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Effects of pH on the growth rate exhibited of the wild-type and Cd-resistant *Dictyosphaerium chlorelloides* strains

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ABSTRACT

Effects of pH on the growth rate exhibited of the wild-type and Cd-resistant *Dictyosphaerium chlorelloides* strains

Environmental factors such as pH affect the growth rate, productivity, and the toxicity of metals in microalgae. This research reports the exponential growth of both wild-type ($Dc1M^{wt}$) and cadmium-resistant (Dc^{RCd100}) strains of *Dictyosphaerium chlorelloides* under different pH ranges at 1 to 15 days of exposure. The results reveal that Dc^{RCd100} strain had a maximum growth rate at pH ranges close to neutrality as time increases (pH 8.0, m = 0.93 on 10^{th} day), besides with limited growth capacity to moderately acidic pH (pH 4.0, m = 0.67 at 1^{st} day; pH 5.0 m = 0.16, 0.13 at 10 and 15^{th} days respectively). Whereas, $Dc1M^{wt}$ strain grows with a maximum growth rate at pH 9.0, with an increase at longer exposure time (m = 2.17 on the 15^{th} day). However, this strain was unable to maintain growth rates at the lowest pH tested at all exposure times. These results suggested that Dc^{RCd100} shows changes in cell division as well as in the photosynthetic activity to cope with slightly acidic pH ranges, although it was unable to grow in alkaline environments.

Key words: *Dictyosphaerium chlorelloides*, cadmium resistance, exponential growth, pH

RESUMEN

***Efectos del pH sobre la tasa de crecimiento exhibido por las cepas tipo salvaje y resistente a cadmio de* Dictyosphaerium chlorelloides**

Factores ambientales como el pH, afectan la tasa de crecimiento, la productividad y la toxicidad de los metales en microalgas. Esta investigación reporta el crecimiento exponencial de dos cepas de Dictyosphaerium chlorelloides*, una tipo salvaje ($Dc1M^{wt}$) y otra resistente a cadmio (Dc^{RCd100}) bajo diferentes rangos de pH en el medio de cultivo en 1 a 15 días de exposición. Los resultados revelan que, la cepa Dc^{RCd100} presentó una tasa de crecimiento máxima en rangos de pH cercanos al neutro a medida que se incrementa en el tiempo (pH 8.0,* m *= 0.93 al décimo día), además, con una capacidad de crecimiento limitada a valores de pH moderadamente ácidos (pH 4.0,* m *= 0.67 al 1er día de exposición, pH 5.0* m *= 0.16, 0.13 a los 10 y 15 días, respectivamente). Mientras que, la cepa $Dc1M^{wt}$ presentó su máximo crecimiento a pH de 9.0, conforme se incrementa en el tiempo de exposición (*m *= 2.17 al día 15). Sin embargo, esta cepa no puede mantener una tasa de crecimiento a los valores de pH más bajos ensayados en todos los tiempos de exposición. Estos resultados sugieren que, la cepa Dc^{RCd100} muestra cambios en la división celular, así como en la actividad fotosintética para hacer frente a las condiciones en los rangos de pH moderadamente ácidos, pero no puede crecer en entornos alcalinos.*

Palabras clave: Dictyosphaerium chlorelloides*, resistencia a cadmio, crecimiento exponencial, pH*

