ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Plant growth stimulating activity of fresh water micro algae

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Manuscript Info

Manuscript History:

Received: 17 March 2015
Final Accepted: 22 April 2015
Published Online: May 2015

Key words:

Microalgae, Cytokine, Growth promoting, *Chlorella*, *Scenedesmus* and *Chlamydomonas*

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Abstract

The objective of this study is to screen the cultivable microalgae for growth promoting activity. Four axenic microalgae (Chlorophyta) strains from three genera (*Chlorella*, *Scenedesmus* and *Chlamydomonas*) were analyzed for endogenous growth promoting activity. The microalgae strains were tested for phyto-toxicity and phyto-stimulation against cucumber seeds. All the microalgae show phyto-stimulating activity and devoid phyto-toxic activity. Cytokinin-like activity was detected using the excised cucumber cotyledon bioassay. Out of 4 strains, two showed high cytokinin-like activity and others have relatively low cytokine-like activity. Although tested algae are not subjected for auxin like activity, but *Chlamydomonas* sp. shows high root stimulation in dark condition. From all tested samples, *Chlamydomonas* sp. has both high root and shoot stimulating activity.

Introduction

Growth hormones are group of plant growth regulators (PGRs) and can be exploited in agriculture for both pre-harvest and postharvest management of leafy vegetables, fruits and cut flowers (Bhore and Sathisha, 2010). Algae produce plant growth regulators (PGRs), similar to higher plants. *Anabaena oryzae* and *Nostoc ellipsoidum* treatments was effective in stimulating the accumulation of mineral in roots and shoots of *Lycopersicon esculentum* L.(Soad, 2002). Cyanobacteria produced variable amount of auxin in the presence of different concentrations of L-Tryptophan (Sumaira and Hasnain, 2011). Algae, particularly the seaweeds, are used as fertilizers, resulting in less nitrogen and phosphorous runoff than the other from the use of livestock manure(Radheyshyam, Khokar, Jat and Khandelwal, 2012). In available literature, freeze-dried and ultrasonicated algal biomass was applied to support the development of certain orchids (Emese, 2011). Planktonic bacteria isolated from littoral and pelagial zones of lake Jeziorak in spring and summer have been carried out. In summer 62.5% of bacteria isolated, and 12.5% of bacteria isolated in spring such organisms were able to produce cytokinin-like substances (Donderski, 2000). Elena and Werner (2005) worked on growth promoting and inhibiting effects of extracellular substances of soil microalgae and cyanobacteria on *Escherichia coli* and *Micrococcus luteus*. Elaborate review is available in support and enhancement of plnat growth by phytohormones of algae (Tarakhovskaya et al., 2007). The *Scytonema hofmanni* (Cyanobacteria) extract was used for in *vitro* propagation of *Lilium alexandrae* (María et al., 2006). A comprehensive review of the effect of various seaweed species and seaweed products on plant growth and development with an emphasis on the use of this renewable bioresource in sustainable agricultural systems (Khan et al., 2009). The cytokinin like compounds like isopentenyladenine and isopentenyladenosine, cis-Zeatin andcis-zeatin riboside occurred at higher concentrations than the trans isomers, whereas trans-zeatin-O-glucoside and trans-zeatin riboside-O-glucoside were dominant over the cis isomers was detected in microalgae (Vince et al., 2004). The present study aims on successful isolation and cultivation of microalgae and their screening for plant growth promoting activity.

Material and Method

Collection of algae

Two strains of algae was obtained BIT, Mesra, Ranchi RB1 (*Chlorella* sp. CB4) and RB2 (*Chlamydomonas* sp. CRP7) and other two was collected from Jalgoan region and isolated at Department of Biotechnology, Moolji Jaitha College, Jalgaon S1 (*Chlorella sorokinian)* and S2 (*Scenedesmus obliquus*).

Cultivation of algae

The isolated algae were cultured in TAP (Tris-Acetate-Phosphate) medium and incubated at room temperature under continuous light. After 5 days the growth of algae was measured by following methods.

