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Evaluation of n-Alkane Contents in *Spirulina platensis* under UV-B Stress by GC-MS

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ABSTRACT

n-Alkanes variability in response to UV-B stress has been investigated in the cyanobacterium Spirulina platensis. Saturated hydrocarbons of UV-B treated and untreated cells of S. platensis were separated and identified by gas chromatography-mass spectrometry (GC-MS) using serially coupled capillary column and obtained light chain n-alkanes (C_9–C_{20}) 78% and heavy chain n-alkanes (C_{21}–C_{34}) 22% in the UV-B treated cells of S. platensis. In contrast, UV-B–untreated cells of S. platensis had dominance of only long chain 71% and 29% light chain n-alkanes.49% of short chain n-alkanes were increased in UV-B treated cells of S. platensis compared to very low level in its UV-B untreated counterpart. Our finding suggest that in S. platensis under UV-B stress the short chain of n-alkane and the level of tetradecane ($C_{14}H_{30}$) and heptadecane ($C_{17}H_{36}$) were also increased compared to UV-B untreated counterpart for protection of the cell in maintaining the vital cellular functions in S. paltensis under UV-B stress.

Key words: *Spirulina, UV-B stress, n-Alkanes and GC-MS*

INTRODUCTION

One of the primary concern regarding possible global climatic changes has been the agronomic consequences of global warming and enhanced solar UV radiation[4]. Cyanobacteria *Spirulina paltensis* are Gram-negative photoautotrophic prokaryotes having plant type oxygenic photosynthesis[17]. The cosmopolitan distribution of cyanobacteria indicates that they can cope with wide spectrum of global environmental stresses such as radiation, heat, cold, dessication, etc[8]. UV-B radiation has been a ubiquitous problem for life that depend on solar radiation for vital activities and induces deleterious effects in all living organisms from prokaryotic bacteria and unicellular aquatic organisms to higher plants and animals[1,11]. They have developed a number of mechanisms by which they defend themselves against environmental stresses[7]. *S. platensis* is a filamentous, spiral, multicellular cyanobacterium with a long history of being used as food supplement and rich in ingredients like essential fatty acids, high protein content with nutritional and biomedical values[12, 13, 14].

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Impact of UV-B radiation on thylakoid membrane alteration, in fatty acid composition in *S. platensis* with variability in short chain n-alkanes was reported by Gupta *et al.*[3, 10]. Hexadecane treatment effectively induces CYP110 expression in cyanobacteria *Anabaena*[19]. The hydrocarbon-degrading bacteria utilizes cyanobacterial alkane in the ocean hydrocarbon cycle[5]. Considering above importance of alkanes, an attempt has been made to evaluate the effect of UV-B treatment on n-alkanes variability in *Spirulina platensis*, in the present investigation.

MATERIAL AND METHODS

Organism and culture condition:

Axenic culture of *Spirulina platensis* was maintained in Zarrouk's medium. The culture was grown at light intensity of 3000 lux with light and dark cycles of 10 and 14 h and incubated at 30 ± 2^0C temperature, respectively. The flasks were shaken for 3 to 4 times during the process of growth. Exponentially growing culture were harvested and transferred to sterile Petri dishes (25 mm diameter) for exposure to artificial UV-B (280–315 nm) radiation, generated from a UV-B lamp (TL 12 20W fluorescent tubes, Phillips, Holland). The intensity of UV-B radiation falling upon the cells was measured by photometer (Type IL 1350, Japan) [3, 10].

Extraction of hydrocarbons

The UV-B treated and untreated *Spirulina platensis* were harvested by centrifugation (6,000 rpmfor10 min) and extracted with pentane/dichloromethane/methanol (40:30:30, v/v)[6]. Non-saponifiable fractions were used for hydrocarbon analysis[2].

GC-MS analysis

Hydrocarbons content of UV-B treated and untreated *Spirulina platensis* were analysed by avarian gas chromatograph with capillary column coupled to mass detector Finnigan mat TSQ (700). GC on HP-5 column (30 m, internal diameter 0.32 mm, film thickness 0.25 mm) was programmed for 2 min at 50^0C; 15^0C min^{-1}- 250^0C min^{-1}; and hold for 5 min at 250^0C. The injector temperature was kept at 250^0C (splitless) and the flow rate of carrier gas was 2 ml min^{-1}. The MS detector was operated at 150^0C with electron impact ionization energy at 70 eV. The scan range was m/z 40–650 and scan rate 0.9 scans s^{-1}. Solvent delay was set at 11 min. Hydrocarbons were identified by comparison with those found in Wiley mass spectral library (7th edition).

