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

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PEER REVIEW

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Comments

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.

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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

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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.

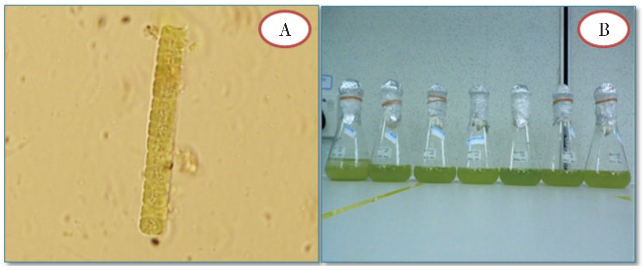

Figure 1. Collected marine microalgae.
A: Confocal microscopical structure of *Lyngbya* sp. B: Marine micro algae mass culture in different salinity concentration (10%-40%).

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 7 890A 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).

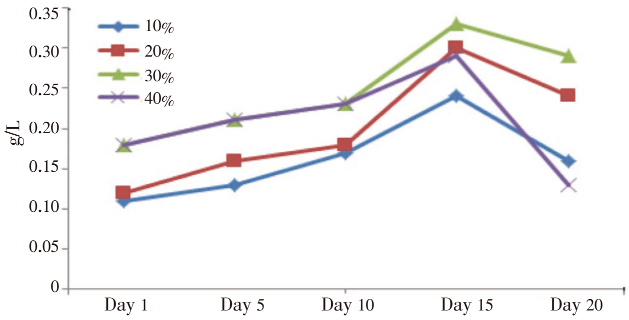

Figure 2. Microalgae *Lyngbya* sp. biomass concentration in different salinity stress conditions.

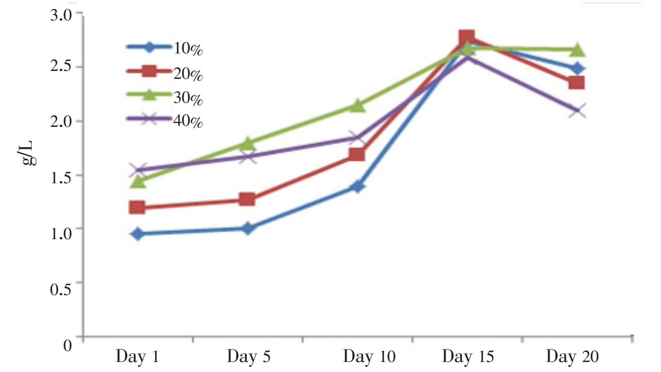

Figure 3. The chlorophyll content of microalgae *Lyngbya* sp. in various salinity stress condition.

