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Combined effects of nitrogen content in media and *Ochromonas* sp. grazing on colony formation of cultured *Microcystis aeruginosa*

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ABSTRACT

To gain insight into the combined effects of nitrogen content in media and flagellate grazing on colony formation of Microcystis aeruginosa*, we added* Ochromonas *sp. to* M. aeruginosa *cultured in different nitrogen content media for 7 days. Results showed that* M. aeruginosa *could be efficiently ingested by* Ochromonas *sp., no matter what nitrogen content media* M. aeruginosa *was cultured in. Colony formation was observed in* M. aeruginosa *in all* Ochromonas *sp. grazing treatments during the experiment. In contrast,* M. aeruginosa *populations in the controls were strongly dominated by unicellular and paired cell forms, and no colonies were observed. Among all* Ochromonas *sp. grazing treatments, the mean numbers of cells per particle of* M. aeruginosa *increased with decreased nitrogen concentration (except 0% N), therefore colony formation of* M. aeruginosa *can be enhanced under lower nitrogen conditions. This suggests that both nitrogen content and* Ochromonas *sp. grazing combine to affect* M. aeruginosa *colony formation. Three-way ANOVA showed a statistically significant interaction between time (day 1, 3, 5, and 7), treatment (with and without* Ochromonas *sp. grazing) and N content (0%, 10%, 25%, and 100% N) on the mean numbers of cells per particle, i.e. the extent of colony formation. At the end of the experiment, the influence of nitrogen content (except 0% N) on the numbers of cells per particle followed a rectangular hyperbolic response. The experiments demonstrated that there exists a combined effect of nitrogen concentration and flagellate grazing on colony formation of* M. aeruginosa *under laboratory conditions.*

Key words: inducible defense, cyanobacterial blooms, phenotypic plasticity, Protozoa

1. INTRODUCTION

Microcystis aeruginosa Kützing, a common bloom-forming cyanobacterium occurring in eutrophic freshwaters worldwide (Reynolds & Walsby 1975; Dokulil & Teubner 2000; Lehman *et al*. 2005), has two physiologically different phenotypic types (Wu & Song 2008; Yang *et al*. 2008): a large colonial morph under natural conditions and single cells or a few paired cells in axenic cultures in the laboratory (Reynolds *et al*. 1981; Bolch & Blackburn 1996; Yang *et al*. 2006; El Herry *et al*. 2009). Colony formation in *M. aeruginosa* may be a phenotypic response of single cells to prevailing environmental conditions, including abiotic and biotic factors. Recent studies have indicated that colony formation in *M. aeruginosa* will occur when an axenic culture was subjected to *Ochromonas* sp. grazing (Burkert *et al*. 2001; Yang *et al*. 2006). These observations suggested that flagellate grazing may be one of the biotic factors responsible for colony formation in *M. aeruginosa*. However, sizes of *M. aeruginosa* colonies induced by flagellates were made up of only several or dozens of *M. aeruginosa* cells and were far smaller than those which occur in natural lakes, indicating that the colony-inducing effects of flagellates is very weak. It is likely that the colony formation of this species *in situ* is also associated with other factors. This means that the large colonies of *M. aeruginosa* occurring in natural lakes may be the synergic effect of abiotic and biotic factors (Yang *et al.* 2006).

It is well recognized that extracellular polysaccharides affect the stickiness of the cell surface and contributes to cell aggregation in algal species (De Philippis & Vincenzini 1998; van Rijssel *et al*. 2000; Thornton 2002; Yang *et al*. 2008). Extracellular polysaccharides may be stimulated under lower nitrogen conditions (Kroen & Rayburn 1984; Arad *et al*. 1992; De Philippis *et al.* 1993; Moreno *et al*. 1998; Guerrini *et al.* 2000; Staats *et al*. 2000; Granum *et al*. 2002; Magaletti *et al*. 2004). Thus, we suggest that colony formation of *M. aeruginosa* may be enhanced when *M. aeruginosa* cultured in low nitrogen conditions was subjected to *Ochromonas* sp. grazing.

