	●	●
Heteroleibleiniaceae	(Komárek & Anagnostidis) J.Komárek, J.Kastovsky, J.Mares & J.R.Johansen	-	-	-	-	●	-	-	-	-
Heteroleibleinia	(L.Geitler) L.Hoffmann	-	-	-	-	●	-	-	-	-
Heteroleibleinia kuetzingii	(Schmidle) Compère	-	-	-	-	●	-	-	-	-
Leptolyngbyaceae	(Anagnostidis & J.Komárek) J.Komárek, J.Kastovsky, J.Mares & J.R.Johansen	●▲	-	-	-	▲	-	-	-	-
Leptolyngbya	Anagnostidis & Komárek	●▲	-	-	-	▲	-	-	-	-
Leptolyngbya gelatinosa	(Woronichin) Anagnostidis & Komárek	▲	-	-	-	-	-	-	-	-
Leptolyngbya laminosa	(Gomont ex Gomont) Anagnostidis & Komárek	●	-	-	-	-	-	-	-	-
Leptolyngbya nostocorum	(Bornet ex Gomont) Anagnostidis & Komárek	●	-	-	-	-	-	-	-	-
Leptolyngbya ochracea	(Thuret ex Gomont) Anagnostidis & Komárek	●	-	-	-	-	-	-	-	-
Leptolyngbya sp.	Anagnostidis & Komárek	▲	-	-	-	▲	-	-	-	-
Leptolyngbya valderiana	(Gomont) Anagnostidis & Komárek	●	-	-	-	-	-	-	-	-
Planktolyngbya	Anagnostidis & Komárek	● ▲	-	-	-	-	-	-	-	-
Planktolyngbya limnetica	(Lemmermann) Komárková-Legnerová & Cronberg	● ▲	-	-	-	-	-	-	-	-
Planktolyngbya sp.	Anagnostidis & Komárek	● ▲	-	-	-	-	-	-	-	-
Merismopediaceae	Elenkin	●	-	●	●	●	-	-	●	●
Aphanocapsa	C.Nägeli	●	-	-	-	●	-	-	-	-
Aphanocapsa delicatissima	West & G.S.West	●	-	-	-	-	-	-	-	-
Aphanocapsa elachista	West & G.S.West	●	-	-	-	●	-	-	-	-
Aphanocapsa grevillei	(Berkeley) Rabenhorst	-	-	-	-	●	-	-	-	-
Aphanocapsa incerta	(Lemmermann) G.Cronberg & Komárek	●	-	-	-	-	-	-	-	-
Aphanocapsa sp.	C.Nägeli	●	-	-	-	-	-	-	-	-
Eucapsis	F.E.Clements & H.L.Shantz	●	-	●	●	-	-	-	●	●
Eucapsis alpina	F.E.Clements & H.L.Schantz	●	-	●	●	-	-	-	●	●
Eucapsis minuta	F.E.Fritsch	●	-	-	-	-	-	-	-	-
Merismopedia	F.J.F.Meyen	●	-	●	●	-	-	-	-	-
Merismopedia glauca	(Ehrenberg) Kützing	●	-	●	-	-	-	-	-	-
Merismopedia sp.	F.J.F.Meyen	●	-	-	●	-	-	-	-	-
Merismopedia tenuissima	Lemmermann	●	-	●	-	-	-	-	-	-
Synechocystis	C.Sauvageau	●	-	-	●	-	-	-	-	-
Synechocystis sp.	C.Sauvageau	●	-	-	●	-	-	-	-	-
Pseudanabaenaceae	K.Anagnostidis & J.Komárek	●▲	-	-	●▲		-	-	●	●
Limnothrix	M.-E.Meffert	●	-	-	-	-	-	-	-	-
Limnothrix planctonica	(Woloszynska) Meffert	●	-	-	-	-	-	-	-	-
Pseudanabaena	Lauterborn	●▲	-	-	●▲	-	-	-	●	●
Pseudanabaena catenata	Lauterborn	●	-	-	-	-	-	-	-	-
