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PRIMARY PRODUCTIVITY OF COCCOID CYANOBACTERIA ISOLATED FROM A COASTAL LAGOON ENVIRONMENT SOUTH OF THE GULF OF CALIFORNIA

Productividad primaria de cianobacterias cocoides aisladas de un ambiente lagunar costero al sur del Golfo de California

RESUMEN. El objetivo de este estudio fue determinar la productividad primaria de cianobacterias cultivadas bajo diferentes fuentes nitrogenadas. Se consideraron 3 tratamientos: 1) sin fuente nitrogenada, 2) con nitrato (NO_3), y 3) con amonio (NH_4). Se realizaron incubaciones bajo condiciones controladas. Las densidades celulares fueron significativamente mayores en el tratamiento con nitratos. Los máximos de biovolumen (19.9 μm^{-3}) y de contenido de carbono (5.4 pg C cel^{-1}) se observaron en la fase estacionaria del tratamiento con amonio. La mayor concentración de clorofila *a* en la fase exponencial se registró en el tratamiento sin nitrógeno (1.038 mg m^{-3}), mientras que el máximo en la fase estacionaria en el tratamiento con nitratos (0.65 mg m^{-3}). La mayor producción primaria se registró en la fase exponencial (23.9 mg C m^{-3} h^{-1}), asociada al tratamiento sin fuente nitrogenada incubado a 150 µ E m^{-2} s^{-1}; en la fase estacionaria el máximo (11.6 mg C m^{-3} h^{-1}) se registró en el tratamiento enriquecido con nitratos a 75 µ E m^{-2} s^{-1}.

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The study of prokaryotes in marine environments has gained considerable importance because some photosynthetic cyanobacteria can prosper under conditions in which other organisms may have their growth or productive capacity limited. Such conditions can foster the growth and diversity of cyanobacteria, allowing them to take on an important role in various biogeochemical reactions that occur in the ocean (Kirchman, 2008). Some coccoid forms smaller than 1 µm may be responsible for 50-90% of the primary production (Lucas & Walsby, 2000) in places where oxygenic photoautotroph production is a common feature of such genera as *Synechococcus*, because they possess chlorophyll *a* and supramolecular compounds of antenna pigments that contain divinyl chlorophyll *a* and *b* (Giovannoni & Rappé, 2000). The cyanobacteria from this study belong to the Order Chrococcales and were isolated from a sample collected in Laguna de Macapule (25º21" and 25º24" N, 108º30" and 108º45" W) in northern Sinaloa, Mexico (Fig. 1).

Analysis began with a density of $10x10^3$ cells ml^{-1} in 500 ml Erlenmeyer flasks with ASN-III medium (Atlas, 2010) that was prepared without adding $NaNO_3$. In all cases, artificial seawater was used to ensure the absence of sources of nutritive nitrogenates. Three treatments were developed:1) no nitrogen source was added; 2) $NaNO_3$ was added as a nitrogenated source; 3) treatment was inoculated by adding NH_4. All cultures were maintained under controlled conditions of irradiance (150 µE m^{-2} h^{-1}), temperature (26±1.5°C), and photoperiod, 13.5:10.5 h. For the counting technique, a Neubauer camera was employed to calculate cell density (Alfonso & Leal, 1998). Cell density, biovolume and carbon content were determined daily.

A non-parametric Kruskal-Wallis analysis was performed to determine whether significant differences existed among the variables considered in the different treatments. A sample of 30 cells was taken from each treatment, and their biovolume was established to later calculate their carbon content following the approach by Verety *el al.* (1992). Likewise, the concentration of chlorophyll *a* was determined at the exponential and stationary phases of the cultures (Strikland & Parson, 1972), together

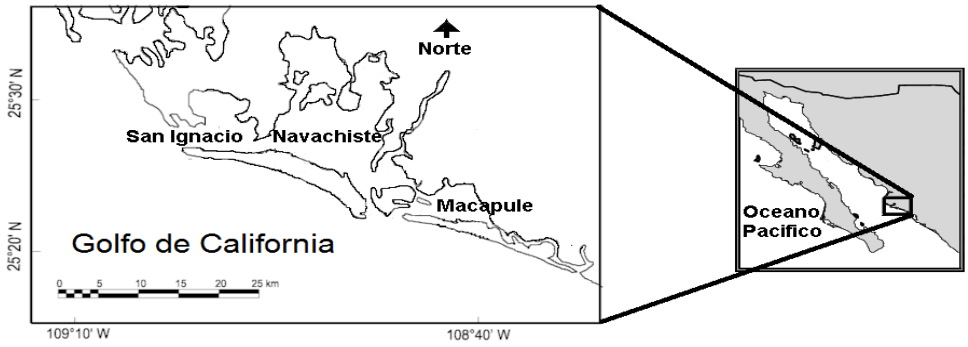

Figure 1.- Collection area of the cyanobacteria.

