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Full Length Research Paper

Chemical analysis of the biomass of a native strain of *Spirulina subsalsa* Oersted ex Gomont 1892 (Spirulinaceae) cultivated in low-cost saline medium

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Spirulina subsalsa, **a filamentous cyanobacterium, was first described by Gomont in 1892. This microorganism has been subject to biotechnological evaluations, due to their high content of proteins and pigments. The objective of this study was to analyze the biochemical composition of the biomass of a native strain of *S. subsalsa* cultivated in low-cost saline medium and harvested in the exponential and stationary phases of growth. The highest protein contents (58.5%) were obtained in the exponential phase; while the highest amounts of carbohydrates (20%), lipids (19.7%), chlorophyll (51.6 µg/ml), total carotenoids (218,215 µg/ml), exopolysaccharides (7.30 ± 0.7 mg/ml) and phycocyanin (25.8 µg/ml) were accumulated in the stationary phase. Additionally, in the biomass of *S. subsalsa*, the presence of saponins and polyphenols was detected in both growth phases, whereas basic alkaloids and flavonoids were detected only in the stationary phase. This article concludes information on the potential future biotechnological applications of the cyanobacterium strain, *S. subsalsa.***

Key words: Cyanobacterium, biotechnology, *Spirulina subsalsa*.

INTRODUCTION

Spirulina subsalsa Oersted ex Gomont is a filamentous cyanobacteria originally described by Gomont (1892, 1893). This microorganism inhabits saline and fresh waters all over the world (Szulbert et al., 2018). In Venezuela, Spirulina has been reported by Rodriguez (2001), Bernal (2002), González et al. (2003) and Petrash et al. (2012).

This cyanobacterium forms mantles on the substrate, usually blue-green in color and has sometimes been observed to be part of the cyanobacteria blooms that cause poisoning in flamingos (Ballot et al., 2004) and shrimp (Lightner, 1978); however, there is no evidence that this cyanobacterium produces any cyanotoxin.

The biotechnological potential of *S. subsalsa* has been little studied, being used as a bioremediator agent of residual contaminants (Jiang et al., 2015), biosensor for

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the evaluation of the toxicity of estuarine waters (Campanella et al., 2001), and producing bioactive metabolites (Mazur-Marzec et al., 2015). In addition, *S. subsalsa* is a source of polyhydroxyalkanoates (PHA), which are biopolymers for construction of implants and artificial tissues (Shrivastav et al., 2010).

Spirulina cultures are usually carried out in fresh water and need expensive culture media, due to the inclusion of a large number of analytical grade salts. Between these means, Zarrouk medium was emphasized (Zarrouk, 1966), *Spirulina* (Aiba and Ogawa, 1977), BG-11 (Rippka, 1988), and some modified media (Amala and Ramanathan, 2013; Kumari et al., 2014a, b). This situation has led to the search for alternative sources of culture media that allow obtaining high yields of biomass at low cost. Furthermore, it is necessary to evaluate new strains of this cyanobacterium, since it has been demonstrated that the responses of microalgae to changes in abiotic factors vary considerably from one species to another, between strains of the same species and even between clones originating from the same unialgal culture, which would be due to morphological and physiological differences, attributable to intraspecific genetic variations (Gómez and González, 2005; Guevara et al., 2016).

The objective of this investigation was to analyze the biomass of a native strain of *S. subsalsa* cultivated in low-cost saline medium and harvested in exponential and stationary phases of growth.

MATERIALS AND METHODS

A native strain of S. subsalsa, isolated from the Clavellino Reservoir, Sucre State, Venezuela (coordinates: between 10° 19 'to 10° 23' Lat. N and between 63° 35 'to 63° 40' Long. O) and deposited in the Algae Germplasm Bank of the Oceanographic Institute of Venezuela, Universidad de Oriente, with the code BGAUDO 161, was cultivated in seawater (9‰) previously treated, according to the methodology of Faucher et al. (1979).

The cultures were carried out in quadruplicate, discontinuously, for 30 days, in 45 cm diameter plastic bags, placed in cylindrical metal frames (Figure 1), containing 100 L of culture medium each with a nitrate concentration of 14 mM, 0.036 mM phosphate, 95.23 mM sodium bicarbonate, 0.0013 mM Fe and 0.0009 mM Mn. The bags were located in a controlled laboratory environment (T: 32 ± 1°C, continuous irradiance of 39 µmol/m^2/s provided by 3 white light lamps of 40 W and photoperiod 12:12) and aerated with plastic hoses and diffuser stones. The salinity and nitrate concentration used were selected according to results in previous experiments (Romero et al., 2018).