INTRODUCTION

Some metals are essential as biological components for metabolism at low concentrations (i.e. Cu, Fe) (Canli & Atli, 2003). However, aquatic ecosystems are often receptors of toxic metals derived by anthropogenic activities (Cd, Pb). They are a source of aquatic pollution with severe environmental implications such as bioaccumulation causing structural changes in populations and planktonic communities with evolutionary consequences (Qian *et al.*, 2009; Sánchez-Fortún *et al.*, 2009). However, some phytoplankton species are sensitive to these contaminants while others become resistant, transforming these signs into extremely harmful to the aquatic food web (Guanzon *et al.*, 1994; Sánchez-Fortún *et al.*, 2009). Toxic metals can induce alterations in the photosynthetic activity and productivity and changes in population growth rate (Yan & Pan, 2002). Resistant phytoplanktonic organisms may survive in chemically contaminated environments because of two different processes: physiological acclimation, which usually results from modifications of gene expression, and genetic adaptation by natural selection due to the occurrence of mutations that provide the resistance to several pollutants (Belfiore & Anderson, 2001; Sánchez-Fortún *et al.*, 2009; Gupta & Sandalio, 2012). According to Macfie & Welbourn, (2014) among the main mechanisms that photosynthetic cells present to maintain concentrations of free metal ions at levels that do not exceed cellular needs include binding in the cell wall, ion exchange, biotransformation, extra and intracellular chelation, or compartmentalization. On the other hand, the uptake and accumulation of metal ions by microalgae generally occurs in two stages: (1) first by a reversible and passive initial adsorption on the cell surface (e.g., to different functional groups OH^-, SH^-, COO^-, PO_4^{3-}, NO_3^-, RNH_2^-, RS^-, and RO^-) through electrostatic interactions, and (2) followed by a much slower, irreversible process, involving the active transport through the cell membrane into the cytoplasm, with subsequent binding to intracellular biomolecules (Monteiro *et al.*, 2012).

In this way, several physical-chemical environmental variables, such as pH fluctuations can influence in the toxicity and resistance to metals in microalgae cells. In aquatic systems, the hydrogen ion concentration has frequently demonstrated to affect metal toxicity, in part through its influence on metal speciation (as a free metal ion in solution M^+, organic metal complexation, and metal precipitation) rather than to the total metal concentration (Macfie *et al.*, 1994). Further, pH is the most important parameter influencing metal adsorption by microalgal biomass (Suresh Kumar *et al.*, 2015). This variable influences the metal resistance in microalgae, it affects cell surface metal binding and microalgae flocculation (Brinza *et al.*, 2007; Liu *et al.*, 2013; Ren *et al.*, 2013). At low pH, cell wall ligands are associated with the hydronium H_3O^- and H^+ ions, thereby restricting the approximation of metal cations due to the repulsive forces (Çetinkaya Dönmez *et al.*, 1999). When the pH increases, functional groups such as carboxyl, phosphate and amino groups are exposed (negative charges) producing attraction of metallic ions via biosorption on the cell surface (Çetinkaya Dönmez *et al.*, 1999). Likewise, several chemical functional groups are available for binding metal cations in according to pH ranges: at pH 5.0-9.0 metals are joined by phosphate groups, whereas at pH 9.0-12.0, carboxyl, hydroxyl and amine groups would also be suitable (Monteiro *et al.*, 2012; Suresh Kumar *et al.*, 2015). Other functional groups, like thiol (R-SH) plays an important role in sorption of metals like Cd at lower pH (>2.0) (Mehta & Gaur, 2005). The study of genetic adaptation of microalgae to extreme environments (i.e. characterized by extreme values of pH and exposure to metals) could be use adequately to assess the origin of favored mutants and the process of microalgal resistance (Sánchez-Fortún *et al.*, 2009). Some researchers have suggested that eukaryotic microalgae are resistant to acidic (pH 1.7–2.5) and metal-rich waters (Costas *et al.*, 2007; López-Rodas *et al.*, 2008; Pereira *et al.*, 2013). The pH fluctuations involve changes in the concentration gradients of H^+ ions, with effects on transport through the cell membrane (i.e. metabolic and photosynthetic activities such as PSII and photorespiration; Weisse & Stadler, 2006; Pereira *et al.*, 2013). Alkaline pH increases the flexibility of the cell wall of mother cells, which prevents its

rupture and inhibits autospore release, thus increasing the time for cell cycle completion (Guckert & Cooksey, 1990). The pH is one of the important parameters that affect the composition and phytoplanktonic production (Cabello *et al.*, 2015). However, the tolerance in green microalgae to changes in environmental variables subsequent to the evolution of microalgae to resistance to metals is still poorly documented, and therefore not sufficiently understood.

The aim of the present study is to assess the effect of pH on the growth rate of the two strains of the Chlorophycean *Dictyosphaerium chlorelloides*, wild-type (Dc1M^{wt}) and Cd-resistant strain (Dc^{RCd100}), under different pH values through the assessment of the maximum growth rate by the Malthusian fitness parameter using the fluorescence emitted by the Chlorophyll of Dc1M^{wt} and Dc^{RCd100} strains. This may explain of mechanisms on the growth rate that rule the tolerance to fluctuations in pH in association with the resistance to cadmium on microalgae.