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with the primary production (Steemann-Nielsen, 1952). Incubations were conducted by inoculating 2 µ Ci of $NaH^{14}CO_3$. The incubation period was 1.5 h at two distinct irradiances (150 and 75 µ E m^{-2} s^{-1}), both in the exponential phase and in the stationary.

The highest densities were observed in the treatment enriched with nitrates ($4x10^6$ cell ml^{-1}). The initial density of 10,000 cell ml^{-1} was based on reports which mention that this commonly occurs in tropical and sub-tropical environments (Partensky *et al*., 1999). We were able to document that the growth of this cyanobacteria responded best to the treatment enriched with nitrates, as its density in the stationary phase doubled the figures recorded for the treatments with ammonium and without nitrogen source. Agawin *et al.* (2007) documented similar behavior for *Cyanothece* sp. when cultured in a nitrate-enriched medium (Fig. 2). The study also registered a significant difference between the growth curve of the treatment with nitrates compared to the one with added ammonium and the one with no nitrogen (p= 0.05).

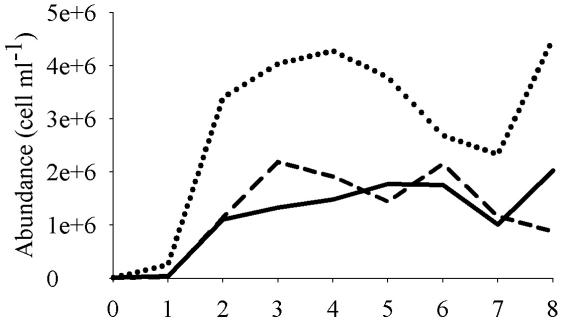

Figure 2.- Growth curves under the different treatments: without nitrogen (dashed line); with nitrates (dotted line); with ammonium (continuous line).

The carbon content of the picofitoplancton seems to be well related to abundance (Buitenhuis *et al*., 2012); however, biovolume and cell carbon content, showed significant differences when the treatment without nitrogen was contrasted to the one with ammonium (p=0.05). In both cases, observations showed that these variables were favored principally in the ammonium-enriched treatment at the onset of the stationary phase. This finding allows us to infer that both, nitrates and ammonium, are essential nutrients that permit the growth and reproduction of these microorganisms (Fig. 3a, 3b).

Concerning chlorophyll *a* concentrations, we recorded the maximum value in the stationary phase (1.03 mg m^{-3}) associated to the treatment without nitrogen (Fig. 4a left), while a minimum of 0.02 mg m^{-3} was recorded for the treatment with ammonium. In the case of the stationary phase, the maximum (0.6 mg $^{-3}$) was recorded in the treatment with nitrates, while the minimum coincided with the treatment with ammonium (Fig 4b left). These results suggest that a higher capacity for chlorophyll synthesis exists when the culture is in the growth phase, while as the days passed before reaching the stationary phase, the strain we cultivated showed a diminished capacity to synthesize this photosynthetic pigment. This condition does not necessarily have a negative effect on the photosynthetic capacity, since authors like Falkowski & Raven (1997); Jonte *et al.* (2007) have documented conditions in which an increase in carotenoid concentrations occurs in response to a reduction in the concentration of phycocyanins and the synthesis of chlorophyll *a*, which could favor the capture of photons through these antenna pigments making it possible to maintain intermediate levels of production. The relationship between chlorophyll concentration and the carbon content in the picofitoplanktons in response to environmental variability is exceptionally variable (Falkowski & La Roche, 1991), for this reason, Westberry *et al*. (2008) recommend estimations of primary production.

Primary productivity reached values of 3.1-23.9 mg C m^{-3} h^{-1} in the exponential phase. The maximum value was recorded in the treatment with no source of nitrogen when incubated at 150 µE m^{-2} s^{-1}, while

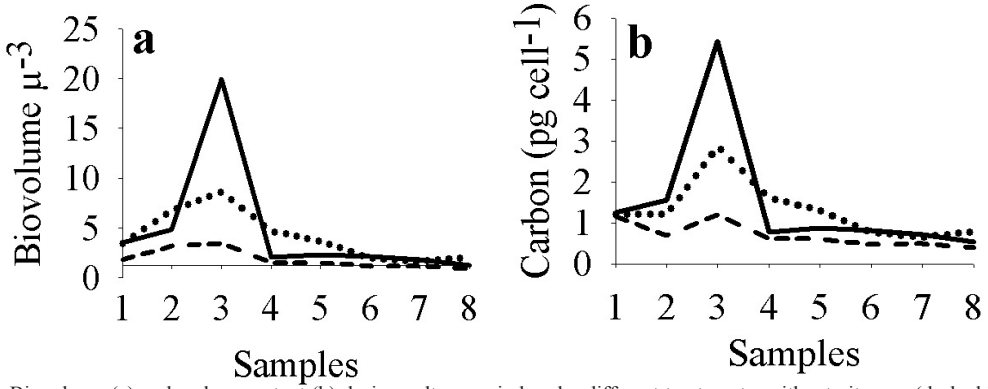

Figure 3. Biovolume (a) and carbon content (b) during culture period under different treatments: without nitrogen (dashed line); with nitrates (dotted line); with ammonium (continuous line).