Pseudanabaena limnetica	(Lemmermann) Komárek	●	-	-	●▲	-	-	-	●	●

Pseudanabaena minima	(G.S.An) Anagnostidis	-	-	-	▲	-	-	-	-	-
Pseudanabaena mucicola	(Naumann & Huber-Pestalozzi) Schwabe	●	-	-	-	-	-	-	-	-
Pseudanabaena sp.	Lauterborn	●▲	-	-	●	-	-	-	●	●
Schizotrichaceae	Elenkin	-	-	●	-	●	●	-	-	-
Schizothrix	Kützing ex M.Gomont	-	-	●	-	●	●	-	-	-
Schizothrix cuspidata	(West & G.S.West) West & G.S.West	-	-	-	-	-	●	-	-	-
Schizothrix fuscescens	Kutzing ex Gomont	-	-	●	-	-	-	-	-	-
Schizothrix lacustris	A.Braun ex Gomont	-	-	●	-	-	-	-	-	-
Schizothrix pallida	(Kützing ex Forti) Geitler	-	-	-	-	●	-	-	-	-
Schizothrix sp.	Kützing ex M.Gomont	-	-	●	-	-	-	-	-	-
Schizothrix symplocoides	(N.L.Gardner) Geitler	-	-	●	-	-	-	-	-	-
Schizothrix telephoroides	Gomont	-	-	-	-	-	●	-	-	-
Schizothrix vaginata	Gomont	-	-	-	-	●	-	-	-	-
Synechococcaceae	J.Komárek & Anagnostidis	●	-	●	-	▲	-	-	-	-
Anathece	(Komárek & Anagnostidis) Komárek, Kastovsky & Jezberová	●	-	-	-	-	-	-	-	-
Anathece clathrata	(W.West & G.S.West) Komárek, Kastovsky & Jezberová	●	-	-	-	-	-	-	-	-
Anathece minutissima	(West) Komárek, Kastovsky & Jezberová	●	-	-	-	-	-	-	-	-
Synechococcus	C.Nägeli	●	-	●	-	-	-	-	-	-
Synechococcus sp.	C.Nägeli	●	-	●	-	-	-	-	-	-
III. Spirulinales	J.Komárek, J.Kastovsky, J.Mares & J.R.Johansen	-	-	-	-	▲	-	-	-	-
Spirulinaceae	(Gomont) L.Hoffmann, J.Komárek & J.Ka	-	-	-	-	▲	-	-	-	-
Spirulina	Turpin ex Gomont,	-	-	-	-	▲	-	-	-	-
Spirulina subsalsa	Oersted ex Gomont	-	-	-	-	▲	-	-	-	-
IV. Chroococcales	Schaffner	●▲	-	●	●	▲	●	-	●	●
Aphanothecaceae	(J.Komárek & Anagnostidis) J.Komárek, J.Kastovsky, J.Mares & J.R.Johansen	●▲	-	-	●	-	-	-	●	-
Aphanothece	C.Nägeli	●	-	-	●	-	-	-	●	-
Aphanothece castagnei	(Kützing) Rabenhorst	-	-	-	-	-	-	-	●	-
Aphanothece microscopica	Nägeli	●	-	-	-	-	-	-	-	-
Aphanothece naegelii	Wartmann in Rabenhorst	●	-	-	-	-	-	-	-	-
Aphanothece nidulans	P.Richter in Wittrock & Nordstedt	●	-	-	-	-	-	-	-	-
Aphanothece pallida	(Kützing) Rabenhorst	●	-	-	-	-	-	-	-	-
Aphanothece saxicola	Nägeli	●	-	-	-	-	-	-	-	-
Aphanothece sp.	C.Nägel	●▲	-	-	-	-	-	-	●	-
Aphanothece stagnina	(Sprengel) A.Braun in Rabenhorst	-	-	-	●	-	-	-	-	-
Gloeothece	C.Nägeli	●	-	-	-	-	-	-	●	-
Gloeothece cystifera	(Hassall) Rabenhorst	●	-	-	-	-	-	-	-	-