MATERIAL AND METHODS

Experimental organisms and culture conditions

Laboratory experiments were performed using two strains of *Dictyosphaerium chlorelloides* (Nauman) Komárek & Perman, wild-type variant (Dc1M^{wt}) and resistant variant to 100 µM cadmium (Dc^{RCd100}) from the Algal Culture Collection of the Environmental Toxicology Laboratory (Chemical-Pharmacobiology School, U.M.S.N.H). Dc1M^{wt} strain (wild-type) grew axenically in culture flasks (Greiner, Bio-One, Longwood, NJ, USA) with 20 ml of BG-11 medium at pH 7.1 (Sigma, Aldrich Chemie, Taufkirchen, Germany), while Dc^{RCd100} strain was cultured in BG-11 medium supplied with 11.57 mg/L cadmium sulfate (CdSO4; Sigma, Aldrich Chemie, Taufkirchen, Germany) which is equivalent to Cd 100 µM concentration.

This value corresponded to $IC_{50(72)}$ i.e. the concentration that inhibited population growth by 50 % after 72h of exposure to $CdSO_4$ in Dc1M^{wt}. Dc^{RCd100} strain obtained from Dc1M^{wt} by selection of spontaneous mutants that showed an increased resistance to Cd in previous studies (Bartolomé *et al.*, 2016). Both strains were maintained at 21 ºC under continuous light of 60 µmol m^{-2} s^{-1} over the 400 to 700 nm waveband in a 16:8h light-dark photoperiod. Cells were maintained in mid-log exponential growth by serial transfers of one-cell inoculums to fresh medium once a fortnight. All tests were performed while both microalgal strains were in exponential growth phase.

pH behavior on microalgal growth rate

To determine the inhibition of the algal growth rates, the changes in Malthusian fitness (*m*) on Dc1M^{wt} and Dc^{RCd100} strains of *D. chlorelloides* were measured. Both strains were tested in 5 ml polystyrene sterile double sealing cultured tubes (Sarstedt Co., Nümbrecht, Germany) filled with BG-11 medium, with initial cell densities adjust-

Table 1. Malthusian fitness parameter values of the different exposure days (1, 3, 6, 10 and 15 days) at the different pH values selected (4.0, 5.0, 6.0, 7.1, 8.0, 9.0 and 10.0) on the rate of growth in wild-type (Dc1M^{wt}) and Cd-resistant (Dc^{RCd100}) strains of *D. chlorelloides*. The results are represented as the sample mean ± standard deviation (n = 6). *Valores del parámetro maltusiano de aptitud biológica de los distintos días de exposición (1, 3, 6, 10 y 15 días) a los diferentes valores de pH seleccionados (4.0, 5.0, 6.0, 7.1, 8.0, 9.0 y 10.0) sobre la tasa de crecimiento en las cepas tipo salvaje (Dc1M^{wt}) y Cd-resistente (Dc^{RCd100}) de* D. chlorelloides*. Los resultados se representan como la media muestral ± desviación estándar (n=6)).*

Malthusian fitness d^{-1} *D. chlorelloides*											
		1st day		3rd day		6th day		10th day		15th day	
pH	*N*	Dc1M^{wt}	Dc^{RCd100}	Dc1M^{wt}	Dc^{RCd100}	Dc1M^{wt}	Dc^{RCd100}	Dc1M^{wt}	Dc^{RCd100}	Dc1M^{wt}	Dc^{RCd100}
4.0	6	-1.18±0.80	0.67±0.15**	-2.40±0.40	-0.28±0.13***	-3.43±1.00	-0.47±0.22**	-4.00±0.51	-0.14±0.10***	-5.24±0.67	-0.51±0.16 ***
5.0	6	-0.11±0.09	-0.28±0.004*	-0.22±0.10	0.005±0.013 **	-0.41±0.13	-0.50±0.14 ns	-0.62±0.19	0.16±0.08 ***	-0.42±0.12	0.13±0.06 ***
6.0	6	0.018±0.005	0.031±0.009*	-0.07±0.19	0.41±0.11**	-0.05±0.08	0.21±0.02 ***	-0.09±0.03	0.75±0.19 ***	0.28±0.08	0.76±0.10 ***
7.1	6	-0.24±0.15	0.089±0.058**	0.10±0.05	0.12±0.04 ns	0.05±0.03	-0.43±0.11***	0.20±0.06	0.03±0.02 **	0.32±0.09	0.12±0.03 **
8.0	6	0.17±0.087	0.19±0.080 ns	0.37±0.22	0.72±0.16*	0.30±0.08	0.35±0.15 ns	0.57±0.11	0.93±0.31 *	0.61±0.20	0.90±0.23 ns
9.0	6	-0.43±0.19	-0.57±0.27 ns	1.05±0.31	0.04±0.14***	1.33±0.59	-0.30±0.31**	1.87±0.88	-1.93±0.40***	2.17±0.24	0.04±0.02***
10.0	6	-0.52±0.12	-0.50±0.24ns	-0.67±0.19	-0.02±0.05 ***	-2.01±0.15	-0.36±0.11***	0.15±0.62	-0.08±0.53 ***	0.68±0.34	-0.079±0.06**