In this study, therefore, we conducted experiments to examine the combined effect of nitrogen content in media and *Ochromonas* grazing on colony formation in *M. aeruginosa*. Specifically, we hypothesize that: (1) Colony formation of *M. aeruginosa* will occur when *M. aeruginosa* is subjected to *Ochromonas* sp. grazing; colony formation will be enhanced when *M. aeruginosa* cultured in lower nitrogen conditions was subjected to *Ochromonas* sp. grazing; (2) there will be an interaction between time, *Ochromonas* sp. grazing, and nitrogen content on colony formation of *M. aeruginosa*. Ultimately, these results may be used to predict the influence of natural mechanisms of grazing and nutrient content on colony formation in *M. aeruginosa*.

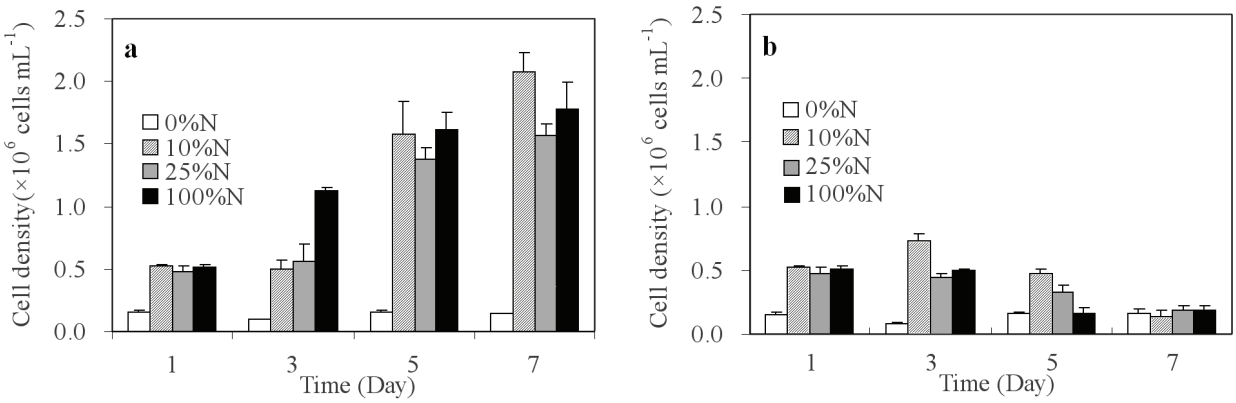

Fig. 1. Changes in cell densities of *M. aeruginosa* in the controls (**a**) and grazing treatments (**b**) over 7 days. Vertical lines represent one SE.

2. METHODS

2.1. Organisms and cultivation

M. aeruginosa Kützing (FACHB 927) was obtained from the Institute of Hydrobiology, Chinese Academy of Sciences. The cyanobacterium was batch cultured axenically in liquid BG-11 medium (Rippka *et al*. 1979) in 1.0 L flasks at 25 ºC and under a fluorescent light intensity of 40 μmol photons m^{-2} s^{-1} with a light-dark period of 12:12 hours. The flagellate *Ochromonas* sp. used in the experiments was taken from established cultures originally isolated from Lake Taihu (119°53'45"-120°36'15"E, 30°55'42"-31°33'50"N), a large eutrophic shallow lake in East China. *Ochromonas* sp. was cultivated in flasks with the addition of *M. aeruginosa* under the conditions as described above.

2.2. Experimental design

To minimize intracellular nutrient storage and nitrogen in the medium, prior to the experimental phase *M. aeruginosa* cultures in exponential growth were harvested by centrifugation and then resuspended in nitrogen-free BG-11 medium for two days. This process was repeated twice in four days. After four days, the suspensions were collected by centrifugation and the pellets were inoculated in the modified BG-11 medium with different proportions of nitrogen content (0%, 10%, 25%, and 100% of N in BG-11). The real nitrogen contents were 0.23, 25.08, 61.63, and 254.73 mg L^{-1} respectively. Inoculum of each of the four groups was grown in batch culture in 500 mL Erlenmeyer flasks containing 200 mL of above liquid medium respectively. On day 7, each culture was distributed to six 100 mL flasks; each flask containing 28 mL suspension of *M. aeruginosa*. In the grazing treatments, 2 mL concentrated *Ochromonas* sp. was added to three flasks (the initial density of *Ochromonas* sp. was approximately 1.33×10^4 individuals mL^{-1}), while in the controls, 2 mL corresponding media was added to the other three flasks. The experiment was run in triplicate for 7 days, and the cyanobacterium cultures within the flasks were shaken three times each day.