The cultures were started with inocula previously acclimated to the mentioned environmental conditions. From the beginning of the test and every 48 h, samples were taken from each of the replicas to determine the pH and population growth according to the criteria of Pelizer and Oliveira (2014).

When the culture reached the exponential phase (2 replicas) and stationary (2 replicas), the entire culture was harvested, filtering it in permaline sleeve. The filtrate was used to quantify the exopolysaccharide content according to the methodology of Vicente et al. (2004). The harvested biomass, after several washes with acidulated water (pH 4), was maintained at low temperatures (-20°C) until the moment of realization, in triplicate, the protein analysis, according to Lowry et al. (1951); total lipids, according to Bligh and Dyer (1959) and Pande et al. (1963); carbohydrates, according to Dubois et al. (1956); secondary metabolites according to Domínguez (1973) and Marcano and Hasegawa (2002), and pigments according to Sharma et al. (2014) and Murugan and Rajesh (2014).

Analysis of the results

The data of the values of exopolysaccharides, proteins, lipids, carbohydrates, and *Spirulina* pigments obtained in the exponential and stationary growth phases were contrasted by a one-way analysis of variance (phases), following recommendations of Sokal and Rolhf (1995).

RESULTS

Growth and pH

Figure 2 shows the population growth of the microalgae *S. subsalsa* in the low-cost culture medium during the 30 days of the trial. It is observed that during the first 6 days, this microalga was in adaptation phase; after which, the culture entered the exponential growth phase till day 12. Followed by and until the end of the trial, the culture remained in the stationary phase, and no signs of a descent phase were observed. The pH of the cultures was between 9 and 10.2.

Expolisaccharides

The exopolysaccharide content obtained in the *S. subsalsa* cultures presented significant differences ($p < 0.05$) between the growth phases (Figure 3). The concentration of these exocompounds was 7.30 ± 0.7 and 5.4 ± 0.4 mg/ml on stationary and exponential phase.

Proteins, carbohydrates, lipids and pigments

The contents of proteins, carbohydrates, lipids and pigments of *S. subsalsa* cultivated in a low-cost saline medium are shown in Table 1. Total proteins showed significant differences ($p < 0.05$) between the phases, reaching their highest contents in the exponential phase (58.5 ± 0.58%). The rest of the analyzed compounds, like the proteins, showed significant differences between the phases ($p < 0.05$), but with the difference that their highest values were obtained in the stationary phase. In this way, carbohydrates, lipids, chlorophyll, phycocyanin and total carotenoids had percentages of 20.0 ± 2.71%, 19.7 ± 1.41%, 51.6 ± 0.64 µg/ml, 25.8 ± 0.40 µg/ml and 218.215 ± 2.27 µg/ml, respectively.

Table 1. Content of proteins, carbohydrates, lipids and pigments of *S. subsalsa*.

Content	Exponential phase	Stationary phase
Proteins (%)	58.5 ± 0.58[a]	41.37 ± 0.90[b]
Carbohydrates (%)	17.1 ± 0.95[a]	20.0 ± 2.71[b]
Lipids (%)	14.1 ± 0.20[a]	19.7 ± 1.41[b]
Pigments (µg/ml)		
Chlorophyll *a*	42.7 ± 0.40[a]	51.6 ± 0.64[b]
Total carotenoids	157.9 ± 2.36[a]	218.215 ± 2.27[b]
Phycocyanin	20.2 ± 1.05[a]	25.8 ± 0.40[b]

[a, b]Different letters between rows denote significant differences ($p < 0.05$).

Table 2. Secondary metabolites in *Spirulina subsalsa* grown in low-cost saline medium and harvested during the phases of exponential and stationary growth.

Secondary metabolite	Crop phase	
	Exponential	Stationary
Saponins	+	+
Alkaloids (basic)	-	+
Anthraquinones	-	-
Flavonoids	-	+
Phenolic compounds (polyphenols)	+	+
Phenolic compounds (tannins)	-	-
Cyanogenic glycosides	-	-
Cardiotonic glycosides	-	-
Pentacyclic triterpenes	-	-
Unsaturated sterols	-	-

Secondary metabolites

As shown in Table 2, the presence of saponins and polyphenols in the fresh biomass of *S. subsalsa* was positive in both phases of growth; however, basic alkaloids and flavonoids were only evidenced in the stationary phase (Table 2).

