Pigment Producing capacity of saline tolerant microalgae Chaetoceros calictrans, Chlorella salina, Isochrysis galbana, Tetraselmis gracilis and its antimicrobial activity: An Comparative Study


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ABSTRACT

Carotenoids are pigments which are gaining importance because of its various applications in food, cosmetics industries and medical importance. In recent years research in micro algae is given importance for the Production of pigments because of its economic feasibility to grow and high yield of pigments in stressed conditions. Four marine micro algae (Chaetoceros calictrans, Chlorella salina, Isochrysis galbana, Tetraselmis gracilis) were used in this study for quantification of total carotenoids. Walnes medium is used for the culturing of the algae. Among the four species the algae Isochrysis galbana showed high amount of total carotenoids 1.62 µg/ml and Chlorella salina showed low amount 0.584 µg/ml of total carotenoids. Spectrophotometry is used to quantify the pigments. The antimicrobial activity of four algae against Aspergillus niger, Escherichia coli, Klebsiella sp., Proteus vulgaris and Pseudomonas aeruginosa were carried out by agar diffusion method. Isochrysis galbana showed the maximum activity and Chlorella salina showed minimum activity and no activity in Chaetoceros calictrans.

Key words: Total Carotenoids, Chaetoceros calictrans, Chlorella salina, Isochrysis galbana, Tetraselmis gracilis, Antimicrobial assay

INTRODUCTION

The biodiversity of microalgae is enormous and they represent an almost untapped resource. It has been estimated that about 200,000- 800,000 species exist of which about 35,000 species are described. Over 15,000 novel compounds originating from algal biomass have been chemically determined [1]. (Cardozo). Most of the microalgae species produce unique products like carotenoids, antioxidants, fatty acids, enzymes, polymers, peptides, toxins and sterols. In Research in microalgae is given much importance for the production of numerous biological products. Carotenoids are natural pigments derived from five-carbon isoprene units that are polymerized enzymatically to form regular highly conjugated 40-carbon structures (with up to 15 conjugated double bonds). One or both ends of the carbon skeleton may undergo cyclization to form ring β-ionone end groups, which additionally may be substituted by oxo, hydroxy or epoxy groups at different positions to form the different xanthophylls [2].(Solomons and Bulux, 1994). At least 600 different carotenoids exercising important biological functions in bacteria, algae,
plants and animals have been identified to date [3], (Polivka and Sundström, 2004). Animals lack the ability to synthesize carotenoids endogenously and thus obtain these compounds through their diet. Carotenoids are essential constituents of the photosynthetic apparatus, primarily in the reaction centers of photosystems where they act: (i) as accessory pigments for light-harvesting processes during photosynthesis, (ii) as structural stabilizers for protein assembly in photosystems, and (iii) as inhibitors of both photo and free radical oxidation provoked by excess light exposure[4]. (Zhang et al., 1999). For human nutritional purposes, some carotenoids offer provitamin A activity [5]. (Mayne, 1996). Provitamin A carotenoids are generally converted to retinal via catalysis by the intestinal enzyme β-carotene 15,15'-monooxygenase [6]. (Lindqvist and Andersson, 2002). In the 1990s, vitamin A deficiency has caused approximately 1.2 million deaths per year in children aged 1–4 years worldwide [7]. (Humphrey et al., 1992). Carotenoids directly provide photoprotection against UV light photooxidation in the skin [8]. (Sies and Stahl, 2004; [9]. Aust et al., 2005). The ketocarotenoid astaxanthin is believed to play a key role in the prevention of several human pathological processes, such as skin UV-mediated photooxidation, inflammation, prostate and mammary carcinogenesis, ulcers due to Helicobacter pylori infection and age-related diseases [10]. (Bennedsen et al., 1999; [11]. Guerrin et al., 2003). Among the benefits of carotenoids to eye health, the occurrence of age-related macular degeneration (AMD) is strongly associated with lower levels of both zeaxanthin and lutein (xanthophylls) in the macula, while prospective epidemiological data showed a 19% lower risk of cataract in men taking high levels of both of these xanthophylls [12]. (Meyer and Sekundo, 2005). Zeaxanthin and lutein are the major carotenoids that accumulate in the macula of human retina and inhibit photooxidative damage to the retina [13]. (Neelam et al., 2005). Many of the positive medical and nutritional trials have speculated that the antioxidant activity of carotenoids could be the key factor in reducing the incidence of many diseases, especially those mediated by light [14]. (Cantrell et al., 2003; [15]. Astley et al., 2004). Although there is a considerable epidemiological evidence linking high dietary intake of carotenoids to a decrease risk of certain cancers such as lycopene against prostate cancer [16]. (Ben-Dor et al., 2005). The present work was carried out to study the pigment producing capacity from marine micro algae (Chaetoceros calcitrans, Chlorella salina, Isochrysis galbana and Tetraselmis gracilis), to determine its growth curve, Carotenoid producing ability and to study the antimicrobial activity.