Gloeothece rupestris	(Lyngbye) Bornet in Wittrock & Nordstedt	●	-	-	-	-	-	-	●	-
Chroococcaceae	Rabenhorst	●▲	-	●	●	●▲	●	-	●	●
Chroococcus	Nägeli	●	-	●	●	●	●	-	●	●
Chroococcus dispersus	(Keissler) Lemmermann	●	-	-	●	-	-	-	-	-
Chroococcus membraninus	(Meneghini) Nägeli	●	-	-	-	-	-	-	-	-
Chroococcus minor	(Kützing) Nägeli	●	-	-	-	-	-	-	-	-
Chroococcus minutus	(Kützing) Nägeli	●	-	-	●	-	-	-	●	-
Chroococcus sp.	Nägeli	●	-	-	-	-	-	-	-	-
Chroococcus tenax	(Kirchner) Hieronymus	●	-	-	-	-	-	-	-	-
Chroococcus turgidus	(Kützing) Nägeli	●	-	●	●	●	●	-	●	●
Chroococcus turicensis	(Nägeli) Hansgirg	-	-	-	-	-	-	-	●	-
Chroococcus westii	J.B.Petersen	●	-	-	-	-	-	-	-	-
Gomphosphaeriaceae	Kützing	●	-	-	-	-	-	-	-	-
Gomphosphaeria	Kützing	●	-	-	-	-	-	-	-	-
Gomphosphaeria sp.	Kützing	●	-	-	-	-	-	-	-	-

Microcystaceae	Lemmermann	●▲	-	●	-	●	-	-	●	●
Gloeocapsa	Kützing	●	-	●	-	●	-	-	●	-
Gloeocapsa atrata	Kützing	●	-	●	-	-	-	-	-	-
Gloeocapsa caldariorum	Rabenhorst	-	-	●	-	-	-	-	-	-
Gloeocapsa compacta	Kützing	-	-	-	-	-	-	-	●	-
Gloeocapsa gelatinosa	Kützing	-	-	●	-	●	-	-	-	-
Gloeocapsa sp.	Kützing	●	-	-	-	-	-	-	-	-
Gloeocapsa quaternata	Kützing	-	-	●	-	-	-	-	-	-
Gloeocapsa rupestris	Kützing	-	-	●	-	●	-	-	-	-
Gloeocapsa thermalis	Kützing	-	-	●	-	-	-	-	-	-
Gloeocapsopsis	Geitler ex Komárek	●	-	-	-	-	-	-	-	-
Gloeocapsopsis magma	(Brébisson) Komárek & Anagnostidis ex Komárek	●	-	-	-	-	-	-	-	-
Microcystis	Lemmermann	●▲	-	●	-	-	-	-	●	●
Microcystis aeruginosa	(Kützing) Kützing	●	-	-	-	-	-	-	●	●
Microcystis flos-aquae	(Wittrock) Kirchner	●▲	-	-	-	-	-	-	●	-
Microcystis pulverea	(H.C.Wood) Forti	●	-	●	-	-	-	-	-	-
Microcystis robusta	(H.W.Clark) Nygaard in Ostenfeld & Nygaard	●	-	●	-	-	-	-	-	-
Microcystis sp.	Lemmermann	●	-	-	-	-	-	-	-	-
V. Pleurocapsales	Geitler	-	-	-	-	-	●	-	-	-
Hyellaceae	Borzì	-	-	-	-	-	●	-	-	-
Cyanosaccus	K.J.Lukas & S.Golubic	-	-	-	-	-	●	-	-	-
Cyanosaccus sp.	K.J.Lukas & S.Golubic	-	-	-	-	-	●	-	-	-
Hyella	É.Bornet & C.Flahault	-	-	-	-	-	●	-	-	-
Hyella caespitosa	Bornet & Flahault	-	-	-	-	-	●	-	-	-
Hyella gigas	Lukas & Golubic	-	-	-	-	-	●	-	-	-
Hyella sp.	É.Bornet & C.Flahault	-	-	-	-	-	●	-	-	-
VI. Oscillatoriales	Schaffner	●▲	●▲	●	●	●▲	●	●	●	●