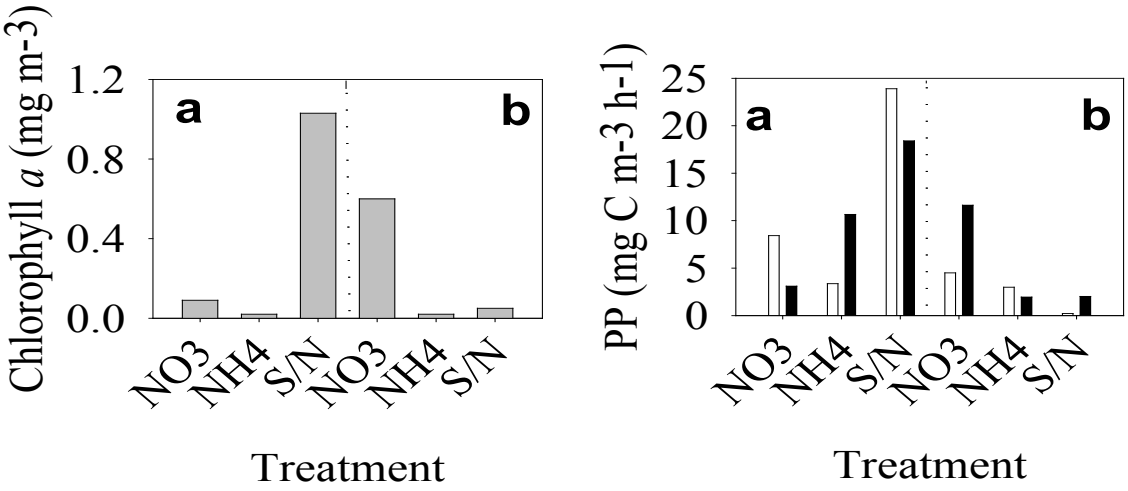

Figure 4.- Chlorophyll concentration (left): (a) exponential phase, and (b) stationary phase. Primary productivity (right): (a) exponential phase; and (b) stationary phase, recorded under the different treatments considered. Irradiances of 150 µE m^{-2} s^{-1} (empty bar) and 75 µE m^{-2} s^{-1} (black bar).

the minimum was found for the treatment with nitrates incubated at an irradiance of 75 µE m^{-2} s^{-1} (Fig. 4a right). Maximum productivity in the exponential phase was recorded for the treatment with no nitrogen added (23.9 mg C m^{-3} h^{-1}), incubated at 150 µE m^{-2} s^{-1}; meanwhile, the relation was inverse in the stationary phase. In the case of the treatment with ammonium, maximum productivity in the exponential phase was reached under the lower irradiance, whereas in the stationary phase the maximum was associated with the higher irradiance. Finally, the treatment with nitrates had a similar effect to the one observed in the treatment without nitrogen, though in larger magnitude in the exponential phase (Fig. 4b right). When the cyanobacteria were in the growth phase, maximum productive capacities were associated with low irradiance; a finding that allows us to infer photoadaptation with a lower index of light saturation. In another result, upon reaching the stationary phase and striving to maintain maximum growth, the demand for photosynthetically active radiation was more significant, as suggested by the fact that the maximum levels of primary production were associated with the higher irradiance. It is important to consider that, in general, cyanobacteria can convert up to 9% of photosynthetically active radiation in biomass, in contrast to 3% efficiency in other phytoplankton groups, which gives them physiological advantages that favor their productive capacity (Dismukes *et al*., 2008; Branco dos Santos *et al*., 2014).

In this regard, the genera like *Cyanothece* have been reported to have low indices of saturation (± 40 µmol photons m^{-2} s^{-1}), while *Synechococcus* and *Synechocystis* are considered to be strong competitors for light since they have the adaptive advantage of broad plasticity in their coefficients of light saturation, as well as diazotrophic activity that allows them to have a constant source of nitrogen species (Agawin *et al.,* 2007). The differences observed in the utilization of distinct sources of nitrogen is one of the adaptive capacities reported in the cyanobacteria, because they can utilize different nitrogen sources according to the environmental conditions of their habitat, as it has been documented for such genera such as *Cyanothece* sp. and *Synechococcus* sp. (Agawin *et al.,* 2007; Masuda *et al.,* 2013).

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