Analysis of Biochemical Composition of Three Stream Macroalgae Species

Aurélio F. Tonetto
Department of Botany
Universidade Estadual de São Paulo
Rio Claro/SP
Brazil

Kassandra S. M. Oliveira
Department of Biology
Universidade Estadual de São Paulo
Assis/SP
Brazil

Tatiana M. Domingues
Department of Biophysics
Universidade Federal de São Paulo
São Paulo/SP
Brazil

Abstract

Species of algae have great interest to society, especially because of their nutritional value in human diets. However, species pertaining to stream habitats have been neglected. Thus, we investigated the biochemical composition of three typical stream species of three different algal groups: Cyanobacteria – Lyngbya majuscula, Rhodophyta – Sirodotia delicatula and Chlorophyta – Oedogonium sp. Contents of protein, total lipid, carbohydrate, gross fiber and mineral composition were assessed. L. majuscula displayed high values for 45% of the compounds analysed. S. delicatula showed notable higher amount of zinc and lower content of gross fiber, while Oedogonium sp. registered higher values of total lipid, carbohydrates and calcium.

Keywords: biochemical composition, stream algae, *Lyngbya majuscula*, *Oedogonium* sp., *Sirodotia delicatula*.

1. Introduction

There are numerous records of historical usage of algae in the human diet (Gantar & Svircev, 2008). Most people unknowingly utilize seaweed products daily in the form of manufactured food items such as processed meat and fruit products, as well as in the form of domestic commodities such as paint, toothpaste, solid air fresheners, and cosmetics (Gressler *et al*., 2011).

Several groups of algae have being used for different purposes. Seaweeds as the green algae (Chlorophyta) *Caulerpa* (Robledo & Freile, 1997) and *Ulva* (Chapman, 1970) have been used by certain populations as vegetables or ingredients for foods (Gressler *et al*., 2011). Cultivations from unicellular *Chlorella* (Chlorophyta) have resulted in high biomass and high content of lipid in cells (Xu *et al*., 2006). Recent trends in drug research from natural sources suggest that algae are a promising group of organisms to provide novel biochemically active substances. The greatest use of red algae (Rhodophyta) is associated to food preparation and technology, and with pharmaceutical industry. Cyanobacteria as *Spirulina* (Burlew, 1953) contain high protein content and have been used in a variety of foods throughout the world (Gantar & Svircev, 2008).

Although the numerous studies published about algal composition, most of them were applied for algal species pertaining to lakes or marine habitats. Species from stream environments have been overlooked. Therefore, the objective of our study was to make an exploratory analysis about biochemical composition of three species (belonging to the group of Cyanobacteria, Chlorophyta and Rhodophyta) found typically in stream systems.

In addition, we compared our results with other studies in an attempt to contribute to current researches about economic value of stream algae and, if warranted, to recommend its use for human activities.

2. Experimental

2.1. Samples

Lyngbya majuscula (Cyanobacteria), *Sirodotia delicatula*, (Rhodophyta) and *Oedogonium* sp. (Chlorophyta) were collected manually from stones of three streams in São Paulo State, Brazil. All samples were cleaned with a tooth brush and water jets to remove epiphytes associated to the talus. After this procedure, they were kept in a refrigerator until biochemical quantification. The species were identified following specialized literature and they were deposited at the herbarium of the Federal University of Paraná (UPCB). The identification numbers of the species *Oedogonium* sp., *L. majuscula* and *S. delicatula* were UPCB76025, UPCB76026 and UPCB76027, respectively.

2.2. Lipid Analysis

Total lipid content was determined based on Erickson, 1993. The extraction was performed using a mixture of $CHCl_3$ and CH_4O (2:1), which was added to a tube containing algae sample (2 min in vortex). This step was repeated to re-extract the residue (30 s in vortex). After that, the supernatants were combined, filtered and concentrated under N_2, using gravimetric analysis to determine the total lipid content (all tests were performed in triplicate).

2.3. Nitrogen Analyses

Total nitrogen was determined by sample digestion in a H_2SO_4 solution, based on the Kjeldhal method, adapted by Umbreit *et al*., 1964. Concentrations were measured from the absorbance read (λ = 690 nm) in a spectrophotometer. Briefly, we pipetted 1 mL of the sample into a tube, and 1 mL of H_2SO_4 2N containing 0.2 g L^{-1} of $CuSeO_3$ was added. Thus, the solution obtained was kept in a digester block for 12 h (100-115 ºC). After this, 2 mL of Nessler reagent and 3 mL of NaOH 2N was added and the sample solution was read in a spectrophotometer. A standard solution of organic nitrogen (0.0472g of $(NH_4)_2SO_4$ in 100 mL of ultrapure water) was used as a control. The analysis was performed in triplicate.

2.4. Protein Analysis

Total protein content was based on a nitrogen-to-protein factor (Lourenço *et al*., 2002). According to Gressler *et al*., 2011, this factor (4.43) can estimate the content of protein quite accurately. The authors found that the protein content of *Plocamium brasiliense* was very similar to methods usually applied. Therefore, we adopted this factor to estimate the protein content of our species.