RESULT AND DISCUSSION

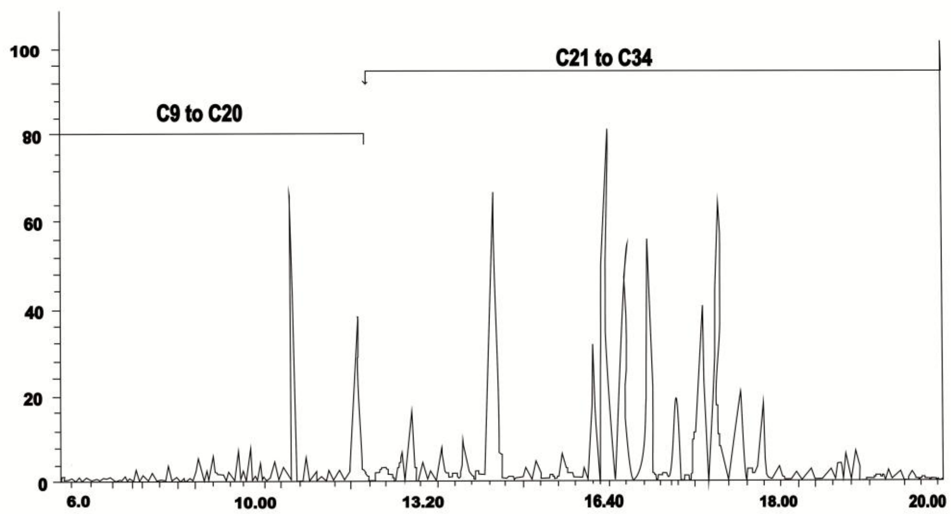
The n-alkanes produced by filamentous cyanobacterium *S. platensis* under UV-B treated and untreated condition were extracted with hexane and separated by serially coupled capillary column to mass detector. Figure 1 a, b shows the complete ion chromatogram of hydrocarbons by GC-MS analysis in *Spirulina platensis*. The solvent delay was 6 min. Therefore, TIC shown in the Fig. 1 is from 6 min. The data of GC-MS analysis of hydrocarbon in UV-B un-treated *S. platensis* cells indicated C_{14}, C_{17}, C_{18}, light chain and C_{21} C_{23},C_{24},C_{26}, C_{28}, C_{30}, C_{32} and C_{34} were the major constituents of heavy chain n- alkane. UV-B treated *S. platensis* cells, in contrast, showed both very light and long n-alkane range from C_9-C_{20}, and C_{21}-C_{30}, respectively. C_9, C_{10}, C_{12}, C_{14}, C_{15}, C_{16}, C_{17}, and C_{18} were Light chain n-alkane and C_{21}, C_{22}, C_{24}, and C_{30} were the constituents of heavy chain n-alkane. Hydrocarbons of variability with similar range of light chain hydrocarbon has also been reported in filamentous cyanobacteria *Anabaena cylindrical* in response to NaCl stress [2]. Hydrocarbon variability in filamentous cyanobacterium *Scytonema* spp. has also been reported by Dembitsky and Srebnik,[7]. MS profile of predominant short n-alkanes revealed ion m/z at 127, 141, 169, 198, 211, 225, 240,253, and 281, corresponding to molecular formula C_9H_{20}, $C_{10}H_{22}$, $C_{12}H_{26}$, $C_{14}H_{30}$, $C_{15}H_{32}$, $C_{16}H_{34}$, $C_{17}H_{36}$, $C_{18}H_{38}$, and $C_{20}H_{42}$, respectively. Similarly, long chain n-alkane revealed ion m/z at 295,309, 323, 337, 366, 392, 421, 449 and 477, corresponding to molecular formula $C_{21}H_{44}$, $C_{22}H_{46}$, $C_{23}H_{48}$, $C_{24}H_{50}$, $C_{26}H_{24}$, $C_{28}H_{58}$, $C_{30}H_{62}$, $C_{32}H_{66}$ and $C_{34}H_{70}$ respectively. The molecular ion peak of straight chain saturated hydrocarbon was always present, though of low intensity for long chain compounds. The fragmentation pattern was characterized by cluster of peak and the corresponding peak of each cluster was 14 (CH_2) mass units apart. The most abundant peak has been observed at m/z 57 that corresponds to $CH_3\ (CH_2)^{3+}$.

