Effect of Myo-inositol Concentration on the Growth of Marine *Porphyridium Cruentum*

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***Abstract*—Microalgae are sources for aquaculture, health foods, food additives, pharmaceuticals natural colorants and bioenergy. Aquaculture is a fast developing area and microalgal culture is a key procedure in fish hatcheries. Various species are cultivated according to demands of different applications Nevertheless, different species need specific conditions such as light, temperature, and adapted culture medium.**

The present study was undertaken to examine effect of myo-inositol on growth of Porphyridium cruentum. Maximum cell densities of control, 100 mg L-1 and 500 mg L-1 groups were found as 101.5±4.5 x 105 cell mL-1, 113±5.4 x 105 cell mL-1, 129.5±12.8 x 105 cell mL-1, respectively. However, no significant difference was found between groups, statistically. Also, mean specific growth rates of control, 100 mgL-1 and 500 mgL-1 groups were calculated as 0.20, 0.21 and 0.22, respectively. This study shows that myo-inositol as an ingredient of medium in microalgae production may provide higher yields.

***Index Terms*—photobioreactor, porphyridium cruentum, myo-inositol, growth rate**

I. Introduction

Microalgae are indispensable sources for biotechnological products of different industrial applications such as feed, health foods, natural colorants, pharmaceuticals and bioenergy [1], [2]. Also, essential feed source for all growth stages of bivalves and for larvae of some crustaceans and fish species in aquaculture as used directly in larval tanks. In this aquaculture feed chain, important nutrients from microalgae are transferred to higher trophic levels via intermediary zooplankton [3], [4].

Microalgae species demand optimum conditions to provide continuous growth. However, as microalgal growth starts slowing down due to any limiting factor, such as light restriction, nutrient depletion, or non-optimal pH, temperature or salinity, lipid and carbohydrate synthesis may be increased [5].

Porphyridium cruentum [6] is a microalga with 4-9 μm diameter which may found as a single cell or irregular colony and has no cell wall [7]. Biochemical composition of *P. cruentum* contains %33 protein, %36 carbohydrates, %7 lipids and %2 phycobiliproteins also may vary by culture conditions [8]. It may accumulate high levels of AA (arachidonic acid, %42) and EPA (eicosapentaenoic acid, %37.5) [9] and it is recommended that might be convenient to use in aquaculture [10].

Inositol is a chemical compound with formula $C_6H_{12}O_6$, a six-fold alcohols of cyclohexane. It exits several stereoisomers and the most prominent form is myo-inositol. Myo-inositol and its multifunctional position in plant biochemistry and physiology, has already been described [11], [12]. Biosynthesis and function of several phosphoinositide stereoisomers have investigated in terms of signaling and plant growth which supports essential importance of inositol lipids in plants [13].

Auxins are a class of phytohormones involved in numerous aspects of plant growth and development. [14]. Auxins were detected in higher plants also in algae. IAA (Indole-3-acetic acid) is the natural auxin commonly occurring in vascular plants and its presence already detected in several microalgae species such as *Chlorella vulgaris, Chlorella pyrenoidosa, Scenedesmus armatus, Scenedesmus acuminatus and Chlamydomonas reinhardtii* [15]. The biological role of IAA-MI esters has not been studied in detail as much as need, and several hypotheses concerning their possible role. The IAA by itself is known to be a growth hormone, and the esterification of Ml by IAA may be involved in the metabolism of this acid in plants. Also, it is known that excessive rates of IAA may inhibit growth. IAA-esters which include IAA-MI, generally regarded as inactive 'storage' forms whereby plants cope with excess auxin production. Alternately, such structures might facilitate transport of auxin within the plant. Apart from the fact that free MI is required for IAA-MI production, little is known regarding the metabolic fate of this moiety. The fact that free IAA is in equilibrium with its precursor may open up new possibilities for controlled plant growth [16], [17].

The main problems of microalgae production are low productivity, low quality and high production cost. Purposes of microalgae production from industrial point of view, are increasing the growth rate and reducing the

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costs of production. The present study was undertaken to examine effect of myo-inositol on growth of *P. cruentum*.

