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BIOMASS PRODUCTION AND NITROGEN FIXATION IN STIGONEMATALES

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Stigonematales are highly evolved group of Cyanobacteria by having heterotrichous habit and other morphological complexities. Present communication deals with the biomass- production (in terms of chlorophyll-a) and nitrogen fixation of ten selected strains of Stigonematales. It was found that *Chlorogloeopsis*-354, *Hapalosiphon*-53, *Hapalosiphon*-384 and *Westiellopsis*-29 were better biomass producing strains and *Hapalosiphon*-53 was best nitrogen fixing strain. Interesting observation was noticed that biomass production was found better in nitrogen enriched medium than in nitrogen deficient medium. *Hapalosiphon*-53 was found to be best biomass producing and fast growing strain in enriched medium and nitrogen deficient medium after 20th day growth intervals.

Key words: Stigonematales, Biomass production, Nitrogen fixation

Nitrogen fixation has been found accelerated by blue-green algae (Watanbe *et al.* 1978, Anonymous 1978). Blue green algae are well known diazotrophs and are frequently used as biofertilizer in rice fields. The strains which are being currently used as biofertilizer in rice cultivation mostly belong to the order Nostocales and the genera are *Nostoc*, *Anabaena*, *Scytonema*, *Tolypothric*, *Calothrix* and *Plectonema*. Comparatively less emphasis has been laid on the members of Stigonematales which are also frequently present in rice fields.

The survey of literature showed a lot of variations have been observed amongst blue-green algal strains in their biomass production and nitrogen fixation (Kolte and Goyal 1986, Roy Chaudhary *et al*. 1986).Limited literature is available on the proper characterization of Stigonematales (Muster *et al.* 1983, Singh and Kumar 1992, Singh *et al.* 1994, Suseela and Goyal 1995). Contribution of blue-green algal strains to the ricefields depends upon their growth behavior (biomass production) and nitrogen fixation. In the present study ten strains of Stigonematales were isolated from various habitats mainly from rice fields of Uttar Pradesh which were distinct from their morphological features. The present paper deals with the evaluation of the biomass production and nitrogen fixation of these selected isolate in different concentration of nitrogen.

MATERIALS AND METHODS

Growth behavior and nitrogen estimation has been estimated in ten selected strains of Stigonematales that includes *Chlorogloeopsis* (1 strain), *Hapalosiphon* (4 strains), *Westiellopsis* (4 Strains) and *Nostochopsis* (1 strain). Which were available in phycology laboratory, Department of Botany, University of Allahabad. These strains were maintained in our germplasm collection of Cyanobacteria under controlled laboratory conditions. In the present study growth behavior (Biomass) and nitrogen fixation of these strains studied with their isolation in unialgal and axenic culture. It was grown in BG-11 medium (Stanier *et al.* 1971 liquid nitrogen enriched medium (+ N medium) in the form of $NaNo_3$ and nitrogen deficient medium (- N medium) without combined nitrogen. Growth behavior (biomass) was estimated in terms of the assessment of chlorophyll-a (by Mackiney 1941) and total nitrogen by (Allen1957). For installation of the experiment exponentially growing cultures were inoculated in equal quantities in triplicates, from which mean of triplicate was considered,

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Table 1 : Biomass production (in terms of chlorophyll-a µg/ml) and Total nitogen of different strains in nitrogen deficient and nitrogen enriched medium

Strains	Chlorophyll-a (µg/ml)		Total nitrogen (µg/ml)	
	20th day		20th day	
	Nitrogen deficient medium (-N Medium)	Nitrogen enriched medium (+N Medium)	Nitrogen deficient medium (-N Medium)	Nitrogen enriched medium (+N Medium)
Chlorogloeopsis-354	2.252	3.002	4.167	27.25
Nostochopsi-368	0.320	0.653	2.39	28.39
Hapalosiphon-53	4.226	5.694	11.21	35.17
Hapalosiphon-196	0.320	1.154	4.602	30.20
Hapalosiphon-350	1.599	2.419	5.87	26.29
Hapalosiphon-384	2.349	4.935	7.79	29.76
Westiellopsis-29	2.572	3.183	9.022	23.15
Westiellopsis-61	1.348	1.007	5.45	35.09
Westiellopsis-370	1.515	1.863	6.9 4	35.16
Westiellopsis-392	2.196	3.000	9.64	17.59

After incubation into culture tube they were incubated in culture chamber under controlled laboratory condition (14: 10 h, L: D 28 ± 2^{0}C at 3000 lux light intensity provided by fluorescent tube).