*, **, ***: significant differences ($p < 0.05$) with respect to Dc1M^{wt}

ed to 10^4 cells/ml for both variants and the control. To estimate the stress induced in both *D. chlorelloides* strains by the pH variation in the medium, HCl and NaOH 0.1M buffers were added to the culture medium achieve final pH ranges of 4.0, 5.0, 6.0, 8.0, 9.0, 10.0 and pH 7.1 as control. In order to estimate the growth rate, the samples of the culture medium were maintained at 0, 1, 3, 6, 10, 15 days' time intervals to keep the exponential growth at 21 ºC in a 16:8h light-dark photoperiod in a thermostatically controlled chamber (Thermo Fisher Scientific Inc., EE.UU.) at 60 μmol m^{-2} s^{-1} over the 400 to 700 nm waveband to ensure exponential algal growth. Each assay was repeated six times (n=6). To estimate the maximal algal growth rate, we estimated the growth rate as the Malthusian parameter m which was calculated as $m = loge\ (Nt/N0)/t$ (Crow & Kimura, 1970). Where N_t are the cell numbers at the end of the experiment, N_0 are the cell numbers at the beginning of the experiment, and t = 0, 1, 3, 6, 10 and 15 days. The Malthusian fitness parameter was estimated through the fluorescence emitted by the chlorophyll of the both strains of *D. chlorelloides* with excitation-emission filters of 485-670 nm using a Tecan Genios plate reader (Tecan Group Ltd., Switzerland).

Statistical analysis

The effects of the pH on the maximum growth rate in Dc^{RCd100} and $Dc1M^{wt}$ strains was determined by the Malthusian fitness parameter described above. Each test was repeated six times (n=6), and of which the sample mean and its standard deviation ($\bar{x}\pm\sigma$) were obtained. D'Agostino and Pearson test assessed data normality. The statistical differences were obtained by applying unpaired T-test by the correction by the Welch test for distinct variances and the differences were considered significant at $p<0.05$. Statistical analysis was performed using the computer software package GraphPad Prism v6.0 (Graph-Pad Software Inc., USA).

RESULTS

After different exposure times, $Dc1M^{wt}$ was unable to maintain exponential growth rates at moderately acidic conditions (pH values of 4.0 and 5.0) showing negative values of m of different magnitude, which increased according to the increase in the exposure times on this strain. $Dc1M^{wt}$ presents statistically significant differences (Table 1) with respect to Dc^{RCd100} ($p<0.001$; $p>0.0001$). At pH 10.0, the strain $Dc1M^{wt}$ does not present growth rate, response that it shares with the Dc^{RCd100} variant at the first, third and sixth day of exposure (Table 1, Fig. 1A-1C). Nevertheless, at 10 and 15 days, $Dc1M^{wt}$ exhibited a growth rate in this pH, with the consequent induction phase initiating division and cell growth. Indeed, its maximal growth rate results in the pH value of 9.0 increasing this fitness to longer exposure time (15^{th} day, m = 2.17, Table 1, Fig. 1E).