Table: 1 n-Alkane variability in UV-B untreated and treated cells of *Spirulina platensis*

n-Alkane	Percent variability	
	UV-B untreated	UV-B treated
C_9-C_{20}	29	78
C_{21}-C_{34}	71	22

Table 1 revealed that UV-B treated cells of *Spirulina platensis* contains 78% light chain n-alkanes (C_9–C_{20}) and 22% heavy chain (C_{21}–C_{34}) saturated alkanes as the major components of total hydrocarbons. In contrast, a UV-B untreated cell of S. platensis contains 29% light chain and71% heavy chain of its total n-alkanes. In Fig. 2 a, b EI-MS spectrum reveals that m/z values 199; $[M-1]^+$ for tetradecane ($C_{14}H_{30}$) and 241$[M-1]^+$ for heptadecane ($C_{17}H_{36}$). In UV-B treated cells of *S. platensis* 49% of short chain n-alkanes has been increased as compared to very low level (29%) in its UV-B untreated counterpart. Under UV-B stress the short chain of n-alkane and the level of tetradecane ($C_{14}H_{30}$) and heptadecane ($C_{17}H_{36}$) in *S. platensis* were increased as compared to UV-B untreated counterpart. Ozademir *et al.*[15] suggested that tetradecane (C_{14}) and heptadecane (C_{17}) has potent antimicrobial activity. GC-MS analysis of volatile components of *S. platensis* indicates the presence of hydrocarbons heptadecane and tetradecane, which also have antimicrobial activity.

It is clear that *S. platensis* cells to shift towards the synthesis of short chain n-alkanes which may be involved in maintaining the vital cellular functions and protect the cell in *S. paltensis* under UV-B stress. The n-alkane content increased in *S. platensis* under UV-B stress for from environmental stress. Similar results were reported in leave of higher plants containing waxy alkanes which is useful for protection against UV-B radiations and photoinhibition[16].



a

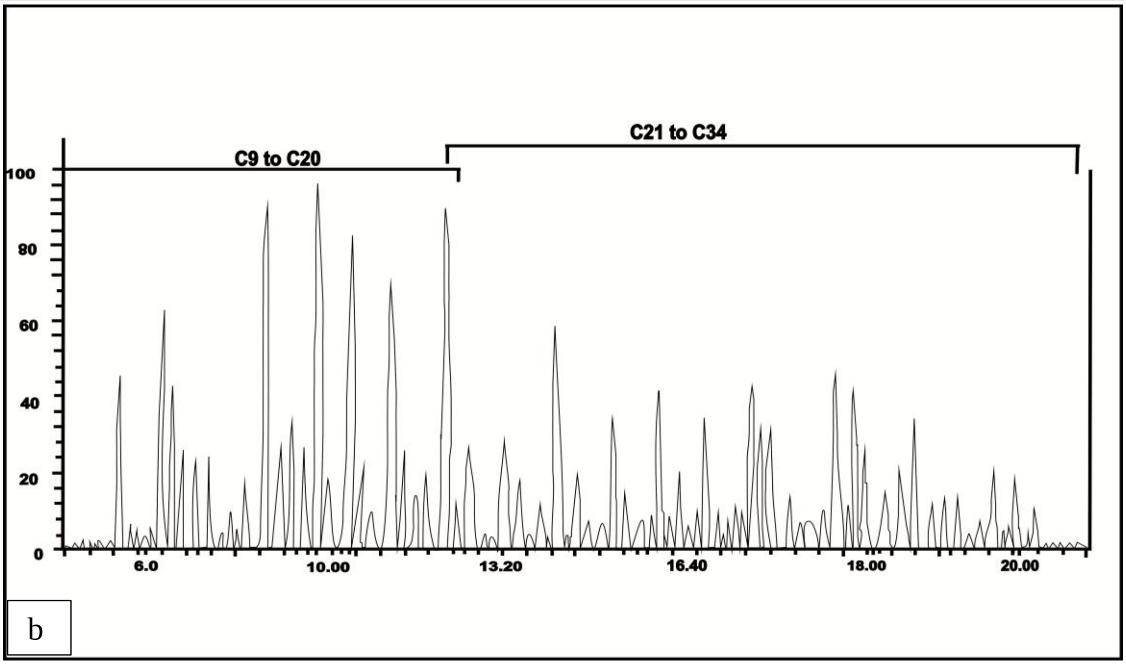
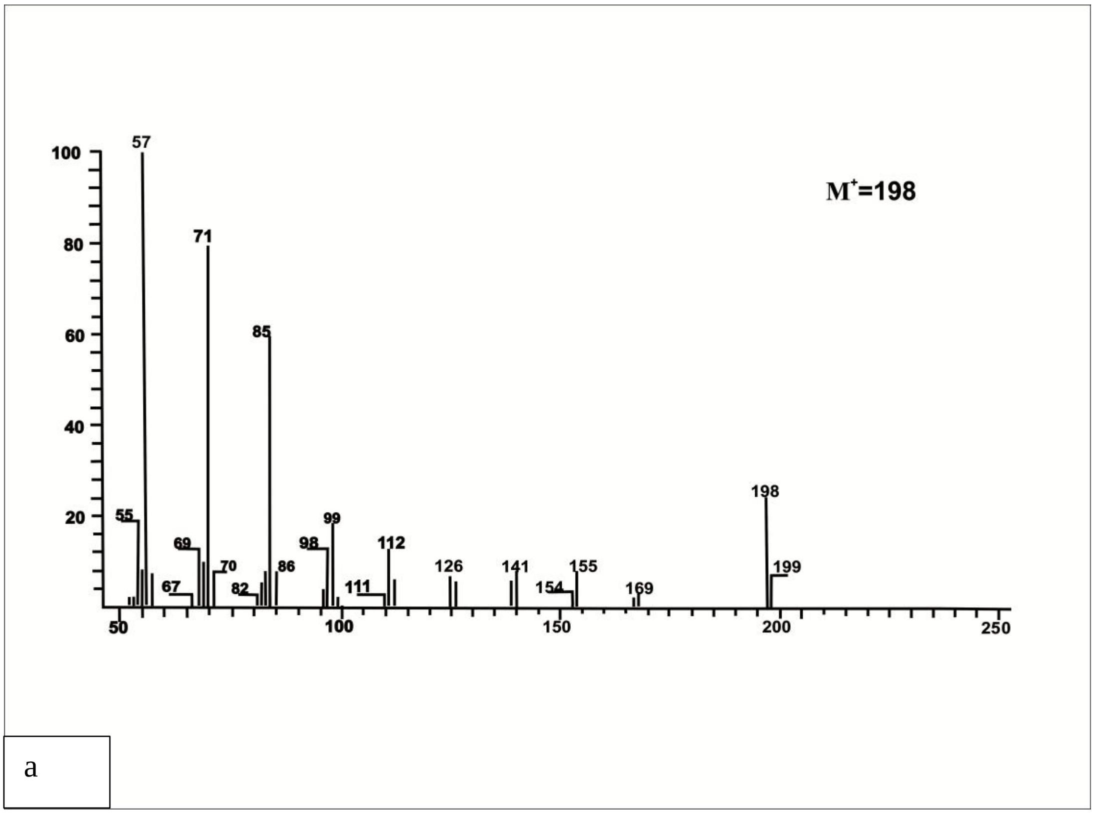