Growth determination of algae

By measuring chlorophyll content: 5 ml of algal culture were subjected for ultra-sonication and centrifuged at 8000 rpm, for 10 minutes at 4^0C. Supernatant were subjected for chlorophyll content at different wavelength.

The estimation of phyto-toxicity and phyto-stimulation activity

The phyto toxicity and phyto stimulating was held by means of method given by Bataeva et al., (2012) test on cucumber seeds. For the experiment on toxicity the cucumber seeds were put into the moist chambers (Petri plates with filter paper and cotton wool), in each chamber, 5 seeds, were moistened with suspension of 0.5 g of experimental biomass of microalgae communities. Seeds, processed with suspension, were couched BOD incubator during 3 days whereas; test seeds were soaked in sterile distilled water. Toxicity of microalgae was determined by following steps:

1) The number of sprouted seeds was calculated;

2) The length of root and stem in cucumber seeds was measured to determine phyto-stimulation activity of suspension; and

3) The ability of microalgae communities-base suspension to promote growth was calculated (as % of the control response).

Cucumber cotyledon bioassay

Cucumber cotyledon bioassay performed by the method described by Fletcher et al., (1992). The cucumber seeds were purchased from local market. Seeds were germinated on tissue paper saturated with autoclaved distilled water in the Petri plates. For germination, seeds were incubated at room temperature in dark for 7 days. Cotyledons were excised from cucumber seedlings (7 day old) that were grown in the dark condition. Cotyledons are pulverized in mortal and pistel in chloroform. The chloroform extract was used for determination of total chlorophyll content. A negative control with sterile distilled water alone and a synthetic cytokine 6-Benzylaminopurine (BAP) at 25ppm is used as positive control.

Extraction and quantitative estimation of chlorophyll content

Cucumber cotyledon samples along with positive and negative control were incubated under fluorescent tube light for 3.5 hours at 22^0C. After the incubation, the cotyledons were collected and grounded with 80% acetone with mortar and pestle. The chlorophyll extract was collected and then centrifuged at 4000 rpm for 10 minutes. The resultant supernatant was analyzed for total amount of chlorophyll estimation using spectrometer (Sadasivam and Manickam, 2010)

Results and Discussion

Cultivation of algae

The collected algae are cultured in TAP medium and incubated at room temperature under continuous light.

Growth estimation of algae

Figure 1 shows the growth of algae in terms of total chlorophyll content. Data was interpreted that RB2 had highest growth rate than RB1.

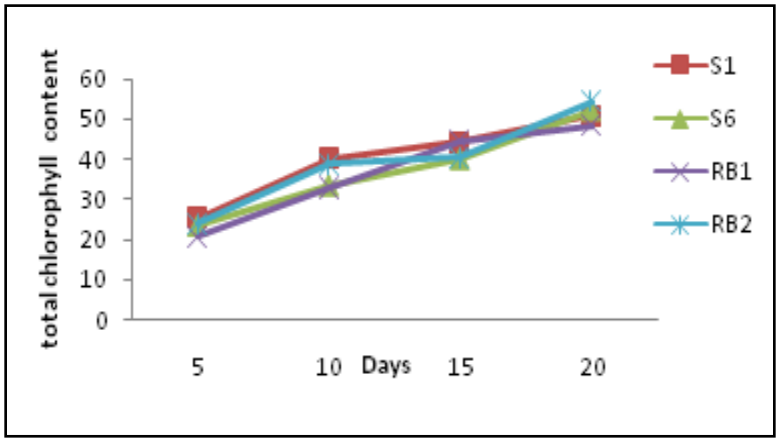

Figure 1 Growth of cultured algae in terms of chlorophyll content

The determination of phyto-toxicity and phyto-stimulation activity

The data arrived from the studying of phyto-toxicity and phyto-stimulation activity shows high growth stimulating activity. Intrenstriengly, the microalgae are free of toxicity for cucumber seeds and results are almost comparable to that of control. All microalgae displayed phytostimulation activity. The highest phytostimulation activity was exhibited by RB2 i.e, 187% and 163% in terms of root and shoot respectively. The highest root stimulating activity 208% was shown by S6 and shoot 163% shown by RB2. The phyto-stimulation activity in concerned with fibrous roots shown by RB2 (374.73%) and S6 (332.63%) in dark. Such activity decreases by 1.94% and 2.6% by RB2 and S6 respectively under light.