**MATERIALS AND METHODS**

**Collection of algae cultures:**
The algae cultures (Chaetoceros calcitrans, Chlorella salina, Isochrysis galbana and Tetraselmis gracilis,) were procured from CMFRI, Tutucorin, Tamilnadu, India.

**Culture maintenance:**
The stock culture was maintained by using Walne’s culture media. The stocks were maintained at temperature of 24 ± 1ºC in a thermostatically controlled room illuminated with cool white fluorescent tubes (Philips 40 W) providing an irradiance of 50 µE/m2/s in a 12h: 12h light/dark regime.

**Mass cultivation of algae:**
Four cultures were cultured separately in four 5 litres Hoffkin’s flask. The Walne’s media was used for cultivation. When an appropriate cell density was reached, the cells were harvested from the culture.

**Growth Curve:**
The growth pattern of Chaetoceros calcitrans, Chlorella salina, Isochrysis galbana and Tetraselmis gracilis were studied by measuring optical density once in two days for 30 days at their λ max.

**Extraction of Pigments:**
The different pigments namely chlorophyll a, chlorophyll b, and total carotenoid of the sample were extracted by using methods of [17]. Dere et al. (1998) and [18]. Yoshii et al. (2004). 0.5 g of dry weight of algal cells were homogenized manually using pestle and mortar and the pigments were extracted in 100% acetone and stored in the dark at -20ºc for 18 hrs. The pellet was discarded and the supernatant was separated which contains the pigments.

**Spectrophotometric analysis of Pigments:**
For Chlorophyll a, Chlorophyll b and total Carotenoids calculation the supernatants were read at the absorbances of 470, 645 and 662 nm respectively by using Hitachi U 2900 spectrophotometer. The pigments were calculated by the following formula: 

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Ca = 11.75×A662 - 2.350×A645
Cb = 18.61×A645 - 3.960×A662
CX+C = (1000×A470 - 2.270×Ca-81.4×Cb) ÷227

Ca = Chlorophyll a
Cb = Chlorophyll b
CX+C = Total carotenoids
A = Absorbance

Antimicrobial Assay:

Cell Harvest:
Algal extract was prepared with three solvents namely Butanol, Distilled water and Isopropyl alcohol. Then the cells were harvested by centrifugation. The supernatant was discarded and the pellets were washed with normal saline and then again centrifuged.

Extract preparation:
About 0.5 gm of pellet was taken in three tubes and 2ml of three solvents were poured in each test tube to get the extract. The cells in each solvent are sonicated using ultrasonicater for 10 min.

Test Organisms:
The strains of Aspergillus niger, Escherichia coli, Klebsiella sp., Proteus vulgaris and Pseudomonas aeruginosa.

Antimicrobial testing:
Antimicrobial activity was evaluated using the agar diffusion technique in Petri dishes. Micro organisms were spread on Muller Hinton agar plates with the help of cotton sticks. Sterile discs are loaded with the extract and placed on the plates. After incubation for overnight at 30°C, a clear around a disc was the evidence of antimicrobial activity. Diameters of the zones of inhibition were measured in millimeter.

RESULTS

Cultivation of Algae:
The pure cultures are grown in the conical flasks which shown in Fig: 1 and are mass cultivated in 5 liter conical flasks which is shown in Fig: 2.

