UNIVERSIDAD NACIONAL DE COLOMBIA
SEDE BOGOTÁ

ACTA BIOLÓGICA COLOMBIANA
http://www.revistas.unal.edu.co/index.php/actabiol

ARTÍCULO DE INVESTIGACIÓN/RESEARCH ARTICLE

COMPARATIVE GROWTH AND BIOCHEMICAL COMPOSITION OF FOUR STRAINS OF *Nostoc* AND *Anabaena* (CYANOBACTERIA, NOSTOCALES) IN RELATION TO SODIUM NITRATE

Comparación del crecimiento y Composición Bioquímica de cuatro cepas de *Nostoc* y *Anabaena* (Cyanobacteria, Nostocales) en relación con el nitrato de sodio

Néstor ROSALES LOAIZA[1], Patricia VERA[1], Cateryna AIELLO-MAZZARRI[2], Ever MORALES[1].

[1] Laboratorio de Microorganismos Fotosintéticos, Departamento de Biología, Facultad Experimental de Ciencias, Universidad del Zulia. Maracaibo, Venezuela.

[2] Laboratorio de Fermentaciones Industriales, Departamento de Ingeniería Bioquímica, Facultad de Ingeniería, Universidad del Zulia. Maracaibo, Venezuela.

For correspondence. nestoralgae@gmail.com

Received: 4th February 2015, **Returned for revision**: 23th July 2015, **Accepted**: 26th October 2015.
Associate Editor: Rafael Riosmena Rodríguez.

Citation / Citar este artículo como: Rosales Loaiza N, Vera P, Aiello-Mazzarri C, Morales E. Comparative growth and biochemical composition of four strains of *Nostoc* and *Anabaena* (Cyanobacteria, Nostocales) in relation to sodium nitrate. Acta biol. Colomb. 2016;21(2):347-354. doi:http://dx.doi.org/10.15446/abc.v21n2.48883

ABSTRACT

Nitrogen concentration is an essential parameter in cyanobacterial cultures to produce enriched biomass with biotechnological purposes. Growth and biochemical composition of *Nostoc* LAUN0015, *Nostoc* UAM206, *Anabaena* sp.1 and *Anabaena* sp.2 were compared at 0, 4.25, 8.5 and 17 mM $NaNO_3$. Cultures under laboratory conditions were maintained for 30 days at a volume of 500 mL. *Anabaena* sp.1 yielded the highest value of dry mass of 0.26 ± 2.49 mg mL^{-1} at 8.5 mM $NaNO_3$. For chlorophyll, phycocyanin and phycoerythrin, maximum values were achieved at 17 mM $NaNO_3$ with 18.09 ± 1.74, 102.90 ± 6.73 and 53.47 ± 2.40 µg mL^{-1}, respectively. *Nostoc* LAUN0015 produced its maximum value of protein 644.86 ± 19.77 µg mL^{-1}, and 890 mg mL^{-1} of carbohydrates in the absence of nitrogen. This comparative study shows that the most efficient strain for the production of protein, carbohydrates and lipids in diazotrophic conditions corresponded to *Nostoc* LAUN0015. However, *Anabaena* sp.1 and *Anabaena* sp.2 required high nitrogen concentrations to achieve higher values of metabolites, comparing with *Nostoc* strains. Nitrogen dependence for the production of pigments and high protein production in strains of *Anabaena* and in diazotrophic conditions for *Nostoc* was demonstrated. *Nostoc* can be cultured under nitrogen deficiency and *Anabaena* in sufficiency, for biomass production enriched with proteins and carbohydrates.
Keywords: *Anabaena*, biochemical composition, biomass, culture, nitrate, *Nostoc*.

RESUMEN

La concentración de nitrógeno constituye un parámetro esencial en cultivos de cianobacterias para la producción de biomasa enriquecida con fines biotecnológicos. Se comparó el crecimiento y composición bioquímica de las cepas *Nostoc* LAUN0015, *Nostoc* UAM206, *Anabaena* sp.1 y *Anabaena* sp.2 a 0, 4,25; 8,5 y 17 mM $NaNO_3$. Los cultivos en condiciones de laboratorio fueron mantenidos durante 30 días a un volumen de 500 mL. En masa seca, *Anabaena* sp.1 obtuvo el mayor valor, con 2,49 ± 0,26 mg mL^{-1} a 8,5 mM $NaNO_3$. Para clorofila, ficocianina y ficoeritrina, los máximos se alcanzaron a 17 mM $NaNO_3$ en *Anabaena* sp.1, con 18,09 ± 1,74; 102,90 ± 6,73 y 53,47 ± 2,40 µg mL^{-1}, respectivamente. *Nostoc* LAUN0015 produjo su máximo valor de proteínas de 644,86 ±19,77µg mL^{-1}, y alrededor de 890 µg mL^{-1} de carbohidratos en ausencia de nitrógeno. El estudio comparativo indica que la cepa más eficiente para la producción de proteínas, carbohidratos y lípidos, en condiciones diazotróficas, correspondió a *Nostoc* LAUN0015. En cambio, las cepas de *Anabaena* sp.1 y sp.2 requieren de elevadas concentraciones de nitrógeno para alcanzar los mayores valores de

metabolitos, respecto a las cepas de *Nostoc*. Se demuestra la dependencia de nitrógeno para la producción de los pigmentos y la alta producción proteica en las cepas de *Anabaena* y en condiciones diazotróficas para *Nostoc*. Esta última puede ser cultivada bajo una deficiencia de nitrógeno y *Anabaena* con suficiencia para la producción masiva de biomasa enriquecida con proteínas y carbohidratos.
Palabras clave: *Anabaena*, biomasa, composición bioquímica, cultivo, nitrato, *Nostoc*.