DISCUSSION

The population growth observed in *S. subsalsa* in this study is related to the results presented by Rodríguez and Triana (2006), who indicated that in the *Spirulina* species, the adaptation phase usually lasts between zero and four days, because the microalga is coupled to the culture conditions and has a low specific growth rate. From there, the growth of the microalga gradually increases, entering the phase of exponential growth, where cell multiplication is at its maximum. This phase continues until it reaches its maximum value (days 12-16), where depletion of nutrients has been observed, hence a decrease in growth. The stationary phase begins, due to the decrease in the rate of growth, increased cellular respiration and accumulation of enhancement of toxic wastes. At this point, it is important to take care of the cultivation conditions to extend the phase and avoid unfavorable conditions that might cause the death of the cells (death phase). In the present study, death of the cells did not occur during the present test.

The amount of maximum biomass obtained in this study was 3.1 mg/ml. This biomass value is higher than those reported by Oliveira et al. (1999), where they determined an amount of 2.4 mg/ml at 30°C, in *Spirulina platensis* and *Spirulina maxima*. This difference may be due to the temperatures used for the culture, in this work the maximum temperature recorded was 32 ± 1°C.

Volkmann et al. (2008) and Licet et al. (2014) obtained higher biomass than those achieved in this research when cultivating *Arthrospira platensis* viz. 4.95 and 3.5 mg/ml, respectively. This difference may be due to the fact that the previous authors used different culture conditions, among these are the irradiance (140 and 390 µmol/m^2/s, respectively), which were greater than those

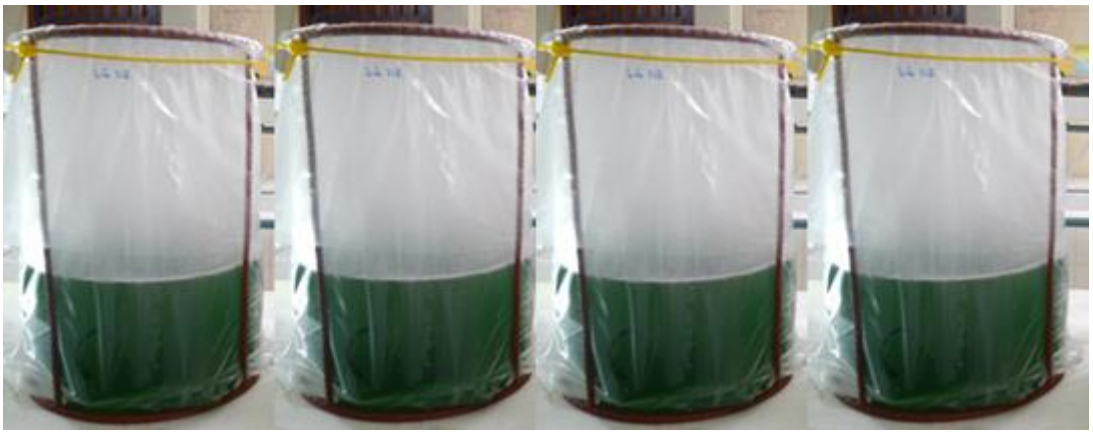

Figure 1. Cultivation of *S. subsalsa* in 45 cm diameter plastic bags, placed in cylindrical metal frames, containing 100 L each of culture medium.