In contrast, Dc^{RCd100} strain on the 1^{st} day of the exposure to pH 4.0 (m = 0.67), had statistically significant growth rate compared to $Dc1M^{wt}$ ($p<0.0001$) (Table 1, Fig. 1A). Nevertheless, at 10 and 15 days (m = 0.16 and 0.13 respectively), Dc^{RCd100} grew at pH 5.0, but the growth was slow due to longer lag phase in the culture medium (Table 1, Fig. 1D and 1E). Even though this strain had limited and slowly growth in moderately acidic environments. However, cell division and growth rate of Dc^{RCd100} started at pH 6.0, peaking at day 10 (m = 0.93) to pH 8.0 (Fig. 1D). The main differences between both strains are that Dc^{RCd100} does not grow in alkaline environments (pH values of 9.0 and 10.0), whereas $Dc1M^{wt}$ preferred alkaline conditions where it shows its maximum growth rate (Table 1, Fig. 1B-1E).

DISCUSSION

The pH of the medium growth is important in the context of metal speciation (i.e. complexes formation of different solubility and toxicity, or in free ion form) and can influence the binding of metals to cell walls. This process involves displacement of protons, which is dependent on the protonation degree as determined by the pH of the surrounding medium (Macfie *et al.*, 1994; Chen *et al.*, 2017). On the other hand, pH parameter can influence the growth of phytoplankton through an increase in the absorption of inorganic

