

Isolation and Growth Characterization of the Fresh Water Algae *Chlorosarcinopsis Eremi* on Different Growth Media

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<http://dx.doi.org/10.22207/JPAM.12.1.46>

(Received: 06 January 2018; accepted: 19 February 2018)

In the present study the freshwater algae *Chlorosarcinopsis eremi* was isolated from the water sample of Kovai Kutralum. The pure culture of the algae was obtained using serial dilution technique followed by streak plate method. The species was observed under light microscopy and identified with the help of algal manuals and also by sequencing 18S rRNA genes. The growth of the algae, *C. eremi* was characterized in different growth media. The different growth media's include Bold's Basal medium, Modified Bourrelly medium, BG 11 medium, Half strength Chu10 medium and Modified Hoagland's medium. The growth was measured by observed optical density of the biomass and chlorophyll "a" content. The experiment was carried out three times and mean values and standard deviation were calculated. From the results it is concluded that growth of *C. eremi* was maximum in Bold's basal medium when compared with other media tested.

Keywords: *Chlorosarcinopsis eremi*; 18SrRNA; Growth characteristic; Bold's basal medium.0

Microalgae are highly diversified and found distributed in both freshwater and marine water. They exist with diverse morphology and in a wide variety of habitats. These diversified microalgae are also rich in secondary metabolites. These Microalgal secondary metabolites are proved to have bacteriostatic, bactericidal, antifungal, antiviral and antitumor activity (Justo *et al.*, 2001). But Isolation and purification of such pharmacologically important secondary metabolites are found difficult due to their low quantity of algal biomass. Formulation of a suitable culture medium is prerequisite in achieving high biomass production. Many commercial media are available, since than is not enough to meet the requirement. Hence much research is needed in this area of biomass production. With this as aim the present study is focused on the isolation and growth

characteristic of pharmacologically important microalgae *Chlorosarcinopsis eremi* in different growth media for its better biomass production.

C. eremi was previously reported to have the presence of Alkaloids, Anthraquinones, Cardiac glycosides, Flavonoids, reducing sugars, Saponins and Terpenoids. It was also found to contain antibacterial activity against *Escherichia coli*, *Klebsiella sp*, *Pseudomonas sp*, *Salmonella*, *Staphylococcus aureus*, *Proteus sp*, *Methicillin-Resistant Staphylococcus aureus*, *Serratia sp* and *Bacillus sp* (Dhanalakshmi and Angayarkanni, 2013).

MATERIALS AND METHODS

Collection, Isolation and identification of microalgae

The water samples were collected from Kovai Kutralam, Siruvani hill ranges of Western Ghats, Coimbatore, TamilNadu, India. The collected water samples were centrifuged at

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5000rpm for 10 minutes. The pellets were subjected to serial dilution (Shivhare *et al.*, 2014) followed by streak plate method. The purity of each colony was observed under light microscopy and identified with the help of algal manuals (Prescott, 1954; Watanabe, 1983).

PCR amplification and sequencing of the 18S rRNA genes

The DNA of the algae was isolated using CTAB method. The ITS genes were amplified by polymerase chain reaction (PCR) using primers ITS2 FP: 5' AGGAGAAGTCGTAACAAGGT'3 ITS2 RP:5' TCCTCCGCTTATTGATATGC 3'. PCR product obtained was gel purified and quantitated and was sequenced. The sequence was analyzed and consensus sequence generated from forward and reverse sequences using aligner

software. This sequence was subjected to BLAST. **Estimation of algal biomass of *C. eremi* in different growth media**

The growth of *Chlorosarcinopsis sp* was measured directly by analysing the biomass of the culture. The isolated algal culture (1mL) previously grown in Bold's Basal medium was inoculated in 100 mL conical flask with 25ml of the medium (Modified Bourelly's medium, Modified Hoagland's medium, Bold's basal medium, half strength Chu 10 medium and BG-11 medium) (Guillard, 2005). The culture flasks were incubated at 25 ± 1°C under 1.2 ± 0.2 lux light intensity with 12:12 h light and dark cycle for a period of 5 days. The growth of the algal biomass was assessed by means of optical density read at 540 nm using UV-Vis spectrophotometer (Miron *et al.*, 2003).

