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Salinity stress on exopolysaccharides production by *Chaetoceros neogracilis*

Estresse salino na produção de exopolissacarídeos pela Chaetoceros neogracilis

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ABSTRACT

Objective: To evaluate the effect of saline stress on microalgae *Chaetoceros neogracilis* farmed for production of photosynthetic pigments and exopolysaccharides. **Method**: Seven different treatments, with different salinity were conducted during seven days. Cellular counts and absorbance measures were daily accessed to analyze the growth rate and cellular density. Photosynthetic pigments and exopolysaccharides concentration was determined by spectrophotometry and alcoholic precipitation, respectively. **Results**:

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Although the great mean absorbance (1.032 ± 0.039 nm) was verified in 25‰ salinity and the greatest mean cellular density (481 ± 29 x 104 cell.mL^{-1}) was obtained in 20‰ salinity. The greatest mean pigment concentration was registered in 15‰ salinity (1.820, 2.566 and 3.915 mg.L^{-1}, for chlorophyll-a, pheophytin-a and carotenoids respectively). Production of exopolysaccharides was directly influenced by growing salinity, with great mean concentration (2.952±0,204 g.L-1) within treatment of 35‰ salinity. **Conclusion**: Despite production of microalgae C. neogracilis is viable in a wide range of salinity, when the main purpose is to focus on the production of exopolysaccharides, one should choose higher values.

Key words: Microalgae; Diatom; Chlorophyll; Carotenoids; Biocompounds.

RESUMO

Objetivo: Avaliar o efeito da salinidade no cultivo da microalga *Chaetoceros neogracilis* para produção de pigmentos fotossintetizantes e exopolissacarídeos. **Método:** Foram realizados sete tratamentos com diferentes salinidades, por sete dias. Diariamente foram realizadas contagens celular e medição da absorbância, para analisar o crescimento e a densidade celular. Os pigmentos fotossintéticos foram determinados por espectrofotometria, e os exopolissacarídeos por precipitação alcoólica do sobrenadante. **Resultados:** Na salinidade 25‰ ocorreu o maior valor médio de absorbância (1.032 ± 0.039 nm), porém a maior densidade celular foi obtida na salinidade 20‰ (481±29 x10^{4}cél.mL^{-1}). A maior concentração de pigmentos foi registrado em salinidade de 15‰ (1,820, 2,566 e 3,915 mg.L^{-1}, para clorofila-a, feofitina-a e carotenoides respectivamente). A produção de exopolissacarídeos foi diretamente influenciada pela salinidade, com maior concentração na salinidade de 35‰ (2.952±0,204 g.L^{-1}). **Conclusão:** A microalga *C. neogracilis* é capaz de ser produzida em uma ampla faixa de salinidades, mas para maximizar a produção de exopolissacarídeos, deve-se utilizar salinidades elevadas.

Palavras-chave: Microalga; Diatomácea; Clorofila; Carotenoides; Biocompostos.

INTRODUCTION

Diatoms of *Chaetoceros* genus are unicellular microalgae widely used in larval culture of crustaceans, fishes and mollusks due to their high growth rates and nutritional value, especially regarded to production of polyunsaturated fatty acids, proteins, carbohydrates, pigments and antioxidants substances[1,2,3,4,5].

The nutritional content of microalgae can be influenced by factors such as cultivation medium, light intensity, temperature, nitrogen, phosphorus and carbon dioxide. Studies have shown that *Chaetoceros* sp. can contain protein and carbohydrate contents of 33-36% and 12-17%, respectively[6,7]. These microalgae still have good lipid levels of 15-22%, especially EPA (C20:5; 9.18%) and DHA (C22:6; 10.47%) and fucoxanthin (0.4 mg L^{-1})[7].

The use of *Chaetoceros* spp. in shrimp and marine fish larvae farming brings several benefits such as increased nutritional quality of the diet, especially in polyunsaturated fatty acids, improved water quality and inhibition of the growth of *Vibrio harveyi*, favoring improved zootechnical performance indices[8,9,10]. A greater economic return can also be achieved when using *Chaetoceros* spp. in association with other microalgae in the larviculture of the mollusk *Mercenaria mercenária*[2].