2.3. Data collection

Samples were taken after agitation every other day. Samples were fixed in Lugol's solution (2%) and cell densities of *M. aeruginosa* and *Ochromonas* sp. were determined using a microscope and a Neübauer haemocytometer. To determine the combined influence of *Ochromonas* sp. grazing and nitrogen content in media on colony formation of *M. aeruginosa*, the number of cells per particle was measured for *M. aeruginosa* in the treatments and the controls during the experiment. The mean numbers of cells per particle were recorded by counting at least 600 particles, which comprised of single cells, two-celled forms, or colony formations. To assess and describe the relationship between nitrogen content and mean number of cells per particle in *Ochromonas* sp. grazing treatments, a rectangular hyperbolic function, $Y = a\times X/(b+X)$, was fit to the data, where Y is the mean number of cells per particle, X is the reciprocal of nitrogen relative contents (except 0% N), a is maximum mean number of cells per particle, and b is a constant.

2.4. Statistical analysis

All data were presented as mean ± 1 SE and were analyzed by two-way or three-way ANOVA ($\alpha = 0.05$). All statistical analyses were carried out with MS-Excel (Microsoft, Seattle, USA) and SigmaPlot 11.0 (SSI, San Jose, California, USA).

3. RESULTS

3.1. Population dynamics of M. aeruginosa *and* Ochromonas *sp.*

Algal cell concentrations increased in all controls (except 0% N) over the course of the experiment (Fig. 1a), whereas in the *Ochromonas* sp. grazing treatments

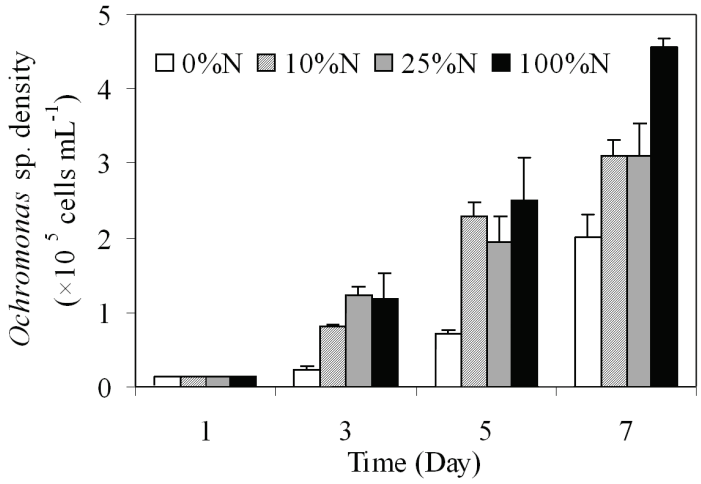

Fig. 2. Changes in densities of *Ochromonas* sp. in the grazing treatments over 7 days. Vertical lines represent one SE.