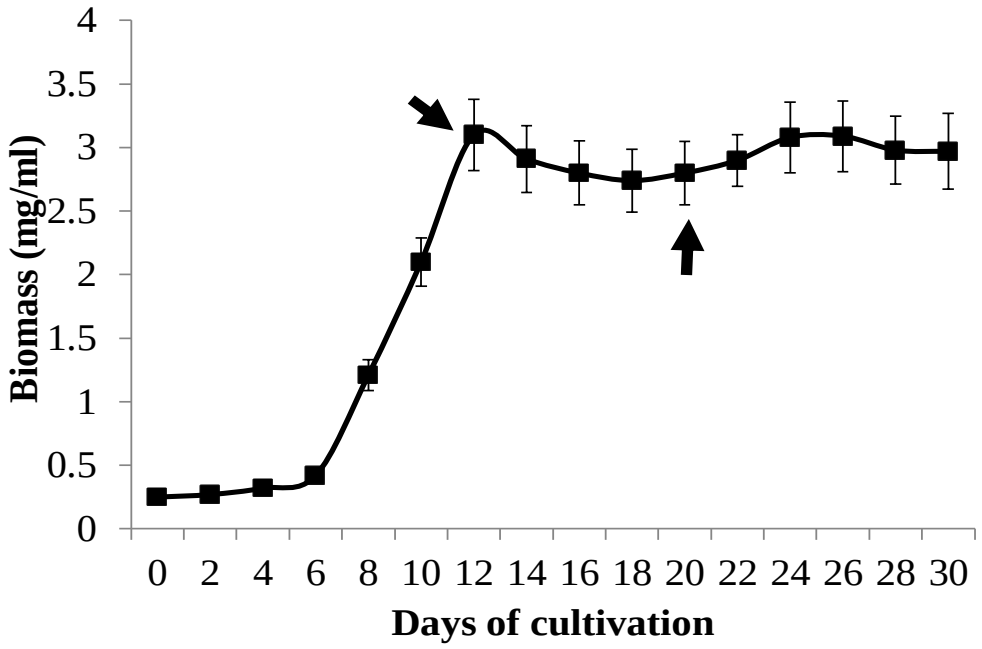

Figure 2. Population growth of *S. subsalsa*. The arrows indicate the days of harvest (exponential at 12 days and stationary at 20 days).

implemented in this research (39 µmol/m^2/s).

The pH of the cultures remained between 9 and 10.2, which is within the values reported for this cyanobacterium, according to the criteria of Rincón et al. (2013).

Several studies have reviewed the ability of cyanobacteria to adapt to variations in salinity (Thajuddin and Subramanian, 2005; Nagle et al., 2010; Joset et al., 1996), but not all cyanobacteria are halotolerant (Blumwald and Tel-Or, 1982). The ability of cyanobacteria to grow at high concentrations of Na^+ may be related to their ability to regulate respiration (Gabbay-Azaria et al., 1992), the flow of Na^+ (Molitor et al., 1986) and the production of osmolytic compounds (Reed et al., 1986), which help the cells to withstand the pressure caused by the large amount of sodium ions present in the medium. One of these compounds are the exopolysaccharides, which are exuded into the environment, as an osmoprotective effect.

The higher content of exopolysaccharides in the

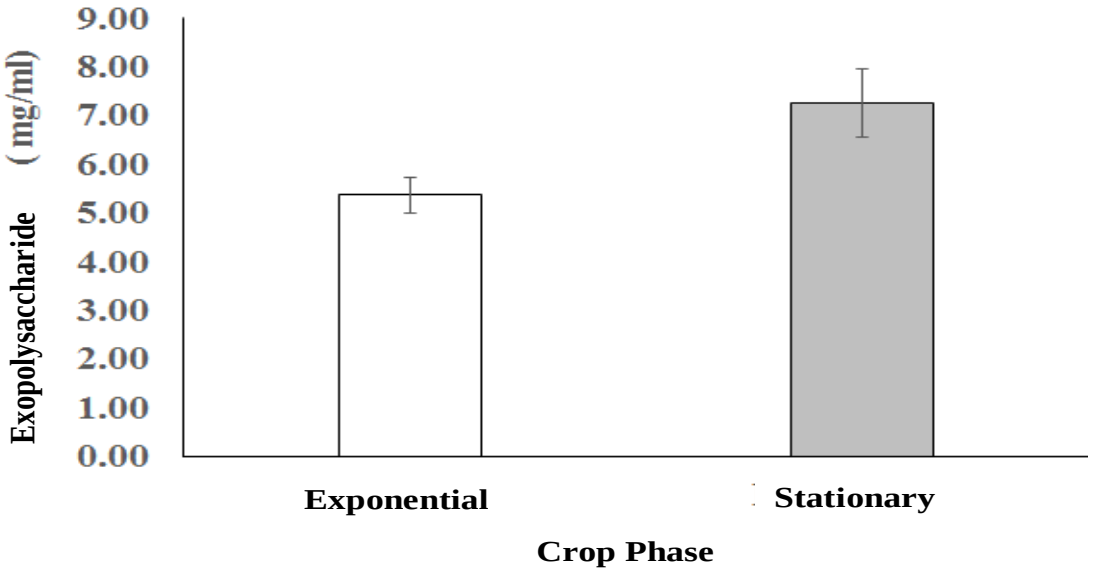

Figure 3. Exopolysaccharide content (mg/ml) of a native strain of *S. subsalsa*, cultured in a low-cost saline medium and harvested in the phases of exponential and stationary growth.

stationary phase may be due to the deficiency of nitrogen that occurs in this phase, as indicated by De Philippis et al. (1993) and Otero and Vincenzini (2003). This situation probably contributes to the increase in the C: N ratio, which promotes the incorporation of carbon in polymers (Otero and Vin-cenzini, 2003; Kumar et al., 2007).

The higher contents of exopolysaccharides together with the growth of the cyanobacterium and increase in the pH of the medium, limit the availability of light, which leads to an increase in the content of accessory pigments and phycobiliproteins, thus reducing the phosphorus and nitrogen content, and subsequently the redirection of the cellular metabolism towards the synthesis of carbohydrates (Laloknam et al., 2010; Magro et al., 2018).

