Induction of bioactive compound composition from marine microalgae (*Lyngbya* sp.) by using different stress condition

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**PEER REVIEW**

**ABSTRACT**

**Objective:** To the effect of salinity stress on the production of microalgae (*Lyngbya* sp.) and chlorophyll pigments in the growth medium.

**Methods:** Stress was investigated by using green algae strains *Lyngbya* sp. in response to change bioactive compounds without any modification of cell growth and biomass production rate. The different stress conditions like 10%–40% were analyzed.

**Results:** During the stress condition, various biochemical and microbiological assays were monitored. The photochemical composition was evaluated by GC–MS studies. The studies expressed that 30% higher salinity stress was suitable for high phytochemical production rate including chlorophyll content.

**Conclusions:** Our study indicates the wide range of salinity stress to enhance the growth on microalgae culture and enhance the production of major secondary metabolites.

**KEYWORDS**

*Lyngbya* sp., Salinity stress, Secondary metabolites, Biomass, Chlorophyll

**1. Introduction**

Recently microalgae used for the production of health oriented pharmaceuticals, nutritional has been widely studied. Apart from that, phytoplankton is mainly involved in biofuel production, oil extraction and ethanol production[1]. Marine algae possesses different classes of biogenic compounds, and its principal group of secondary metabolites inhibit the growth of bacteria, fungi and viruses[2]. As a response to salinity stress, green algae usually undergoes metabolic acclimatization which always results in fluctuations of the cellular composition of macromolecules. Micronutrients factors like nitrogen limitation frequently results in reduced protein content and relatively enhanced carbohydrate or lipid storage[3]. Therefore, the biochemical structure of green algae is linked with the growth rate, and reflects the physiological potential of the primary productivity.

Salinity stress is the main limiting factor in plant productivity in aquatic, natural and trophically modified environment. This is the reason why the identification of physiological responses to the interactive effects of high salt concentration is an important requirement for the selection of tolerant and highly productive plant ecotypes under varying environmental conditions[4]. Salt stress can induce enhancement of antioxidant and
antiviral efficiency of *Spirulina platensis* because salt stress conditions can cause a raise in production of biologically active compounds and an alteration of algal metabolism\(^5\). *Lyngbya* sp. marine cyanobacterium is from the family Oscillatoriaceae. It has been shown to be a diverse source of bioactive compounds, some of which possess antiviral, antifungal and antimicrobial properties\(^6\).

The study was mainly focused on the effect of salinity stress on the improvement of the secondary metabolite production from marine microalgae. Different concentrations of salinity stress was effectively synthesized in various clinically related secondary metabolites from marine algae.

2. Materials and methods

2.1. Collection of marine microalgae

The marine microalgae samples were collected from the Kuantan east coast region, in Pahang state of Peninsular Malaysia between latitude of 3°55’31” N and longitude of 103°22’23” E. The study area is rich in biodiversity source with a high degree of variation of life forms within an ecosystem. There was no previous record reported about the marine microalgae in this particular study area. This was the first report for identification of microalgae from the Kuntan coastal region, Malaysia.

2.2. Identification and mass cultivation

The identified microalgae was subjected to further mass cultivation (Figure 1). The stock culture of microalgae was maintained in conical flasks containing 250 mL of BG-11 medium (direct culture medium) and assigned randomly in an automatic oscillating shaker for two weeks at room temperature in normal fluorescent light. All operations were conducted in the biohazard safety cabinet to prevent contamination.

![Figure 1. Collected marine microalgae.](image)

A: Confocal microscopical structure of *Lyngbya* sp. B: Marine microalgae mass culture in different salinity concentration (10%–40%).

2.3. Salinity stress condition

The BG-11 culture medium with different salinity concentration (10%, 20%, 30% and 40%) were prepared. Different ranges of salinities were obtained by dissolving correct amount of sodium chloride (NaCl) with appropriate amounts of distilled water\(^7\).

2.4. Biomass measurement

The microalgae biomass was measured by counting the number of cells in test samples using haemocytometer. The concentration of cell volume was determined with a Genesys 10S UV-Vis spectrophotometer by measuring culture turbidity at A\(_{687}\) nm\(^8\).

2.5. Estimation of chlorophyll content

The growth of algal can be determined by observing the expression of pigments in a period of time. The extraction of pigments from the algal cell can be done by using acetone extraction. The absorption (A) reading of the pigment extract at particular wavelength against a solvent blank in a spectrophotometer is defined as chlorophyll concentration\(^9\).