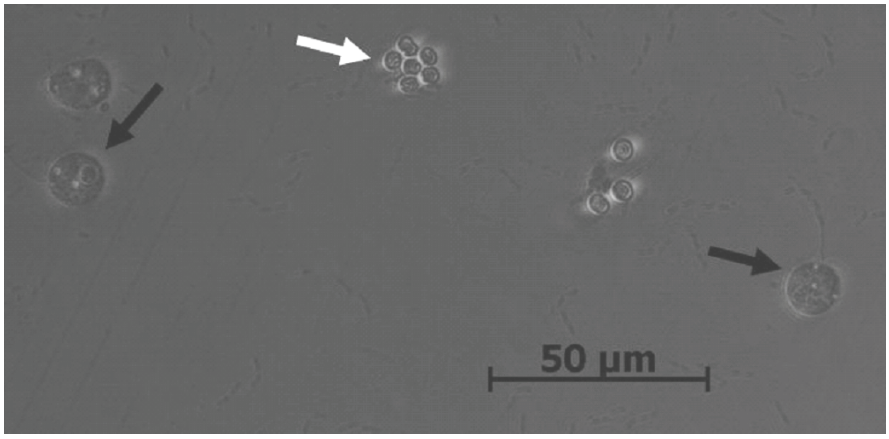

Fig. 3. *Ochromonas* sp. and *M. aeruginosa* in the grazing treatment. The black arrows show *Ochromonas* sp.; the white arrow shows *M. aeruginosa*.

M. aeruginosa decreased gradually (Fig. 1b) and was significantly lower than those in the grazing treatments. *Ochromonas* sp. densities increased in all treatments (Fig. 2 and Fig. 3).

3.2. Colony formation in M. aeruginosa

Colony formation was observed in *M. aeruginosa* in all *Ochromonas* sp. grazing treatments during the experiment. Some large colonies occurred in the grazing treatments. In contrast, *M. aeruginosa* populations in the controls were strongly dominated by unicellular and paired cell forms, and no colonies were observed. At the beginning of the experiment, the *M. aeruginosa* cultures comprised about 78% unicells, while in *Ochromonas* sp. grazing treatments the unicells reduced to approximately 10% and the proportion of cells in colonies increased from 0% to 18.2% (0% N), 81.2% (10% N), 67.7% (25% N), 47.9% (100% N) of the population at the end of the experiment (Fig. 4). The mean numbers of cells per particle of *M. aeruginosa* in the flagellate grazing treatments were significantly higher than those in the controls (Fig. 5). Among all *Ochromonas* sp. grazing treatments, the mean numbers of cells per particle of *M. aeruginosa* increased with decreased nitrogen content (except 0% N), and the mean number of cells per particle in 10% N medium was significantly higher than that in the BG-11 standard medium at the end of the experiments (Fig. 5). At the end of the experiment, the influence of nitrogen content on the numbers of cells per particle followed a rectangular hyperbolic response within the range of nitrogen content (except 0% N) we used in the experiment (Fig. 6). Three-way ANOVA indicated that there is a statistically significant combined effect of nitrogen content in media and *Ochromonas* sp. grazing on the mean numbers of cells per particle of *M. aeruginosa*, i.e. the extent of colony formation. Also, there is a statistically significant interaction between time (day 1, 3, 5, and 7), treatment (with and without *Ochromonas* sp. grazing) and N content (0%, 10%, 25%, and 100% N) on colony formation (Tab. 1).

