

Molecular Identification of Green Algae, *Spirogyra Porticalis*, along Parts of River Kaduna and its Potential for Singlecell Protein (SCP) Production

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Abstract

The freshwater alga *spirogyra porticalis*, a *filamentous chlorophyte*, was collected and identified on the basis of morphology and molecular characteristics. The nutritional composition of the Alga (protein, carbohydrate, moisture, fat, ash) were determined using the AOAC, Official Methods of Analysis. The components of Single Cell Protein (SCP) were determined using method of proximate analysis. The total content of protein (% dry weight) ranged from 12.46-16.89%, carbohydrate from 34.72-39.25%, moisture from 12.97-16.75%, fat from 20.17-22.03%, ash from 10.78-15.98%. The result revealed that this Macroalga has an appreciable amount of protein, carbohydrate, moisture, fat, and ash. Amino acids composition determines the protein quality of Algae. Hence, the amino acids present in the algal sample were extracted and estimated using an automated amino acids Analyzer. The results have shown the presence of the following amino acids; Leu, Lys, Ile, Phe, Val, His, Arg, Met, Trp, Thr, Pro, Tyr, Gly, Glu, Asp, Cys, Ser, Ala. The essential amino acids (Leu, Lys, Ile, Phe, Val, His, Arg, Met, Trp, Thr) profile of the alga as compared with conventional protein sources and the WHO/FAO reference pattern of essential amino acids (g per 100 protein), reveals that the levels of the essential amino acids in the alga were either similar to or greater than the levels of the same amino acids in the reference foods and WHO/FAO reference pattern. Thus, results of the present study shows that *S.porticalis* is a potential source of single cell protein, and may be of use to the food industry as a source of ingredients with high nutritional value. *S. porticalis* can provide a dietary alternative due to its nutritional value and its commercial value can be enhanced by improving the quality and expanding the range of freshwater macroalgae based products.



Introduction

The term single-cell protein (SCP) is used to describe protein derived from pure or mixed cultures of microorganisms such as yeast, fungi, algae and bacteria which are used as a substitute for the conventional protein sources exploited for human and animal consumption (Najafpour, 2007).

Population growth combined with increasingly limited resources of arable land and fresh water has resulted in a need for alternative protein sources. Nigeria with a population of approximately 196 million people, in a million Km², needs to brace up to such challenges, and exploit all sources of food production, especially protein production. Macroalgae (seaweed) and microalgae are examples of under-exploited “crops”. Algae do not compete with traditional food crops for space and resources (Stephen *et al.*, 2017).

The global population is expected to increase by over a third (2.3 billion people) by 2050, requiring an estimated 70% increase in food production (Godfray *et al.*, 2010). A combination of improved agricultural food production methods and an increase of average per capita income have led to a decrease in global hunger over the last half-century, despite a doubling of the world's population (Godfray *et al.*, 2010). The term SCP was coined by Carol L. Wilson in 1966. Single cell protein is dried cells of microorganism, which are used as protein supplement in human foods or animal feeds (Rasoul-Amini *et al.*, 2009).

Besides high protein content (about 60-82% of dry cell weight), SCP also contains fats, carbohydrates, ash ingredients, moisture, nucleic acids, vitamins and minerals. Another advantage with SCP is that it is rich in certain essential amino acids like lysine and methionine which are limiting in most plant and animal foods (Jamel *et al.*, 2008).

Generally, products from macroalgae are considered as low calorie foods with high contents of minerals, vitamins, proteins and carbohydrates (Kumari *et al.*, 2009). Most of the figures published in the literature on concentration of algal proteins, dominantly enzymatic proteins, are based on estimates of so called crude proteins, commonly used in evaluating food and feed. These figures are the result of hydrolysis of the algal biomass and estimation of the total nitrogen (Rasoul-Amini *et al.*, 2009).

Algae synthesize all of the amino acids necessary for protein synthesis and some unique ones too, including D-alanine, D-glutamine and β -amino acids are found in microcystin, a toxic principle in the cyanobacterium *Microcystis*

aeruginosa.

Algae are generally regarded as a viable protein source, with EAA composition meeting FAO requirements and they are often on par with other protein sources, such as soybean and egg (Fleurence 1999). The advantages in using algae include simple cultivation, effective utilization of solar energy, faster growth and high protein and nutrient content (Rajaet *al.*, 2008).