Table 1 Phyto-stimulation activity microalgae in the bioassay with cucumber seeds

Sample number	Length of cucumber seedling						% of the control response	
	Root			Stem			Root	Stem
	M	SD	V	M	SD	V		
Dw	0.96	0.61	0.373	4.56	1.69	2.58	100	100.00
RB1	1.58	0.60	0.367	5.12	1.26	1.59	164.5	112.2
RB2	1.8	0.38	0.145	7.44	2.03	4.14	187.5	163.15
S1	1.68	0.32	0.107	7.44	1.81	3.29	175	163.15
S6	2	0.64	0.415	4.56	1.69	2.85	208.3	100.00

Note: N= 5, M= Mean, SD= Standard deviation, V= Variance, The value of Distilled water is taken as 100%

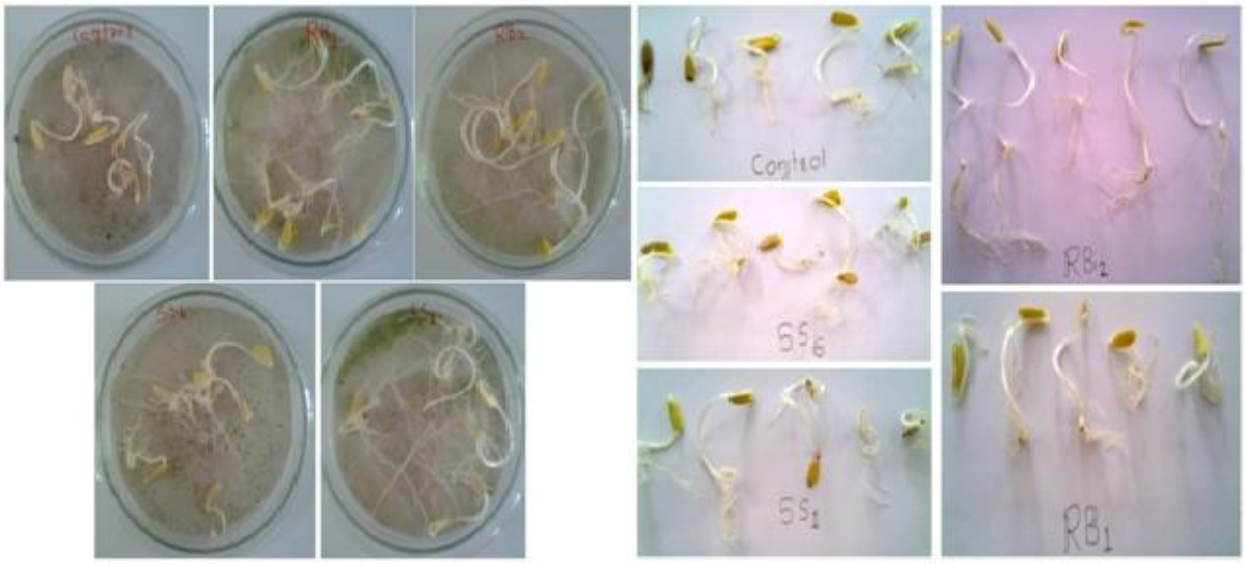

Figure 2 Phyto-stimulation activities of microalgae in the bioassay with cucumber seeds

Table 2 Number of fibrous roots in germinated seeds

Sample number	Number of fibrous roots present						% of the control response	
	Dark			Light			Dark	Light
	M	SD	V	M	SD	V		
Dw	4.75	2.06	4.25	7.5	4.20	17.66	100	100
RB1	8.8	3.96	15.7	7.6	2.07	4.3	185.26	101.3
RB2	17.8	5.11	26.2	14.4	6.80	46.3	374.73	192.0
S1	14	4.06	16.5	11.8	2.86	8.2	294.73	157.3
S6	15.8	5.26	27.7	8.4	4.03	16.3	332.63	112.0

