The cyanobacteria are gram negative prokaryotes showing oxygenic photosynthesis. These organisms have the potential to produce light harvesting pigments namely chlorophyll-a, carotenoids and phycobiliproteins. Chlorophylls function as pivotal pigments with carotenoids and phycobilins as accessory pigments. Chlorophylls are key compounds for trapping light energy for photosynthesis, thus, their quantitative determination is of great importance in studies of photosynthesis and biomass production (Hertzberg et al. 1971). Accessory pigments confer extended ability to harvest light for photosynthesis and in some cases protection from UV and other light induced cell damage. The association of carotenoids with chlorophylls prevents the formation of highly reactive singlet oxygen radicals that would otherwise cause irreversible damage to lipids, proteins and other molecules (Bartley and Scolnick 1995). A wide variety of carotenoids are found in these including α, β-carotene, echinenone, zeaxanthin, myxoxanthophyll and oscillaxanthin (Clayton and King 1990). Phycobilins are water-soluble pigments located on the periphery of the thylakoid membrane (Humm and Wick 1980).

**MATERIALS AND METHODS**

Cyanobacterial cultures namely *Oscillatoria, Lyngbya, Anabaena* and *Microchaete* were isolated from paddy fields of Uttar Pradesh were used in the study. Increase in light intensity enhanced pigment production in general; however the effect of varying temperature and CO₂ concentration was variable for pigment production. Production of pigments under optimised conditions in selected cyanobacterial genera may be used in biotechnological applications.

**Key words:** Cyanobacteria, pigments, enhancements, cultural manipulations.

Four cyanobacterial genera namely *Oscillatoria, Lyngbya, Anabaena* and *Microchaete* isolated from paddy fields of Rohilkhand region of India following standard enrichment culture technique. Their unialgal population was raised in nitrogen free for heterocystous and nitrogen supplemented for non-heterocystous genera in BG-11 medium (Stanier et al. 1995) in a culture room at 52-55µmole photon/m²/s light intensity with 16/8 light and dark period and 28± 2°C temperature.

Cyanobacterial cultures were incubated under varying temperatures (25, 30 and 35°C), light intensity (4, 5, 6 kLux), and CO₂ incubation (350, 550 and 750ppm) separately in different growth chambers of National Phytotron Facilities of IARI, New Delhi. In addition, the effect of ZnSO₄ (0.5 mg/L) and glutamine (1mM) either alone or in combination was also studied on the pigment production.

Known volume of exponentially growing cultures from different treatments was centrifuged and pellet was used for the determination of photosynthetic pigments.
namely chlorophyll, carotenoids and phycobiliproteins (Phycocyanin, allophycocyanin and phycoerythrin). Cultures were grown in conical flasks with initial volume of 100 mL capacity in triplicates under varying cultures conditions. Growth was measured in terms of dry weight and chlorophyll was extracted in methanol and absorbance was read at 650nm and 665nm for estimation of chlorophyll (McKinney et al. 1941). Carotenoids were extracted in acetone and analysed by taking absorbance at 450nm (Jensen et al. 1978). Phycobilins were extracted in phosphate buffer and quantified from the absorbance read at 562, 615 and 652nm (Bennet and Bogard 1973).

RESULTS AND DISCUSSION

Table 1: Effect of glutamine (1 mM) and ZnSO\(_4\) 0.5mg/L on pigments (µg/g dry weight) in selected cyanobacterial genera isolated from paddy fields of Uttar Pradesh (Each value is mean±SD)