Chaetoceros spp. secrete a long variety of substances during its life cycle, such as exopolysaccharides, proteins, nucleic acids and lipids[11,12]. Such substances can be found connected to cell surface as well as in extracellular medium, providing physical and chemical protection against stress factors, contributing this way to a stable environment[13,14].

Exopolysaccharides (EPS) are carbohydrates polymers, composed of monosaccharides connected by glycosidic bonds, generally known as non-toxic metabolites[15]. They present peculiar chemical properties required in food industry as to stabilizers, emulsifiers and bulking agents, and also if pharmaceutical industry, due to their antioxidant, anti-inflammatory, antibiotic, immunomodulation and antiviral activity[14,15,16,17,18,19,20].

In the food industry, microalgae EPS can be used as natural preservatives, stabilizers, gelling agents and texturizers[21,22]. The application of microalgae EPS as a food thickener is due to the ability of these high molecular weight polymers to form hydrocolloids in aqueous solution, conferring high intrinsic viscosity[23,24].

One of the applications of microalgae EPS is in the fight against cancer. Cells that cause cervical cancer can have their genes regulated by microalgal EPS, reducing resistance to apoptosis and consequently cell death[25]. Furthermore, *Phormidium* sp. EPS demonstrated anti-inflammatory action in zebrafish by markedly attenuating the activity of NF-κB transcription factors linked to inflammation[26].

In aquaculture dietary supplementation with polysaccharide improved growth rates, food efficiency, digestive enzymes activity, antioxidant activity, blood count, and virus inhibition in different species of fish and crustaceans[27,28,29,30,31].

Production of EPS by microalgae is triggered by a number of environment factors, such as temperature, light intensity, pH, nutrients and salinity[14,32,33]. Among those factors, salinity variation may affect, most importantly, photosynthetic rates, respiration and cell membrane permeability, as they are compelled to adjust energy expenditure[34,35]. In doing such, microalgae secrete EPS to protect its cellular structure and thrive through saline stress.

The change in salinity in the *Porphyridium cruentum* culture medium interfered with the accumulation of starch and 2-α-O-D-galactopyranosyl glycerol so that at low salinity it favored a greater concentration of starch[36]. Possibly, salinity was able to initiate specific biosynthetic pathways for the EPS precursors of *P. cruentum*, triggering the remobilization of intracellular carbon concentration for polysaccharide synthesis[36]. Green algae Chlorophyta when subjected to different saline concentrations present different responses such as increased cell size, significant reduction in EPS production, lipid accumulation and increased cell wall thickness[37].

Microalgae farming conditions in Brazil changes according to the region and salinity figures among those multiple factors. For example, the expansion of *Penaeus vannamei* shrimp farming in regions further away from the Brazilian coast, where water salinity is lower, led to a change in management strategies, especially feeding. Thus, the aim of this study was to analyze salinity effect on farming of microalgae *Chaetoceros neogracilis* for production of EPS and photosynthetic pigments.

METHODS

Farming in salty water

The study was conducted in Planctology Laboratory of Federal University of Ceará in Fortaleza, Ceará, Brazil (3°44'S e 38°34'W), where *Chaetoceros neogracilis* (VanLandingham 1968) strain was obtained. Experimental design was composed of seven treatments, in which seven different salinities had been matched as the following: T1 - 5‰;

T2 - 10‰; T3 - 15‰ T4 - 20‰; T5 - 25‰; T6 - 30‰: T7 - 35‰. Each treatment had six repetitions. Treatment T4 20‰ was undertaken as the control one. Culture was performed in bioreactors with 5.0 L capacity and useful volume of 4.0 L, which was filled up with 25% of microalgae inoculum and 75% of culture medium. The culture medium chosen was Guillard f/2 modifiyed, which nutritional composition as describid by Lourenço[38] (table 1). Culture medium was autoclaved at 121 ºC and 1 atm for 30 min prior to utilization.

Table 1. Nutritional composition of culture medium Guillard f/2 modifiyed used in *Chaetoceros neogracilis* culture under different salinities.