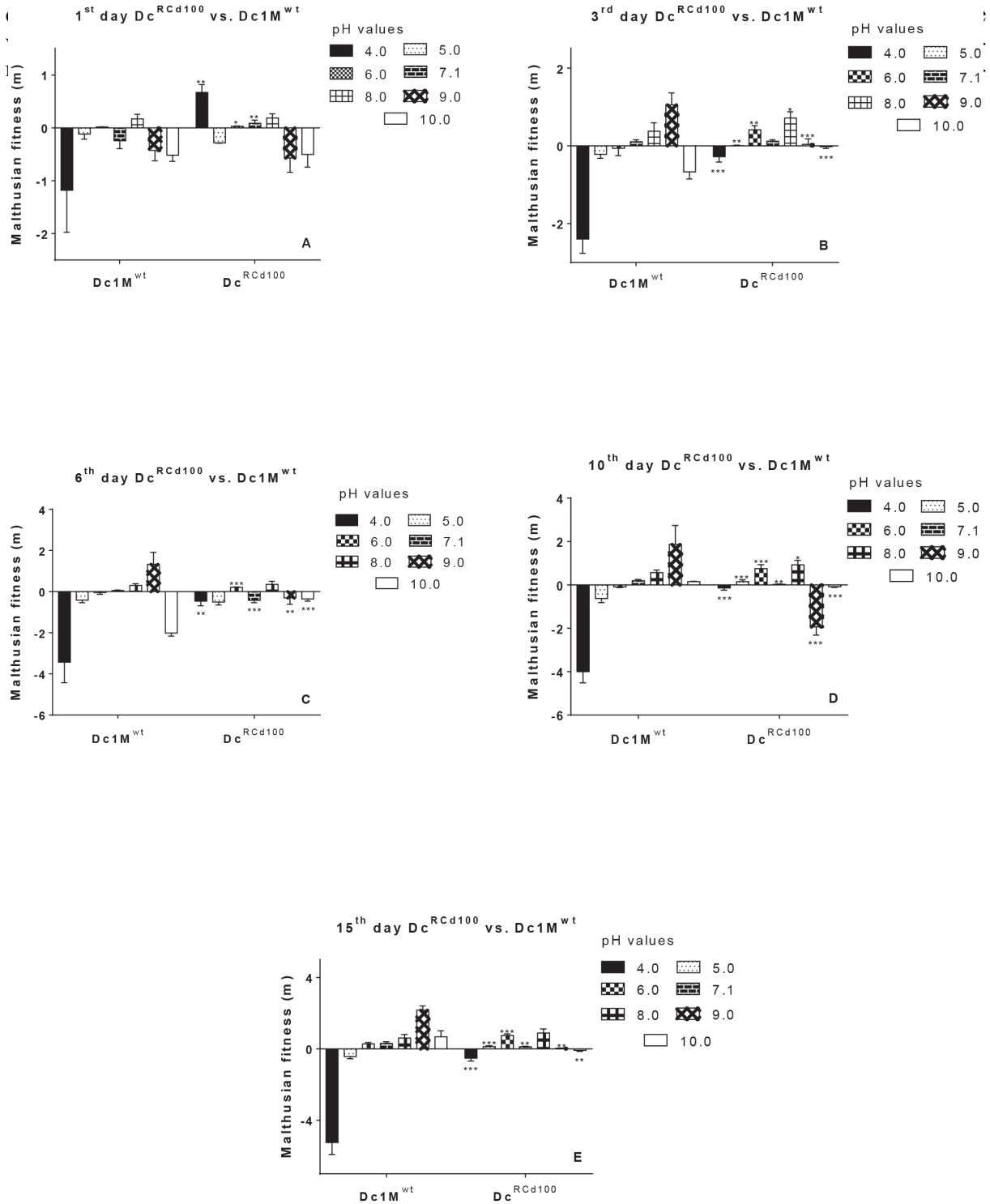

Figure 1. pH fluctuation effect of culture medium on fitness of Cd-resistant strain (Dc^{RCd100}) vs. wild type strain of *D. chlorelloides* ($Dc1M^{wt}$). (A): First day of exposure (1st Day), (B): 3rd day, (C): 6th day, (D): 10th day, (E): 15th day. (n=6). *Efecto de la fluctuación del pH en el medio de cultivo sobre la aptitud biológica de la cepa Cd-resistente (Dc^{RCd100}) vs. cepa tipo salvaje ($Dc1M^{wt}$) de* D. chlorelloides*. (A): Primer día de exposición (1er día), (B): 3er día, (C): 6to día, (D): 10mo día, (E) 15to día. (n=6).*

gae (e.g. *Chlorella*) can grow under extreme pH and temperature conditions (Visviki & Santikul, 2000; Sakarika & Kornaros, 2016).

Our results show that Dc^{RCd100} strain did not grow in alkaline conditions and exhibits its maximum growth rate in the pH range closer to neutrality (6.0 and 8.0) with limited capacity to survive in moderately acidic conditions (pH 4.0 and 5.0). On the other hand, the $Dc1M^{wt}$ strain preferred alkaline (pH 9.0 and 10.0) and neutral environments. The results obtained in this study are consistent with other studies where most species of microalgae grow maximally around neutral pH (7.0–7.6), although the optimal pH is the initial culture pH at which microalgae adapts to grow (lag phase; Visviki & Santikul, 2000; Hansen, 2002). Bartley *et al.*, (2014), investigated the pH influence on the growth and lipid accumulation in *Nannochloropsis salina* and showed that the highest growth rates were at pH 8.0 and 9.0 (0.19 ± 0.008 and 0.19 ± 0.011, respectively), but this microalga was not able to grow at pH 10.0, and the maximum cell densities were reached around 21 days into the experiment.

Several authors reported the effect of pH in different genera of both freshwater and marine microalgae (*Thalassiosira pseudonana* and *Chlorella pyrenoidosa*), namely on the growth rate and photosynthetic productivity from pH values of 5.7, 6.5, 8.3 to 8.8; nonetheless, above pH 9.0 growth could not be maintained (Chen & Durbin, 1994, Tan *et al.*, 2016.) Therefore, the changing of pH in the media may limit algal growth via metabolic inhibition (Juneja *et al.*, 2013). In bioassays performed by Touloupakis *et al.*, (2016), using a cyanobacterium strain *Synechocystis* sp. PCC 6803, it was determined the photosynthetic productivity, growth yield, and biomass in light energy declined by 32, 28, and 26 % respectively at pH 11.0. In our study, the pH 7.1 is the optimum pH value in the $Dc1M^{wt}$ strain showed growth after being exposed to the lowest values. However, the maximum growth rate was at pH 9.0, which agrees with Eaton-Rye *et al.*, (2003) and Kurian *et al.*, (2006), which showed that the optimal pH for microalgal growth is higher than 7.5 and close to 10.0. In this context, Moheimani, (2013) informed in green microalgae *Chlorella* sp. and *Tetraselmis suecica*, that the highest biomass productivity had the following order: pH 7.0 > pH > 7.5 > pH 6.5 > pH 8.0 > pH 6.0 > pH 5.5. For *Dunaliella salina* it was reported that the maximum growth rate on the 2nd to 5th day of exposure at pH 7.0-8.0 was of 0.5 μ_m/day (specific growth rate/day); whereas, from 5 to 10 days the specific maximum growth rate reached a low value of $0.1\mu_m$/day (Ying *et al.*, 2014).