INTRODUCTION

Cyanobacteria or blue-green algae are photosynthetic microorganisms that can be used to produce high-value compounds (Vincent, 2009). These include high protein content; capacity to synthesize all amino acids (and provide the essential ones to humans and animals); presence of carbohydrates composed of starch, glucose, sugars and non-digestible polysaccharides (agar, carrageenan and alginate); lipids in the form of glycerol and fatty acids of the ω-3 and ω-6 families; and a valuable content of many essentials vitamins, minerals and antioxidant substances (Harun *et al*., 2010). With this biochemical composition is not a surprise that this microorganism can be used as a food source for animal and humans (Gantar and Svirčev, 2008; Cunningham and Joshi, 2010).

Dried microalgal biomasses typically contain 46–63 % protein, 8–17 % carbohydrates, and 4–22 % lipids, as well as a wide range of vitamins and other biologically active substances such as bioactive peptides and pigments (Gantar and Svirčev, 2008).

Nostoc, an edible blue-green alga, is a cyanobacterium that has been grown and cultivated for medicinal uses for centuries (Gantar and Svirčev, 2008). Recent studies have indicated that *Nostoc* contains cryptophycin, a compound that inhibits cancer cell growth, as well as anti-viral compounds (Cunningham and Joshi, 2010; Sharma *et al*., 2011).

Filamentous cyanobacteria *Nostoc*, *Spirulina*, *Arthrospira*, *Anabaena*, *Aphanizomenon*, *Rivularia,* and many others are particularly attractive for the production of high quality biomass, because they represent a source of protein and a variety of chemicals and pharmaceuticals (Gantar and Svirčev, 2008).

Despite the great interest in culturing microalgae and cyanobacteria, it is estimated that only 10 % of existing species have been studied in order to know their physiology and their potential as producers of biocompounds, especially in relation to tropical strains (Rasmussen and Morrissey, 2007). Therefore, the aim of this work was to evaluate the gross biochemical characteristics of two stains of *Nostoc,* and two strains of *Anabaena*, cultivated in different nitrogen concentrations in order to prove their potential use as food or supplement, especially as a protein source.

MATERIALS AND METHODS

Filamentous heterocystous cyanobacteria studied were: (1) *Nostoc* LAUN 0015, isolated form a humid environment in Bogota, Colombia; (2) *Nostoc* UAM206, isolated from an inundated rice field in Valencia, Spain; (3) *Anabaena* sp.1, from activated sludge of a treatment plant; and (4) *Anabaena* sp.2 from an oil pit in Venezuela.

Cultures by triplicate were maintained in 1 L flasks with 500 mL culture medium composed of sterilized tab water enriched BG-11 culture medium (Rippka *et al*., 1979). Flasks were inoculated to an absorbance of 0.08 at 750 nm, and incubated at 29 ± 2 °C under a 12h-light/12h-dark cycle with a light intensity of 156 µmol of photons s^{-1} m^{-2} and constant aeration of 4.95 ± 0.03 mL s^{-1}.

Growth was evaluated at four different sodium nitrate concentrations by using 0, 4.25, 8.5 and 17 mM $NaNO_3$, equivalent to 0, 25, 50 and 100 % of nitrate concentration present in BG-11 culture medium.

Growth was determined by turbidity (OD750 nm). Biomass was harvested by centrifugation to 10 x10^3 g for 10 min. Frozen biomass, stored to -20 ºC, were used for all the biochemical analyses, except for pigments and dry weight, for which fresh biomass samples were used. The protein content was determined by the modified Folin-Lowry method (Herbert *et al*., 1971). Pigments were extracted in methanol (99 %) at 4 °C overnight and measured by spectrophotometric methods (Strickland and Parsons, 1972; Marker and Jinks, 1982). Carbohydrates were measured by the phenol-sulfuric acid method (Kochert, 1978). Dry weight was determined using a Millipore© filtration system, with 0.45 µm fiberglass filter, by the method of Utting (1985).

Statistical analyses were performed with SPSS 15.0, using analysis of variance (ANOVA) and Sheffé's test to examine differences in cellular density and biochemical composition between different nutrient concentrations.

RESULTS

For biomass production there were different patterns for every strain of cianobacteria (Table 1). *Nostoc* strains produced higher biomass values to lower sodium nitrate concentrations, with maximum of 1.32 ± 0.12 and 1.56 ± 0.16 mg mL^{-1}, at 0 mM $NaNO_3$, for *Nostoc* LAUN0015 and *Nostoc* UAM206, respectively ($p < 0.05$). *Anabaena* strains enhanced biomass production increasing nitrogen concentration until 8.5 mM $NaNO_3$. *Anabaena* sp.1 reached the highest biomass production of 2.49 ± 0.26 mg mL^{-1} ($p < 0.05$).

Chlorophyll *a* and carotenoids production from filamentous cyanobacteria to different nutrient concentrations are shown in Figure 1. Production of these pigments seems not to be influenced for nitrogen concentration, except for *Nostoc* LAUN0015. This strain showed a diminishing of pigment

Table 1. Dry weight (mg mL^{-1}) from *Nostoc* and *Anabaena* strains cultured to different nitrogen concentration (mM $NaNO_3$).

	0	4.25	8.5	17
Nostoc LAUN005	1.32 ± 0.12[a]	1.25 ± 0.13[a]	1.20 ± 0.14[a]	0.98 ± 0.13[b]
Nostoc UAM206	1.56 ± 0.16[a]	1.38 ± 0.07[b]	1.31 ± 0.10[b]	1.09 ± 0.12[c]
Anabaena sp.1	1.89 ± 0.21[a]	2.38 ± 0.26[b]	2.49 ± 0.26[c]	2.40 ± 0.30[b]
Anabaena sp.2	1.86 ± 0.13[a]	1.95 ± 0.13[a]	2.01 ± 0.12[a]	1.96 ± 0.17[a]

Values obtained in stationary phase. Letters correspond to groups with statistical differences ($p < 0.05$) for each strain.