Materials and methods

Kaduna, capital of Kaduna state, lays at Latitude 10.2N and Longitude 7.23E. It has a huge human population and a lot of both Industrial and Agricultural activities. This has led to great amount of wastes, especially from large number of textile industries, being dumped in neighbouring water bodies, one of which is River Kaduna (Ohioma *et al.*, 2009). The Algal samples collected from River Kaduna were transferred to labeled transparent 1litre plastic bottles. The samples were transported to laboratory for species identification. The algal species was washed with distilled water to remove macro/microscopic contaminations as described by Tipnee *et al.*, (2015).

The alga sample was placed as a thin smear of the sample on a glass slide with a few drops of water and covered with a cover slip. The algal sample was observed under light microscope and photographs were taken with attached digital camera. The Alga was identified by comparing the morphology with taxonomic keys and literature as given by Prescott (2007). According to its morphology and macro/microscopic observations, it was identified as *Spirogyra porticalis*

DNA Extraction

The DNA of *Spirogyra porticalis* macroalga was extracted using Bioland mini prep extraction kits. A hundred mg of the plant tissue (microalgae) was grounded in a 2.0 mL microcentrifuge tube, 600 μ l Buffer PL1 was added; The mixture was incubated at 65°C for 10 min and was mixed twice during incubation by inverting the tube, A 140 μ l Buffer PL2 was added and mixed by vortexing for 10s followed by centrifugation at 11,000 rpm for 10 min. The supernatant was carefully transferred to a clean 2.0 mL tube.

18S rDNA primer sets purchased from Bioneer, SS5 (forward, 5'-GGTGATCCTGCCAGTA

GTCATATGCTTG-3') and SS3 (reverse, 5'-GATCCTTCCGCAGGTTACCTACGGAAACC-3') were used for PCR reaction

(Grzebyk *et al.*, 1995). Each PCR sample consist of a solution of DNA template (10–20 μ g), 1 μ l of each primer (Bioneer), with a master mix which consist of Taq polymerase, dNTPs, buffer and cations. The best PCR conditions, based on PCR product quality results were determined for the microalgae sample. The first uses 50°C for 1 minute and the second run was at 60°C for 2minutes.

Following PCR reactions, the quality of the PCR products were determined through 1% standard Agarose gel electrophoresis. The DNA was isolated from the gel using Bioland mini prep extraction kits according to the manufacturer's instructions. The concentration of the DNA that was isolated was determined before proceeding for sequence analysis. The DNA obtained was sequenced, using BECKMAN COULTER CEQ 2000XL DNA analysis system and the sequences obtained were compared for homology against sequences on the GenBank at the NCBI website for Identification of the Alga.

Sample preparation

A total of 50grams of wet weight of *Spirogyra porticalis* was harvested. The alga was rinsed with fresh water to remove sand and debris and again with distilled water and manually sorted to remove epiphytes (Tipnee *et al.*, 2015).

The sample was dried using the oven drying technique. Thick slurry of wet biomass was placed in an oven overnight at temperature of 60°C for 12hours as described by Lee, *et al.*, (2010).

The dried biomass was crushed using a mortar and pestle and the dried algal powder stored in desiccators to avoid absorption of moisture prior to use.

Protein measurement

The standard AOAC (2006) procedure was used for protein analysis. Kjeldahl nitrogen method is one of the most widely recognized methods employed for crude protein determination. Crude protein was determined as total nitrogen by a conversion factor 6.25. This method involves the three (3) stages, namely, Digestion, Distillation and Titration.

Determination of moisture content

Moisture content is the amount of moisture present in a food product. This test is based on LOD (Loss on drying) at an oven temperature of 105°C. Besides water, other matter volatile at

105°C will also be lost.

A dried cooled platinum dish was weighed (w_1) and 3g of the test sample was introduced into the dish and weighed accurately (w_2). The dish was transferred and its content into an oven at 105°C to dry for about 3 hours and the dish was removed and weighed (w_3) (AOAC, 2006).

Determination of Fat content

2g of the sample was weighed into a boiling tube. 10ml of conc. HCl was added and put in a boiling water bath until solid particles dissolve and until mixture becomes brown. It was then taken off and cooled, then transferred into a separating funnel. 10ml of ethanol and 30ml of diethyl ether were added and shaken to dissolve; it was then allowed to stand for some minutes so as to separate. A clean dried conical flask (w_1) was weighed and the ether layer was transferred into the flask. The extraction was repeated twice with 25ml of diethyl ether and the extract was evaporated in a water bath. The fat was dried at 105°C in an oven, cooled and weighed (w_2) (AOAC, 2006).