Coleofasciculaceae	J.Komárek, J.Kastovsky, J.Mares & J.R.Johansen	●	●	●	●	-	-	-	●	-
Anagnostidinema	Strunecký *et al.*	●	-	●	-	-	-	-	●	-
Anagnostidinema amphibium	(C.Agardh ex Gomont) Strunecký, Bohunická, J.R.Johansen & J.Komárek	●	-	●	-	-	-	-	●	-
Geitlerinema	(Anagnostidis & Komárek) Anagnostidis	●	●	-	●	-	-	-	-	-
Geitlerinema ionicum	(Skuja) Anagnostidis	-	●	-		-	-	-	-	-
Geitlerinema splendidum	(Greville ex Gomont) Anagnostidis	●	-	-	●	-	-	-	-	-
Homoeotrichaceae	Elenkin	-	-	-	-	●	-	-	-	-
Homoeothrix	(Thuret ex Bornet & Flahault) Kirchner	-	-	-	-	●	-	-	-	-
Homoeothrix africana	G.S.West	-	-	-	-	●	-	-	-	-
Microcoleaceae	O.Strunecky, J.R.Johansen & J.Komárek	●▲	●▲	●	●	●▲	●	-	●	●
Kamptonema	O.Strunecký, J.Komárek & J.Smarda	●	●	●	-	-	-	-	-	-
Kamptonema formosum	(Bory ex Gomont) Strunecký, Komárek & J.Smarda	●	●	●	-	-	-	-	-	-
Microcoleus	Desmazières ex Gomont	●	-	●	-	-	-	-	●	-
Microcoleus amoenus	(Gomont) Strunecky, Komárek & J.R.Johansen	●	-	-	-	-	-	-	●	-
Microcoleus autumnalis	(Gomont) Strunecky, Komárek & J.R.Johansen	●	-	-	-	-	-	-	●	-
Microcoleus lyngbyaceus	(Gomont) Strunecky, Komárek & J.R.Johansen	-	-	●	-	-	-	-	-	-
Phormidium	Kützing ex Gomont	●▲	-	●	●	●▲	●	-	-	●
Phormidium aerugineo-caeruleum	(Gomont) Anagnostidis & Komárek	●	-	-	-	-	-	-	-	-
Phormidium allorgei	(Frémy) Anagnostidis & Komárek	-	-	●	-	-	-	-	-	-
Phormidium breve	(Kützing ex Gomont) Anagnostidis & Komárek	●	-	-	-	-	-	-	-	-
Phormidium durum	N.L.Gardner	-	-	-	-	●	-	-	-	-
Phormidium irriguum	(Kützing ex Gomont) Anagnostidis & Komárek	-	-	●	-	●	-	-	-	-
Phormidium pachydermaticum	Frémy	-	-	●	-	●	-	-	-	-
Phormidium retzii	Kützing ex Gomont	●	-	-	-	●	●	-	-	-

Phormidium rotheanum	Itzigsohn in Rabenhorst	-	-	-	-	●	-	-	-	-
Phormidium sp.	Kützing ex Gomont	●▲	-	-	●	▲	●	-	-	●
Phormidium terebriforme	(C.Agardh ex Gomont) Anagnostidis & Komárek	●	-	-	-	-	-	-	-	-
Planktothrix	K.Anagnostidis & J.Komárek	●	-	-	-	-	-	-	-	-
Planktothrix agardhii	(Gomont) Anagnostidis & Komárek	●	-	-	-	-	-	-	-	-
Pseudophormidium	(Forti) Anagnostidis & Komárek	-	▲	-	-	-	-	-	-	-
Pseudophormidium pauciramosum	(Anissimova) Anagnostidis	-	▲	-	-	-	-	-	-	-
Symploca	Kützing ex Gomont	●	-	-	-	-	-	-	-	-
Symploca dubia	Gomont	●	-	-	-	-	-	-	-	-