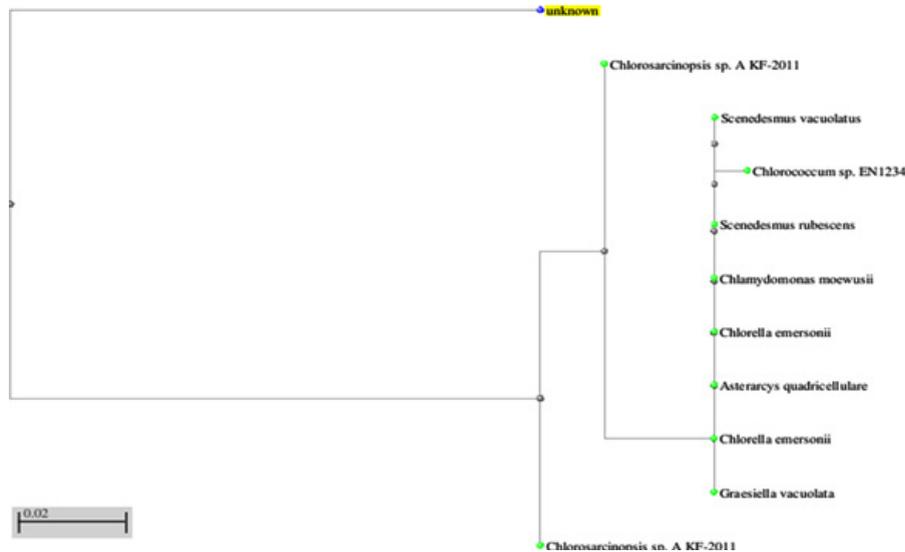


Fig. 1. Phylogenetic tree constructed for 18SrRNA gene sequence of *Chlorosarcinopsis eremi*

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1 cccgtgggta ctgcgggagg acattcaata ttttgtttgg ttgtttgggt ttttttttct
61 ggaaggggat cgccttttgt ctctgacctt ttggagtacc ttgtgccctt tgcagggcaa
121 tgagggaaat gaagggaaag agcttgagga acgcctctcc tgagacaatg gaacaaagct
181 gtacaatgga tgcctaggct cccattacga tgaaaaacat agcgaatgat gaagggagtg
241 tgcattgcaa atctttttga acactacgtc tttgaatgca gacattgqgc caggactacc
301 tggcaagttt gattgactgc tagttgtata tcgaataact aaaaggatag gaaatggaag
361 gagcttggtc ttttgaccac cccacathtt tctttgaggt tggttcgta aagagaagct
421 ttgtctttcc ttgggtctct tqtctctgat gaggagacga gagggagag gagggatgga
481 gtagtacctt ttgcaactgg cgtcagttaa gctagacgac cgggtgaact taagcatatc
541 aataaggcgg gaggaacct
//
    
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Fig. 2. 18SrRNA sequence of *Chlorosarcinopsis eremi*

Growth pattern was checked at every 24h interval. Growth rate and division times were calculated as mentioned earlier. The experiment was carried out three times and mean values and standard deviation were calculated.

Estimation of chlorophyll 'a' content of *C. eremi* in different growth media

The algal culture of 1ml was inoculated uniformly in different growth media. The inoculated culture flasks were incubated at $25 \pm 1^\circ\text{C}$ under 1.2 ± 0.2 lux light intensity with 12:12 h photoperiod of 5 days. Homogenous algal suspension (5ml) was centrifuged at $5000 \times g$ for 10 minutes. The pellets were washed twice in distilled water. After washing the pellets were resuspended in 4ml of methanol (80%) and vortexed thoroughly. The test tubes were incubated for 1 h at 60°C in water bath with occasional shaking. The tubes were then cooled and the contents were centrifuged at $5000 \times g$ for 5 minutes. The supernatant was transferred to

another tube and once again 4 ml of the solvent was added to pellet and the process was repeated. The supernatant was pooled and made up to 10mL with methanol (to compensate the solvent loss during heating). The absorbance was read at 663nm in a spectrophotometer against methanol blank (Mac kinney *et al.*, 1945).

$$\text{Chlorophyll a} = A_{663} \times 12.63 \times \text{volume of sample} / \text{Volume of methanol mg/ml}^{-1}$$

A_{663} = Absorbance at 663

12.63 = Correction factor

The experiment was carried out three times, mean values and standard deviations were calculated statistically.