Solutions	**Reagents**	**Stock solution ($g.L^{-1}$)**	**Volume (mL) used for 1 L of culture medium**
I	Sodium nitrate	75.0	1.0
II	Sodium phosphate	5.0	1.0
III	Sodium silicate	30.0	1.0
IV	Trace metal solution	-	1.0
	Copper sulfate	0.0098	-
	Zinc sulfate	0.022	-
	Manganese chloride	0.180	-
	Cobalt chloride	0.01	-
	Sodium molybdate	0.0063	-
	Na_2EDTA	0.00436	-
	Ferric chloride	0.0315	-
V	Vitamins	*	0.5

*Vitamins were added from a couple of ampoules of vitaminic compound (B1 and B6, 100 mg; B12 5 mg) in 50 mL destiled water previously autoclaved.

Bioreactors were mainteined under mean temperature of 27°C, constant aeration, air flux of 3 $L.min^{-1}$, coupled to a filter made of perlon, cotton and activated charcoal. Culture was subjected to 24 hours of photoperiod and illuminance of 5.000 Lux, provided by four tubular LED of 18 W each.

All material used in the experiment that did not withstand autoclaving was subjected to 2.0% chlorinated water and rinsed with distilled water.

Standard salinity used to set each treatment salinity was the one in the original strain 20‰. Treatments were added then with salt water 40‰, filtered and autoclaved at 121°C and 1 atm for 30 min.

Salinity was mesured with manual refractometer ATAGO and dilutions to reach each treatments salinities folowed the Solution Dilution Equation described as:

$$\boldsymbol{C1.V1 + C2.V2 = CF.VF}$$

where: C_1 = Salinity of microalgae inoculum; C_2 = Salinity required to dilution; C_F = Salinity of the treatment; V_1 = Volume of microalgae inoculum; V_2 =Volume of culture medium and V_F = Volume of the treatment.

Inoculation and monitoring of culture

One litre of initial strain with 200 cel.mL^{-1} desity was inoculated in each treatment. Groth rate was observed daily and mesured in Neubauer chamber, by cellular counting in using optical microscope with phase contrast (OLYMPUS BX-41). Optic density was mesured by absorbance in spectrophotometer (HACH DR 2000) at λ= 680 nm. Daily data were collected to produce growth curve for each treatment.

Maximum cellular density (DCM) was difined as the great number of cellular count throughout microalgae culture and growth velocity (k) was calculated by the following equation:

$$\boldsymbol{k = \frac{log_2\left(\frac{N_t}{N_0}\right)}{\Delta t}}$$

where: k = cellular division per day; N_t = number of cells at the end of time interval; N_0 = number of cells at the begining of time interval; Dt = time interval.

Quantification of chlorophyll-a, pheophytin-a and total carotenoids

To estimate microalgae biomass and define physiological state of culture pigment content (chlorophyll-*a* pheophytin-*a* and total carotenoids) was determined Golterman *et al.*[39] according to Lourenço[38] and Cetesb[40]. To do so, aliquots of 10 mL (n=3) of each treatment were vacuum filtered in 0.45 µm mesh. Meshes were then placed in Falcon tubes, added 25 mL acetone (90%), covered with aluminum foil to avoid light contact and photo oxidation and

stored under 4 °C refrigeration for 24 hours. After this period, extracts were centrifuged at 3.000 rpm for 10 min.

After centrifugation, the supernatant was subjected to absorbance reading in optical path cuvettes of 2.5 cm in 750 nm wave length to determine turbidity and in 664 nm to determine chlorophyll-*a* quantification. To determine total carotenoids quantification the absorbance reading was made in 480 and 510 nm wave length. Each sample was then added with hydrochloric acid (HCl) 0.1 M and read again in 750 nm and 664 nm wave length to determine quantification of pheophytin-*a*.

Pigment concentrations were determined by the following equations[38]:

$$\boldsymbol{chlorophyll-a\ (\mu gL^{-1})} = \boldsymbol{26.73x[(A_{664nm} - A_{750nm}) - (Aa_{664nm} - Aa_{750a}nm)]xv(Vxc)}$$

$$\boldsymbol{pheophytin-a(\mu gL^{-1})} = \boldsymbol{26.73x[1.8x(A_{664nm} - A_{750nm}) - (A_{a664nm} - A_{a750nm})]xv(Vxc)}$$

where: 26.73 = absorption coefficient; v = acetone volume (mL); V = filtered culture volume (L); c = optical path cuvettes (cm); A = absorbance and a = absorbance after acid addition.

$$\boldsymbol{Total\ carotenoids\ (\mu gL^{-1})} = \boldsymbol{7.6xA_{480nm} - (3.0xA_{750nm}) - (1.49xA_{510nm}) - (2.0xA_{750nm})xv(Vxc)}$$

where: the values 7.6; 3.0; 1.49 and 2.0 = absorbance coefficients; v = acetone volume (mL); V = filtered culture volume (L); c = optical path cuvettes (cm) and A = absorbance.