Symploca thermalis	Gomont	●	-	-	-	-	-	-	-	-
Oscillatoriaceae	Engler	●▲	-	●	●	-	●	●	●	●
Lyngbya	C.Agardh ex Gomont	●	-	●	●	-	-	-	●	-
Lyngbya lutea	Gomont ex Gomont	-	-	●	-	-	-	-	-	-
Lyngbya martensiana	Meneghini ex Gomont	●	-	●	-	-	-	-	-	-
Lyngbya sp.	C.Agardh ex Gomont	●	-	-	●	-	-	-	●	-
Oscillatoria	Vaucher ex Gomont	●▲	-	●	●	-	●	●	●	●
Oscillatoria planctonica	Woloszynska	●	-	-	-	-	-	-	-	-
Oscillatoria princeps	Vaucher ex Gomont	-	-	●	-	-	-	-	-	-
Oscillatoria sancta	Kützing ex Gomont	●	-	-	-	-	-	-	-	-
Oscillatoria sp.	Vaucher ex Gomont	●	-	●	●	-	-	-	●	-
Oscillatoria tenuis	C.Agardh ex Gomont	●▲	-	●	●	-	●	●	●	●
Plectonema	Thuret ex Gomont	-	-	-	-	-	●	-	-	-
Plectonema endolithicum	Ercegovic	-	-	-	-	-	●	-	-	-
Plectonema terebrans	Bornet & Flahault ex Gomont	-	-	-	-	-	●	-	-	-
VIII. Nostocales	Borzi	●▲	●▲		●▲	●▲			●▲	●
Aphanizomenonaceae	Elenkin	●▲	-	-	●▲	-	-	-	●	●
Anabaenopsis	V.V.Miller	-	-	-	-	-	-	-	●	-
Anabaenopsis circularis	(G.S.West) Woloszynska & V.Miller in V.Miller	-	-	-	-	-	-	-	●	-
Anabaenopsis sp.	V.V.Miller	-	-	-	-	-	-	-	●	-
Aphanizomenon	A.Morren ex É.Bornet & C.Flahault	●▲	-	-	●▲	-	-	-	●	-
Aphanizomenon flos-aquae	Ralfs ex Bornet & Flahault	●	-	-	●	-	-	-	●	●
Aphanizomenon gracile	Lemmermann	●	-	-	●	-	-	-	●	-
Aphanizomenon manguinii	Bourrelly in Bourrelly & Manguin	-	-	-	▲	-	-	-	-	-
Aphanizomenon sp.	A.Morren ex É.Bornet & C.Flahault	●▲	-	-	-	-	-	-	-	-
Dolichospermum	(Ralfs ex Bornet & Flahault) P.Wacklin, L.Hoffmann & J.Komárek	●	-	●	●▲	-	-	-	●	●
Dolichospermum affine	(Lemmermann) Wacklin, L.Hoffmann & Komárek	●	-	-	●	-	-	-	-	-
Dolichospermum delicatulum	(Lemmermann) Wacklin, Hoffmann & Komárek	●	-	-	●	-	-	-	●	●
Dolichospermum planctonicum	(Brunnthaler) Wacklin, L.Hoffmann & Komárek	●	-	-	●	-	-	-	-	-
Dolichospermum scheremetieviae	(Elenkin) Wacklin, L.Hoffmann & Komárek	●	-	-	●	-	-	-	●	●
Dolichospermum sigmoideum	(Nygaard) Wacklin, L.Hoffmann & Komárek	●	-	-	●	-	-	-	-	-
Dolichospermum solitarium	(Klebahn) Wacklin, L.Hoffmann & Komárek	●	-	●	●	-	-	-	●	-
Dolichospermum sp.	(Ralfs ex Bornet & Flahault) P.Wacklin, L.Hoffmann & J.Komárek	-	-	-	▲	-	-	-	-	-
Dolichospermum spiroides	(Klebhan) Wacklin, L.Hoffmann & Komárek	●	-	-	-	-	-	-	-	-
