2. Materials and Methods

The present work entitled Isolation, Molecular identification and Hydrocarbon analysis of Cyanobacteria and Green algae in Paddy fields of Rasipuram area, Namakkal Dt, Tamil Nadu carried out in the Department of microbiology, Muthayammal College of arts and Science, Rasipuram from February, 2007 to June, 2012.

2.1 Growth condition

A time course study was carried out on the growth of isolates. The experiment was carried out in the 250ml Erlenmeyer flask containing each 50ml BG11 medium (Cyanobacteria) (Rippka et al., 1979) and 50 ml AF6 medium (Green algae) (Sheehan et al., 1998) for a period of 4 weeks. The culture flasks were inoculated (0.05 initial OD value) and incubated at 25 ± 2°C with cool white fluorescent tubes emitting 2.5 Klux light for 18 hrs a day for Cyanobacteria (Gomathi et al., 2011) and under 1.2±0.2 Klux light with 16:8 hrs light dark cycle for Green algae. All the experiments were carried out in triplicates (Dayananda et al., 2010).

2.2 Measurement of growth rate

The growth rate of isolate was measured by optical density at 680nm for 4 weeks at 7 days intervals (Chang and Yang, 2003).

2.3 Effect of pH on growth of algae

The effect of pH on growth of the isolates such as Green algae in AF6 medium and Cyanobacteria in BG11 medium was studied using different pH viz., of 5.0, 6.0, 7.0, 8.0 and 9.0. The pH of the medium was optimized before autoclaving. All the flasks were inoculated uniformly at (0.05 OD value) inoculums of 2weeks old culture. The flasks were incubated for 4weeks and growth was calculated (Dayananda et al., 2007).

2.4 Effect of Temperature on growth of algae

Cyanobacteria and green algae were grown at different temperatures (23°C, 28°C and 33°C) and incubated for four weeks under 16:8h light and dark cycle (Vitova et al., 2011; Jodlowska and Latala, 2013).
2.5 Effect of light on growth of algae

Isolates were grown at different incident light intensities viz., 1.5, 2.0 and 2.5 Klux to study the effect of light on growth of algae (Vitova et al., 2011; Jodlowska and Latala, 2013). The intensity of light was measured using quantum meter with a cosine collector. In the culture unit, dimmable fluorescent tubes were used to adjust irradiance. The cultures were incubated under a 16:8h light and dark cycle for four weeks.

2.6 Effect of Carbon dioxide on growth of algae

The Effect of Carbon dioxide on growth of the isolates such as Cyanobacteria in BG11 medium (Rippka et al., 1979) and Green algae in AF6 medium (Sheehan et al., 1998) were studied using various concentration of sodium bicarbonate such as 0.5, 1.0, 1.5 and 2% respectively as per method described by Tripathi et al., (2001). The mixture of carbonate (3M) and bicarbonate (3M) solutions were added (100ml) to the culture flasks and the culture flasks were incubated for four weeks under 16:8hrs light and dark cycle with 1.2±0.2 Klux light intensity at 25±2ºC. The experiments were carried out in triplicates. The growth was analysed.

2.7 Standardization of media for isolated algal strains

Collected isolates were grown autotrophically in two different media such as BG11 medium (Rippka et al., 1979) and AF6 medium (Sheehan et al., 1998). Further they were inoculated uniformly at 0.05 (OD value) inoculums and were incubated at 25±1ºC under 1.2± 0.2 Klux light intensity with 16:8hrs light and dark cycle. All experiments carried out were replicated thrice. Cultures were incubated for a period of four weeks, then harvested and analysed for growth. (Bhuvanesh et al., 2010).

2.8.1 Estimation of Chlorophyll

A known volume of culture was centrifuged 8000 rpm for 10 min and the pellet was treated with known volume of methanol and kept in water bath for 30 min at 60ºC. Absorbance of the pooled extracts was measured at 652 and 665nm and chlorophyll (a+b) was estimated using Lichtenthaler equations (Lichtenthaler, 1987).
Chlorophyll a = \frac{(13.36A_{665} - 5.19A_{652})\times8.1}{DW} \text{ (mg g}^{-1}\text{dw)}

Chlorophyll b = \frac{(27.43A_{652} - 8.12A_{665})\times8.1}{DW} \text{ (mg g}^{-1}\text{dw)}

Chlorophyll (a+b) = \frac{(5.24A_{665} + 22.24A_{652})\times8.1}{DW} \text{ (mg g}^{-1}\text{dw)}

Where,

$A_{652} =$ Absorbance at 652 nm

$A_{665} =$ Absorbance at 665 nm

$DW =$ dry weight of the sample (mg)

2.8.2 Estimation of Carotenoids

A known quantity of algal dry biomass was homogenized and extracted repeatedly with acetone. The pooled extracts absorbance was read at 470nm and total carotenoids contents were quantified according to Lichtenthaler equations (Lichtenthaler, 1987).

Total Carotenoids (Cx+c) = \frac{(4.785 A_{470} + 3.657A_{665} - 12.76A_{652})\times8.1}{DW} \text{ (mg g}^{-1}\text{dw)}

Where

$A_{470} =$ Absorbance at 470 nm

$DW =$ dry weight of the sample (mg)

2.8.3 Estimation of Phycobilin pigments

The phycobilin pigments, C-phycocyanin, C-phycoerythrin and allophycocyanin were estimated as per the method described by Bennet and Bogoard, (1973). Ten ml of homogenized suspension was pelleted, washed with 2-3ml of 0.5M phosphate buffer (pH 6.8) and subjected to alternate freezing and
thawing. The pigments were extracted by centrifugation. The procedure was repeated until the supernatant became colourless. The supernatant was made up to 100ml with 0.05M phosphate buffer and absorbance was measured at 562, 615 and 652nm in UV spectrophotometer against 0.05M phosphate buffer as a blank. The Phycobilin pigments were calculated and expressed as mg g⁻¹ dry weight of the cultures.

\[
\text{C- Phycocyanin} = \frac{A_{615}-0.474(A_{652})}{5.34} \quad \text{(mg g}^{-1}\text{dw)}
\]

\[
\text{Allophycocyanin(APC)} = \frac{A_{652}-0.208(A_{615})}{5.09} \quad \text{(mg g}^{-1}\text{dw)}
\]

\[
\text{C- Phycoerythrin(PE)} = \frac{A_{662}-2.41(\text{PC})-0.849(\text{APC})}{9.62} \quad \text{(mg g}^{-1}\text{dw)}
\]

Where,

\( A = \) Absorbance at specific wavelength

2.9 Statistical analysis

Results obtained were subjected to statistical analysis using Statistical package for social sciences (SPSS) version 17.5. Comparison of proportion was done for different pH with Paired Samples Test (t-Test), different temperature with Friedman Repeated Measures Analysis of Variance on Ranks and All Pair wise Multiple Comparison Procedures (for Cyanobacteria - Turkey Test), Green algae with One Way Repeated Measures Analysis of Variance and All Pair wise Multiple Comparison Procedures (for green algae Holm-Sidak method) and for different Carbon dioxide concentration with Paired Samples Test (t-Test) while P value less than 0.5 were considered significant.