Exopolysaccharides (EPS) quantification

At the last day of culture, aliquots of 100 mL of each treatment were taken into Falcon tubes and underwent centrifugation at 7280 g for 15 minutes to extract EPS. Supernatants were added of commercial alcohol 92.8%, in proportion of 2:1 (alcohol: supernatant) and samples were refrigerated as -16°C for 72 hours, so EPS could precipitate, according to Guzmán-Murillo & Ascencio[41] methodology.

After this period, samples were centrifuged at 7280 g for 10 minutes and supernatant was discarded. Pellets were rinsed twice with commercial alcohol 80% and centrifuged. After centrifugation, the supernatant was discarded and pellet was dried up in drying oven with circulation at 60 °C for 12 hours. Mean yield was expressed in $g.L^{-1}$.

Sulfated exopolysaccharides (EPS)

Metachromasia assay methodology proposed by Aranzadi *et al.*[42] was used in this study to check sulfation of EPS. EPS samples from each treatment were diluted in distilled water to achieve concentration of 15 $mg.mL^{-1}$. Aliquots of 3 µL from diluted solution were sequentially added to 2 ml Eppendorf's tubes containing 1 mL of blue dye (1,9-dimethylmethylene, ADM) until color changed to pale pink. Color change meant the existence of sulfation of Eps.

Statistical analysis

Results from growth curve, maximum cellular density (DCM), growth velocity (k), quantification of photosynthetic pigments and EPS were subjected to variance (ANOVA)(α=0.05). In case of significant differences between means, Tukey's test was applied.

RESULTS

Culture in salt water

Among treatments, T1 presented the lowest absorbance (0,308 ± 0,01, Fig. 1) since the second day of culture. Rising salinity influenced positively absorbance values, being T5 the one with greatest values along seven culture days, with exception of day 3 to day 5. The greatest value of absorbance was verified in T5 1,032 ± 0,066, during the 7^{th} day of culture and was statistically similar to T4 0,986 ± 0,021. Therefore, all salinity values promoted microalgae growth, but the best performance occurred with 25‰ (T5).

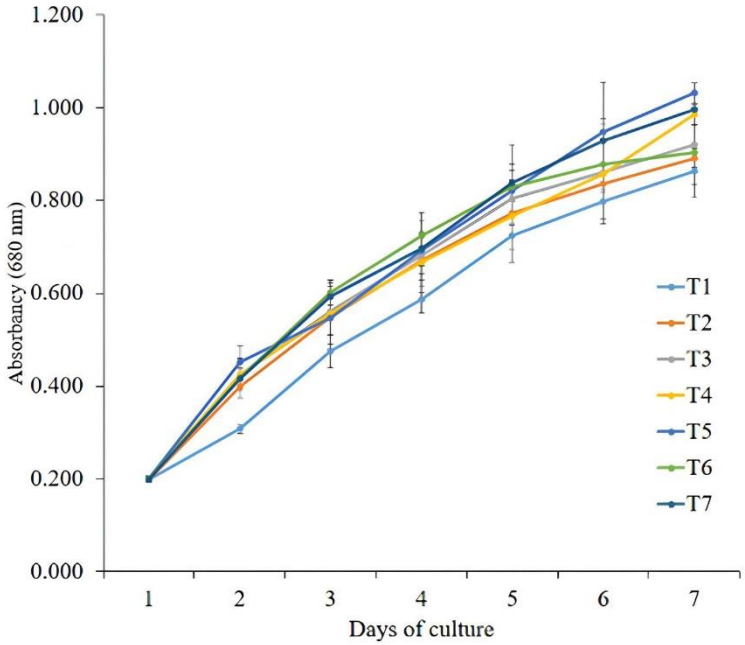

Figure 1 – Optic density (absorbance 680 nm) in different salinities treatments culture of diatom *Chaetoceros neogracilis* cultivated under different salinities (T1 – T7).