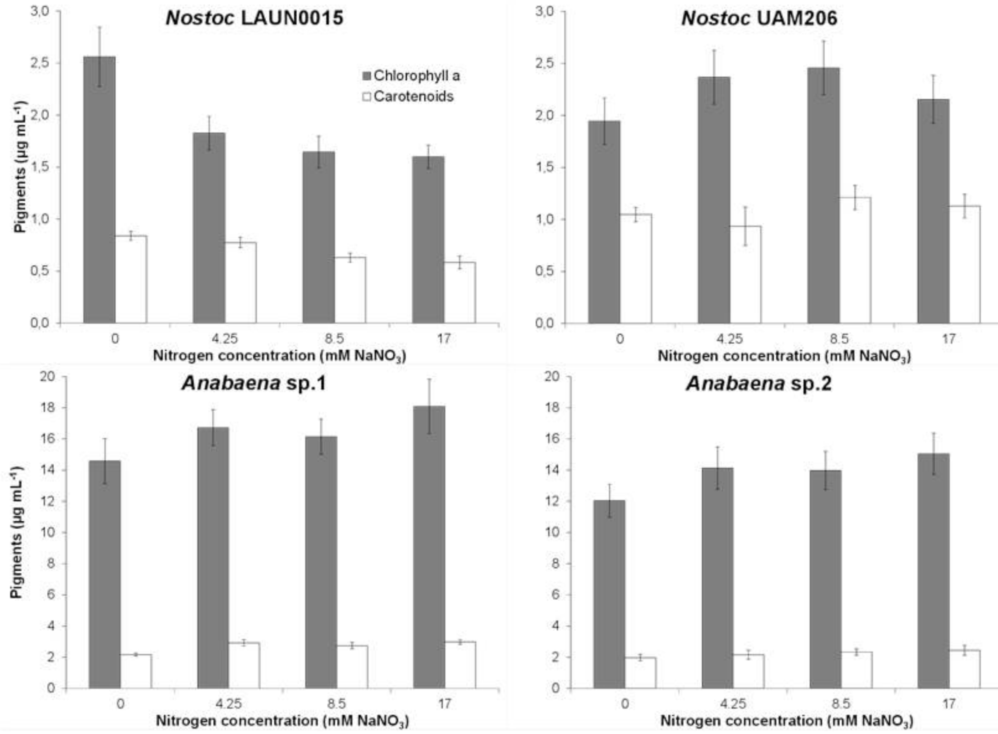

Figure 1. Liposoluble pigments: chlorophyll *a* and carotenoids (µg mL^{-1}) from *Nostoc* and *Anabaena* strains cultured to different nitrogen concentration.

production with an increase of nitrogen. Maximal production for *Nostoc* LAUN0015 was obtained at 0 mM $NaNO_3$ with 2.56 ± 0.28 and 0.84 ± 0.04 µg mL^{-1} for chlorophyll *a* and carotenoids, respectively ($p < 0.05$).

The remaining strains showed higher levels of pigments with increasing nitrogen concentration. *Nostoc* UAM206 obtained 2.37 ± 0.26 and 2.46 ± 0.26 µgmL^{-1} for chlorophyll *a* at 4.25 and 8.5 mM $NaNO_3$, respectively; with no significant differences ($p > 0.05$). In contrast, carotenoids reached their peak between 8.5 and 17 mM $NaNO_3$ with 1.21 ± 0.12 and 1.13 ± 0.11 µg mL^{-1}, respectively ($p > 0.05$).

Anabaena strains obtained their higher values of liposoluble pigments to the highest nitrogen concentration. *Anabaena* sp.1 produced 1.74 ± 18.09 and 2.99 ± 0.15 µg mL^{-1} of chlorophyll *a* and carotenoids, respectively. *Anabaena* sp.2 achieved 15.04 ± 1.33 µg mL^{-1} of chlorophyll *a* and 2.44 ± 0.32 µg mL^{-1} of carotenoids. These values were obtained at 17 mM $NaNO_3$, although there was no significant difference ($p > 0.05$) with respect to treatments 4.25 and 8.5 mM $NaNO_3$.

Figure 2 shows production of hydrosoluble pigments (phycocyanin and phycoerythrin). *Nostoc* LAUN0015 (Fig. 2) showed for phycoerythrin, the same trend observed in the liposoluble pigments, with the highest value of 23.68 ± 2.33 µg mL^{-1} in the absence of nitrogen ($p < 0.05$). Highest values of phycocyanin, 14.04 ± 0.83 and 13.97 ± 0.23 µg mL^{-1}, were obtained at 0 and 4.25 mM $NaNO_3$, respectively; with no significant differences ($p > 0.05$).

Nostoc UAM206 showed statistical homogeneity ($p > 0.05$) for phycocyanin, phycoerythrin production from 0 to 8.5 mM $NaNO_3$. Maximum values of phycocyanin and phycoerythrin 11.55 ± 1.05 and 13.10 ± 1.04 µg mL^{-1} were obtained at 8.5 and 0 mM $NaNO_3$.