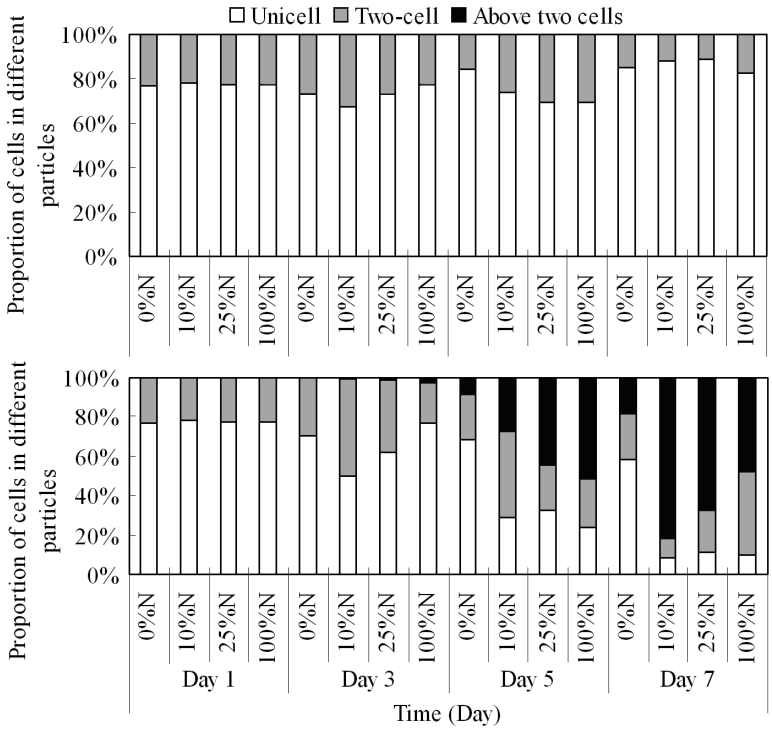

Fig. 4. Changes in proportion of cells in different particles in the controls and grazing treatments over 7 days. The upper panel is *M. aeruginosa* in the controls; the lower panel is *M. aeruginosa* in the grazing treatments.

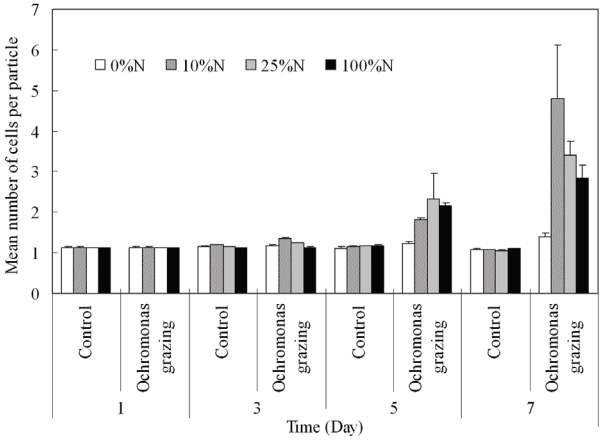

Fig. 5. Changes in mean number of cells per particle of *M. aeruginosa* in the controls and grazing treatments over 7 days. Vertical lines represent one SE.

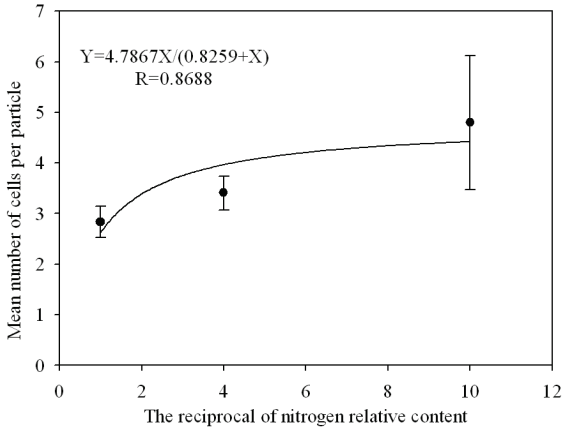

Fig. 6. Relationship between mean number of cells per particle of *M. aeruginosa* in the grazing treatments and nitrogen contents in the media. The formula in the figure follows a rectangular hyperbolic function. Vertical lines represent ±SE.

Tab. 1. Summary of three-way ANOVA on the interactions between time, treatment, and N content on mean number of cells per particle.

Effect	DF	SS	MS	F	P
time (day 1, 3, 5, 7)	3	14.027	4.676	20.626	<0.001
treatment (grazing, without grazing)	1	11.889	11.889	52.447	<0.001
N content (0%, 10%, 25%, 100% N)	3	3.650	1.217	5.367	0.002
time × treatment	3	16.000	5.333	23.528	<0.001
time × N content	9	6.463	0.718	3.168	0.003
treatment × N content	3	3.374	1.125	4.962	0.004
time × treatment × N content	9	6.517	0.724	3.194	0.003

4. DISCUSSION

From the population dynamics of *M. aeruginosa* in *Ochromonas* sp. grazing treatments, it was obvious that *M. aeruginosa* could be efficiently consumed by *Ochromonas* sp., as evidenced by the sharp decline in *M. aeruginosa* populations under grazing. Based on the results, *Ochromonas* sp. could be used as a potential grazer to effectively control growth of *M. aeruginosa* in the field.