Nodularia	Mertens ex Bornet & Flahault	-	-	●	-	-	-	-	-	●
Nodularia harveyana	Thuret ex Bornet & Flahault	-	-	●	-	-	-	-	-	●
Raphidiopsis	F.E.Fritsch & F.Rich	●	-	-	-	-	-	-	●	●
Raphidiopsis curvata	F.E.Fritsch & M.F.Rich	●	-	-	-	-	-	-	●	●
Sphaerospermopsis	Zapomelová, Jezberová, Hrouzek, Hisem, Reháková & Komárková	-	-	-	●▲	-	-	-	-	-
Sphaerospermopsis aphanizomenoide	Zapomelová, Jezberová, Hrouzek, Hisem, Reháková & Komárková	-	-	-	●	-	-	-	-	-
Sphaerospermopsis sp.	Zapomelová, Jezberová, Hrouzek, Hisem, Reháková & Komárková	-	-	-	▲	-	-	-	-	-
Chlorogloeopsidaceae	Mitra & Pandey	▲	-	-	-	-	-	-	-	-

Chlorogloeopsis	A.K.Mitra & D.C.Pandey	▲	-	-	-	-	-	-	-	-	
Chlorogloeopsis fritschii	(A.K.Mitra) A.K.Mitra & D.C.Pandey	▲	-	-	-	-	-	-	-	-	
Fortieaceae	Komárek, Kastovsky, Mares & J.R.Johansen	●▲	-	-	▲	-	-	-	▲	-	
Coleospermum	O.Kirchner ex A.B.Frank	●▲	-	-	▲	-	-	-	▲	-	
Coleospermum goeppertianum	Kirchner ex Frank	●	-	-	-	-	-	-	-	-	
Coleospermum sp.	O.Kirchner ex A.B.Frank	▲	-	-	▲	-	-	-	▲	-	
Fortiea	De Toni	-	-	-	▲	-	-	-	-	-	
Fortiea sp.	De Toni	-	-	-	▲	-	-	-	-	-	
Gloeotrichiaceae	J.Komárek, J.Kastovsky, J.Mares & J.R.Johansen	●	-	-	-	-	-	-	-	-	
Gloeotrichia	J.Agardh ex Bornet & Flahault	●	-	-	-	-	-	-	-	-	
Gloeotrichia pisum	Thuret ex Bornet & Flahault	●	-	-	-	-	-	-	-	-	
Hapalosiphonaceae	Elenkin	●▲	▲	●	-	●	-	-	●	●	
Hapalosiphon	Nägeli ex É.Bornet & C.Flahault	●	▲	●	-	●	-	-	●	●	
Hapalosiphon hibernicus	West & G.S.West	●	-	-	-	-	-	-	●	●	
Hapalosiphon intricatus	West & G.S.West	-	-	●	-	●	-	-	-	-	
Hapalosiphon pumilus	Kirchner ex Bornet & Flahault	-	▲	-	-	-	-	-	●	-	
Mastigocladus	Cohn ex O.Kirchner	●▲	-	-	-	-	-	-	-	-	
Mastigocladus laminosus	Cohn ex Kirchner	●▲	-	-	-	-	-	-	-	-	
Mastigocoleus	Lagerheim ex É.Bornet & C.Flahault	-	-	-	-	-	●	-	-	-	
Mastigocoleus testarum	Lagerheim ex Bornet & Flahault	-	-	-	-	-	●	-	-	-	
Nostocaceae	Eichler	●▲	●	●	●▲	●	●	-	●	●	
Anabaena	Bory ex Bornet & Flahault	●	-	●	●	-	-	-	●	●	
Anabaena aspera	Frémy	●	-	●	-	-	-	-	-	-	
Anabaena augstumalis	Schmidle	-	-	-	-	-	-	-	●	-	
Anabaena cylindrica	Lemmermann	●	-	-	-	-	-	-	-	-	
Anabaena inaequalis	Bornet & Flahault	●	-		●	-	-	-	●	-	
Anabaena sp.	Bory ex Bornet & Flahault	●▲	-	●	●	-	-	-	●	●	