Cucumber cotyledon bioassay (CCGB)

The crude free broth of each isolated microalgae was tested using CCGB to identify cytokinin-like compound producing isolates. All the microalgae showed positive result in CCGB. The results of CCGB are shown in Table 3. The total amount of chlorophyll content in cucumber cotyledons which were exposed to microalgae was more than negative

control. Our results are good in agreenent with the reports of Stirk et al. (2002) and Zhao et al., (1992) regard to the cytokinin and auxin like activity.

Table 3 Cucumber cotyledon bioassay of tested microalgae

Sample number	Number of sprouted seeds	% of the control response	Length of seedlings		% of the control response	
			Roots	Stem	Roots	Stem
Dw	3	100	1.8	3.4	100	100
RB1	4	133.3	2.1	3	116.6	88.23
RB2	5	166.6	3.2	6	177.7	176.47
S1	4	133.3	2.9	4.2	161.1	123.52
S6	4	133.3	3	2.4	166.6	70.58
BAP	4	133.3	2.6	2.8	144.4	82.35

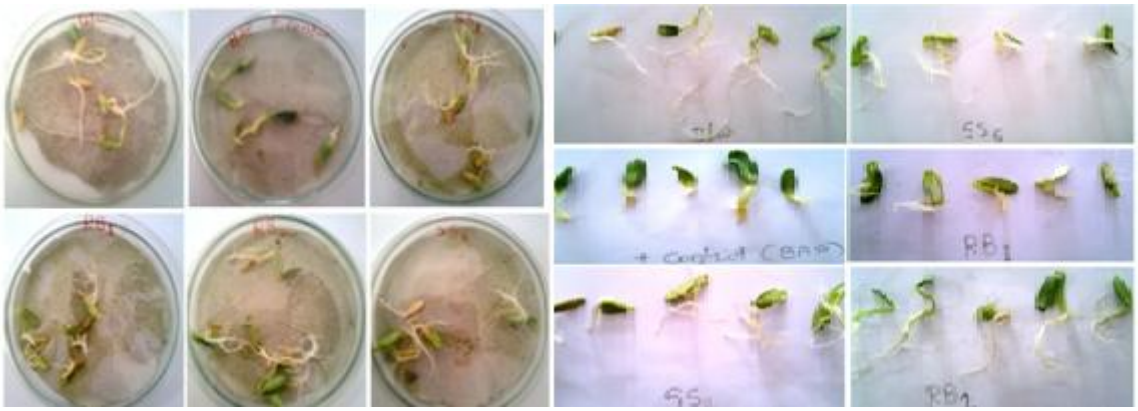

Figure 3 Cucumber cotyledon bioassay of tested microaglae

Table 4 Chlorophyll content of germinated seeds by cucumber cotyledon bioassay

Sample number	OD at 663	OD at 645	Total chlorophyll content
Dw	0.836	0.813	23.40
RB1	0.496	0.123	10.99
RB2	1.753	0.556	39.86
S1	1.216	1.468	36.33
S6	1.301	0.305	28.72
BAP	1.363	0.950	35.14

Conclusion

The present study focused on successful isolation and cultivation of microalgae and their screening for plant growth promoting activity. The microalgae are screened for cytokinin like compounds using crude cell free broth and CCGB. The algal extract of all the tested organisms shows root and shoot stimulatory property for Cucumber plants. The *Chlamydomonas* sp. shows highest plant stimulating activity than other organisms. Since, *Chlamydomonas* sp. may be one of the rich sources of cytokinin and auxin like compounds. Nonetheless, our present research finding could serve as the foundation for the further research work screening of microalgae for cytokinin and auxin like compounds.

Acknowledgement

The Authors are grateful to the Principal, Dr. Uday Kulkarni, Moolji Jaitha College, Jalgaon for providing library and laboratory facilities to carry out this work.

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