2.6. Bioactive compounds analysis using gas chromatography and mass spectrometry

Bioactive compounds were analysed in gas chromatography system using Agilent 7890A model equipped with a DB-1 column (30.00 m×0.25 mm ID, 0.25 µm film thickness, Agilent 122–0132) and a mass spectrometer detector with Triple–Axis Detection, using helium as a carrier gas at 1.0 mL/min.

3. Results

Figure 2 shows growth rates of microalgae with different salinity conditions. The salinity stress had a significant effect on growth rates of microalgae. Similarly the growth rates are decreased in accordance with the decrease of the concentrations of salinities. Optimum growth rate was observed at 30% salinities in the study. It was observed that chlorophyll, a content of microalgae, was fluctuated in all the tested concentrations of salt stress (10% to 20% and 40% salinity) (Figure 3). The chlorophyll yields gradually increased to 30% salinity and the optimum yields were achieved.

The GC–MS results show the compound yields by *Lyngbya* sp. (Table 1). The clinically important compound are yield by marine microalgae strains in different salinity concentration. The GC–MS spectrum of microalgae culture reveals characteristic of the capability of cells to accumulate chemical compounds in saline environment. The least compound accumulates by microalgae was in 10% salinity concentration, where undecane was observed. This followed by 20%, 30% and 40% salinity concentration. In microalgae 10% and 20% salinity concentration were observed. Microalgae in 30% salinity concentration is recognized to accumulate the highest number of compounds with 22 compounds in order to tolerate with the environment (Figure 4).
Figure 2. Microalgae *Lyngbya* sp. biomass concentration in different salinity stress conditions.

Table 1

Higher percentage of compounds accumulated from *Lyngbya* sp. in different salinity concentration.

<table>
<thead>
<tr>
<th>Salt concentration</th>
<th>Retention time (min)</th>
<th>Name of compound</th>
<th>Group of compound</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10%</td>
<td>28.351</td>
<td>Hexadecanoic acid, methyl ester</td>
<td>Fatty acid</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>24.791</td>
<td>1,5-Diphenyl-2H-1,2,4-triazole-3-thione</td>
<td>Ketone</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>27.49</td>
<td>2-(4-(Dimethylamino)-1-naphthyl) naphthoquinone</td>
<td>Hydrocarbon</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>30.349</td>
<td>Acetamide, 2-chloro-</td>
<td>Acetic acid</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>59.663</td>
<td>5H-Thiazolo [5,2-alpyrimidin-3-one,6,7-dihydro-</td>
<td>Hydrocarbon</td>
<td>18</td>
</tr>
<tr>
<td>20%</td>
<td>33.017</td>
<td>Methyl stearate</td>
<td>Fatty acid</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>5.797</td>
<td>Undecane</td>
<td>Hydrocarbon</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>24.833</td>
<td>1,3-Diphenyl-4H-1,2,4-triazoline-5-thione</td>
<td>Hydrocarbon</td>
<td>43</td>
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<td></td>
<td>27.501</td>
<td>4-[4-[p-[n-Hexyloxyphenyl]butylamino]-1,2-naphthoquinone</td>
<td>Hydrocarbon</td>
<td>16</td>
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<tr>
<td></td>
<td>28.351</td>
<td>Hexadecanoic acid, methyl ester</td>
<td>Fatty acid</td>
<td>98</td>
</tr>
<tr>
<td>30%</td>
<td>5.797</td>
<td>Undecane</td>
<td>Hydrocarbon</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>28.351</td>
<td>Hexadecanoic acid, methyl ester</td>
<td>Fatty acid</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>24.801</td>
<td>Benzimidazol-5-amine, 1-(4-ethoxyphenyl)</td>
<td>Hydrocarbon</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>27.48</td>
<td>Silane diethyl (2-chloro-5-methylphenoxy) octyloxy</td>
<td>Hydrocarbon</td>
<td>14</td>
</tr>
<tr>
<td>40%</td>
<td>32.401</td>
<td>9,12,15-Octadecatrien-1-ol, (Z,Z,Z)</td>
<td>Alcohol</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>28.351</td>
<td>Tridecanoic acid, methyl ester</td>
<td>Fatty acid</td>
<td>94</td>
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<tr>
<td></td>
<td>33.007</td>
<td>Methyl stearate</td>
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<td>49.927</td>
<td>2-((Acridin-9-ylamino)-3-phenyl-propionic acid</td>
<td>Hydrocarbon</td>
<td>32</td>
</tr>
</tbody>
</table>

Figure 3. The chlorophyll content of microalgae *Lyngbya* sp. in various salinity stress condition.