Fig: 1 Culture maintenance in the laboratory
Fig: 2 Mass culturing in the Laboratory

Fig: 3 Growth Curve

<table>
<thead>
<tr>
<th></th>
<th>Chaetoceros calictrans</th>
<th>Chlorella salina</th>
</tr>
</thead>
<tbody>
<tr>
<td>OD at 470 nm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of Days</td>
<td>2 6 10 14 18 22 26 30</td>
<td>2 4 6 8 10 12 14 16 18 20 22 24 26 28 30</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Isochrysis galbana</th>
<th>Tetraselmis gracilis</th>
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<tr>
<td>OD at 470 nm</td>
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</tr>
</tbody>
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Growth Curve:
The growth of each alga was studied by measuring their absorbance values at their respective $\lambda_{\text{max}}$ values for 30 days and the readings were taken once for two days. The exponential phase of algal cells was observed for first 13 days. (Fig: 3)

Extraction of Pigments and Quantification:
The chlorophyll a, chlorophyll b and total carotene were extracted by using 100% Acetone shown in Fig: 4 and the read Spectrophotometrically at 470 nm, 645 nm and 662 nm respectively calculated using the above mentioned formula and the final values were shown in Table: 1. *Tetraselmis gracilis* showed highest chlorophyll a content (5.17), chlorophyll b (10.71) and *Isochrysis galbana* showed highest total carotene (1.62).

![Fig: 4 Pigment Extracts from four algae](image)

**Table: 1 Total quantity of Pigments**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Algae</th>
<th>Chlorophyll a (µg/ml)</th>
<th>Chlorophyll b (µg/ml)</th>
<th>Total Carotene (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Chaetoceros calcitrans</td>
<td>3.33</td>
<td>5.64</td>
<td>0.89</td>
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<tr>
<td>2.</td>
<td>Chlorella salina</td>
<td>2.58</td>
<td>3.58</td>
<td>0.58</td>
</tr>
<tr>
<td>3.</td>
<td>Isochrysis galbana</td>
<td>4.81</td>
<td>6.39</td>
<td>1.62</td>
</tr>
<tr>
<td>4.</td>
<td>Tetraselmis gracilis</td>
<td>5.17</td>
<td>10.71</td>
<td>1.04</td>
</tr>
</tbody>
</table>

Antibacterial assay:
The antibacterial assay showed highest zone of inhibition in algae *Isochrysis galbana* against the pathogens *Klebsilla sp, proteus vulgaris* which was 15 mm, 16 mm respectively in Butanol extract (Fig: 5). Four algae showed activity against the pathogens in the extracts from Butanol, distilled water and isopropyl alcohol which is shown in Fig: 6.
DISCUSSION

Carotenoids are pigments which are commercially important which is used in industries like Cosmetics, Food as natural colourant, Medical etc. The algae living in the Marine environment were able to withstand salinity which indicates the algae are potent strains which can be exploited. We used four marine algae Chaetoceros calicetans, Chlorella salina, Isochrysis galbana, Tetraselmis gracilis for the amount of pigments present in algae especially carotenoids. The growth curve was also studied for the above mentioned four algae. The antibacterial activities against the pathogens Aspergillus niger, Escherichia coli, Klebsiella sp., Proteus vulgaris and Pseudomonas aeruginosa were studied. Isochrysis galbana was found to produce high amount of total carotene than the other three marine algae and it also showed good antibacterial activity against the pathogens Klebsiella Sp., Proteus vulgaris in Butanol extract. This shows that this algae is a potent algae which can be exploited further for the carotenoids production by inducing external stress like increasing the salinity, light intensity, Nitrogen depletion etc. The contamination with the other algae is a problem which can be achieved by optimizing the media and culture conditions.
CONCLUSION

The algae Chaetoceros calcitrans, Chlorella salina, Isochrysis galbana and Tetraselmis gracilis were cultured in the laboratory. Mass production of algae was done by using walne’s media. The solvent extraction using acetone showed high amount of chlorophyll a (5.17 µg/ml), chlorophyll b (10.71 µg/ml) from Tetraselmis gracilis and high total carotene (1.62 µg/ml) from Isochrysis galbana and low amount of total carotene (0.584 µg/ml) quantified from Chlorella salina. The antibacterial assay showed high activity against the pathogens Klebsiella Sp., Proteus vulgaris in Butanol extract from I.galbana, C.salina showed low activity against the Klebsiella Sp., Pseudomonas aeruginosa and no antimicrobial activity in C.calcitrans.

REFERENCES