Anabaena torulosa	Lagerheim ex Bornet & Flahault	-	-	-	-	-	-	-	-	●	
Cylindrospermum	Kützing ex É.Bornet & C.Flahault	●	-	-	●	●	●	-	●	-	
Cylindrospermum licheniforme	Kützing ex Bornet & Flahault	●	-	-	-	-	-	-	-	-	
Cylindrospermum majus	Kützing ex Bornet & Flahault	●	-	-	-	●	●	-	●	-	
Cylindrospermum sp.	Kützing ex É.Bornet & C.Flahault	-	-	-	●	-	-	-	-	-	
Nostoc	Vaucher ex Bornet & Flahault	●▲	●	●	●▲	●	●	-	●	●	
Nostoc carneum	C.Agardh ex Bornet & Flahault	-	-	-	-	-	●	-	-	-	
Nostoc commune	Vaucher ex Bornet & Flahault	-	-	-	●▲	-	-	-	-	-	
Nostoc ellipsosporum	Rabenhorst ex Bornet & Flahault	●	-	-	-	-	-	-	-	●	
Nostoc paludosum	Kützing ex Bornet & Flahault	●	●	-	-	-	-	-	-	-	
Nostoc punctiforme	Hariot	●▲	-	-	-	-	-	-	-	-	
Nostoc sp.	Vaucher ex Bornet & Flahault	-	-	-	-	-	-	-	-	-	
Nostoc sphaericum	Vaucher ex Bornet & Flahault	●	-	-	-	●	●	-	●	-	
Nostoc sphaeroides	Kützing ex Bornet & Flahault	-	-	-	-	●	●	-	-	-	
Nostoc verrucosum	Vaucher ex Bornet & Flahault	●	-	●	-	-	-	-	-	-	
Trichormus	(Ralfs ex É.Bornet & C.Flahault) J.Komárek & K.Anagnostidis	●	-	-	-	-	-	-	-	-	
Trichormus variabilis	(Kützing ex Bornet & Flahault) Komárek & Anagnostidis	●	-	-	-	-	-	-	-	-	
Nostochopsidaceae	Geitler	●	-	-	-	-	-	-	-	-	
Nostochopsis	H.C.Wood ex É.Bornet & C.Flahault	●	-	-	-	-	-	-	-	-	
Nostochopsis lobatus	H.C.Wood ex Bornet & Flahault	●	-	-	-	-	-	-	-	-	
Rivulariaceae	Bornet & Flahault	▲●	-	●	▲	▲	●	-	●▲	●	
Calothrix	C.Agardh ex Bornet & Flahault	●	-	-	▲	▲	-	-	●▲	-	
Calothrix breviarticulata	West & G.S.West		-	-	-	-	-	-	▲	-	
Calothrix castellii	Bornet & Flahault	▲	-	-	▲	-	-	-	-	-	

Calothrix parietina	Thuret ex Bornet & Flahault	●	-	-	-	-	-	-	-	-	
Calothrix sp.	C.Agardh ex Bornet & Flahault	▲	-	-	-	▲	-	-	●	-	
Dichothrix	G.Zanardini ex É.Bornet & C.Flahault	-	-	-	-	-	-	-	●	●	
Dichothrix baueriana	Bornet & Flahault	-	-	-	-	-	-	-	-	●	
Dichothrix orsiniana var. africana	Frémy	-	-	-	-	-	-	-	●	-	
Kyrtuthrix	Ercegovic	-	-	-	-	-	●	-	-	-	
Kyrtuthrix dalmatica	Ercegovic	-	-	-	-	-	●	-	-	-	
Microchaete	Thuret ex Bornet & C.Flahault	●▲	-	-	▲	-	-	-	-	-	
Microchaete bulbosa	J.Copeland	▲	-	-	-	-	-	-	-	-	
Microchaete tenera	Thuret ex Bornet & Flahault	●	-	-	▲	-	-	-	-	-	
Rivularia	C.Agardh ex Bornet & Flahault	●	-	●	-	-	-	-	●	-	