Initial cell density was the same for all treatments 50 ± 2 x 10^4 cell. mL^{-1}, however at the end of seven culture days maximum cell densities (DCM) values were different among them. The smallest one was in T1 (311 ± 38 x 10^4 cell. mL^{-1}) and the greatest one in T4 (481 ± 29 x 10^4 cell. mL^{-1}), which was statistically similar to T3 (405 ± 26 x 10^4 cell. mL^{-1}) and T7 (420 ± 34 x 10^4 cell. mL^{-1}).

These similarities are evidence that *C. neogracilis* adapt well to this salinity values (15‰, 20‰, 35‰). Growth velocity (K) behavior was similar to DCM, being T4 the treatment that presented greatest value (0.557 ± 0.018 division.dia^{-1}), statistically similar to T3 (0.513 ± 0.022 division.dia^{-1}) and T7 (0.500 ± 0.026 division.dia^{-1} (Tab. 2).

Table 2 – Maximum cellular density – DCM (x10^4 cell. mL^{-1}) and Growth velocity – K (cellular division. day^{-1}) of diatom *Chaetoceros neogracilis* cultured in different salinities (T1 – T7). Initial cell number was the same for all treatments (50 ± 2 x 10^4 cell. mL^{-1}). Different letters in columns indicates significative statistic difference ($p \leq 0.05$) among treatments.

Treatment	DCM (x10^4 cell mL^{-1})	K (Cellular division. day^{-1})
T1	311 ± 38 [b]	0.465 ± 0.048 [b]
T2	392 ± 10 [b]	0.504 ± 0.008 [a]
T3	405 ± 26 [a]	0.513 ± 0.022 [a]
T4	481 ± 29 [a]	0.557 ± 0.018 [a]
T5	345 ± 62 [b]	0.457 ± 0.043 [b]
T6	344 ± 81 [b]	0.422 ± 0.066 [b]
T7	420 ± 34 [a]	0.500 ± 0.026 [a]

Quantification of chlorophyll-a, pheophytin-a and total carotenoids

Quantification values of photosynthetic pigments from *C. neogracilis* cultivated in different salinities are presented in Fig. 2A-C. The greatest chlorophyll-*a* concentration (Fig. 2A) was verified in T3 (1.820 ± 0.175 $mg.L^{-1}$), which was statistically similar T1 (1.632 ± 0.125 $mg.L^{-1}$), T2 (1.495 ± 0.150 $mg.L^{-1}$) and T4 (1.425 ± 0.145 $mg.L^{-1}$). Treatment 5 presented the smallest chlorophyll-*a* concentration (1.178 ± 0.17 $mg.L^{-1}$) statistically similar to T2 (1.495 ± 0.150 $mg.L^{-1}$), T4 (1.425 ± 0.145 $mg.L^{-1}$), T6 (1.378 ± 0.08 $mg.L^{-1}$) and T7 (1.413 ± 0.170 $mg.L^{-1}$).

Pheophytin-*a* concentration behavior was similar to chlorophyll-*a* concentration (Fig. 2B) and the greatest value was found in T3 (2.566 ± 0.137 $mg.L^{-1}$), being statistically similar to T1 (2.304 ± 0.279 $mg.L^{-1}$), T2 (2.458 ± 0.093 $mg.L^{-1}$) and T4 (2.353 ± 0.049 $mg.L^{-1}$).

Regarding total carotenoids (Fig. 2C), the greatest concentration was also observed in T3 (3.915 ± 0.087 $mg.L^{-1}$), followed by T2 (3.489 ± 0.163 $mg.L^{-1}$), T1 (3.411 ± 0.019 $mg.L^{-1}$) and T4 (3.265 ± 0.444 $mg.L^{-1}$). Among these treatments there wasn't a statistical difference.

This study showed that *C. neogracilis* produced more pigments when cultivated in salinities lower than 20‰, especially in 15‰, in which carotenoids concentration was twice

as much as chlorophyll-*a* concentration, and about 1.5 time greater than concentration of pheophytin-*a*.