Although the characterization of the obtained exopolysaccharides was not satisfied in the development of this work, some authors have managed to isolate and identify some sulphated type of *Spirulina* polysaccharides, called spirulan calcium Ca-SP, in which antiviral (*in vitro* and *ex vitro*) microbiological tests has inhibited the replication of HIV, Herpes simplex, human cytomegalovirus, influenza A virus, mumps and measles (Chamorro et al., 2002). *In vitro* studies suggest that the polysaccharides, unique to *Spirulina*, improve the enzymatic activity of the cell nucleus and the synthesis and repair of DNA (Premkumar et al., 2004).

The highest total protein contents of *S. subsalsa*, cultivated in low-cost saline medium, were obtained in the exponential phase (58.5%). These results may be due to the fact that in this phase, the culture medium did not present nutrient limitations, which favors protein synthesis. In addition, the salinity used in crops does not represent extreme stress levels that can interfere with protein accumulation.

Andrade et al. (2018) have observed protein content in *Spirulina* between 50 and 70%. These differences in biochemical composition, including proteins, are attributed to the variation between genera and species, and in the culture conditions (availability of nutrients, pH, light, temperature) of a particular species (Colla et al., 2007).

The highest contents of lipids (19%) and carbohydrates (20%) were observed in the stationary phase; this could be due to the fact that in this phase, the supply of nutrients usually decreases and the irradiance received by the culture becomes less, motivated by the overshadowing caused by the massive growth of this cyanobacterium, which have been referred to as stimulants of the accumulation of lipids and carbohydrates (Möllers et al., 2014).

Similar to carbohydrates and lipids, the pigment contents showed their highest values in the stationary phase. Chlorophyll *a* reached contents of 51.6 µg/ml and total carotenoids of 218.215 µg/ml. These results differ from that reported by Marrez et al. (2013), who obtained values of chlorophyll *a* and total carotenoids of 147.43 and 139.88 µg/ml, respectively for *S. platensis*. The discrepancies may be due to the dissimilarity of the salinities, since 9‰ was used in the present investigation and the mentioned authors cultivated salinities of 4.83‰.

Senthilkumar and Jeyachandran (2006) reported that the cultivation of cyanobacteria with high salt concentrations significantly affects the chlorophyll content. The results of Ayachi et al. (2007) supports this, who

observed that the inhibition of chlorophyll synthesis under salt stress is due to a decrease in the energy level caused by the pumping of sodium ions entering the cell, and that also causes a significant inhibition of the chain of electron transport and transport of electrons in the photosystem (PS-II), due to damage in the PS-II reaction center and alterations in the water oxidation complex (Pulz and Gross, 2004).

The highest values of phycocyanin were 25.8 µg/ml, which is lower than those reported (55.37 µg ml^{-1}) by Marrez et al. (2013) obtained in *S. platensis* and cultivated in SHU medium. It is evident here that the composition of the culture medium exerts influence on the chemical composition of cyanobacteria (Marrez et al., 2014). The optimization of the culture conditions to maximize the accumulation of phycocyanin is due to the fact that this compound is indicated as being responsible for the antioxidant activity of this cyanobacterium (Ahmed et al., 2014).

The presence of saponins and flavonoids in both phases of cultivation, and basic alkaloids and flavonoids in the stationary phase, coincides with that reported by Borowitzka (1995), who proposes that almost all biologically active compounds of interest are secondary metabolites, thereby tending to be more abundant in the stationary phase or in slow-growing crops.

Some reports show that microalgae and cyanobacteria can contain many kinds of phenolic compounds, such as flavonoids (Klejdus et al., 2010). Hamouda and Doumandji (2017) performed the phytochemical analysis of *S. platensis*, testing with some solvents: acetone, methanol, ether, dichloromethane and hexane, and found the presence of flavonoids, phenolic compounds, alkaloids and cardiac glycosides.

Although no calculations were made to estimate the production costs of *S. subsalsa* with the culture medium used in this research, it can be inferred that this medium is less expensive, since it only has 5 commercial grade salts, while the zarrouk medium, the most widely used in the cultivation of *Spirulina*, has 21 analytical grade salts, with which 1000 L of medium can be prepared at a price of US$ 79.5 (Raoof et al., 2006).

The results obtained on the growth, as well as the contents of proteins, lipids, carbohydrates and pigments in the native strain of *S. subsalsa* when cultivated in low-cost saline medium, permit us to suggest the use of this cyanobacterium in the biotechnological industries with a view to their use as food in aquaculture and in humans, making it necessary to specify the degree of toxicity, since some strains can be toxic in certain culture conditions.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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