2.5. Carbohydrate Analysis

Algae sample was extracted using 70% C_2H_6O (v/v) at 70 °C, and it was then centrifuged (5 min at 5000 x g) (Karsten *et al*., 1999). After that, the total carbohydrate concentrations were assessed using a colorimetric method (Masuko *et al*., 2005). Briefly, a concentrated solution of H_2SO_4 and, in sequence, 5% C_6H_6O (w/w, in water) was added to the sample. The solution obtained was kept in water bath (90 °C) for 5 min, after that its temperature was reduced to the room temperature. The measurements of sample absorbance was performed at λ = 490 nm using a microplate reader. The glucose was used as standard solution and all tests were performed in triplicate.

2.6. Gross Fiber Analysis

In order to obtain the sample digestion we added 100 mL of 1.25% H_2SO_4 (v/v) in 1 g of dry sample. After 30 min, the sample was filtered and the digestion process was repeated using 1.25% NaOH (v/v) for more 30 min. In sequence, we filtered the sample again and kept it at 100-110 °C for 4 h. Thus, we obtain the dry mass value from the sample and, after calcination, the ashes weight was subtracted from this value of dry mass, resulting in the fiber content (MAA, 1998). The analysis was performed in triplicate.

2.7. Mineral Analysis

The minerals Co, B, Ni, Cr and Pb were quantified through calcination of 2.5 g of the sample at 550 °C for 3 h. After that, we added 25 mL of 1 mol HNO_3 and the concentrations of Co (λ = 240.7 nm), Ni (λ = 232.0 nm), Cr (λ = 357.9 nm) and Pb (λ = 217.0 nm) were measured using atomic absorption spectrometry, and the concentration of B (λ = 420.0 nm) was obtained using a spectrophotometer.

In turn, the minerals Ca, Mg, P, K, S, Fe, Na, Mn, Zn and Cu were quantified through the digestion of 0.5 g of sample in a solution containing a mixture of HNO_3 and $HClO_4$ (2:1) for 3 h. After this procedure, we measured the concentrations of each mineral using atomic absorption spectrometry (Ca, λ = 422.7 nm; Mg, λ = 285.2 nm; K, λ = 766.5 nm; Fe, λ = 372.0 nm; Na, λ = 589.0 nm; Mn, λ = 403.1 nm; Zn, λ = 213.9 nm and Cu, λ = 324.8 nm), and the concentrations of P (λ = 420.0 nm) and S (λ = 277.5 nm) were obtained using a spectrophotometer (MAA, 1998). The sample concentrations were read after calibration of both equipment using a standard curves. The analysis was performed in triplicate.

3. Results and Discussion

In the three algal species studied we observed a variation of all biochemical compounds analysed. Table 1 shows total lipid, protein, carbohydrate and gross fiber values for *L. majuscula*, *S. delicatula* and *Oedogonium* sp.. *L. majuscula* displayed the highest values of protein and gross fiber content (264.73 g Kg^{-1} and 194.40 g Kg^{-1}, respectively). High protein content was registered for other Cyanobacteria species (Gantar & Svircev, 2008). In fact, isolates from *Nodularia* and *Nostoc* are commonly used as food due to their high nutritional value (Ciferre, 1983). In addition, fiber intake through the consumption of foods rich in this dietary component is associated with reductions in LDL-cholesterol, attenuating glycemic and insulin response and improving laxation (Schneeman, 1999). Foods such as fresh vegetables, fruits, whole grains, and nuts present high contents of fiber (Tungland, 2002) and it is required for a healthy diet. Thus, *L. majuscula* could be more studied to become an option of fiber source to healthy food industry.

S. delicatula, although its lower protein content in comparison to *L. majuscula,* it presented values of protein higher than those found for marine red algae (Gressler *et al*., 2011), which are largely used in food industry. This result shows that the non-studied stream algae have a great potential for economic application and deserve more attention. Carbohydrates, on the other hand, presented lower content for *S. delicatula* in comparison with marine species. In marine habitats, the increase of sugar contents may be considered an adaptive measure under saline conditions to maintain the osmotic regulation of cell (Kirrolia *et al*., 2011). In addition, the cell wall of eukaryotic algae, as Rhodophyta, contains ~10% carbohydrates, such as cellulose, mannose, and xylose (Robinson & Toerien, 1982). The presence of these polysaccharides in the cell wall of red algae imply in a more hardily digestible biomass and therefore less acceptable for human consumption (Richmond, 1980). For this reason, the absence of these compounds in Cyanobacteria makes them more suitable.

The lipid content in the biomass of algae varies among different algal divisions and depends on ecological factors applied during growth (Gantar & Svircev, 2008). Here, we observed the highest value for *Oedogonium* sp. (19.2 g Kg^{-1}), a representative specie of Chlorophyta. Aaronson *et al*., 1980; stated that high lipid content is characteristic of Chlorophyta, followed by Crysophyceae and Bacillariophyceae. Hossain *et al*., 2008, stated that biodiesel can be produced from green macroalgae because of lipid contents, although it has lower lipid content than microalgae. Rorrer *et al*., 1999, proposed bioreactor systems for marine macroalgae to produce bioactive metabolites. These systems can be applied for stream macroalgae with the same objective, and because of its great occurrence and low harvest cost, it can be an economical option for bioenergy.

