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Assessment of Genetic Variation and Optimizing Growth Conditions for High Lipid Content in Cyanobacteria

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ABSTRACT

Cyanobacteria or blue green algae occupy a unique position since they possess an autotrophic mode of growth like eukaryotic plant cells and a metabolic system as that of bacteria. Cyanobacterial strains were collected from different location and isolated by batch culture, spread and streak plate methods.The isolated cyanobacteria were identified as *Oscillatoria, Phormidium*and *Spirulina sp.*Cyanobacteria were grown in BG-11 media and then lipid contentwas analyzed at different intervals. Maximum lipid content was observed on 24th day (0.31 mg / ml) in *Oscillatoria sp*. In case of *Phormidium sp,*lipid content increasedup to 28th day (0.44 mg / ml) and later declined. The highest lipid content recorded on 20th day, 0.14 mg / ml in *Spirulina sp.* beyond twenty days, the lipid content decreased. Better biomass production was observed in case of *Spirulina sp.*(48mg / 100 ml) in BG-11 medium.

CYANOBACTERIA are a major group of bacteria that occur throughout the world.They are also known as blue-green algae. They store reserve food materials which can be used as the source of pigments, lipids, vitamins, proteins and certain secondary metabolites (Cardozo *et al*., 2007). They are widely used in food industries and in few biotechnological applications.

Limitations of phenotypic characters have highlighted the requirement for more reliable methods and promoted molecular approaches in cyanobacterial taxonomy, including DNA base composition (Kaneko *et al*., 2001) and PCR fingerprinting (Rasmussen and Svenning, 1998).

In the present study cyanobacterial strainwere isolated from various, water and soil sample from different locations.The isolated cyanobacteria were then screen for high lipid content and the growth requirementsare optimized.The details of collection location and source ispresented in Table I.

The collected samples were serially dilutedand inoculated in BG-11 mediumand later incubatedunder light assembly for two weeksin 3000 lux light and incubation at 28±2^{o}C temperature. The positive plates werepurified tillobtaining the monocultures.Standard spread and streak plating method were carried out to purify the culture.

Morphological study was carried out by observing the culture in light microscopeunder 40Xmagnification. Micrometry was used to measure thecell shape and size and documented as microphotograph.

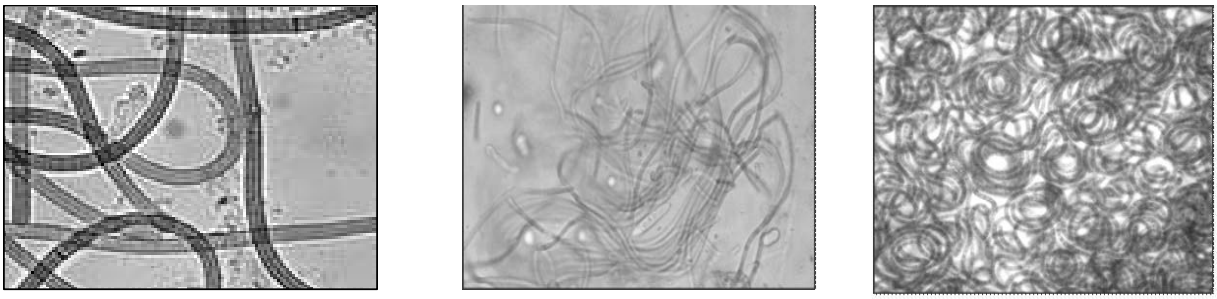
The identified species*Oscillatoria sp, Phormidium sp and Spirulina sp.*were cultured in five different growth media (ASM, Fogg's, BG-11, Modified BG-11 and MN medium), and grown for two weeks as batch cultures in the laboratory. The appearance of bluish green color was observed as an indication of growth on the 12th day. The selected culture media were inoculated and incubated. The biomass was harvested on 12th day by filtration and placed in the oven at 60°C for 12 hr to estimate the dry weight and lipid content. Further harvesting was done at four day intervals up to 36 days to determine the lipid contentby the sulpho-phosphovanillin method (Barnes and Blackstock, 1973).

Isolated DNA samples from all the strains (Smoker and Barnum, 1998) were subjected to RAPD analysis with eight random primers. Agarose gel electrophoresis was performed to resolve the amplified products. The bands were manually scored '1' for the presence and '0' for the absence and the binary data were used for statistical analysis. The scored band data (Presence or absence) was subjected to cluster analysis-using STATISTICA.

TABLE I

Samples collected from different locations

Collection sites	Habitat	Coding name
Thirthahalli Western Ghats	Water sample from Small pond soil sample from open field	TWG
Addheri Western Ghats	Water sample from stored tank soil sample from open field	AWG
Western ghats deep forest hosanagar	Water sample from open deep forest pond and soil sample from open field	HWG
Western Ghats Rippen pet	Water sample from open well and soil sample from open field	RWG
Biotech Rice Field	Water sample from Standing waterand soil sample from open field	BRF
Mavallipura near BBMP wastage	Water sample from well Mavallipura pond Water sample from pond	MWW MWP
Marketing Department pond, GKVK	Water sample from pond	MDG
Botanical garden pond, GKVK	Water sample from pond	BGG
Hebbal, Bengaluru	Water sample from lake	HLB
Allalsandra, Bengaluru	Water sample from lake	ALB



Oscillatoria sp *Phormidium* sp *Spirulina* sp

Fig.1: Cyanobacteria Samples in Microscopic View

Results reveal thatisolated algae from different sources were identified as *Oscillatoria sp, Phormidium sp, Spirulina sp.*based on cell morphology and colonial characteristics (Desikachary, 1959) and are documented as microphotographs (Fig.1) and Taxonomic characters recorded are, *Oscillatoria sp.*: Thallus blue-green, unbranched filamentous,Cells are broad and long. *Phormidium sp.*: Thallus blue-green, thin, trichome straight and densely entangled. Cells longer than broad. *Spirulina sp.*: unbranched filaments, walls regularly spirally coil.