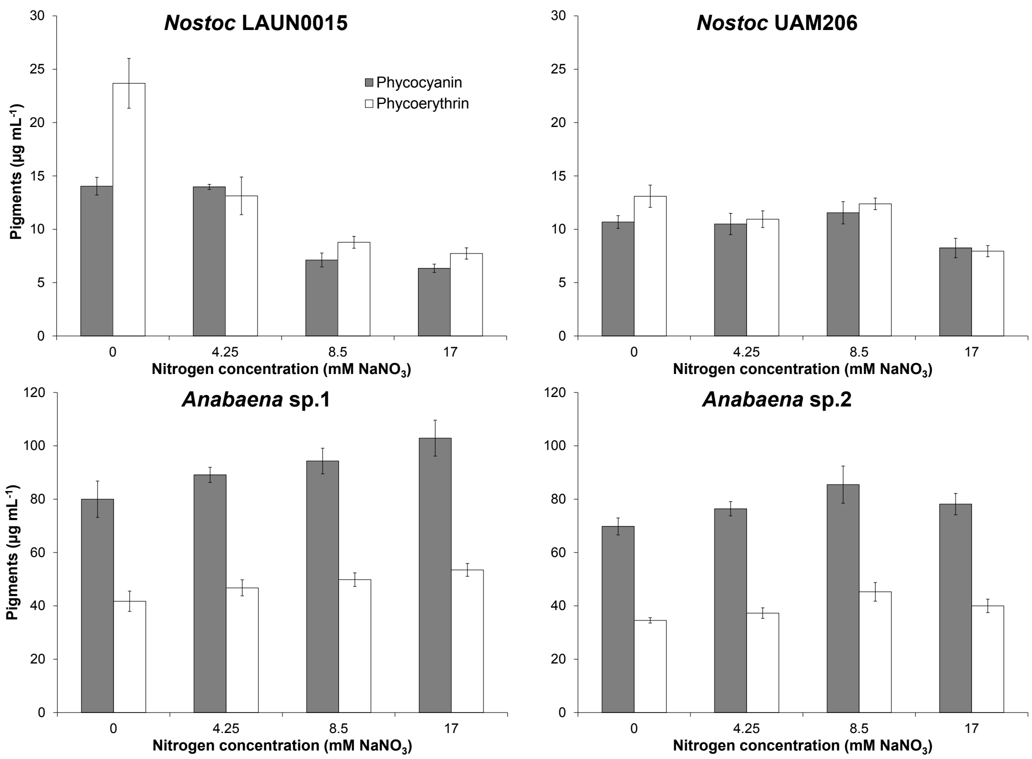

Figure 2. Hydrosoluble pigments: phycocianin and phycoerythrin (µg mL^{-1}) from *Nostoc* and *Anabaena* strains cultured to different nitrogen concentration.

Anabaena strains showed an increase in hydrosoluble pigments with nitrogen concentration. *Anabaena* sp.1 (Fig. 2) reached the highest values at 8 and 17 mM $NaNO_3$ with 94.31 ± 4.82 and 102.90 ± 6.73 µg mL^{-1} and 49.83 ± 2.54 and 53, 47 ± 2.40 µg mL^{-1} for phycocyanin and phycoerythrin, with no significant difference ($p > 0.05$). Meanwhile, *Anabaena* sp.2 produced 85.46 ± 9.95 and 45.27 ± 3.49 µg mL^{-1} for phycocyanin and phycoerythrin to a nitrogen concentration of 8.5 mM $NaNO_3$ ($p < 0.05$).

Nostoc LAUN0015 produced highest phycocyanin and phycoerythrin values at 0 and 4.25 mM $NaNO_3$, compared to *Nostoc* UAM206. *Anabaena* strains produced higher content of these pigments, compared to *Nostoc*. Highest phycocyanin and phycoerythrin production in *Anabaena* sp.1, at 17 mM $NaNO_3$, were 7.33 and 2.26 times higher than the highest productions achieved for *Nostoc* LAUN0015, growing with no nitrogen. The production order for phycobiliproteins was: *Anabaena* sp.1 > *Anabaena* sp.2 > *Nostoc* LAUN0015 > *Nostoc* UAM206.

In general, protein production seemed to be enhanced at medium to high nitrogen concentrations (Fig. 3). On the other hand, *Nostoc* LAUN0015 achieved maximum value in the absence of nitrogen with 686 ± 19.77 mg mL^{-1}($p <$ 0.05). Maximal values for protein concentration for *Nostoc* UAM206, *Anabaena* sp.1 y *Anabaena* sp.2 of 442.14 ± 17.09, 897.64 ± 46.94 and 758.13 ± 11.53 mg mL^{-1}were achieved at 8.5 mM $NaNO_3$ ($p < 0.05$).

Carbohydrate production for filamentous cyanobacteria under study at different nitrogen concentrations were summarized in Table 2. *Nostoc* and *Anabaena* strains

Table 2. Carbohydrate production (µg mL^{-1}) from *Nostoc* and *Anabaena* strains cultured to different nitrogen concentration (mM $NaNO_3$).

	0	4.25	8.5	17
Nostoc LAUN005	895.12 ± 6.74 ^{a}	603.70±17.05 ^{c}	736.20±34.73 ^{b}	572.18±24.53 ^{c}
Nostoc UAM206	892.43±34.59 ^{a}	844.15±30.27 ^{a}	809.32±38.52 ^{b}	649.63±30.29 ^{c}
Anabaena sp.1	549.84±25.17 ^{a}	912.61±65.98 ^{b}	910.88±63.99 ^{b}	801.63±53.54 ^{b}
Anabaena sp.2	582.08±53.26 ^{a}	742.16±29.00 ^{b}	817.49±38.24 ^{b}	788.66±41.57 ^{b}

Values obtained in stationary phase. Letters correspond to groups with statistical differences ($p < 0.05$) for each strain.

