STUDIES ON ENVIRONMENTAL STRESS ON FATTY ACIDS AND β-CAROTENE OF SELECTED CYANOBACTERIAL STRAINS

THESIS
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JAMIA MILLIA ISLAMIA
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BY:
SABEENA SULTAN

DEPARTMENT OF BIOSCIENCES
JAMIA MILLIA ISLAMIA
NEW DELHI - 110 025
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SUMMARY

Cyanobacteria are the most primitive, oxygenic photosynthetic organisms possessing a number of microbial characters that influence fast growth rate, variable metabolism which responds rapidly to the environmental changes. Microalgae (including cyanobacteria) also play a very significant role in the food chain as producers of essential fatty acids like $\gamma$-linolenic acid. Micro algae are the essential components of the diet of humans and animals and are also becoming important feed additives in aquaculture, crustaceans and sea fish. Since zooplankton, fish and other higher fauna are incapable of denovo synthesis of these, they must obtain from the diet of phytoplankton or benthic algae.

The entire work for the thesis entitled “Studies on environmental stress on fatty acid and $\beta$-carotene of selected cyanobacterial strains” is being presented in seven chapters as follows:

Chapter 1: Contains the introduction showing objectives as well as significance of the work.

Chapter 2: Deals with a brief literature review on lipids, fatty acids and $\beta$-carotene especially with reference to cyanobacteria.
Chapter 3: Includes materials and methods which were employed during present investigation.

Chapter 4: Pertains to the results and discussion which is divided into four parts as follows:

A. Screening of the cyanobacterial strain(s) for lipids and fatty acids, specially polyunsaturated fatty acid (PUFA).

B. Studies on the effects of various environmental stress conditions on the lipid and fatty acid profile of the selected cyanobacterial strains (Hapalosiphon hibernicus ARM 488 and Nostoc calcicola ARM 261).

C. Screening of cyanobacterial strains for high yielding of β-carotene.

D. Studies on the effects of various environmental stress conditions on the β-carotene content of the selected cyanobacterial strain (Phormidium fav Nikolov ARM 695).

Chapter 5: Presents the conclusion of the above study.

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Above chapters are followed by bibliography, tables, figures and publications.
Screening of 21 cyanobacterial strains was studied for lipids, fatty acids and \( \beta \)-carotene production. Strains were procured from the culture collection maintained at National Facility for Blue Green Algae. The cultures were illuminated by cool day light fluorescent tubes providing a light intensity of 1800 lux with 16:8 hour’s light:dark regime.

For biochemical analysis, 10\textsuperscript{th} day old biomass was harvested by centrifugation and filtration (Spirulina platensis and Phormidium faecolarum ARM 695) through fine polyester cloth and dried. It was found that the levels of total lipids, fatty acids as well as \( \beta \)-carotene varied with the species and growth conditions. The results of the present study can be summarised as follows:

1. The gas chromatographic analysis of the methyl esters of the studied cyanobacterial fatty acids was carried out for saturated, monounsaturated and polyunsaturated fatty acids.

a. Among the saturated fatty acids caprylic acid (8:0) was present in abundance in (59.97\%) followed by palmitic acid (16:0, 42.28\%), stearic acid (18:0, 29.28\%), myristic acid (14:0, 15.01\%), lauric acid (12:0, 9.75\%), capric acid (10:0, 6.21\%) and aracidic acid (20:0, 5.97\%).
b. In comparison to saturated fatty acids, monounsaturated fatty acids (MUFA) were relatively present in lower amounts. Surprisingly, eicosenoic acid (21:1) was present in higher amounts (53.73%) which is not reported in any cyanobacteria till date. Palmitoleic acid (16:1) ranged from 0.19% to 42.30%. Plectonema boryanum ARM 350 had the highest content of palmitoleic acid (42.30%). Oleic acid (18:1) was the least represented MUFA and was highest in Anabaena variabilis ARM 41 (26.17%).

c. In all the strains polyunsaturated fatty acids (PUFA) was remarkably less in comparison to saturated and unsaturated fatty acids. Hapalosiphon hibernicus ARM 488 had the highest amount of linoleic acid (18:2, 11.68%). Linoleic acid was often present alone as PUFA. Linolenic acid (18:3) was mostly present along with linoleic acid (18:2) and ranged from 0.30% to 13.02%. Spirulina platensis had the highest amount (13.02%) followed by Nostoc calcicola ARM 261.

2. Hapalosiphon hibernicus ARM 488 with the highest content of linoleic acid (11.68%) and Nostoc calcicola ARM 261 with the second highest content of linolenic acid (7.96%), were further
studied under various environmental stress conditions (pH, temperature, salt, nitrogen source and heavy metals) for obtaining maximum production of these nutritionally important polyunsaturated fatty acids. *Spirulina platensis* with highest content of linolenic acid (13.02%) was not selected for further studies, since it has already been studied extensively.

3. Estimation of β-carotene content of 21 cyanobacterial strains had shown a wide range. The highest β-carotene content was found for *Phormidium faveolarum* ARM 695 (643.615 µg/g dry wt.) followed by *Spirulina platensis* (204.33 µg/g dry wt.). The lowest was obtained for *Aulosira* ARM 719 (61.12 µg/g dry wt.).

4. *P. faveolarum* was selected as the best strain with maximum β-carotene content and further studied in detail under various environmental stress conditions (pH, salinity, nitrogen source, temperature and heavy metals) in order to find out best possible condition for maximum β-carotene productivity.

5. The optimal conditions obtained for *Hapalosiphon hibernicus* ARM 488 and *Nostoc calcicola* ARM 261, for high production
of their PUFA and Phormidium faveolarum ARM 695 for high production of β-carotene are as follows:

<table>
<thead>
<tr>
<th>Stress</th>
<th>PUFA</th>
<th>β-carotene</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. hibernicus ARM 488 (18.2)</td>
<td>N. calcicola ARM 261 (18.3)</td>
<td>P. faveolarum ARM 695</td>
</tr>
<tr>
<td>Nitrogen source</td>
<td>1.5 g/l NaNO3</td>
<td>0.075 g/l Urea</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.15 M</td>
<td>0.15 M</td>
</tr>
<tr>
<td>pH</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Temperature</td>
<td>15°C</td>
<td>15°C</td>
</tr>
<tr>
<td>Heavy metal*</td>
<td>5 ppm (Cr)</td>
<td>--</td>
</tr>
</tbody>
</table>

* Because of heavy metal bioaccumulation and biomagnification problem it is advisable to avoid the use of heavy metals.