Confirmatory test of different media shows that, growth of *Oscillatoria* was restricted to three culture media, and that of*Phormidium*to four growth media. The rest of the four media were favorable to the growth of *Spirulina*. The growth of three species in three selected growth media showed that *Oscillatoria*

produced a biomass of 45 mg in BG-ll on the 12th day, *Phormidium*produced 42 mg, and *Spirulina*had a yield of 48 mg, which is quite higher than the growth in other media (Table II). Hence BG-ll was selected as the growth medium for the isolated three species.

Estimated lipid content of *Oscillatoria sp.* showed an increasing tendency up to 24th day and then it was decreasing up to 36th day. Maximum lipid was obtained on 24th day (0.31 mg/ml). In case of *Phormidium sp.*lipid content showed increased tendency up to 28th day (0.44 mg/ml) and later declined. The highest lipid content was recorded on 20th day (0.14 mg/ml) in *Spirulina sp.* beyond twenty days, the lipid content decreased (Fig.2). Lipid content of

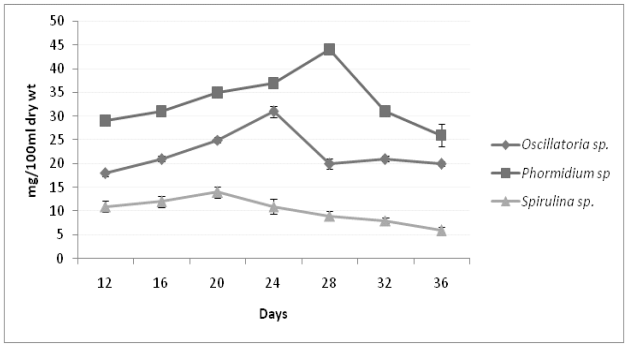


Fig. 2: Lipid concentration of Oscillatoria, Phormidium and Spirulina sp. at different time intervals

three Cyanobacterial species from l2th day wet and dry biomass is given in the Table III.

From the obtained RAPD analysis dendrogram was constructed by Ward's method of clustering using minimum variance algorithm (Fig.3). The dissimilarity matrix was developed using Squared Euclidean Distance (SED), which estimated all the pair wise differences in the amplification product. The band sizes were determined by comparing with the 100 bp DNA ladder.

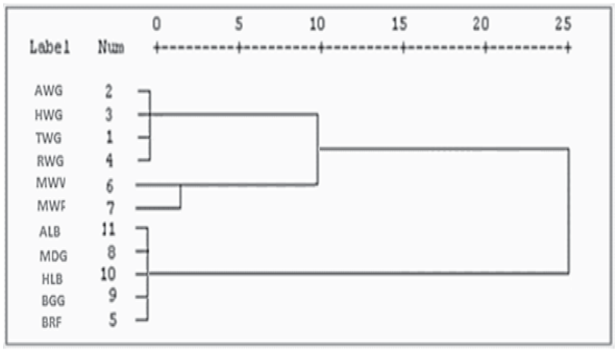


Fig. 3: Dendrogram based on RAPD primer profile obtained from 11 different samples

The number of bands scored for each primer varied from 1 to 10 with an average of 9.3 bands per

TABLE II

Yield of Oscillatoria, Phormidium and Spirulina sp. after 12 days of inoculation. The data represents the means± Standard error of three replications for each species and medium

Culture medium	(mg Dry weight / 100ml) ± SE		
	Oscillatoria sp	*Phormidium sp*	*Spirulina sp*
Fogg's	24 ± 1.53	32 ± 0.58	27 ± 0.58
Modified BG-11	10 ± 1.00	38 ± 0.58	39 ± 1.00
BG-11	45 ± 1.00	42 ± 1.53	48 ± 0.58

TABLE III

Lipid content of three species of cyanobacteria from 12th day wet and dry** biomass*

Parameters	*Oscillatoria sp.*		*Phormidium sp.*		*Spirulina sp.*	
	Wet biomass	Dry biomass	Wet biomass	Dry biomass	Wet biomass	Dry biomass
Lipid(mg / ml)	0.18	0.17	0.33	0.29	0.14	0.10

*Wet biomass= immediately harvested product **Dry biomass= sample dried in oven at 60°C for 12hr

primer. Out of 22 different sizes of amplification bands, 6 bands (28.19 %) were monomorphic and 18 bands (81.81%) were shared polymorphic, which were informative in revealing the relationship among the strains. The Cluster analysis based on 22 RAPD bands revealed that the eleven cyanobacteria isolates were examined. Dendrogram clearly depicted that all the isolates formed two major clusters. Isolates HLB, ALB, BRF, BGG and MDG formed the first sub cluster Iwhich contain *Phormidium sp*. and the isolates MWW and MWP formed the first sub clusters II contain *Spirulina sp.*and isolates TWG, AWG, HWG and RWG formed in second cluster has *Oscillatoria sp*. Linkage distance was almost equal between two clusters.

Three species of filamentous Cyanobacteria were identified and developed into pure cultures in BG-11 medium.They were *Oscillatoria sp, Phormidium sp and Spirulina sp*. They showed better biomass production in BG-11 medium compared to other four media.

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