Sixteen different minerals were evaluated for all studied species. *L. majuscula* showed the highest concentration for seven of them, in descending order: nitrogen, iron, phosphorous, manganese, chrome, nickel and cobalt. *S. delicatula*, in comparison with the other species, displayed the biggest values for five minerals: potassium, sulfur, magnesium, zinc and boron. In addition, *Oedogonium* sp. presented higher values for calcium, sodium, copper and lead. The mineral composition of all species is described in Table 2. The ability of algae to absorb metals has been recognized for many years (Dwivedi, 2012). In natural environments, algae play a major role in controlling metal concentration in lakes and oceans (Dwivedi, 2012). Various studies have been carried out to show the role of algae in the bioremediation of heavy metals. *Cladophora glomerata* and *Oedogonium rivulare* are able to remove some metals such as Cu, Pb, Cd, Co from environment (Dwivedi, 2012). Dwivedi (2012) commented about the possibility of using live *Spirulina* to remove aqueous lead in low concentration and 74% of the metal was biologically adsorbed very fast (0-12 min) in experimental studies (Dwivedi, 2012). Metal pollutants, due to great industrialization, can easily penetrate the food chain and reach the human consumption, which has several dangers consequences. Thus, algae, such as those of the present study (each one with their particularity) could be a possible way to reduce the concentration of heavy metals in stream habitats.

In general, stream species investigated here displayed a potential use for humans as other species has already demonstrated. However, more attention must be given for benthic algae, commonly found in streams. Benthic (attached) freshwater algae may have an advantage over planktonic (suspended) algae in the ease of separation and recovery of algal biomass from an aqueous stream (Wilkie & Mulbry, 2002). There are several ways for cultivating benthic algae (Skadovski, 1961; Wood, 1987; Rectenwald & Drenner, 2000). Thus, further exploratory studies should be done with stream species to reveal potential applications of algae. In addition, other investigations with more precise analyses of biochemical composition of them should also be made in order to create a more detailed information and, thus, favor future applications.

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Table 1: Comparison of the Approximate Compositions of Three Stream Macroalgal Species (G Kg^{-1} Dry Weight, *N* = 3)

Species	Total Lipid	Protein	Carbohydrate	Gross Fiber
L. majuscula	6.8 ± 1.3	264.73 ± 1.0	160.12 ± 1.6	194.40 ± 4.5
S. delicatula	3.4 ± 1.5	217.15 ± 2.4	170.31 ± 1.7	50.80 ± 2.3
Oedogonium sp.	19.2 ± 5.3	135.24 ± 0.6	220.73 ± 0.5	177.10 ± 3.8

Table 2: Comparison of the Approximate Mineral Compositions of Three Stream Macroalgal Species (Dry Weight, *N* = 3)

Minerals	*L. majuscula*	*S. delicatula*	*Oedogonium* sp.
N (g Kg^{-1})	59.76 ± 0.5	49.02 ± 1.2	30.53 ± 0.3
Ca (g Kg^{-1})	3.70 ± 0.02	3.20 ± 0.2	6.40 ± 0.1
Mg (g Kg^{-1})	1.50 ± 0.03	2.80 ± 0.03	1.90 ± 0.02
P (g Kg^{-1})	2.59 ± 0.03	2.39 ± 0.02	1.62 ± 0.01
Po (g Kg^{-1})	2.02 ± 0.01	17.30 ± 0.2	10.50 ± 0.03
S (g Kg^{-1})	3.60 ± 0.04	3.90 ± 0.03	2.71 ± 0.02
Fe (g Kg^{-1})	14.80 ± 0.1	7.10 ± 0.2	11.00 ± 0.03
Na (g Kg^{-1})	72.00 ± 3.5	378.00 ± 4.8	437.00 ± 8.3
Mn (mg Kg^{-1})	1740.00 ± 11.2	980.00 ± 9.7	820.00 ± 12.4
Zn (mg Kg^{-1})	43.00 ± 2.1	172.00 ± 4.2	58.01 ± 3.4
Cu (mg Kg^{-1})	65.00 ± 1.3	23.00 ± 2.1	73.00 ± 4.1
Co (mg Kg^{-1})	11.47 ± 0.1	9.77 ± 0.09	8.15 ± 0.2
B (mg Kg^{-1})	3.35 ± 0.02	12.85 ± 0.2	8.01 ± 0.1
Ni (mg Kg^{-1})	12.48 ± 0.2	6.78 ± 0.03	8.87 ± 0.3
Cr (mg Kg^{-1})	16.05 ± 0.5	13.00 ± 0.4	14.41 ± 0.9
Pb (mg Kg^{-1})	4.16 ± 0.02	3.96 ± 0.02	4.44 ± 0.03