As we expected, colony formation was observed in *M. aeruginosa* in all *Ochromonas* sp. grazing treatments during the experiment, in accordance with previous studies, and has been considered as an inducible defense against flagellate grazing (Yang *et al*. 2006). However, the extent of colony formation of *M. aeruginosa* cultured in different nitrogen content media is significantly different. Reflecting on the mean numbers of cells per particle, the values increased with decreased nitrogen content (except 0% N), which indicated that colony formation of *M. aeruginosa* can be enhanced under lower nitrogen conditions. It is well known that extracellular polysaccharides play an important role on affecting the stickiness of the cell surface and contributing to cell aggregation in algal species (De Philippis & Vincenzini 1998; van Rijssel *et al*. 2000; Thornton 2002; Yang *et al*. 2008) and that extracellular polysaccharides may be stimulated under low nitrogen (Kroen & Rayburn 1984; Arad *et al*. 1992; De Philippis *et al.* 1993; Moreno *et al*. 1998; Guerrini *et al*. 2000; Staats *et al.* 2000; Granum *et al*. 2002; Magaletti *et al*. 2004). Thus, in this experiment, we suggested that increased colony formation under decreased nitrogen may be due to more polysaccharides produced by *M. aeruginosa* under lower nitrogen conditions and thus an increase in stickiness of the cell surface. However, the colony formation in the 0% N media was weaker than those in the higher nitrogen media, which suggested that perhaps metabolism of *M. aeruginosa* has been severely affected under nitrogen deficiency and as a result can not respond to grazing actively.

In addition, *M. aeruginosa* cultures with no *Ochromonas* sp. grazing pressure can not form colonies, which indicated *Ochromonas* sp. grazing on solitary cells could induce colony formation in *M. aeruginosa*; colony formation can be enhanced under low nitrogen conditions, suggesting there exists a combined effect of nitrogen content and flagellate grazing on colony formation of *M. aeruginosa*. Three-way ANOVA demonstrated that three factors (time, grazing, and nitrogen content) significantly interact together on colony formation of *M. aeruginosa*. At the end of the experiment, the influence of nitrogen content on the number of cells per particle of *M. aeruginosa* followed a rectangular hyperbolic response, which suggested that colony formation can be enhanced under low nitrogen conditions, but the enhancement is not unlimited. Although colony formation in *M. aeruginosa* induced by *Ochromonas* sp. grazing can be enhanced under low nitrogen content conditions, the induced colonies are still far smaller than those colonies in natural eutrophic waters. The mechanism of large colony formation of *M. aeruginosa* occurring in natural lakes need to be further investigated.

5. CONCLUSIONS

In conclusion, colony formation was observed in *M. aeruginosa* in all *Ochromonas* sp. grazing treatments during the experiment. Among all *Ochromonas* sp. grazing treatments, colony formation of *M. aeruginosa* can be enhanced under lower nitrogen conditions. The experiments demonstrated that there exists a combined effect of nitrogen content in media and *Ochromonas* sp. grazing on colony formation of *M. aeruginosa* under laboratory conditions.

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REFERENCES

Arad, S.M., Y.B. Lerental & O. Dubinsky. 1992. Effect of nitrate and sulfate starvation on polysaccharide formation in *Rhodella reticulata. Bioresour. Technol*., 42: 141-148.

Bolch, C.J.S. & S.I. Blackburn. 1996. Isolation and purification of Australian isolates of the toxic cyanobacterium *Microcystis aeruginosa* Kütz. *J. Appl. Phycol*., 8: 5-13.

Burkert, U., P. Hyenstrand, S. Drakare & P. Blomqvist. 2001. Effects of the mixotrophic flagellate *Ochromonas* sp. on colony formation in *Microcystis aeruginosa. Aquat. Ecol*., 35: 9-17.

De Philippis, R. & M. Vincenzini. 1998. Exocellular polysaccharides from cyanobacteria and their possible applications. *FEMS Microbiol. Rev*., 22: 151-175.