Determination of Ash content

A dry cool platinum dish was accurately weighed as (w_1) and about 3g of the food sample was spread evenly in the dish and weighed as (w_2). If the substance is moist, dry using water bath and char over hot plate in the fume cardboard until no more sooth is given off. Then it was transferred using a pair of tongs into a muffle furnace at 550°C until fully ashed (colour changes to gray) and weigh as (w_3) (AOAC, 2006). Also determined were, **Total carbohydrate**, **Amino Acid Profile** and **Nitrogen Content**.

Statistical Analysis

All the values or readings are the result of mean of three replicates. Data is reported as mean \pm standard deviation (SD). Statistical analyses were performed using Microsoft Excel.

Results

Morphological Identification of Alga

The green alga was identified based on their morphology. The morphology of algal specimen was studied carefully and the specimen was identified as *Spirogyra porticalis* (Müller) Dumortier. Figure 1, show the morphology of *S. porticalis* observed under a light microscope.

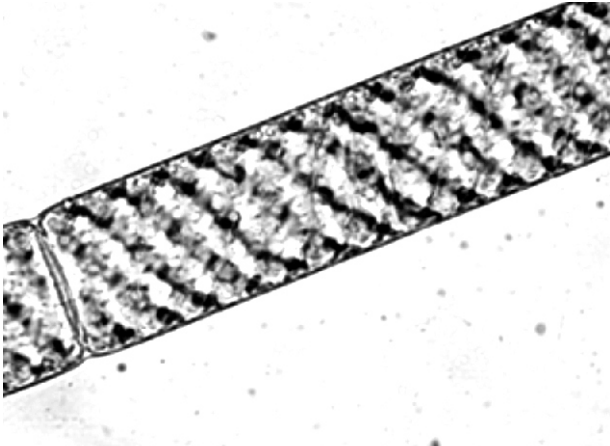


Figure 1(a) Microscopic picture of macro algae *Spirogyra porticalis* (X10 Magnification)

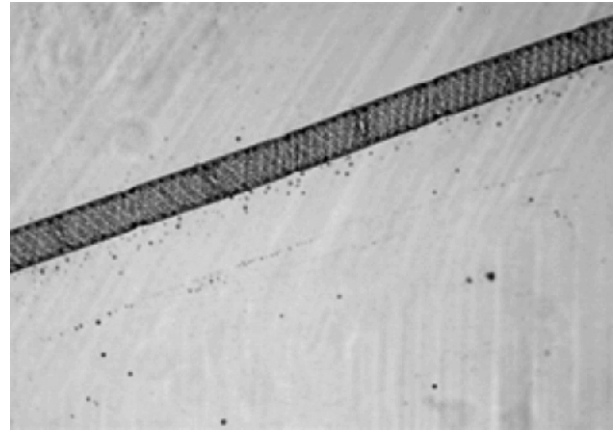


Figure 1(b) - Microscopic picture of macro algae *Spirogyra porticalis* (X40 Magnification)

Molecular Identification

DNA extraction of *Spirogyra porticalis*-

The genomic DNA was extracted and quantified. DNA concentration ranged from 1- 10 μ g. DNA band that showed high molecular weight and brightness were selected for PCR analysis.

Gel electrophoresis of the PCR product

The run made, resulted in a double band size of 0.3kb and 1.8kb on lane B when matched with the DNA molecular weight ladder on lane A as presented in Plate 4.1

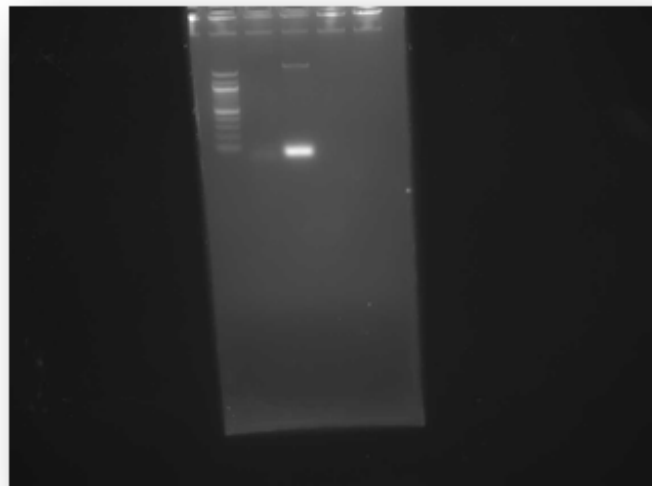


Plate 4.1 Showing Gel electrophoresis results of *Spirogyra porticalis* PCR products

Legend-

Lane A: DNA molecular weight ladder.

