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INFLUENCE OF METAL IONS ON GROWTH AND C-PHYCOCYANIN PRODUCTION IN *ARTHROSPIRA* (*SPIRULINA*) *PLATENSIS*

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Abstract

The present investigation was performed to analyze the influence of metal ions on growth and pigmentation of *A. platensis*. The metal ions in sulfate forms in five different concentrations were utilized for experiments. $MgSO_4$ at concentrations of 1g l^{-1} showed optimum cell mass productivity 37.5 mg L^{-1} day^{-1} with total chlorophyll and C-PC concentrations 27.0 and 22.0 mg g^{-1}. Zinc and copper sulphate proved to be lethal with maximum depletion in growth as well as pigment synthesis of the cyanobacterium, at 1 g l^{-1} the cell mass productivity was reduced to 9.2 and 4.2 mg l^{-1} day^{-1} with 1.6 and1.8 mg $g^{-1}$of total chlorophyll. The C-PC concentration was reduced to 6.3 and 6.0 mg g^{-1}. Higher concentration of $CuSO_4$ exhibited toxicity resulting in death of the isolate. $NiSO_4$ in lowest concentration showed 19.6 % decrease in cell mass productivity with 42.6% and 30.14% decrease in total chlorophyll and C-PC content respectively whereas $MnSO_4$ exhibited 10.97%, 36.06% and 2.29% decrease at minimum concentration.

Keywords: *A. platensis*, metal ions, cell mass productivity, pigment concentration
Abbreviations: cmp- Cell mass productivity, T ch.- Total chlorophyll and C-PC- C Phycocyanin

Introduction

The *Spirulina platensis* is a muticellular filamentous cyanobacterium. The habitat of the microorganism ranges from sea water, brackish water and fresh water in some cases lake water. The worldwide production of *A. platensis* has been increasing since 1980; currently up to 3,000 tons of *A. platensis* has been produced (Borowitzka, 1999; Pulz and Gross, 2004 and Spolaore et al., 2006). *Spirulina* is a phototroph and hence several parameters influence its growth such as light intensity, pH, aeration etc. (Richmond and Grobbelaar, 1986). *Spirulina* has been used as food since many centuries; presently it has become industrially important not only due its nutritional value but also caretenoids, Phycobiliproteins, γ linolenic acid which has got several biotechnological, pharmacological and medicinal applications (Belay, 2002). Phycocyanin (PC) is a blue, light-harvesting pigment in cyanobacteria and in the two eukaryote algal genera, Rhodophyta and Cryptophyta. PC is water soluble, strongly fluorescent and has antioxidant properties (Romay et al., 2003 and Eriksen, 2008). PC and related phycobiliproteins are utilised in a number of applications in foods and cosmetics, biotechnology, diagnostics and medicine. The present investigation was carried to analyze the effect of metal ions on growth and C-phycocyanin production of *Spirulina platensis*.

Materials and Methods

Microbial Source: The *Spirulina platensis* was isolated from hypersaline, alkalophilic habitat of Lonar Crater Lake. The isolate was identified by 16S- rRNA sequencing.

Growth Conditions: The isolate was maintained in CFTRI medium (Venkatraman and Becker, 1985). The pH of the medium was kept at 9.1 with 1 N NaOH. The 500 ml Erlenmeyer Flasks were used containing 200ml of the medium. Continuous aeration was provided by aerator. The flaks were exposed to illuminations of fluorescent cool white lamp (40W, Philips). 12-12 hours of light and dark cycles were maintained. The temperature was maintained at 28° C.

Experimental Design: The five different concentrations ranging 1.0, 1.5, 2.0, 2.5, 3.0 g l^{-1} of selected metal ions viz. $MgSO_4$, $ZnSO_4$, $CuSO_4$, $NiSO_4$, and $MnSO_4$ were incorporated in Zarrouk's medium. 500 ml flasks containing 250 ml media were inoculated with A. *platensis*. The culture of *A. platensis* in exponential growth phase was utilized for inoculation with 20% inoculum size. The inoculated flasks were kept under growth conditions for 12 days. The 20 ml media was used for each analytical test i.e. determination of dry weight, chlorophyll content and C-PC concentration. The analysis was performed after 72 hrs of time interval. The mat formation was restricted by manual agitation thrice a day.

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Growth Measurements: Algal growth was spectrophotometrically measured as described by Payer (1971). The calculated biomass (the mean of three experiments) was used to obtain maximum specific growth rates (μmax) from the log phase of the growth curves by exponential regression. Productivities was calculated from the equation P= (Xi -X0) / ti, where P = productivity (mg L-1day-1), X0 = initial biomass density (mg L-1), Xi = biomass density at time i (mg L-1) and ti =time interval (h) between X0 and Xi (Colla et al., 2007).