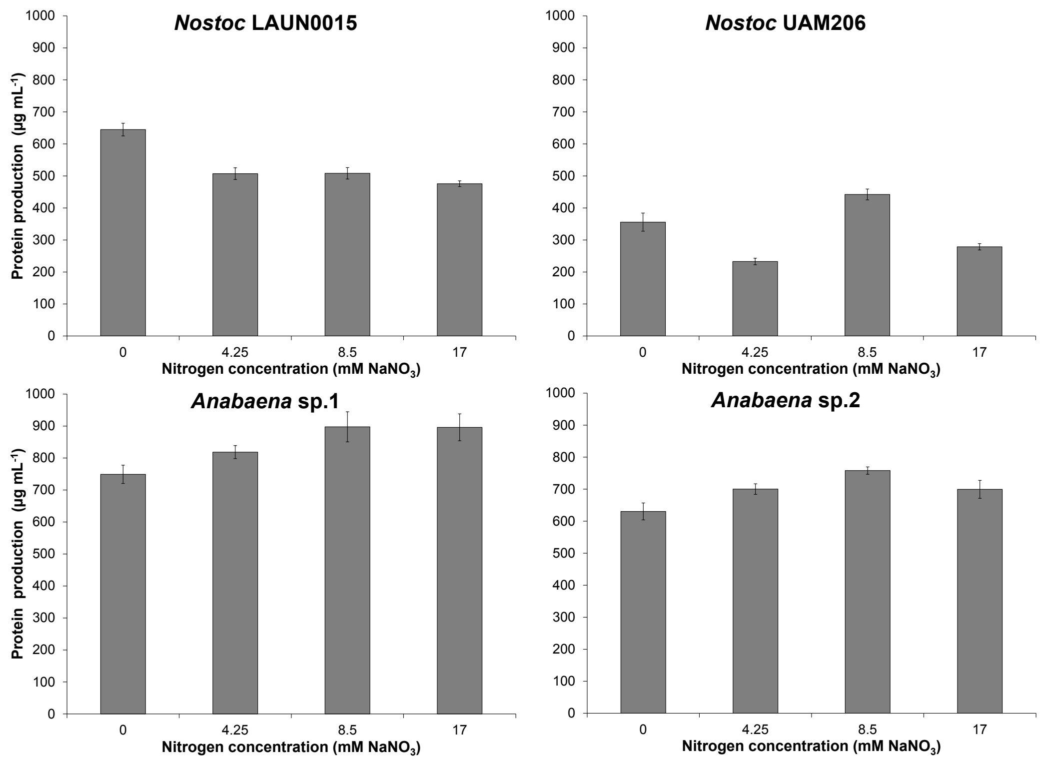

Figure 3. Protein production (µg mL^{-1}) from *Nostoc* and *Anabaena* strains cultured to different nitrogen concentration.

presented two different patterns. *Nostoc* seems to accumulate carbohydrates under absence of nitrogen in the culture, while *Anabaena* accumulates carbohydrates in high nitrogen concentrations.

Carbohydrate maximum production for *Nostoc* LAUN0015 and *Nostoc* UAM206 were 895.12 ± 6.74 to 893.43 ± 34.59 mg mL^{-1} at 0 mM $NaNO_3$, with no statistical differences ($p > 0.05$). For *Anabaena* maximum values were reached at 4.25 and 8.5 mM $NaNO_3$ with 912.61 ± 65.98 and 817.49 ± 38.24 mg mL^{-1} for *Anabaena* sp.1 and *Anabaena* sp.2, respectively. These values did not differ significantly with respect to the treatments where nitrogen was added to the culture ($p > 0.05$), but did respect to the absence of nitrogen ($p < 0.05$).

DISCUSSION

In *Synechococcus*, *Synechocystis*, *Oscillatoria agardhii* and *O. redekei*, a positive correlation between nitrogen concentration and growth, using low concentrations, from 0 to 2.5 mM $NaNO_3$ have been described (Foy, 1993; Hu *et al*., 2000). Increased biomass production from *Nostoc* strains at low nitrogen concentrations seems not to be related neither with increased cellular production nor intracellular accumulation of metabolites, but with the increase in exopolysaccharide production; which is very common in cyanobacteria strains under nutritional stress conditions (Otero and Vincenzini, 2004). Because there is no simple method to determine dry weight from free cells, with no capsular polysaccharide, it was included within the value of dry weight.

Nostoc and *Anabaena* strains were capable of diazotrophic growth, which means that these strains do not need a nitrogen source in the culture medium for growth (Whitton and Potts, 2012). Nitrogen-fixing cyanobacteria are widespread among filamentous heterocyst-forming genera, such as *Anabaena*, *Nostoc*, *Rivularia*, *Stigonema* and *Scytonema*, among others (Tsygankov, 2007). Its ability to grow at different nitrogen concentrations shows its physiological versatility to adapt to diverse environments, even when nitrogen is limiting or absent. Therefore, its growth at low nitrogen concentrations is supported by the nitrogen-fixing process (Loreto *et al.*, 2003).

Similar studies has been verified that cyanobacteria grow better with higher levels of nitrogen (Jonte *et al.*, 2003; Loreto *et al*., 2003; Rosales *et al.*, 2006; Fuenmayor *et al.*, 2009; Rosales Loaiza and Morales, 2013). However, it should be noted that this characteristic is not present in all cyanobacterial cultures. Growth differences, in relation to environmental conditions such as light, temperature, and pH, can be found even within strains of the same specie (Jonte *et al.*, 2003; Vonshak and Torzillo, 2004).

Results from chlorophyll *a* provide important tool that helps to quantify the growth of a phototrophic organism.

There is considerable evidence that supports the fact that the amount of chlorophyll is positively correlated with cell density or biomass (Serpa and Calderon, 2006). Results showed that *Anabaena* sp. 1 is an excellent source of chlorophyll *a*, with commercial interest, especially for its antioxidant properties (Lanfer-Marquez *et al*., 2005). The order of production of both chlorophyll *a* and total carotenoids was as follows: *Anabaena* sp.1 > *Anabaena* sp.2 > *Nostoc* LAUN0015 > *Nostoc* UAM206.

Also, it was demonstrated that pigment production is suitable at low and intermediate nitrogen concentrations. In *Anabaena*, highest value was found at 17 mM $NaNO_3$, but this value was just 1.06 times higher than chlorophyll concentration obtained at 4.25 mM $NaNO_3$; despite the fact that nitrogen concentration was increased three times. This finding has great importance, especially for industrial purposes, because the use of low nitrogen concentrations yields a very good chlorophyll *a* production, without causing great expenses.