II. Material and Methods

Porphyridium cruentum was obtained from the Microalgal Biotechnology Laboratory at Ben-Gurion University, Israel (Fig. 1).

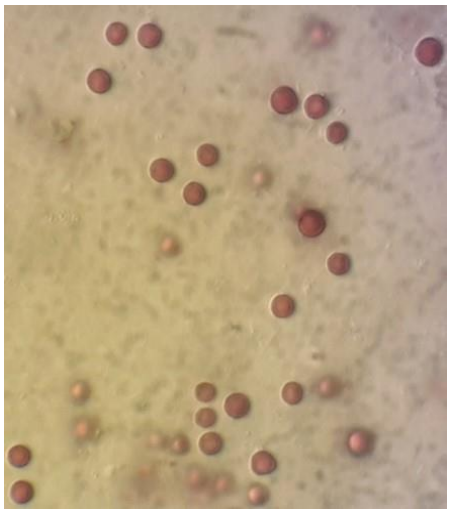


Figure 1. Porphyridium cruentum cells under light microscope.

Erlenmeyeres and seawater were sterilized by autoclave at 121 ℃ with 20 minutes. Autoclave tapes were used to be sure the sterilization process was successful. F/2 medium (Table I & II) [18] was used as culture medium and trials were done with 1 L erlemenyers. Philips MASTER TL-D Reflex 36W/865 1SL/25 fluorescent lamps provided 84 µmol.m-2s-1 illumination with 24:0 photoperiod. Salinity of all experimental groups was adjusted to 35 ‰ and initial pH of experimental groups were adjusted as 7.5. Cultures were stirred by air without any CO2 addition. 0.2 µ Sartorius Midisart 2000 filters were used to avoid contamination by aeration. Experiments were done in a room with 20±1 ℃ temperature which maintained by air-conditioner.

TABLE I. F/2 Medium

Quantity	Component	Stock Solution	Molar Concentration
1 mL	$NaNO_3$	75g L^{-1}	882 µmol L^{-1}
1 mL	NaH_2PO_4	0.5 mg L^{-1}	363 µmol L^{-1}
1 mL	f/2 trace metal	Table II	

TABLE II. F/2 Medium Trace Metal Solution

$FeCl_3.6H_2O$	3.15 g
$Na_2EDTA.\ 2\ H_2O$	4.36 g
$CuSO_4.\ 5\ H_2O$	0.0098 g
$Na_2MoO_4.2\ H_2O$	0.0063 g
$ZnSO_4.7\ H_2O$	0.022 g
$CoCl_2.6\ H_2O$	0.01 g
$MnCl_2.4\ H_2O$	0.18 g

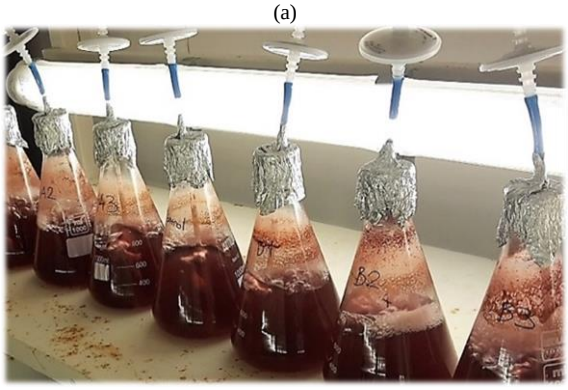
Myo-inositol were purchased from Sigma-Aldrich. Inositols are not suitable to be sterilized by autoclave and are degradable under high temperatures. Therefore, myo-inositols were firstly dissolved in sterilized distilled water and then filtered through a 0.2 µ membrane filter (Whatman Anodisc 47mm) to avoid of any contamination.

100 mg/L and 500 mg/L myo-inositol concentrations with control group (Table III) were chosen in this study and experiments were done in triplicate (Fig. 2).

