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EFFECT OF PHYTOHORMONES ON FATTY ACID PROFILE OF SPIRULINA PLATENSIS

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ABSTRACT

The present work was proposed to study the effect of plant growth hormones on the production of free fatty acids present in the microalgae *Spirulina platensis*. Phytohormones are the chemical messengers that coordinate together and help in the growth and development of plants. Since like plants microalgae also involve in photosynthesis, an attempt was made to study the effect of these plant growth regulators on microalgae. Among the different plant growth hormones used 1-Naphthaleneacetic acid (NAA) has enhanced the gamma linolenic acid production by 1.7 fold. Undecanoic acid and heptadecanoic acid were increased by 1.57 fold and 9.9 fold respectively by 6-Benzylaminopurine (6-BAP). Gibberelic acid showed 1.12 fold enhancement of undecanoic acid compared with control. Phytohormones also showed enhanced effect on biomass productivity. Treatment with 1-Naphthaleneacetic acid (NAA) has resulted in highest biomass production with 1.55 fold increase over control.

KEYWORDS: **Spirulina platensis, Phytohormones, Free fatty acids, Naphthaleneacetic acid, Benzylaminopurine**.

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INTRODUCTION

Microalgae being potent to synthesize high value products are drawing much commercial interest since 1950. The low productivities of these valuable products has confined the large scale production only to certain products like beta- carotene and astaxanthin. Much of the ongoing research is mainly focused on enhancing the production of these microlagal products by different biochemical and genetic engineering strategies. Spirulina platensis is a well known cyanobacterium that grows in freshwater with high nutritional value and is of much commercial importance. Certain free fatty acids present in Spirulina platensis like gamma linolenic acid are having good pharmaceutical importance. Enhancing these free fatty acids is one of the important area of research. There are various strategies fallowed to enhance the production of total lipids like nutrient stress, abiotic stress[1] genetic manipulations[2] etc but there is little work done for improving the production of free fatty acids in microalgae using biochemical approach. Phytohormones play very prominent role in improving the productivities in higher plants[3]. Microalgae with similar growth physiology like that of higher plants involving chlorophyll development, cell division and cell enlargement, can be most likely to study the effect of the various phytohormones that are proved to stimulate these growth processes in higher plants[4]. Thus the present study is done to understand the effect of plant growth hormones on the production free fatty acid profile in Spirulina platensis.

MATERIALS AND METHODS

Materials

Spirulina pletensis culture was procured from Spirulina Nutritech Foundation, madhurai, India. All the solvents are of hplc grade, the solvents and chemicals used in the study were procured from Merck India Ltd. The fatty acid mix was bought from Sigma Algrich, India.

Culturing of *Spirulina platensis*

Spirulina platensis was cultured in Zarrouk's medium[5] ($NaHCO_3$-16.8g/L, $NaNO_3$-2.5g/L, NaCl-1 g/L, K_2SO_4-1g/L, K_2HPO_4-0.5, $MgSO_4.7H_2O$-0.2, $FeSO_4.7H_2O$-0.01g/L, $CaCl_2.2H_2O$- 0.04g/L, EDTA-0.08g/L, A5 solution-1ml; A5 solution: H_3BO_4- 2.86g/L, MnCl2.4H_2O -1.81g/L, $ZnSO_4.7H_2O$- 0.22g/L, MoO_3-0.01g/L, $CuSO_4.5H_2O$-0.079g/L). The pH of the medium was maintained at 8.5 and grown at 25^{0}C for 5 days.

Effect of phytohormones

The effect of phytohormones on fatty acid profile of *Spirulina platensis was* studied with different plant growth regulators that include : gibberellic acid, indole -3- butyric acid, Kinetin, 6-benzylaminopurine , 1-Naphthaleneacetic acid. Each hormone was added at 4mg/L concentration to different conical flasks containing Zarrouk's medium and the pH was adjusted to 8.5.

Harvesting of microlagal culture

The culture was harvested at the end of the growth phase by flocculation method[6], using alum (Hydrated potassium aluminum sulphate) as the precipitant. The biomass precipitate was collected after decanting the supernatant and centrifuged at 7000rpm for 5mins using thermo Sorvall Xtr Refrigerated Centrifuge. The pellet thus obtained was micro oven dried and made into fine powder.