The situation is more evident with *Nostoc* LAUN0015 which the best result for liposoluble pigments achieved in total absence of nitrogen. Chlorophyll *a* and carotenoids production was 1.6 and 1.5 times higher under complete absence of nitrogen compared to the highest concentration of 17 mM $NaNO_3$, which verifies the high diazotrophic capacity for growth and pigment production in this strain.

Pigment content depends on the nitrogen source and concentration (Simeunović *et al.*, 2013). In fact, for nitrogen non-fixing strains, the first biomolecule degraded in the process of cellular acclimation under absence of nitrogen are phycobiliproteins (Baier *et al.*, 2001; Simeunović *et al.*, 2013).

Synthesis of pigments, especially phycobiliproteins, is particularly susceptible to environmental influences. In general, the low phycocyanin production in nitrogen-limited cultures obeys to degradation processes in order to mobilize this chromoprotein, for most primary processes such as growth (Lewitus and Caron, 1990); but results showed that at least for *Nostoc*; nitrogen limited cultures actually produce more phycobiliproteins than non-limited cultures. This can be explain through nitrogen fixation.

Heterocystous cyanobacteria, such as *Nostoc* and *Anabaena*, are capable to fix the atmospheric nitrogen, to produce chlorophyll, carotenoids and phycobiliproteins in significant quantities, and that can be seen in the results, especially with *Nostoc* strains. Therefore, production of these microorganisms represents a metabolic strategy with great biotechnological interest, for being produce under diazotrophic conditions. This non-nitrogen culture conditions, it would produce savings in biomass production, since much of the cost of mineral nutrients (i.e. fertilizers) is in the transport of their mass (Stephens *et al*., 2012). Nutrient supply constitutes a primary limitation for mass production for food and fuel (Stephens *et al.*, 2012; Acien *et al.*, 2015).

Decrease in carotenoid content in nutrient-limited cultures, suggests that these strains do not accumulate carotenoids under nitrogen deficiency, such as *Pseudanabaena, Oscillatoria*, *Chlorella* and *Dunaliella* (Canto de Loura *et al.*, 1987; Hu, 2004). Previous reports stated an increase in pigment and protein production with high nitrate concentration in *Gleotrichia* sp. (Pattnaik and Singh, 1978), *Merismopedia tenuissima* (Konopka and Schnur, 1981), *Chroococcidiopsis* sp. (Billi and Grilli, 1996), *Anabaena* sp. PCC 7120 (Loreto *et al.*, 2003) and *Synechococcus* sp. (Rosales *et al.*, 2006).

Nostoc LAUN0015 seems to improve pigment production in total absence or low nitrogen concentrations in the culture medium. This case seems that nitrogen fixation is a more effective than nitrogen assimilation from the surrounding environment.

Increase in biomass and protein production by increasing nitrogen concentration, as it seen in result of *Nostoc* and *Anabaena,* has been widely supported by various reports from *Pseudanabaena* (Leal *et al.*, 2001), *Anabaena* (Loreto *et al.*, 2003), *Oscillatoria* (Saha *et al.*, 2003; Fuenmayor *et al.*, 2009), *Chaetoceros* (Leonardos and Geider, 2004), *Synechococcus* (Rosales *et al.*, 2006), *Dunaliella* (Rosales Loaiza *et al.*, 2007) and *Spirulina platensis* (Colla *et al.*, 2007).

It has been shown that several environmental factors including nutrients status, light, salinity, among others, not only affect photosynthesis and productivity of algal cells, but also influence the overall metabolic activity and cellular composition (Hu, 2004; Guschina and Harwood, 2009).

Microalgae and cyanobacteria are known to modulate the production of both exopolysaccharides and endopolysaccharides in response to various environmental factors, such as salinity stress, high irradiances, and nitrogen deficiency (Moreno *et al.*, 1998; Hu, 2004). Meanwhile, capsular polysaccharide (PSC) production seems to be influenced by nitrogen absence in the culture medium. Numerous studies also show that lipid accumulation is one of the main responses of microalgae and cyanobacteria in nitrogen limited culture (Arias Peñaranda *et al.*, 2013). Also, carbohydrate accumulation occurs frequently (Hu, 2004).

Nitrogen deficiency stimulates polysaccharide production, as it has been demonstrated in *Merismopedia tenuisima* (Konopka and Schnur, 1981), *Synechococcus* (Roux, 1996), *Cyanothece* sp. (De Philippis *et al.*, 1998), *Anabaena* (De Philippis and Vincenzini, 1998; Singh and Das, 2011), *Nostoc* (Otero and Vincenzini, 2003; Otero and Vincenzini, 2004; Singh and Das, 2011) and *Oscillatoria* (Jindal, 2011).

CONCLUSIONS

This comparative study indicates that protein, carbohydrates and liposoluble pigments production increases under diazotrophic conditions for *Nostoc* LAUN0015. Instead, *Anabaena* sp.1 and *Anabaena* sp.2 require high nitrogen concentrations to reach the highest values of metabolites, including pigments and biomass. Growth and production followed the order: *Anabaena* sp.1 > *Anabaena* sp.2 > *Nostoc* LAUN0015 > *Nostoc* UAM206. These results showed that nitrogen concentration between 0 and 17 mM $NaNO_3$ modulate the production of biomolecules in both strains of *Nostoc* and *Anabaena*.

REFERENCES

Acien F, Fernandez J, Molina-Grima E. Economics of microalgae biomass production. In: Pandey A, Lee D, Chisti Y, Soccol C, editors. Biofuels from alga. Oxford, UK: Elsevier; 2014. p. 313-325.

Arias Peñaranda M, Martínez Rondán A, Cañizares Villanueva R. Producción de biodiesel a partir de microalgas: Parámetros del cultivo que afectan la producción de lípidos. Acta biol Colomb. 2013;18(1):43-68.