Determination of dry weight: Twenty ml from the different cultures were filtered using Whatman GF/C filter of 47 mm diameter in vacuum. The filtered cell mass was dried at 70° C for 30 mins (Rafiqul et al., 2005). The dried filter paper then kept in desiccators for 20 min for cooling and weighed.

Determination of Chlorophyll: One gram of *S. platensis* was homogenized in acetone (20 ml, 80%) and allowed to stand overnight in dark at 4°C for complete extraction followed by centrifugation at 10,000 xg for 5 min (Ei-Baky et al., 2008). The contents of total chlorophyll (TChl), chlorophyll a (Chl-a) and chlorophyll b (Chl-b) in the supernatant were spectrophotometrically determined according to Vonshak and Richmond (1988) method.

Determination of C-PC: The content of C-Phycocyanin in the isolated *Arthrospira platensis* was determined by the method described by the Boussiba and Richmond (1979).

Results and Discussion

The effect of five different concentrations of $MgSO_4$ on cell mass productivity, chlorophyll content and C- Phycocyanin concentration is depicted in table 1. The $MgSO_4$ concentrations 1 and 1.5 g L^{-1} showed positive effect with 0.092 and 0.090 growth rates, 37.5 and 35.0 mg L^{-1} $days^{-1}$ cell mass productivity. The total chlorophyll and C-PC concentrations were found to be 27.0, 21.3 and 22.0, 19.3 mg g^{-1} respectively. Its is noteworthy that the concentration of $MgSO_4$ in standard composition of CFTRI medium 1.2 g l^{-1} showed growth rate of 0.091day^{-1} and cell mass productivity 37.2 mg L^{-1} $days^{-1}$; it also revealed maximum total chlorophyll and C-PC concentrations 26.9 and 21.9 mg g^{-1}. This confirms that 1 g l^{-1} of $MgSO_4$ is more efficient influential than 1.2 g l^{-1} present in the standard CFTRI medium composition. The control with no $MgSO_4$ showed decline productivity in every aspect considered in the present study. Chaudhari et al. (1980) have reported 0.1 and 0.2 g l^{-1} of magnesium sulphate as optimum concentration for growth of *Spirulina* when sewage is used as a medium, growth retardation of was observed at concentration higher than 0.2 g l^{-1}.

Table 2 reveals the impact of $ZnSO_4$ on growth and pigment production of *Spirulina platensis* isolate. $ZnSO_4$ exhibited the detrimental effect on the isolate as 1 g L^{-1} of its concentration resulted in 0.033 growth rate, cell mass productivity was restricted up to 09.2 mg $L^{-1}$$days^{-1}$ reflecting the decrease in total chlorophyll and C-PC concentrations. It is observable that as the concentration of ZnSO4 increases cell mass production and pigment production decreases. Table 3 illustrates the influence of $CuSO_4$; it has exhibited the lethal effect. Its very initial concentration 1 g L^{-1} reduced the maximum specific growth rate to 0.025, cell mass productivity to 04.2 mg L^{-1} day^{-1} and chlorophyll and C-PC content up to 1.8 and 06.0 mg g^{-1} respectively. The degree of lethality increased optimally when $CuSO_4$ concentration reached to 2.5 g L^{-1} that *Spirulina* isolate was not able to survive.

The effect of $NiSO_4$ on the *Spirulina platensis* is demonstrated in table 4. The growth rate and cell mass productivity 0.085 day^{-1} and 30.00 mg $L^{-1}$$day^{-1}$ were maximum at minimal concentration of $NiSO_4$ 1 g L^{-1} with total chlorophyll 15.4 mg g-1 and C-PC concentration 15.3 mg g^{-1} were recorded. The differentiating effect of $NiSO_4$ was observed, at concentration of 1.5 g L^{-1} the concentration of total chlorophyll decreased significantly up to 13.4 mg g^{-1} but the alteration in C-PC concentration was negligible with 15.0 mg g^{-1}. When the concentration of $NiSO_4$ increased further the productivity of studied components was decreased. Table 5 reveals the influence of $MnSO_4$; it was observed that after the $MgSO_4$ among the selected metal ions $MnSO_4$ shared the positive effect on the *Spirulina platensis*. At 1 g L^{-1} concentration of manganese sulfate, the maximum growth rate of isolate and its cell mass productivity 0.89 day^{-1} and 32.12 mg L^{-1} day^{-1} were recorded with total chlorophyll reaching up to 17.2 mg g^{-1} and C-PC concentration 21.4 mg g^{-1}. Simultaneous depletion was recorded in growth and pigment synthesis of the isolate as the concentration of $MnSO_4$ increased. The growth rate and cell mass productivity were minimized to 0.08 day^{-1} and 0.09 mg L^{-1} day^{-1}. In similar fashion the chlorophyll and C-PC concentrations were drop down to 0.33and 0.003 mg g^{-1} respectively. Investigation made by Ahuja et al. (2001) reveals the irreversible adaptation and shorter lag phase in *Oscillatoria anguistissima* in presence of $ZnSO_4$, Ni^{2+}, Co^{2+}, Cu^{2+} and Cd^{2+} metal ions.