De Philippis, R., M.C. Margheri, E. Pelosi & S. Ventura. 1993. Exopolysaccharide production by a unicellular cyanobacterium isolated from a hypersaline habitat. *J. Appl. Phycol*., 5: 387-394.

Dokulil, M.T. & K. Teubner. 2000. Cyanobacterial dominance in lakes. *Hydrobiologia*, 438: 1-12.

El Herry, S., H. Nasri & N. Bouaicha. 2009. Morphological characteristics and phylogenetic analyses of unusual morphospecies of *Microcystis novacekii* forming bloom in the Cheffia Dam (Algeria). *J. Limnol*., 68: 242-250.

Granum, E., S. Kirkvold & S.M. Myklestad. 2002. Cellular and extracellular production of carbohydrates and amino acids by the marine diatom *Skeletonema costatum*: diel variations and effects of N depletion. *Mar. Ecol. Prog. Ser*., 242: 83-94.

Guerrini, F., M. Cangini, L. Boni, P. Trost & R. Pistocchi. 2000. Metabolic responses of the diatom *Achnanthes brevipes* (Bacillariophyceae) to nutrient limitation. *J. Phycol*., 36: 882-890.

Kroen, W.K. & W.R. Rayburn. 1984. Influence of growth status and nutrients on extracellular polysaccharide synthesis by the soil alga *Chlamydomonas mexicana* (Chlorophyceae). *J. Phycol*., 20: 253-257.

Lehman, P.W., G. Boyer, C. Hall, S. Waller & K. Gehrts. 2005. Distribution and toxicity of a new colonial *Microcystis aeruginosa* bloom in the San Francisco Bay Estuary, California. *Hydrobiologia*, 541: 87-99.

Magaletti, E., R., Urbani, P. Sist, C.R. Ferrari & A.M. Cicero. 2004. Abundance and chemical characterization of extracellular carbohydrates released by the marine diatom Cylindrotheca fusiformis under N- and P-limitation. *Eur. J. Phycol*., 39: 133-142.

Moreno, J., M.A. Vargas, H. Olivares, J. Rivas & M.G. Guerrero. 1998. Exopolysaccharide production by the cyanobacterium *Anabaena* sp. ATCC 33047 in batch and continuous culture. *J. Biotechnol*., 60: 175-182.

Reynolds, C.S. & A.E. Walsby. 1975. Water blooms. *Biol. Rev*., 50: 437-481.

Reynolds, C.S., G. Jaworski, H. Cmiech & G. Leedale. 1981. On the annual cycle of the blue-green alga *Microcystis aeruginosa* Kütz. Philos. *Trans. Roy . Soc. London B – Biol. Sci*., 293: 419-477.

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

Staats, N., L.J. Stalb & L.R. Mura. 2000. Exopolysaccharide production by the epipelic diatom *Cylindrotheca closterium*: effects of nutrient conditions. *J. Exp. Mar. Biol. Ecol*., 249: 13-27.

Thornton, D.O. 2002. Diatom aggregation in the sea: mechanisms and ecological implications. *Eur. J. Phycol*., 37: 149-161.

van Rijssel, M., I. Janse, D.J.B. Noordkamp & W.W.C. Gieskes. 2000. An inventory of factors that effect polysaccharide production by *Phaeocystis globosa*. *J. Sea Res*., 43: 297-306.

Wu, Z.X. & L.R. Song. 2008. Physiological comparison between colonial and unicellular forms of *Microcystis aeruginosa* Kütz. (Cyanobacteria). *Phycologia*, 47: 98-104.

Yang, Z., F.X. Kong, X.L. Shi & H.S. Cao. 2006. Morphological response of *Microcystis aeruginosa* to grazing by different sorts of zooplankton. *Hydrobiologia*, 563: 225-230.

Yang, Z., F.X. Kong, X.L. Shi, M. Zhang, P. Xing & H.S. Cao. 2008. Changes in the morphology and polysaccharide content of *Microcystis aeruginosa* (Cyanobacteria) during flagellate grazing. *J. Phycol*., 44: 716-720.

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