Baier K, Nicklisch S, Grundner C, Reinecke J, Lockau W. Expression of two nblA-homologous genes is required for phycobilisome degradation in nitrogen-starved *Synechocystis* sp. PCC6803. FEMS Microbiol Lett. 2001;195(1):35-39. Doi:10.1111/j.1574-6968.2001.tb10494.x

Billi D, Grilli M. Effects of nitrogen limitation and starvation on *Chroococcidiopsis* sp. (Chroococcales). New Phytol. 1996;133(4):563-571. Doi:10.1111/j.1469-8137.1996.tb01925.x

Canto de Loura I, Dubacq J, Thomas J. The effect of nitrogen deficiency on pigments and lipids of cyanobacteria. Plant Physiol. 1987;83(4):838-843. Doi: 10.1104/pp.83.4.838

Colla L, Oliveira C, Reichert C, Vieira Costa J. Production of biomass and nutraceutical compounds by *Spirulina platensis* under different temperature and nitrogen regimes. Biores Technol. 2007;98(7):1489-1493. Doi:10.1016/j.biortech.2005.09.030

Cunningham S, Joshi L. Algal Biotechnology: An Emerging Resource with Diverse Application and Potential. In: Kole C, Michler C, Abbott A, Hall T, editors. Transgenic Crop Plants, Vol. 1: Principles and Development. Berlin and Heidelberg, Germany: Verlag-Springer; 2010. p. 343-357. Doi:10.1007/978-3-642-04812-8_9

De Philippis R, Margheri M, Materassi R, Vincenzini M. Potential of unicellular cyanobacteria from saline environments as exopolysaccharide producers. Appl Environ Microbiol. 1998;64(3):1130-1132.

De Philippis R, Vincenzini M. Exocellular polysaccharides from cyanobacteria and their possible applications. FEMS Microbiol Rev. 1998;22(3):151-175. Doi: 10.1111/j.1574-6976.1998.tb00365.x

Foy R. The phycocyanin to chlorophyll *a* ratio and cell components as indicators of nutrient limitation in two planktonic cyanobacteria subjected to low-light exposure. J Plank Res. 1993;15(11):1263-1276. Doi: 10.1093/plankt/15.11.1263

Fuenmayor G, Jonte L, Rosales-Loaiza N, Morales E. Efecto de la salinidad y la concentración de nutrientes sobre el crecimiento y composición bioquímica de la cianobacteria autóctona *Oscillatoria* sp. MOF-06. Ciencia. 2009;17(1):50-57.

Gantar M, Svirčev Z. Microalgae and cyanobacteria: Food for thought. J Phycol. 2008;44(2):260-268. Doi:10.1111/j.1529-8817.2008.00469.x

Guschina I, Harwood J. Algal lipids and effect of the environment on their biochemistry. In: Arts M, Brett M, Kainz K, editors. Lipids in aquatic ecosystems. Dordrecht, The Netherlands: Springer Academics; 2009. p. 1-24. Doi:10.1007/978-0-387-89366-2_1

Harun R, Singh M, Forde G, Danquah M. Bioprocess engineering of microalgae to produce a variety of consumer products. Renew Sust Energ Rev. 2010;14(3):1037-1047. Doi:10.1016/j.rser.2009.11.004

Herbert D, Phipps P, Strange P. Chemical analysis of microbial cells. In: Norris J, Ribbons D, editors. Methods in microbiology Vol 5B. London, UK: Academic Press; 1971. p. 209-344.

Hu Q, Westerhoff P, Vermaas W. Removal of nitrate from groundwater by cyanobacteria: Quantitative assessment of factors influencing nitrate uptake. Appl Environ Microbiol. 2000;66(1):133-139. Doi:10.1128/AEM.66.1.133-139.2000

Hu Q. Environmental effects on cell composition. In: Richmond A, editor. Handbook of microalgal culture, biotechnology and applied phycology. Oxford, UK: Blackwell Publishers; 2004. p. 83-94. Doi:10.1002/9780470995280

Kochert G. Carbohydrate determination by the phenol-sulfuric acid method. In: Hellebust J., Craigie J, editors. Handbook of phycological methods. Physiological and biochemical methods. Cambridge, UK: Cambridge University Press; 1978. p. 95-97.

Konopka A, Schnur M. Biochemical composition and photosynthetic carbon metabolism of nutrient limited cultures of *Merismopedia tenuissima* (Cyanophyceae). J Phycol. 1981;17(2):118-122. Doi:10.1111/j.1529-8817.1981.tb00829.x

Jindal N, Singh D, Khattar J. Kinetics and physico-chemical characterization of exopolysaccharides produced by the cyanobacterium *Oscillatoria formosa*. World J Microbiol Biotechnol. 2011;27(9):2139-2146. Doi:10.1007/s11274-011-0678-6

Jonte L, Rosales N, Briceño B, Morales E. La salinidad y la irradiancia modulan el crecimiento de la cianobacteria *Synechocystis minuscula* en cultivos discontinuos. Multiciencias. 2003;3(1):1-13.

Lanfer-Marquez U, Barros R, Sinnecker P. Antioxidant activity of chlorophylls and their derivatives. Food Res Int. 2005;38(8-9):885-891. Doi:10.1016/j.foodres.2005.02.012

Leal M, Ortiz N, Mora R, Morales E. Caracterización de la cianobacteria *Pseudanabaena galeata* en condiciones de laboratorio. Bol Centro Invest Biol. 2001;35:223-241.