Table 1

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

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

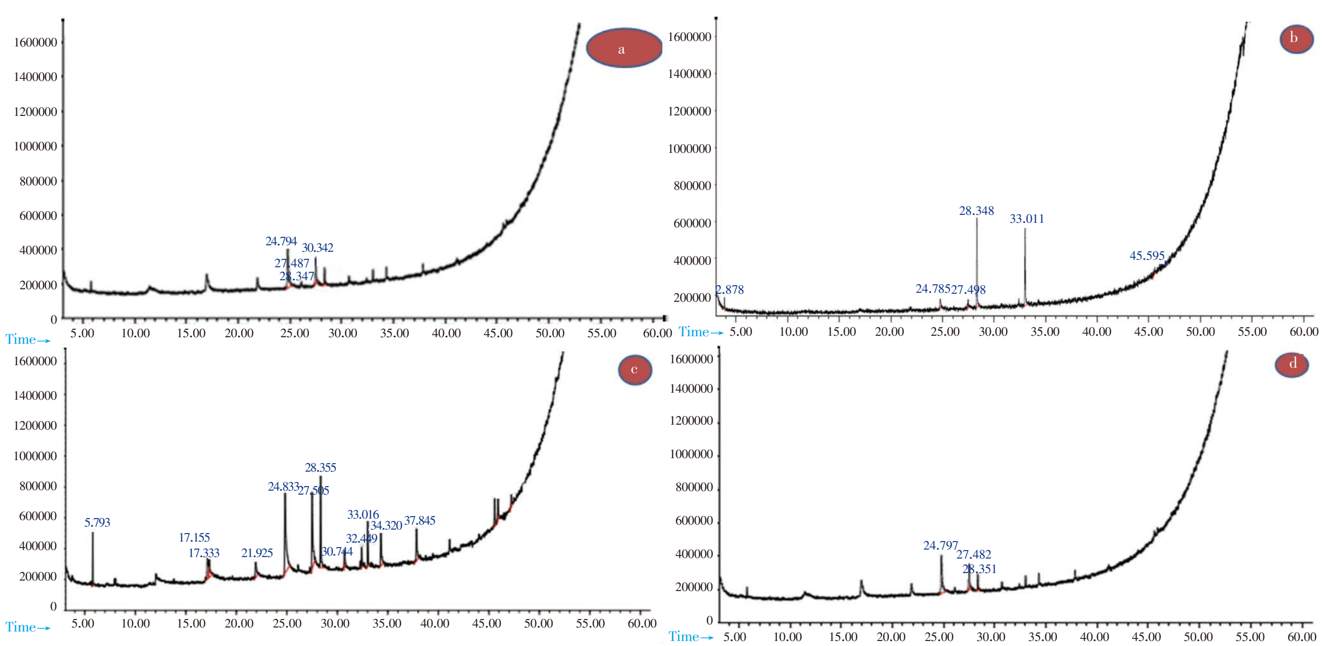

Figure 4. GC-MS chromatogram spectrum of different salinity stress induced *Lyngbya* Sp. sample: (a) control, (b) 10% salinity, (c) 20% salinity, (d) 30% salinity, (e) 40% salinity.

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 *Spirulia 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 opoliensis* 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 secrets 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.

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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 antrophically 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.

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References

[1] Andersen RA. *Algal Culturing Techniques*. California: Academic Press; 2005, p. 1-64.

[2] Pahlich E, Kerres r, Jäger HJ. Influence of water stress on vacuole/extravacuole distribution of proline in protoplasts of *Nicotiana rustica*. *Plant Physiol* 1983; **72**: 590-591.

[3] Radjenović J, Petrović M, Ventura F, Barceló D. Rejection of pharmaceuticals in nanofiltration and reverse osmosis membrane drinking water treatment. *Water Res* 2008; **42**: 3601-3610.

[4] Bhagavathy S, Sumathi P, Bell IJS. Green algae *Chlorococcum humicola*-a new source of bioactive compounds with antimicrobial activity. *Asian Pac J Trop Biomed* 2011; **1**(Suppl 1): S1-S7.

[5] Shalaby EA, Shanab SMM, Singh V. Salt stress enhancement of antioxidant and antiviral efficiency of *Spirulina platensis*. *J Med Plants Res* 2010; **4**(24): 2622-2632.

[6] Burja AM, Banaigs B, Abou-Mansour E, Burgess JG, Wright PC. Marine cyanobacteria-a prolific source of natural products. *Tetrahedron* 2001; **57**: 9347-9377.

[7] Bano A, Siddiqui PJA. Characterization of five marine cyanobacterial species with respect to their pH and salinity requirements. *Pak J Bot* 2004; **36**(1): 133-143.

[8] Jahnke LS, White AL. Long-term hyposaline and hypersaline stresses produce distinct antioxidant responses in the marine alga *Dunaliella tertiolecta*. *J Plant Physiol* 2003; **160**(10): 1193-1202.

[9] Shanab SM, Mostafa SS, Shalaby EA, Mahmoud GI. Aqueous extracts of microalgae exhibit antioxidant and anticancer activities. *Asian Pac J Trop Biomed* 2012; **2**(8): 608-615.

[10] Eggert A, Nitschke U, West JA, Mchalik D, Karsten U. Acclimation of the intertidal red alga *Bangiopsis subsimplex* (Stylonematophyceae) to salinity changes. *J Exp Mar Biol Ecol* 2007; **343**: 176-186.

[11] Sudhir P, Murthy SDS. Effects of salt stress on basic processes of photosynthesis. *Photosynthetica* 2004; **42**(4): 481-486.

[12] Fernandes TA, Iyer V, Apte SK. Differential response of nitrogen-fixing cyanobacteria to salinity and osmotic stresses. *Appl Environ Microbiol* 1993; **59**: 899-904.

[13] Rao AR, Dayananda C, Sarada R, Shamala TR, Ravishankar GA. Effect of salinity on growth of green alga *Botryococcus braunii* and its constituents. *Bioresour Technol* 2007; **98**(3): 560-564.

[14] Asulabh KS, Supriya G, Ramachandra TV. Effect of salinity concentrations on growth rate and lipid concentration in *Microcystis* sp., *Chlorococcum* sp. and *Chaetoceros* sp. In: National Conference on Conservation and Management of Wetland Ecosystems. School of Environmental Sciences, Mahatma Gandhi University, Kottayam, Kerala; 2012.

[15] Raghavan G, Haridevi CK, Gopinathan CP. Growth and proximate composition of the *Chaetoceros calcitrans*, f. *pumilus* under different temperature, salinity and carbon dioxide levels. *Aquac Res* 2008; **39**: 1053-1058.

[16] Dujjanutat P, Kaewkannetra P. Effects of wastewater strength and salt stress on microalgal biomass production and lipid accumulation. *World Acad Sci Eng Technol* 2011; **60**: 1-6.