TABLE III. Myo-inositol Concentrations

Species	*Porphyridium cruentum*
Group 1	F/2 and 100 mg/L myo-inositol
Group 2	F/2 and 500 mg/L myo-inositol
Group control	F/2

(a)



(b)

Figure 2. Porphyridium cruentum cultures (First day (a) and last day (b))

Samples were collected daily for cell count. Cell density was measured via Improved Neubauer hemocytometer and at the same time, contamination was checked daily through visual observation. Growth rates (µ) were calculated with this equation;

$$\mu = \frac{\ln(N_t) - \ln(N_0)}{t - t_0}$$

where Nt is biomass at time (t) and No is the beginning biomass at time to.

The Kolmogorov-Smirnov test was used to verify the normality and homogeneity of variances. Data were analyzed using ANOVA. The sources of significant differences were determined using the Tukey test [19].

III. Results

Initial cell densities were arranged as 11 x 105 cell mL-1 for all groups. Maximum cell density of the control group was measured as 101.5±4.5 x 105 cell mL-1 at the day 11. 100 mg L-1 and 500 mg L-1 myo-inositol added groups were also reached their maximum cell densities at the day 11 and were measured as 113±5.4 x 105 cell mL-1 and 129.5±12.8 x 105 cell mL-1, respectively (Fig. 3). However, no significant difference was found between control and experimental groups (P<0.05).

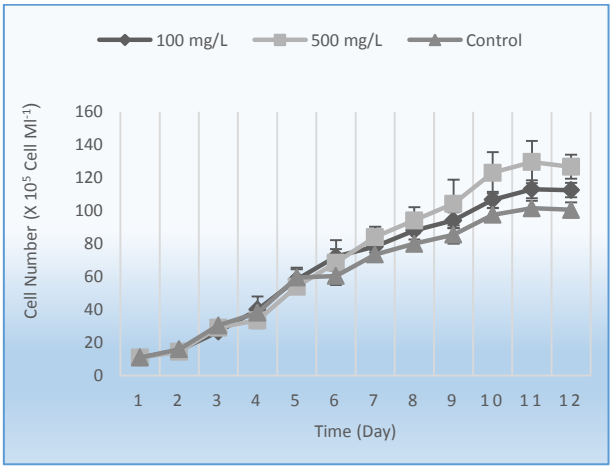


Figure 3. The cell densities of Porphyridium cruentum for different myo-inositol concentration and control group.

Mean specific growth rates were calculated for control, 100 mg L-1 myo-inositol and 500 mg L-1 myo-inositol added groups as 0.20, 0.21 and 0.22, respectively (Fig. 4).

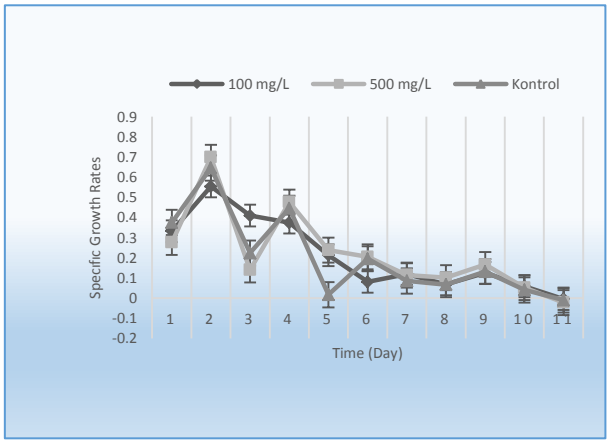


Figure 4. Specific growth rates of Porphyridium cruentum for different myo-inositol concentration and control group

IV. Discussion

In some countries, microalgal biomass is added to improve nutrient profile of human food product like a noodle, such in Japan and Chile [20], [21]. Microalgae is considered as a valuable artificial baby food since its component similar to mother milk [22]. The biochemical composition of *P. cruentum* shows that it is rich in many important compounds, i.e. the protein content of *P. cruentum* ranges from 28 to 39% and the carbohydrate content vary between 40 and 57% and the total lipids may reach 9-14% of dry weight and its biomass contains tocopherol, vitamin K and large amounts of carotenes [23].