Leonardos N, Geider R. Responses of elemental and biochemical composition of *Chaetoceros muelleri* to growth under varying light and nitrate: phosphate supply ratios and their influence on critical N: P. Limnol Oceanogr. 2004;49(6):2105-2114. Doi: 10.4319/lo.2004.49.6.2105

Lewitus A, Caron D. Relative effects of nitrogen and phosphorus depletion and light intensity on the pigmentation, chemical composition and volume of *Pyrenomonas salina* (Cryptophyceae). Mar Ecol Progr Ser. 1990;61:171-181.

Loreto C, Rosales N, Bermúdez J, Morales E. Producción de pigmentos y proteínas de la cianobacteria *Anabaena* PCC 7120 en relación a la concentración de nitrógeno e irradiancia. Gayana Bot. 2003;60(2):83-90.

Marker A, Jinks S. The spectrophotometric analysis of chlorophyll *a* and pheopigments in acetone, ethanol and methanol. In: Rai H, Marker A, editors. The measurement of photosynthetic pigments in freshwaters and standardization of methods. Arch Hydrobiol Beih Ergebn Limnol. 1982;16:3-17.

Moreno J, Vargas M, Olivares H, Rivas J, Guerrero M. Exopolysaccharide production by the cyanobacterium *Anabaena* sp. ATCC 33047 in batch and continuous culture. J Biotechnol. 1998;60(3):175-182. Doi:10.1016/S0168-1656(98)00003-0

Otero A, Vincenzini M. Extracellular polysaccharide synthesis by *Nostoc* strains as affected by N source and light intensity. J Biotechnol. 2003;102(2):143-152. Doi:10.1016/S0168-1656(03)00022-1

Otero A, Vicenzini M. *Nostoc* (Cyanophyceae) goes nude: extracellular polysaccharides serve as a sink for reducing power under unbalanced C/N metabolism. J Phycol. 2004;40(1):74-81. Doi:10.1046/j.1529-8817.2004.03067.x

Pattnaik U, Singh P. Effect of nitrate nitrogen on the growth, heterocyst differentiation and nitrogen fixation in rice field blue-green alga *Gleotrichia* sp. Arch Hydrobiol. 1978;51:318-327.

Rasmussen R, Morrissey M. Marine biotechnology for production of food ingredients. Adv Food Nutr Res. 2007;52:237-292. Doi:10.1016/S1043-4526(06)52005-4

Rippka R, Deruelles J, Waterbury J, Herdman M, Stanier R. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J Gen Microbiol. 1979;111:1-61.

Rosales N, Jonte L, Morales E. Crecimiento y composición bioquímica de *Synechococcus* sp. modulados por el nitrato de sodio. Bol Centro Invest Biol. 2006;40(2):120-132.

Rosales-Loaiza N, Avendaño D, Otero A, Morales E. Crecimiento, producción de pigmentos y proteínas de la microalga *Dunaliella viridis* (Chlorophyta) en cultivos semicontinuos. Bol Centro Invest Biol. 2007;42(3):323-334.

Rosales Loaiza N, Morales E. *Synechococcus* sp. En función de parámetros y sistemas de cultivo. Cultivo de una cianobacteria de ambientes tropicales y la verificación de su potencial biotecnológico. Barcelona, Spain: Editorial Académica Española; 2013. 109 p.

Roux J. Production of polysaccharide slime by microbial mats in the hypersaline environment of a Western Australian solar saltfield. Int J Salt Lakes Res. 1996;5(2):103-130.

Saha S, Uma L, Subramanian G. Nitrogen stress induced changes in the marine cyanobacterium *Oscillatoria willei* BDU 130511. FEMS Microbiol Ecol. 2003;45(3):263-272. Doi:10.1016/S0168-6496(03)00162-4

Sharma N, Prakash Tiwari S, Tripathi K, Rai A. Sustainability and cyanobacteria (blue-green algae): facts and challenges. J Appl Phycol. 2011;23(6):1059-1081. Doi:10.1007/s10811-010-9626-3

Serpa R, Calderón A. Efecto de diferentes fuentes de nitrógeno en el contenido de carotenoides y clorofila de cuatro cepas peruanas de *Dunaliella salina* TEOD. Ecol Apl. 2006;5(1,2):93-99.

Simeunović J, Bešlin K, Svirčev Z, Kovač D, Babić O. Impact of nitrogen and drought on phycobiliprotein content in terrestrial cyanobacterial strains. J Appl Phycol. 2013;25(2):597-607. Doi:10.1007/s10811-012-9894-1

Singh S, Das S. Screening, production, optimization and characterization of cyanobacterial polysaccharide. World J Microbiol Biotechnol. 2011;27(9):1971-1980.

Stephens E, Wagner L, Ross I, Hankamer B. Microalgal production systems: Global impact of industry scale-up. In: Posten C, Walter C, editors. Microalgal biotechnology: Integration and economy. Boston, USA: De Gruyter; 2012. p. 267-306.

Strickland J, Parsons T. A practical handbook of seawater analysis. Fish Res Board Can Bull. 1972;167:310.

Tsygankov A. Nitrogen-fixing cyanobacteria: a review. Appl Biochem Microbiol. 2007;43(3):50-259. Doi:10.1134/S0003683807030040

Utting S. Influence of nitrogen availability on the biochemical composition of three unicellular marine algae of commercial importance. Aquacul Engin. 1985;4(3):175-190. Doi:10.1016/0144-8609(85)90012-3

Vincent W. Cyanobacteria. In: Likens G, editor. Encyclopedia of inland waters. San Diego, USA: Elsevier; 2009. p. 226-232.

Vonshak A, Torzillo G. Environmental stress physiology. In: Richmond A, editor. Handbook of microalgal culture: Biotechnology and applied phycology. Oxford, UK: Blackwell Publishing; 2004. p. 57-82.

Whitton B, Potts M. Introduction to the cyanobacteria. In: Whitton B, Potts M, editors. The ecology of cyanobacteria II: Their diversity in time and space. Dordrecht, The Netherlands: Kluwer Academic Publishers; 2012. p. 1-14.