RESULTS AND DISCUSSION

The organism isolated from Kovai Kutralam, Siruvani hill ranges, Western Ghats,

Table 1. Estimation of algal biomass (OD_{540}) of *Chlorosarcinopsis eremi* in different growth media

Day of observation	Bold's Basal medium (mg/mL)	Modified Bourrelly medium (mg/mL)	BG 11 medium (mg/mL)	Half strength Chu10 medium (mg/mL)	Modified Hoagland's medium (mg/mL)
1	0.029 ± 0.002	0.026 ± 0.000	0.024 ± 0.000	0.021 ± 0.002	0.021 ± 0.000
2	0.06 ± 0.001	0.03 ± 0.002	0.03 ± 0.001	0.04 ± 0.002	0.03 ± 0.001
3	0.08 ± 0.002	0.03 ± 0.000	0.03 ± 0.001	0.05 ± 0.001	0.03 ± 0.002
4	0.05 ± 0.002	0.01 ± 0.000	0.03 ± 0.002	0.05 ± 0.001	0.01 ± 0.002
5	0.05 ± 0.001	0.01 ± 0.002	0.03 ± 0.010	0.04 ± 0.001	0.01 ± 0.000
Specific growth rate	1.220588	1.1	1.151515	1.204082	1.09375

Data are given as mean \pm standard deviation of triplicate experimental culture

Table 2. Estimation of Chlorophyll 'a' content (mg/ml) of *Chlorosarcinopsis eremi* from different growth media

Day of observation	Bold's Basal medium (mg/mL)	Modified Bourrelly medium (mg/mL)	BG 11 medium (mg/mL)	Half strength Chu10 medium (mg/mL)	Modified Hoagland's medium (mg/mL)
1	1.596 ± 0.005	1.556 ± 0.005	1.576 ± 0.001	1.508 ± 0.001	1.502 ± 0.001
2	1.846 ± 0.002	1.742 ± 0.002	1.753 ± 0.002	1.792 ± 0.000	1.734 ± 0.003
3	2.301 ± 0.001	2.094 ± 0.007	2.094 ± 0.001	2.203 ± 0.001	2.020 ± 0.001
4	2.281 ± 0.000	1.002 ± 0.001	2.128 ± 0.001	2.214 ± 0.001	1.003 ± 0.001
5	1.914 ± 0.001	1.00 ± 0.00	1.506 ± 0.001	1.763 ± 0.003	1.114 ± 0.001

Data are given as mean \pm standard deviation of triplicate experimental culture

Coimbatore, TamilNadu, India was identified as *C. eremi* (Chantanachat, S and Bold, H.C), is a multicellular representative of Chlorococcales under the light microscope and with the help of algal manuals. The ITS gene sequences (559bp) were subjected to BLAST search (fig 1 and 2) and proved to have high affinity to *Chlorosarcinopsis eremi* (93%). The phylogenetic tree was constructed based on neighbour joining method. The partial nucleotide sequence has been submitted in NCBI GenBank nucleotide sequence database with the accession number KM114872.

C. eremi was grown in five different growth medium for the growth characteristic study. The growth characteristic study was carried out for a period of five days. The biomass was estimated by measuring the optical density under UV/VIS spectrophotometer at 540nm. Among the five different media tested the algal biomass was found to be maximum in bold's basal medium.

The chlorophyll 'a' content of *C. eremi* was also studied as a parameter for growth characterization. The optical density of chlorophyll 'a' content were measured at 663nm using UV/VIS spectrophotometer. The growth characteristic study was carried out in five different growth media for a period of five days. The chlorophyll 'a' content of *C. eremi* was also found to be maximum in Bold's basal medium when compared with other medium tested (Table 2). Similar results were observed previously by AL-Shatri *et al.* (2013). He reported Bold's basal medium as optimal growth media for *Scenedesmus dimorphus*. Similarly Illavarasi *et al.*, 2011, reported the growth and total chlorophyll content of *Scenedesmus sp* was found to be significant in Bold' basal medium.

CONCLUSION

From the present study it was concluded that the optimal growth medium for the culture of *Chlorosarcinopsis eremi* is Bold's Basal medium both in case of biomass and Chlorophyll 'a' content. The other four growth media tested, Modified Bourrelly medium, BG 11 medium, Half strength Chu10 medium and Modified Hoagland's medium did not support for the biomass production of the algae *C. eremi*. From the result it is also inferred that the presence of optimal concentration of NaCl,

10-fold increase of boric acid and calcium chloride in Bold's basal medium may improved the growth of the algae.

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