Fig. 1: Gas chromatography-Mass spectrometry study of n-alkane in
a) *S. platensis* control b) *S. platensis* under UV-B stress



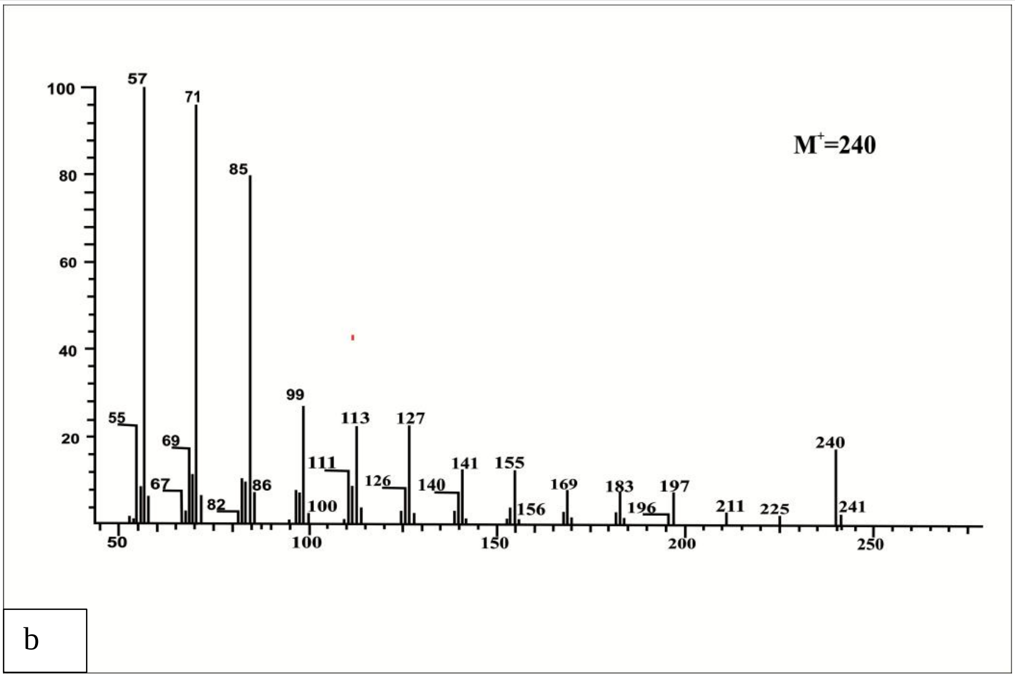


Fig. 2: EI-MS of identified peak of hydrocarbon n-tetra decane (a) and n-heptadecane (b) of *Spirulina platensis*

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