P.cruentum contains valuable fatty acids such as 20:5(n-3) and 20:4(n-6), which are important nutrition for human, as well as animal nutrition. Further studies on the effects of environmental conditions such as high and low temperatures, suboptimal and supra-optimal light intensities, low availability of essential nutrients and etc., on the fatty acid composition of *P. cruentum* should be performed.

The variation in cell density of *P.cruentum* using different myo inositol concentration shown that 100 mg L-1 myo-inositol and 500 mg L-1 myo-inositol added groups were reached 1.11 and 1.28 fold cell densities than control groups, respectively. Still, significant difference was not found according to statistical tests (P<0.05). Results shows that higher concentration of myo-inositol may be more effective on P. cruentum cultures. The cultivation for 12 days was carried and the cell density of *P.cruentum* increased rapidly all groups between 1.01 cells mL-1 to 1.29x107 cells mL-1 on day 10 without any apparent lag phase. The on set of the culture phases was between 0 and 10 days, between 10 and 12 days, respectively for culture exponential and stationary phases. It was recorded that maxsimum specific growth rate of alga was 0.24 day-1 at exponential phase. Similar experiment with *Dunaliella salina* was done previously and as reported maximum cell density was obtained from 500 mg L-1 myo-inositol added groups which was 1.4 fold higher than control group [24] In the same study, neutral lipid content of myo-inositol added *D. salina* group indicated that was 1.34 times higher than control group. Also, carotenoids were compared with control group and no significant difference was reported [24].

Unfortunately, no lipid composition data was obtained of *P. cruentum* trials. Also, pigment composition of *P. cruentum* was not analyzed.

V. Conclusion

P. cruentum has a big importance in aquaculture hatcheries because its specifications (fatty acid and pigment composition, cell size... etc.). This study shows that myo-inositol use in microalgae production could provide higher yields of *P. cruentum* cultures. This experiment should be considered as pre-study for inositol effect on *P. cruentum* cultures. Results showed that different concentrations of myo-inositol or different inositol derivatives may affect *P. cruentum* cultures efficiently. Also, effect of myo-inositol on biochemical composition of *P. cruentum* should be analyzed.

Nowadays, microalgae are produced intensely and researchers still looking forward to optimize its culture medium. Main purpose is the increase biomass while no loss in valuable biomolecules such as fatty acids and pigments. Yet, we have no information how myo-inositol may affect its fatty acid composition. Also, optimum myo-inositol concentration should be determined with further studies.