Quantification of exopolysaccharides (EPS)

Exopolysaccharides concentrations varied from 0.422 ± 0.122 $g.L^{-1}$ in T1, to 2.952 ± 0.204 $g.L^{-1}$ in T7, being the last seven folds superior to the first (Fig. 2D). Among treatments T5, T6 and T7, the greatest values, variation in EPS concentration was more discrete. Treatments T6 (2.790 ± 0.1 $g.L^{-1}$) and T7 (2.952 ± 0.204 $g.L^{-1}$) were statistically similar, whereas T5 (2.525 ± 0.242 $g.L^{-1}$) and T7 (2.952 ± 0.204 $g.L^{-1}$) were different. The rising salinity was directly proportional to EPS concentration, statistically different ($p \leq 0.05$), presented in Fig. 2D.

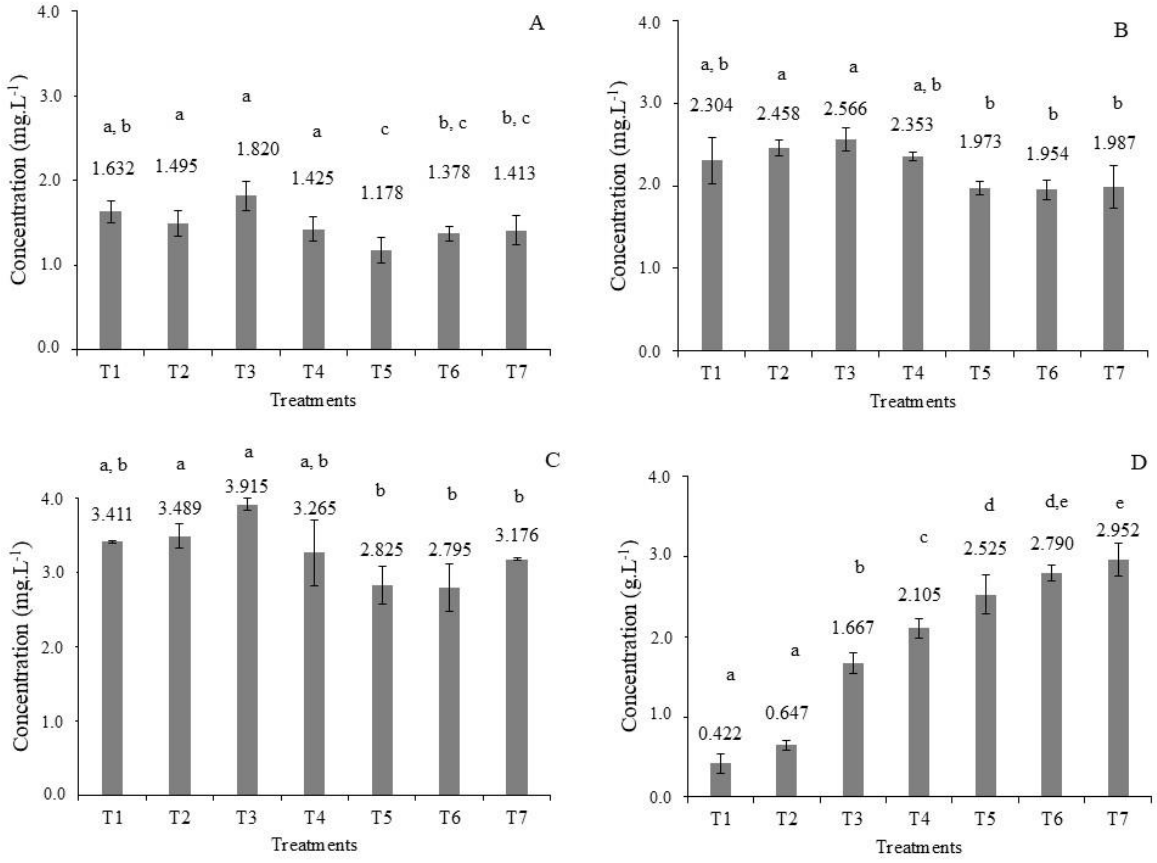

Figure 2 – Concentration of Chlorophyll-*a* (A), pheophytin-*a* (B), total carotenoids (C) in mg L^{-1} and exopolyssacharides (D) in g L^{-1} from *Chaetoceros neogracilis* cultivated in different salinities. Different letters indicate statistical difference ($p \leq 0.05$) between treatments.