Table 6 illustrates the percent decrease in cell mass productivity (P 288), total chlorophyll and C-PC concentrations due to influence of metal ions. The present study confirms that at $MgSO_4$ concentrations 1.2 g L^{-1} (growth medium) the % decrease was 0.8 %, 0.38 % and 0.46 % in cmp and T ch and C-PC concentrations respectively. Control with no $MgSO_4$

also caused 48.27%, 87.78% and 50 % confirming the necessity of $MgSO_4$ for synthesis of chlorophyll and C-PC. The $ZnSO_4$ and $CuSO_4$ were found to be responsible for maximum % decrease at lowest concentration where as higher concentration of $NiSO_4$ and $MnSO_4$ exhibited detrimental effect resulting in huge % decrease in cmp, T ch and C-PC.

Table 1 Influential behavior of $MgSO_4$ on growth and C-PC production of *A. platensis*

$MgSO_4$ Conc. ($g\ L^{-1}$)	μ max (day^{-1})	*P288* ($mg\ L^{-1}\ day^{-1}$)	Total Chl. ($mg\ g^{-1}$)	C-Phycocyanin ($mg\ g^{-1}$)
1.0	0.092 ± 0.002	37.5 ± 0.017	27.0 ± 0.015	22.0 ± 0.001
1.5	0.090 ± 0.003	35.0 ± 0.013	21.3 ± 0.012	19.3 ± 0.013
2.0	0.068 ± 0.005	21.2 ± 0.005	15.0 ± 0.007	12.2 ± 0.008
2.5	0.041 ± 0.002	18.6 ± 0.008	13.1 ± 0.014	08.3 ± 0.016
3.0	0.032 ± 0.004	09.0 ± 0.014	1.5 ± 0.020	06.2 ± 0.004
1.2(Growth medium)	0.091 ± 0.004	37.2 ± 0.020	26.9 ± 0.022	21.9 ± 0.018
Control	0.045 ± 0.008	19.4 ± 0.010	3.3 ± 0.017	11.0 ± 0.015

Table 2 Influential behavior of $ZnSO_4$ on growth and C-PC production of *A. platensis*

$ZnSO_4$ Conc. ($g\ L^{-1}$)	μ max (day^{-1})	*P288* ($mg\ L^{-1}\ day^{-1}$)	Total Chl. ($mg\ g^{-1}$)	C-Phycocyanin ($mg\ g^{-1}$)
1.0	0.033 ± 0.002	09.2 ± 0.003	1.6 ± 0.034	6.3 ± 0.014
1.5	0.021 ± 0.014	04.0 ± 0.006	1.3 ± 0.008	4.2 ± 0.005
2.0	0.012 ± 0.004	02.2 ± 0.018	0.9 ± 0.008	1.9 ± 0.017
2.5	0.011 ± 0.005	02.1 ± 0.016	0.7 ± 0.003	1.9 ± 0.012
3.0	0.008 ± 0.002	01.2 ± 0.012	0.33±0.008	1.8 ± 0.013
Control	0.091 ± 0.004	37.2 ± 0.020	26.9 ± 0.022	21.9 ± 0.018

Table 3 Influential behavior of $CuSO_4$ on growth and C-PC production of *A. platensis*

$CuSO_4$ Conc. ($g\ L^{-1}$)	μ max (day^{-1})	*P288* ($mg\ L^{-1}\ day^{-1}$)	Total Chl. ($mg\ g^{-1}$)	C-Phycocyanin ($mg\ g^{-1}$)
1.0	0.025 ± 0.014	04.2 ± 0.017	1.8 ± 0.022	06.0 ± 0.009
1.5	0.018 ± 0.010	02.8 ± 0.009	1.0 ± 0.018	5.8 ± 0.032
2.0	0.006 ± 0.004	0.8 ± 0.005	0.04± 0.034	1.2 ± 0.004
2.5	0.000	00.00	00.00	00.00
3.0	0.000	00.00	00.00	00.00
Control	0.091 ± 0.004	37.2 ± 0.020	26.9 ± 0.022	21.9 ± 0.018