<table>
<thead>
<tr>
<th>Strain</th>
<th>Control</th>
<th>Glutamine</th>
<th>ZnSO(_4)</th>
<th>Glutamine+ ZnSO(_4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oscillatoria</td>
<td>506±20.05</td>
<td>560.2±26.50</td>
<td>181.6±57.71</td>
<td>1500.0±51.72</td>
</tr>
<tr>
<td>Lyngbya</td>
<td>379.6±23.56</td>
<td>314.6±17.19</td>
<td>192.6±36.55</td>
<td>99.39±34.59</td>
</tr>
<tr>
<td>Anabaena</td>
<td>455.1±22.92</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Microchaete</td>
<td>142.6±2.07</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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<tr>
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<th>ZnSO(_4)</th>
<th>Glutamine+ ZnSO(_4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oscillatoria</td>
<td>58±3.32</td>
<td>38±3.73</td>
<td>19±2.11</td>
<td>35±3.67</td>
</tr>
<tr>
<td>Lyngbya</td>
<td>41±2.09</td>
<td>37±2.58</td>
<td>17±1.84</td>
<td>13±1.11</td>
</tr>
<tr>
<td>Anabaena</td>
<td>1544±50.56</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Microchaete</td>
<td>998±33.67</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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<th>ZnSO(_4)</th>
<th>Glutamine+ ZnSO(_4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oscillatoria</td>
<td>1420±23.6</td>
<td>2064±65.71</td>
<td>767±29.56</td>
<td>7625±330.7</td>
</tr>
</tbody>
</table>

Chlorophyll content of Oscillatoria and Microchaete enhanced with varying temperatures of 25°C, 30°C and 35°C. In Lyngbya and Anabaena, the increase in temperature from 25°C to 30°C enhanced chlorophyll content followed by a reduction at 35°C. Carotenoids enhanced with increase in
temperature in *Lyngbya*, *Anabaena* and *Microchaete*, however, 30°C was inhibitory to the carotenoids in *Oscillatoria* as compared to 25°C. A preparation of *Spirulina* chlorophyll in a mixture containing iron oxide and higher alcohol was patented as a strong deodorant (Yamaguchi 1981). Carotenoids have commercial applications as food colouring and feed additives to enhance flesh colour of salmonoid fish, as well as the colour of the egg yolk. These also improve the health and fertility of cattle (Borowitzka 1988). However increase in temperature of 35°C showed an enhancement in the level of carotenoids. The effect of varying temperatures on phycocyanins was variable. The highest phycocyanin content was recorded at 30°C in *Oscillatoria, Lyngbya* and *Microchaete*. On the other hand 25°C was optimum for enhanced production of phycocyanin in *Anabaena*. Allophycocyanin showed a reduction with 25°C. A preparation of Spirulina chlorophyll in enhanced temperature in *Oscillatoria* and a mixture containing iron oxide and higher variable response in other three cyanobacteria. Alcohol was patented as a strong deodorant (Yamaguchi 1981). Carotenoids have content was also variable in four commercial applications as food colouring and cyanobacterial genera. All naturally occurring feed additives to enhance flesh colour of salmonoid fish, as well as the colour of the egg yolk. These also improve the health and fertility of cattle (Borowitzka 1988). However increase in temperature of 35°C showed an enhancement in the level of carotenoids. The effect of varying temperatures on phycocyanins was variable. The highest phycocyanin content was recorded at 30°C in *Oscillatoria*, *Lyngbya* and *Microchaete*. On the other hand 25°C was optimum for enhanced production of phycocyanin in *Anabaena*. Allophycocyanin showed a reduction with 25°C. A preparation of Spirulina chlorophyll in enhanced temperature in *Oscillatoria* and a mixture containing iron oxide and higher variable response in other three cyanobacteria. Alcohol was patented as a strong deodorant (Yamaguchi 1981). Carotenoids have content was also variable in four commercial applications as food colouring and cyanobacterial genera. All naturally occurring feed additives to enhance flesh colour of salmonoid fish, as well as the colour of the egg yolk. These also improve the health and fertility of cattle (Borowitzka 1988). However increase in temperature of 35°C showed an enhancement in the level of carotenoids. The effect of varying temperatures on phycocyanins was variable. The highest phycocyanin content was recorded at 30°C in