Rivularia bullata	Berkeley ex Bornet & Flahault	●	-	-	-	-	-	-	-	-	
Rivularia nitida	C.Agardh ex Bornet & Flahault	-	-	-	-	-	-	-	●	-	
Rivularia sp.	C.Agardh ex Bornet & Flahault	-	-	●	-	-	-	-	-	-	
Scytonemataceae	Rabenhorst ex Bornet & Flahault	●	-	●	●	●	-	-	●	-	
Petalonema	M.J.Berkeley ex C.Correns	-	-	-	-	-	-	-	●	-	
Petalonema velutinum	Migula	-	-	-	-	-	-	-	●	-	
Scytonema	C.Agardh ex É.Bornet & C.Flahault	●	-	●	●	●	-	-	●	-	
Scytonema amplum	West & G.S.West	●	-	-	-	-	-	-	-	-	
Scytonema dilatatum	Bharadwaja	-	-	●	-	-	-	-	-	-	
Scytonema guyanense	Bornet & Flahault	●	-	-	-	-	-	-	●	-	
Scytonema hofmannii	C.Agardh ex Bornet & Flahault	●	-	-	-	●	-	-	-	-	
Scytonema javanicum	Bornet ex Bornet & Flahault	-	-	-	-	-	-	-	●	-	
Scytonema mirabile	Bornet	●	-	-	●	●	-	-	●	-	
Scytonema myochrous	C.Agardh ex Bornet & Flahault	●	-	-	-	-	-	-	-	-	
Scytonema sp.	C.Agardh ex É.Bornet & C.Flahault	-	-	-	-	-	-	-	●	-	
Scytonema stuposum	Bornet ex Bornet & Flahault	-	-	-	-	-	-	-	●	-	
Stigonemataceae	(Bornet & Flahault) Borzì	●	-	●	-	●	-	-	●	-	
Stigonema	C. Agardh ex Bornet et Flahault	●	-	●	-	●	-	-	●	-	
Stigonema hormoides	Bornet & Flahault	●	-	●	-	●	-	-	●	-	
Stigonema informe	Kützing ex Bornet & Flahault	-	-	●	-	●	-	-	-	-	
Stigonema mamillosum	C.Agardh ex Bornet & Flahault	●	-	-	-	●	-	-	-	-	
Stigonema minutum	Hassall ex Bornet & Flahault	●	-	●	-	●	-	-	●	-	
Stigonema multipartitum	N.L.Gardner	-	-	-	-	●	-	-	-	-	
Stigonema ocellatum	Thuret ex Bornet & Flahault	●	-	●	-	-	-	-	●	-	
Stigonema panniforme	Bornet & Flahault	●	-	-	-	-	-	-	-	-	
Stigonema robustum	N.L.Gardner	-	-	-	-	●	-	-	-	-	
Stigonema tomentosum	Hieronymus	●	-	-	-	●	-	-	-	-	
Tolypothrichaceae	Hauer, Bohunická, J.R.Johansen Mares & Berrendero-Gomez	●▲	-	-	-	-	-	-	●	-	
Tolypothrix	Kützing ex É.Bornet & C.Flahault	●▲	-	-	-	-	-	-	●	-	
Tolypothrix distorta	Kützing ex É.Bornet & C.Flahault	●	-	-	-	-	-	-	-	-	
Tolypothrix lanata	Wartmann ex Bornet & Flahault	●	-	-	-	-	-	-	-	-	
Tolypothrix sp.	Kützing ex É.Bornet & C.Flahault	●▲	-	-	-	-	-	-	-	-	
Tolypothrix tenuis	Kützing ex Bornet & Flahault	-	-	-	-	-	-	-	●	-	

Signal key: ● other authors; ▲ this work; - no records.

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