Table 4 Influential behavior of $NiSO_4$ on growth and C-PC production of *A.platensis*

$NiSO_4$ Conc. ($g\ L^{-1}$)	μ max (day^{-1})	*P288* ($mg\ L^{-1}\ day^{-1}$)	Total Chl. ($mg\ g^{-1}$)	C-Phycocyanin ($mg\ g^{-1}$)
1.0	0.085 ± 0.007	30.00 ± 0.024	15.4 ± 0.003	15.3 ± 0.007
1.5	0.082 ± 0.005	23.22 ± 0.007	13.4 ± 0.004	15.0 ± 0.005
2.0	0.065 ± 0.002	18.77 ± 0.003	13.0 ± 0.005	09.0 ± 0.008
2.5	0.018 ± 0.005	3.65 ± 0.002	01.3 ± 0.003	02.0 ± 0.007
3.0	0.015 ± 0.005	3.33 ± 0.003	01.2 ± 0.003	00.5 ± 0.005
Control	0.091 ± 0.004	37.2 ± 0.020	26.9 ± 0.022	21.9 ± 0.018

Table 5 Influential behavior of $MnSO_4$ on growth and C-PC production of *A. platensis*

$MnSO_4$ Conc. ($g\ L^{-1}$)	μ max (day^{-1})	*P288* ($mg\ L^{-1}\ day^{-1}$)	Total Chl. ($mg\ g^{-1}$)	C-Phycocyanin ($mg\ g^{-1}$)
1.0	0.89 ± 0.024	33.12 ± 0.021	17.2 ± 0.038	21.4 ± 0.009
1.5	0.73 ± 0.011	19.07 ± 0.002	15.2 ± 0.023	15.0 ± 0.002
2.0	0.34 ± 0.004	3.40 ± 0.002	1.2 ± 0.034	02.12 ± 0.017
2.5	0.12 ± 0.014	3.12 ± 0.002	1.1 ± 0.043	02.00 ± 0.003
3.0	0.08 ± 0.004	0.09 ± 0.004	0.33 ± 0.004	0.003 ± 0.001
Control	0.091 ± 0.004	37.2 ± 0.020	26.9 ± 0.022	21.9 ± 0.018

P288= Productivity on 12th day= 288 hrs
μmax= Maximum specific growth rate

Table 6 Percent decrease in cell mass productivity, total chlorophyll and C-Phycocyanin concentrations in presence of metal ions

Metal ions	Concentrations ($g\ L^{-1}$)	P 288 Cell Mass Productivity (% decrease)	Total Chlorophyll (% decrease)	C-Phycocyanin (% decrease)
$MgSO_4$	1.5	6.67	21.12	12.28
	2.0	43.47	44.45	44.55
	2.5	50.4	51.49	62.28
	3.0	76.0	94.45	71.82
	1.2 (GM)	**0.8**	**0.38**	**0.46**
	Control	**48.27**	**87.78**	**50.00**
$ZnSO_4$	1.0	75.27	94.06	71.24
	1.5	89.25	95.17	80.83
	2.0	94.09	96.66	91.33
	2.5	94.36	97.4	91.33
	3.0	96.78	98.78	91.79
$CuSO_4$	1.0	88.71	93.31	72.61
	1.5	92.42	96.29	73.52
	2.0	97.85	98.52	94.53
	2.5	100	100	100
	3.0	100	100	100
$NiSO_4$	1.0	19.36	42.76	30.14
	1.5	37.59	50.19	31.51
	2.0	49.55	51.68	58.91
	2.5	90.19	95.17	90.87
	3.0	91.05	95.54	97.72
$MnSO_4$	1.0	10.97	36.06	**2.29**
	1.5	48.74	43.50	31.51
	2.0	90.87	95.54	90.32
	2.5	91.62	95.92	90.87
	3.0	99.75	98.78	99.98

Conclusion

The findings of the present study confirm that metal ions have significant influence on cell mass production, total chlorophyll content and C-PC production in *S. platensis.* It also suggests the essentiality of $MgSO_4$ and toxicity of $ZnSO_4$ and $CuSO_4$ in growth and pigment synthesis of *S. platensis*.

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