<table>
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</tr>
</thead>
<tbody>
<tr>
<td><em>Oscillatoria</em></td>
<td>1058±35.67</td>
<td>1684±40.89</td>
<td>402±15.91</td>
<td>3441±108.6</td>
</tr>
<tr>
<td><em>Lyngbya</em></td>
<td>939±33.89</td>
<td>1612±36.09</td>
<td>762±28.33</td>
<td>372±13.75</td>
</tr>
<tr>
<td><em>Anabaena</em></td>
<td>999.7±16.74</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Microchaete</em></td>
<td>1009±21.73</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
</thead>
<tbody>
<tr>
<td><em>Oscillatoria</em></td>
<td>422±21.34</td>
<td>944±45.23</td>
<td>393±23.42</td>
<td>6214±209.8</td>
</tr>
<tr>
<td><em>Lyngbya</em></td>
<td>88±4.98</td>
<td>4041±100.9</td>
<td>2164±55.82</td>
<td>944±45.73</td>
</tr>
<tr>
<td><em>Anabaena</em></td>
<td>4309±119.7</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Microchaete</em></td>
<td>1562±89.91</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
</thead>
<tbody>
<tr>
<td><em>Oscillatoria</em></td>
<td>2901</td>
<td>4693</td>
<td>1563</td>
<td>17281</td>
</tr>
<tr>
<td><em>Lyngbya</em></td>
<td>2314</td>
<td>10609</td>
<td>5562</td>
<td>2561</td>
</tr>
<tr>
<td><em>Anabaena</em></td>
<td>2385</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Microchaete</em></td>
<td>3437</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
till 6 decreased chlorophyll content significantly in *Oscillatoria*, *Lyngbya* and *Anabaena* while in *Microchaete* the light intensity of 5 is inhibitory. Carotenoids also showed a significant reduction with increase in light intensity. The effect of varying light intensities on phycobilins was variable. Total phycobilins exhibited a varied response towards increase in light intensity as well as temperature. The effect of varied levels of CO$_2$ depicted variable pattern in terms of chlorophyll, carotenoids and phycobilins. Incubation of *Oscillatoria* at 550 ppm CO$_2$ level enhanced chlorophyll content as compared to the incubation at 350 ppm. The chlorophyll content reduced significantly at 750 ppm of CO$_2$ incubation in *Oscillatoria*. In *Lyngbya* enhanced CO$_2$ levels increased chlorophyll content and in *Anabaena* and *Microchaete* 750 ppm was optimum. Incubation of cyanobacterial cultures at 750 ppm enhanced carotenoids as compared to 350 ppm. On the other hand incubation at 550 ppm was inhibitory. In *Lyngbya* 550 ppm CO$_2$ appeared to be optimum. Enhanced levels of CO$_2$ exhibited an inhibitory influence on phycocyanin in all four genera and allophycocyanin in *Oscillatoria* and *Lyngbya*. 350 ppm CO$_2$ was optimum for allophycocyanin production in *Anabaena* and *Microchaete*. The effect of varying levels of CO$_2$ on phycocerythrin content was variable with the highest production recorded at 350 ppm CO$_2$ in all the four genera examined. Increased in CO$_2$ concentration decreased total phycobilins in *Oscillatoria*, *Lyngbya* and *Anabaena*. There was a significant reduction in total phycobilins at 550 ppm CO$_2$ as compared to 350 ppm CO$_2$ followed by an increase at 750 ppm of CO$_2$ incubation in *Microchaete*. The ranking of strains of PC, APC and PE content exhibits their potential utilisation as colouring agents, phycoflour probes or as additives in a range of cosmetics and pharmaceutical products (MacColl and Guard-Frair 1987). Studies conducted on the role of glutamine, ZnSO$_4$ either taken alone or in combination showed that *Anabaena* and *Microchaete* did not grow in glutamine and ZnSO$_4$ supplemented medium. On the other hand, the chlorophyll enhanced significantly with glutamine and ZnSO$_4$ as compared to control in *Oscillatoria* and *Lyngbya*. When glutamine and ZnSO$_4$ were taken together, there was a marked enhancement in chlorophyll content of *Oscillatoria*. In *Lyngbya*, glutamine supplemented medium showed highest chlorophyll followed by ZnSO$_4$ supplemented medium and glutamine together with ZnSO$_4$ supplemented medium. Chlorophyll was significantly lower under control grown cultures in standard