Sulfated exopolysaccharides (EPS)

Treatments T6 and T7 presented slightly sulfation (Fig. 3). Treatment T7 changed color with 27 µL of EPS solution sample, while T6 took 50 µL of Eps solution sample. Therefore, T7 presented more sulfation than T6.

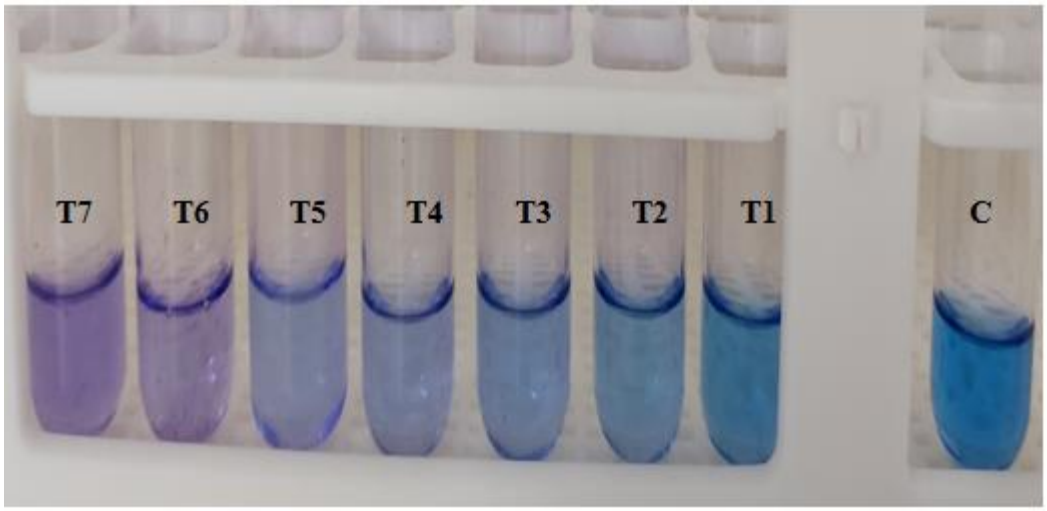

Figure 3 – Exopolysaccharides (EPS) sulfation from diatom *Chaetoceros neogracilis* from different salinities culture using metachromasia assay with 1,9-dimetilmetileno ADM dye. Blue color means no sulfation, while pale pink color means sulfation existence.

DISCUSSION

Diatom *C. neogracilis* presented excellent adaptation to different salinities, as it grew in all treatments. Asulabha *et al.* (2012)[43], corroborate the present study, evidencing *Chaetoceros* sp grew even with salinity stress much higher (5 to 10 times greater) than the ones used in the present study (35‰). *Chaetoceros muelleri*, also under salinity stress (7, 20 e 34‰), presented best cell density in 20‰ salinity[31], the same as in the present study. Espinal *et al* . (2015)[44] reported that poor results were dued to hiposmotic conditions, which was also observed in the present work.

Lopes *et al*. (2020)[45] cultivating *C. gracilis*, under similar conditions to the present study (Guillard f/2, 5,000 lux, 30 °C temperature and salinities of 5, 10, 15, 20, 25 and 35‰), although observing growth in all salinities, pointed out that the best culture performance

(greater absorbance, λ=700 nm) was in 10‰ salinity, while in the present study best absorbance (λ=680nm) was observed in 25‰ salinity.

Among commercial cultivated species *Chlorella* sp. and *Dunaliella salina* are the most fit to salinity changes[46,47,48]. Fitness to salinity differences may extend eligible cultivation areas, enlarging even more these species production either to produce biomass or bioactive substances, such as EPS.

In a study to evaluate salinity stress on culture of *C. gracilis,* with salinities varying from 1 a 70‰, Liang *et al*. (2014)[49] observed that when in 5 and 40‰ there wasn't statistical difference in cell growth, nor in relative chlorophyll content. As for the other salinities values, authors point that growth was negatively affected, and as in consequence, chlorophyl concentration was also affected. Do *et al*. (2023)[50], working with *Graesiella emersonii,* demonstrated that production of chlorophyl-*a* and total carotenoids were negatively affected by salinity changes, but the raise in salt concentration and luminous stress for *Dunaliella tertiolecta* cultivation benefited carotenoids production[51]. It becomes clear that interspecific differences have enormous influence in determining optimum cultivation features.