RESULT AND DISCUSSION

In the present study out of ten selected strains of Stigonematales *Hapalosiphon*-53 was found best biomass produced by having best chlorophyll-a (4.226 µg/ml) production in nitrogen deficient medium and (5.694 µg/ml) in nitrogen enriched medium at 20th day growth intervals. However, best chlorophyll-a production (4.395 µg/ml) was also found in *Hapalosiphon*-384 in nitrogen enriched medium. *Nostochopsis*-386 was found least biomass producer in both nitrogen enriched and nitrogen deficient medium. By having least chlorophyll-a production (0.320 µg/ml) in nitrogen deficient and (o.653 µg/ml) in nitrogen enriched medium. Whereas *Hapalosiphon*-350 (1.599 µg/ml and 2.419 µg/ml) and *Westiellopsis*-61(1.348 µg/ml and 1.807 µg/ml) showed poor performance in chlorophyll-a production.

On the point of nitrogen fixation *Hapalosiphon*-53 showed best performance in nitrogen fixation(11.21 µg/ml) in nitrogen deficient medium and (35.17 µg/ml) in nitrogen enriched medium while *Nostochopsis*-368 showed minimum nitrogen fixation. *Nostochopsis*-368 showed poor performance of nitrogen (2.39 µg/ml) in nitrogen deficient medium. Whereas *Westiellopsis*-61 showed maximum nitrogen fixation while *Westiellopsis*-392 showed minimum nitrogen fixation.

It was found interesting when ratio of production of a particular alga was calculated , strain which were showing poor biomass production were found better nitrogen fixer as compared to chlorophyll-a synthesis than the better biomass producing strains. Most of the species of *Hapalosiphon* showed better biomass

production than *Westiellopsis* in presence of nitrogen i.e. nitrogen enriched medium. These results are comparable with that of Kaushik (1987) who calculated that *Hapalosiphon* fontinals is better suited to a variety of nitrogen sources as compared to *Westiellopsis* prolific in term of chlorophyll-a synthesis. He also pointed out that in general nitrogen and urea stimulated (11-13%) pigment synthesis.

The additive effect of alga in presence of high level of chemical nitrogen fertilizers suggested, but does not poor that growth promoting substances produced by thee alga may have a role (Aiyer *et al.* 1972, Venkataraman 1979, 1981, Rodgers and Reynand 1979), direct proof can come only from suitable experiments to partition the effects of nitrogen and these substances (Venkataraman1993). Most of the previous studies were based on determination of growth nitrogenase activity and total nitrogen fixation in nitrogen deficient medium. The most interesting feature of the present experiment was the determination and comparison of nitrogen fixation (Total nitrogen) in nitrogen enriched medium with that of nitrogen deficient medium along with growth behavior of Stigonematales. Fogg (1974) also suggested that (suppression of heterocysts formation by ammonium salt is complete but by nitrate may be only partial, however found appreciable nitrogen fixation in Lake Windermere England, even when nitrate was its high winter level). Our results are compared in each alga in both the concentration of nitrogen (i.e. nitrogen enriched and nitrogen deficient medium) it was noticed that all the strains showed best performance in nitrogen enriched medium. When ratio of total nitrogen and chlorophyll-a production was compared in both the concentration it is noticed that *Hapalosiphon*-196 *Hapalosiphon*-350, *Hapalosiphon*-384 shows better nitrogen fixation as compared to chlorophyll-a synthesis. In this comparison also nitrogen deficient medium showed poor nitrogen synthesis compare to nitrogen deficient medium. It is suggested from the increase of total nitrogen in nitrogen enriched medium that initially the biomass production by algae in nitrogen enriched medium is more due to the presence of nitrogen in the medium, Heterocysts production and nitrogen was poor in earlier day but due to the assimilation of nitrogen present in medium by alga, depletion of nitrogen in medium take place. So the presence of more biomass creates more nitrogen fixation. Most of the previous studied were based on determination of growth and total nitrogen in nitrogen deficient medium along with growth behavior of Stigonematales. Earlier it was assumed that Stigonemalean forms do not grow as fast as the other forms of Cyanobacteria like *Aulosira*, *Nostoc*, *Anabaena* etc, but the present study revealed that these forms also shows better biomass and nitrogen fixation like other forms which are used as biofertrtilizer in Paddy fields. This may be attracted the attention of Cyanobacteriologists to utilized these forms at efficient nitrogen fixing organisms and they may also be used as biofertilizer in rice cultivation.

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