BG-11 medium. Maximum carotenoids were recorded in control grown cultures of *Oscillatoria* and *Lyngbya*. Supplementation of medium with glutamine and ZnSO$_4$ either alone or in combination showed an inhibitory effect on the levels of carotenoids in these two genera. Glutamine supplementation either alone or in combination with ZnSO$_4$ enhanced phycocyanin in *Oscillatoria* in comparison to control. ZnSO$_4$ had an inhibitory effect on phycocyanin of *Oscillatoria*. On the other hand glutamine and ZnSO$_4$ supplementation in the growing medium separately showed an enhanced production of phycocyanin in *Lyngbya* while these two together influenced phycocyanin content as compared to control in *Lyngbya*. Allophycocyanin was reduced with ZnSO$_4$ in growing medium as compared to control in *Oscillatoria*, however, this together with glutamine enhanced allophycocyanin content. Glutamine alone also exhibited positive influence on allophycocyanin content in
Figure 1: Comparative chlorophyll and carotenoids (µg/g dry weight) at different temperatures (25°C, 30°C, and 35°C), light intensities (4KLux, 5KLux and 6KLux) and CO₂ concentrations (350ppm, 550ppm and 750ppm) in selected cyanobacterial genera isolated from paddy fields of Uttar Pradesh (Each value is mean±SD); 1-Oscillatoria, 2-Lyngbya, 3-Anabaena and 4-Microchaete.
Figure 2: Comparative phycocyanin and allophycocyanin (µg/g dry weight) at different temperatures (25°C, 30°C, and 35°C), light intensities (4KLux, 5KLux and 6KLux) and CO₂ concentrations (350ppm, 550ppm and 750ppm) in selected cyanobacterial genera isolated from paddy fields of Uttar Pradesh (Each value is mean±SD); 1- Oscillatoria, 2- Lyngbya, 3- Anabaena and 4- Microchaete.
Figure 3. Comparative phycoerythrin and total phycobilins (µg/g dry weight) at different temperatures (25°C, 30°C, and 35°C), light intensities (4KLux, 5KLux and 6KLux) and CO₂ concentrations (350ppm, 550ppm and 750ppm) in selected cyanobacterial genera isolated from paddy fields of Uttar Pradesh (Each value is mean±SD); 1- Oscillatoria, 2- Lyngbya, 3- Anabaena and 4- Microchaete.
comparison to control grown cultures. In *Lyngbya* the highest allophycocyanin was recorded in glutamine supplemented medium. Inclusion of ZnSO$_4$ either alone or with glutamine decreased the allophycocyanin in *Lyngbya* as compared to control. Phycoerythrin enhanced with glutamine supplementation alone in *Oscillatoria* and *Lyngbya* in comparison to control grown cultures. ZnSO$_4$ alone depicted an inhibitory effect in *Oscillatoria*. Glutamine and ZnSO$_4$ taken together enhanced phycoerythrin content significantly in *Lyngbya* as compared to control. Highest phycoerythrin was recorded in glutamine supplemented medium followed by ZnSO$_4$ supplemented medium. In general glutamine and ZnSO$_4$ together enhanced total phycobilins as compared to control in *Oscillatoria* whereas in *Lyngbya* significant enhancement in total phycobilins was observed with glutamine or with ZnSO$_4$ compared to control. These together did not influence total phycobilins in two genera. The amount of high levels of phycocyanin and carotenoids under specific optimised condition can have a biotechnological potential in the utilization of natural colours. These pigments represent 20 percent of the total dry weight in cyanobacterial genera (Borowitzka 1988, Walsh 1998). This indicates the possibility of using Zn and glutamine for scaling up the production of phycobilins in this culture as these pigments are increasingly being used in diagnostic and biomedical research including fluorescence immune assays (Romay *et al.* 2003). Studies have also indicated that phycobilins of cyanobacteria have antioxidant, anti-inflammatory and neuroprotective properties (Kumar *et al.* 2003 and Romay *et al.* 2003). Some strains of *Anabaena* and *Nostoc* are rich in phycoerythrin (Guerrero *et al.* 1990) and these can be used as pigments or colourants in food industry as well as for cosmetics.

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