Normally, the increase in salt content in culture medium stimulates accumulation of carotenoids, but when salinity is excessive cell metabolism may be damaged[52]. Hindarti *et al.* (2020)[53] obtained 3.4 $mg.L^{-1}$ of chlorophyl-*a* concentration cultivating *C . gracilis* in 31‰, almost twice the concentration value of chlorophyl-*a* in the present study, in the most successful salinity treatment.

Dunaliella salina proved to be very tolerant to salinity changes[35], producing more chlorophyl-*a* as a response to raising salinity, and therefore improving photosynthetic activity. In contrast, *C. vulgaris* presented high concentrations of chlorophyl-*a* in low salinity (12‰) and retarded growth and reduced chlorophyl-*a* concentration in higher salinities (18 - 24‰)[54].

Pugkaew *et al.* (2019)[55] determined that the ideal salinity for cultivation of *Tetraselmis suecica* varies from 20 until 60‰, which coincides with high values of concentration of carotenoids and chlorophyl-*a*, whilst photosynthetic rates decreases in low salinities.

I regard exopolysaccharides production, Chaisuwan *et al.,* (2020)[56], indicate that age, inoculum size, composition of culture medium and physical-chemical parameters, especially salinity, are the most important features that influence yield and biological activities of EPS [44,45,46].

The increase of EPS production and its accumulation in microalgae cultivation generally occurs starting from the moment when there is nutrients limitation and/or shifting in physical-chemical parameters [47]. Bemal & Anil (2017)[34] evaluated EPS production from *Synechococcus* sp cultivated in different salinities, and observed that as salinity increased EPS also increased, but growth rate decreased in salinities greater than 10‰, the same behavior presented by *C. neogracilis* in the present work.

Porphyridium cruentrum, a Rhodophycea known for its polysaccharides production, presents increasing values of EPS concentration until it reaches marine salinity (32‰). Form this point ahead, until very high salinities (50‰), EPS production reduces[13]. However, Decamp *et al.*, (2023)[36] stated that, although *P. cruentum* was capable to grow in a wide range of salinity, EPS production reaches the maximum point at 25‰ salinity.

When cultivated in salinity zero and 12‰, *Nostoc* sp. HK-01 and *Anabaena* sp PCC7120 produced different concentrations of EPS, increasing it in salinity stress (12‰), 2.3-fold for Nostoc sp. and 5.7-fold *Anabaena* sp.[61]. Usmonkulova (2022)[62] reported that A*nabaena variabilis* cultivated under 12‰ salinity produced about 5 times more EPS than in fresh water (zero salinity), the same occurring to *Botryococcus brauniifoi 63,64, Phaeodactylum tricornutum, Nitzschia frustulum*[14,65,66] *and Dunaliella salina, which increased its EPS production 16 times under salinity stress*[67,68].

Concórdio-Reis *et al*. (2023)[69] investigated EPS production in five marine microalgae *Coolia monotis, Amphidinium carterae*, *Emiliania huxleyi*, *Tisochrysis lutea* e *Dunaliella tertiolecta.* Among them, *T. lutea*, stood out producing the highest values of EPS, with high sulfation rates in all five species tested.

CONCLUSION

Microalgae *Chaetoceros neogracilis* is capable of grow in a wide range of salinity, presenting tendency of better growing in salinities over 15‰, especially 20 and 25‰. When in low salinities (5 and 10‰), it presented reduced growth, probably due to an overload in metabolism. Production of pigments (chlorophyl-*a*, pheophytin-*a* and total carotenoids) was best in 15‰ salinity. Production of EPS was directly proportional to increasing salinity and a

discrete sulfation was observed in 30 and 35‰ salinities. Sulfation wasn't observed in lower salinities.

The results presented here are starting points to further investigation regarding EPS structure and how salinity influences expression of genes related to EPS sulfation in *C. neogracilis.* It is clear that *C. neogracilis* carries great potential to EPS production and is a promising species still little explored.

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