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REFERENCES

[1] W. L. Chu, "Biotechnological applications of microalgae," *IeJSME*, vol. 6, no. 1, pp. S24-S37, 2012.
[2] R. B. Dixit and M. R. Suseela, "Cyanobacteria: Potential candidates for drug discovery," *Antonie van Leeuwenhoek*, vol. 103, no. 5, pp. 947-961, 2013.
[3] M. R. Brown, M. Mular, I. Miller, C. Farmer, and C. Trenerry, "The vitamin content of microalgae used in aquaculture," *J. Appl. Phycol.*, vol. 11, no. 3, pp. 247-255, 1999.
[4] R. Vismara, S. Vestri, C. Kusmic, L. Barsanti, and P. Gualtieri, "Natural vitamin E enrichment of Artemia salina fed freshwater and marine microalgae," *J. Appl. Phycol.*, vol. 15, no. 1, pp. 75-80, 2003.
[5] Z. Cohen, "Porphyridium cruentum," in *Chemical from Microalgae*, Z. Cohen, Ed., London, UK: Taylor& Francis Ltd., 1999, pp. 41-56.
[6] C. Nägeli, *Gattungen Einzelliger Algen: Physiologisch und Systematisch Bearbeitet*, Friedrich Schulthess, 1849.
[7] A. Vonshak, "Porphyridium," in *Microalgal Biotechnology*, M. A. Borowitzka and L. J. Borowitzka, Eds., Cambridge University Press, 1988, pp. 122-134.
[8] R. B. Román, J. M. Alvarez-Pez, F. A. Fernández, and E. M. Grima, "Recovery of pure B-phycoerythrin from the microalga *Porphyridium cruentum*," *J. Biotechnol*., vol. 93, no. 1, pp. 73-85, 2002.
[9] Z. Cohen, A. Vonshak, and A. Richmond, "Effect of environmental conditions on fatty acid composition of the red alga *Porphyridium cruentum:* Correlation to growth rate," *Journal of Phycology*, vol. 24, pp. 328-332, 1988.
[10] Y. Durmaz, M. Monteiro, N. Bandarra, Ş. Gökpinar, and O. Işik, "The effect of low temperature on fatty acid composition and tocopherols of the red microalga, *Porphyridium cruentum*," *J. Appl. Phycol.*, vol. 19, no. 3, pp. 223-227, 2007.
[11] F. A. Loewus and M. W. Loewus, "Myo-inositol: Its biosynthesis and metabolism," *Ann. Rev. Plant Physio.*, vol. 34, no. 1, pp. 137-161, 1983.
[12] D. J. Morre, W. F. Boss, and F. A. Loewus, *Inositol Metabolism in Plants*, New York, NY: Wiley-Liss, 1990, vol. 9.
[13] J. M. Stevenson, I. Y. Perera, I. Heilmann, S. Persson, and W. F. Boss, "Inositol signaling and plant growth," *Trends Plant Sci*., vol. 5, no. 6, pp. 252-258, 2000..
[14] A. Piotrowska-Niczyporuk and A. Bajguz, "The effect of natural and synthetic auxins on the growth, metabolite content and antioxidant resp onse of green alga Chlorella vulgaris (Trebouxiophyceae)," *Plant Growth Regul.*, vol. 73, no. 1, pp. 57-66, 2014.
[15] W. A. Stirk, P. Bálint, D. Tarkowská, O. Novák, M. Strnad, V. Ördög, and J. V. Staden, "Hormone profiles in microalgae: Gibberellins and brassinosteroids," *Plant Physiol. Bioch.*, vol. 70, pp. 348-353, 2013.
[16] E. M. Orekhova, V. I. Shvets, I. N. Gracheva, and B. A. Klyashchitskii, "Biologically active nonlipid derivatives of myo-inositol and prospects for their use in drug design (a review)," *Pharm Chem. J.*, vol. 30, no. 3, pp. 151-163, 1996.
[17] F. A. Loewus and P. P. Murthy, "Myo-Inositol metabolism in plants," *Plant Sci.*, vol. 150, no. 1, pp. 1-19, 2000.
[18] R. R. Guillard, "Culture of phytoplankton for feeding marine invertebrates," in *Culture of Marine Invertebrate Animals*, Springer US, 1975, pp. 29-60.
[19] J. H. Zar, *Biostatistical Analysis*, 4th ed., Upper Saddle River, New Jersey, USA: Prentice Hall, 1999, pp. 231-272.
[20] M. M. Fuentes, G. G. Fernandez, J. A. Perez, and J. J. Guerrero, "Biomass nutrient profiles of the microalga *Porphyridium cruentum*," *Food Chemistry*, vol. 70, pp. 345-353, 2000.
[21] A. Ginzberg, M. Cohen, U. A. Sod-Moriah, S. Shany, A. Rosenshtrauch, and S. M. Arad, "Chickens fed with biomass of the red microalga *Porphyridium sp.* havereduced blood cholesterol level and modified fatty acid composition in egg yolk," *Journal of Applied Phycology*, vol. 12, pp. 325-330, 2000.
[22] Z. Cohen, "Preparation of Eicosapentanoienoic Acid (EPA) concentrate from *Porphyridium cruentum*," *J. Am. Chem. Soc.*, vol. 68, pp. 16-19, 1991.
[23] E. W. Becker, *Microalgae Biotechnology and Microbiology*, Cambridge: Cambridge University Press, 1994.
[24] K. Cho, K. N. Kim, N. L. Lim, M. S. Kim, J. C. Ha, H. H. Shin, and T. Oda, "Enhanced biomass and lipid production by supplement of myo-inositol with oceanic microalga *Dunaliella salina*," *Biomass Bioenerg*, vol. 72, pp. 1-7, 2015.

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