In acidic conditions the nutrient uptake can altered and thus affect the microalgal growth (Juneja *et al.*, 2013). *Chlamydomonas acidophila*, exposed to pH 4.4 denature V-lysine, a proteolytic enzyme that facilitates the release of daughter cells from the parental wall (Visviki & Palladino, 2001). Visviki & Santikul, (2000) reported the tolerance of *Chlamydomonas applanata* at pH 3.4 with mucilage production, presenting an optimal exponential growth rate after 5 days at pH 7.4. However, the tolerance reached at low pH for our resistant strain differs from reported by Pereira *et al.*, (2013), in the wild-type and Cr-resistant *D. chlorelloides* strains, both are able to survive in acidic pH conditions (pH 4.25). Our Dc^{RCd100} strain was able to grow, though limited capacity at moderately low pH levels, but exhibited a better growth rate than $Dc1M^{wt}$ when both were subjected to pH 4.0 and 5.0 in the culture medium. Several authors (Gehl & Colman, 1985, Juneja *et al.*, 2013 and Cabello *et al.*, 2015), reported that for maintaining an intracellular neutral pH in acidic environments, additional energy cost are required to pump protons out of the cell (e.g. the acid-tolerant microalga *Chlorella saccharophila* can change intracellular pH in response to changing external pH), or in correlation to low irradiances, the release of O_2 is not generated indicating that sufficient ATP is not obtained from photophosphorylation to compensate for the energy needs of the cells, including the maintenance of a high H^+ extracellular gradient.

Another adaptation was observed by Tatsuzawa *et al.*, (1996) under acidic conditions in *Chlamydomonas* sp., with an increase in saturated fatty acid content, which reduces membrane fluidity and inhibits high proton concentrations at pH 2.7. In previous studies, Lavoie *et al.*, (2012) the changes in the permeability of the cell membrane as a function of pH were evaluated in three species of green algae (*Chlamydomonas*

reinhardtii, *Pseudokirchneriella subcapitata* and *Chlorella fusca*) estimating that these microalgae were adapted to pH 5.5 with a lower rate of growth than at pH 7.0. For Gerloff-Elias *et al.*, (2006) the accumulation of heat-shock proteins (Hsp) in *Chlamydomonas acidophila* was dependent on changes at low levels of pH (pH 2.6-6.0) and high levels of temperature (29 °C). These results suggest that heat-shock proteins might play a role in the adaptation of *C. acidophila*, and possibly other microalgae to acidic environments.

Despite the lack of information on tolerance to extreme pH values with respect to resistance to metals in microalgae, other researchers reported that *Scenedesmus* sp. strain R-16 exhibited strong tolerance to a wide range of pH (4.0-11.0), and informed that this strain has the potential for using for the wastewater treatment in a wide pH range (Ren *et al.*, 2013).

Therefore, the present study showed that the strain Dc^{RCd100} in relation to $Dc1M^{wt}$ exhibited a maximum growth rate at pH near the optimum (pH 8.0) and a higher fitness in slightly acidic conditions than $Dc1M^{wt}$, the wild-type strain presented its maximum growth rate at alkaline pH values (pH 9.0), unlike Dc^{RCd100}, which begins to grow at pH 9.0 at 15d (stationary phase) showing a low value of the growth rate but not grow at pH 10.0.

CONCLUSIONS

In summary, the results obtained in this study showed that the Cd-resistant strain of *D. chlorelloides* at different times of exposure tends to grow slowly in acidic and slightly alkaline environments. However, its maximum biological fitness at each exposure were observed at pH values close to the optimum (6.0 and 8.0).

For the wild-type strain ($Dc1M^{wt}$) it is evident that its growth rate intensified in neutral and alkaline pH. Therefore, it is not suitable for growing in acid environments, with clear differences with the resistant strain of *D. chlorelloides*. Therefore, it requires the development of new research to reveal the mechanism in the correlation of metal resistance in their influence on tolerance extreme environmental parameters (such as pH, temperature, high light intensity, among others). This provides an alternative for the development of biotechnological models in the environmental monitoring (such as development biosensor using metal resistant and wild-type strains of several genera of microalgae) of toxic metals presented in the polluted waters.

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