Figure 4. GC-MS chromatogram spectrum of different salinity stress induced *Lyngbya* Sp. sample: (a) control, (b) 10% salinity, (c) 20% salinity, (d) 30% salinity, (e) 40% salinity.
4. Discussion

Salt stress is nothing but increasing the inorganic ion concentration in the growth medium. It leads to impair the osmotic balance cell and medium resulted exosmosis will occur. Similarly, which is the growth of *Spirulina platensis* was slightly affected by low salt concentration (0.02 mol/L NaCl) and a marked and progressive inhibition of growth was observed in increasing salinity (0.04 and 0.08 mol/L). These findings revealed that marine microalgae did not tolerate lower salinity and high salinity led to a strong reduction in growth rate\[^{10}\]. This result agreed the report by Murthy and Sudhir *et al*.\[^{11}\] whose stated that in some cyanobacteria were various responses of chlorophyll content to salinity stress. In general, the chlorophyll increased at higher salinities up to 30% and decreased at lower salinities. Likewise, the salinity was a factor of significant accumulation of compatible solutes in marine microalgae. It acts as enzyme producers, stabilizing the structure of macromolecules and organelles\[^{2}\].

In simultaneously the increasing exogenous ion concentration is given some pressure for cells, tighten the electrochemical cellular signaling. Finally stimulate the secondary metabolic pathways in the cells. The freshwater micro algae species *Scenedesmus opolensis* can adapt to high salt concentrations. Under these conditions, the rate of cell multiplication shows the cells develop very small antennae and they excrete high amounts of mucilage\[^{12,13}\].

Although several species of microalgae are resisting to variations in salinity level. At the same time, chemical composition of algae is vigorously affected. The difference in the growth rate with inversely proposal to the concentration of salinity. Our finding suggests genetic and physiological variations of algae was identified in the extremely moderate salinity concentration according to that salinity stress activates the secondary metabolite production\[^{14}\]. The algae secretes some bioactive metabolites to acclimatize to salt stress and also to balance as per the surroundings osmotic pressure\[^{15}\]. Salt stress is mainly generated by two important reason: 1. Sodium chloride is essential factor and highly relation with aquatic habitats and 2. Salt resistance is developing in the field of renewable biomass production\[^{16}\].

The present study demonstrated that salinity is an important parameter in controlling the growth of marine microalgae *Lyngbya* Sp. through its effects on the chlorophyll content and growth rate. The microalgae showed considerable differences in their stress metabolites with respect to changes in salinity pattern during the experimental period. Marine microalgae are differing in their adaptability to salinity environment. Hence, it can be suggested that the chlorophyll and other metabolite production of *Lyngbya* Sp. is optimum in 30% salinity. The growth rates and the cell count showed the maximum number on Day 15 in 30% salinity and followed by the 40% salinity. The major number of compounds accumulated by the tested microalgae strain was accumulated in 30% salinity. This is the reason for we mainly choosen the salt stress of marine micro algae to findings the novel bioactive compound with high biomass production in salt induces microalgae sample.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

**Background**

Recently micro algae are vastly used in the production of health oriented pharmaceuticals. Salinity stress is the main limiting factor in plant productivity both in aquatic, natural and antropically modified environment. The study mainly focused the effect of salinity stress to improve the secondary metabolite production from marine microalgae.

**Research frontiers**

The study was designed in such a way that by increasing salinity stress in the growth medium. There is an increase in the production of biomass and chlorophyll pigments of the marine micro algae (*Lyngbya* Sp.). The different concentration of salinity stress effectively synthesized various clinically related secondary metabolites from marine algae.

**Related reports**

Salt stress enhancement of antioxidant and antiviral
efficiency of spirulina platensis by under salt stress conditions cause a raise or production of biologically active compounds beside an alteration of algal metabolism Lyngbya Sp., a marine cyanobacterium from the family Oscillatoriaceae. It has been shown to be a diverse source of bioactive compounds, some of which possess antiviral, antifungal and antimicrobial properties.

Innovations and breakthroughs

Marine algae possesses different classes of biogenic compounds, that principal group of secondary metabolites are inhibiting the growth of bacteria, fungi and viruses. Stress was investigated by using green algae strains Lyngbya Sp. in response to changing phytochemical constituents without any modification of cell growth and biomass production rate.

Applications

Marine microalgae are differing in their adaptability to salinity environment. The present study demonstrated that salinity is an important parameter in controlling the growth of marine microalgae Lyngbya sp. through its effects on the chlorophyll content, and growth rate. The microalgae showed considerable differences in their stress metabolites with respect to changes in salinity pattern during the experimental period.

Peer review

This research work has been a superior masterpiece in marine algae. The author has done an excellent job on collection of marine algae and worked on its salinity stress on assorted aspects of biomass, chlorophyll content and GC–MS analysis. The results are exhibited in a remarkable style.

References