Lane B: DNA Sample

Sequencing and BLAST analysis

Upon Sequencing and BLAST revealed 95% identity with *Spirogyra porticalis* with an accession number: KC 701473.1 18S ribosomal RNA gene.

Nutritional Constituents (%) Of Green Algae *Spirogyra porticalis*

The chemical analyses of *Spirogyra porticalis* from River Kaduna, revealed it's nutritive value constituents as shown in Table 1. Protein content ranged between 12.46% and 16.89%. Carbohydrate content from 34.72% to

39.25% fat content ranged from 20.17 to 22.03%, moisture content ranged from 12.97 to 16.75%, and ash content ranged from 10.78 to 15.98%.

Determination of Amino Acid Profile

The known sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Applied Biosystems PTH Amino Acid Analyzer.

The results have shown the presence of both essential and non-essential amino acids, as presented in Table 2.

Table 1: Nutritional Constituents (%) Of *Spirogyra porticalis*

S/NO	Parameter	Mean \pm SD (%)	Range (%)
1	Protein	14.68 \pm 2.21	12.46-16.89
2	Fat	21.02 \pm 0.94	20.17-22.03
3	Moisture	14.75 \pm 1.89	12.97-16.75
4	Ash	13.23 \pm 2.61	10.78-15.98
5	Total carbohydrate	36.32 \pm 2.53	34.72-39.25

Table 2: Amino Acids Profile of Green Alga *Spirogyra porticalis*

S/NO	Amino Acids	Concentration: g/100g protein
1	Leucine (<i>Leu</i>)*	8.58
2	Lysine*	6.52
3	Isoleucine (<i>Ile</i>)*	5.20
4	Phenylalanine(<i>Phe</i>)*	4.16
5	Tryptophan (<i>Trp</i>)*	1.10
6	Valine <i>Val</i> (*)	5.26
7	Methionine (<i>Met</i>)*	1.60
8	Proline (<i>Pro</i>)	2.50
9	Arginine (<i>Arg</i>)*	6.02
10	Tyrosine (<i>Tyr</i>)	3.96
11	Histidine (<i>His</i>)*	1.53
12	Cystine (<i>Cys</i>)	0.24
13	Alanine (<i>Ala</i>)	6.82
14	Glutamic acid (<i>Glu</i>)	12.43
15	Glycine (<i>Gly</i>)	3.80
16	Threonine (<i>Thr</i>)*	4.44
17	Serine (<i>Ser</i>)	4.59
18	Aspartic acid (<i>Asp</i>)	9.86

Key: *Essential amino acids

The DNA extraction showed DNA concentration as ranging from 1 -10 μ g. The molecular weight of the PCR product was 1.8kb using a set of 18S r DNA primer sets. This is consistent with the result of Reynaldo, (2012). Sequencing and BLAST revealed 95% identity with *Spirogyra porticalis* 18S ribosomal RNA gene.

Discussion

The freshwater alga used in this study was identified as *Spirogyra porticalis* filamentous green alga. The macroalgae, *S. porticalis* green algae, commonly available in River Kaduna, was analyzed for its nutritional and amino acids composition. The nutritional and amino acids composition of *S. porticalis* reveals that this macroalgae has an appreciable amount of dietary protein, carbohydrate, moisture, fat and ash ingredient which are the components of single cell protein (SCP).

The amino acid composition shows it as rich in *Glutamic acid* (12.4g/100g protein). Study of the amino acid profile of this green alga, has shown that eight amino acids occurred in high concentration in all of them. These were *Lysine*, (8.58 g/100g protein), *Isoleucine* (5.20 g/100g protein), *Aspartic acid* (9.86 g/100g

protein), *Arginine* (6.02). *Leucine* (6.52 g/100g protein), *Alanine* (6.82 g/100g protein), *Valine* (5.26 g/100g protein) *cystine* is the least in all the assessed amino acids (0.24 g/100g protein).

The essential Amino acid profile of the alga as compared with conventional protein sources and the WHO/FAO reference pattern of essential amino acids (g per 100 protein), reveals that the levels of the essential amino acids in the alga were either similar to or greater than the levels of the same amino acids in the reference foods and WHO/FAO reference pattern (WHO/FAO, 2013).

Therefore algae as a protein source can readily substitute for egg or other animal protein. Their amino acid profile is far superior to that of soybean and to that of other plant sources when considering the essential amino acid content. Searching for algae isolates with high rate of biomass potential with essential amino acids

could expand the scope of source of relevant food supplements for humans and animal feed.

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