Fame preparation

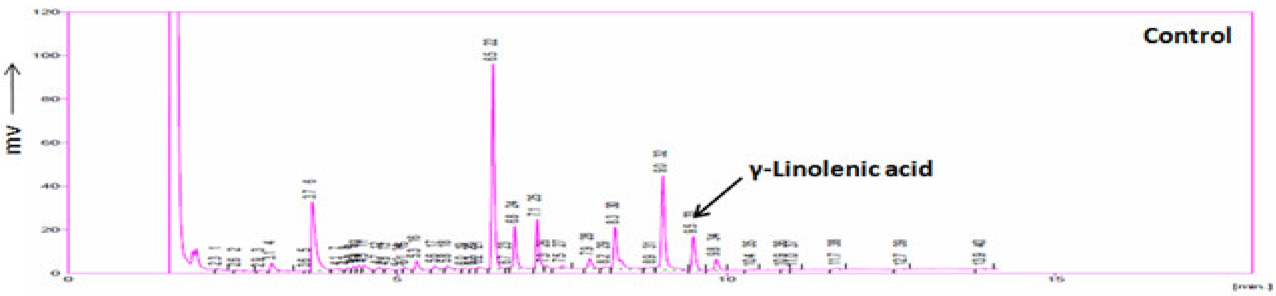
Free fatty acid methylation was done according to Lepage and Roy[7]. Transesterification reagent:20ml (methanol/acetyl chloride, 95:5 v/v) was added to 100mg of dried algal biomass in screw capped bottle and kept on water bath at 85°C 1 h. The mixture was cooled to room temperature and 2-3mL of water was added. Hexane (twice the volume reaction mixture) was added in order to extract methylated FFAs and mixed well. Hexane phase was separated and evaporated to 1mL for GC analysis.

Fatty acids analysis by gas chromatography

Gas chromatography (Thermo Scientific 8610) with a flame ionizing detector was used to detect total fattyacids. A fused silica capillary column with a cyanopropyl polysiloxane stationary phase equivalent to 70% cyanopropyl (SGE, BPX-70, 25 m length x 0.32 mm ID x 0.25 µm film thickness) was employed for the detection of fatty acid methyl ester (FAME). The injection and detector ports were maintained at 240 and 250^{o}C respectively. Fatty acid analysis was performed by injecting 0.5 µL of the sample in split mode (1:50) with nitrogen as a carrier gas. The following temperature program was adopted for detection of FAME: initial temperature 100^{o}C, 1 min hold; ramp at 10^{o}C min^{-1} until 180^{o}C with 1 min hold; ramp at 10^{o}C min^{-1} until 240^{o}C, with a 2 min hold.

RESULTS AND DISCUSSION

All the phytohormones hormones showed varying effect on fatty acid profile of *Spirulina platensis.* 1-Naphthaleneacetic acid (NAA) showed 1.7 fold rise (Fig.1) in the percentage of gamma linolenic acid production, that is having beneficial beneficial effects on bone[8-9] and the expression of various genes is also effected by its metabolites[10].



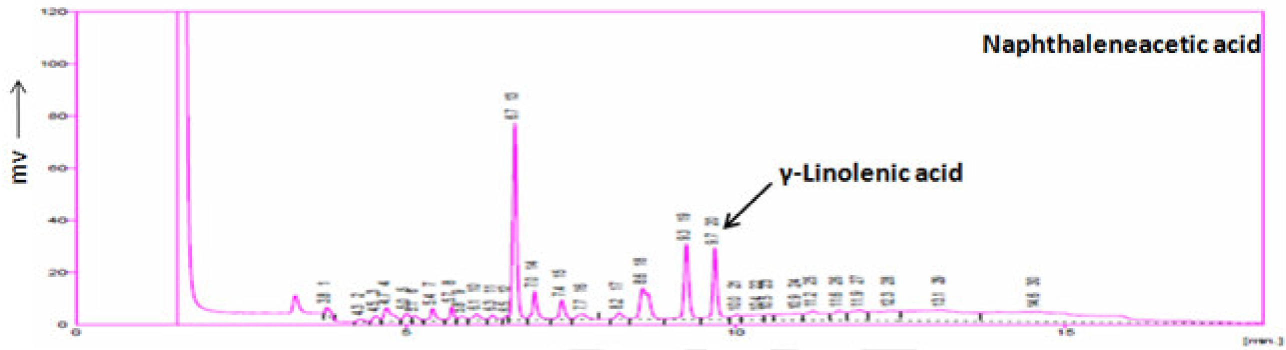


Figure 1
Comparision of gas chromatograms showing the enhanced gamma linolenic acid peak area of spirulina platensis treated with 1-Naphthaleneacetic acid (NAA) over that of control.

6-Benzylaminopurine (6-BAP) showed increase in undecanoic acid and heptadecanoic acid by 1.57 fold and 9.9 fold respectively (Fig.2) over control. Gibberelic acid showed 1.12 fold enhancement (Fig. 2) of undecanoic acid compared with control. Heptadecanoic acid showed reduced risk of developing multiple sclerosis11, these findings are paving way for the growing scientific evidence that some unsaturated fats may not be completely harmful. Undecenoic acid is proved to be one of the best known antifungal fatty acid and is also found to show inhibitory effect on the growth of Candida albicans by inhibiting the hyphal growth12. There are some free fatty acids that are reduce due to the effect of ceratin phytohormones take include palmitoleic acid by 6-BAP, arachidic acid by the effect of gibberelic acid. The other phytohormones (Kinetin and indole -3- butyric acid)used did not show significant effect on the fatty acid profile compared with the control

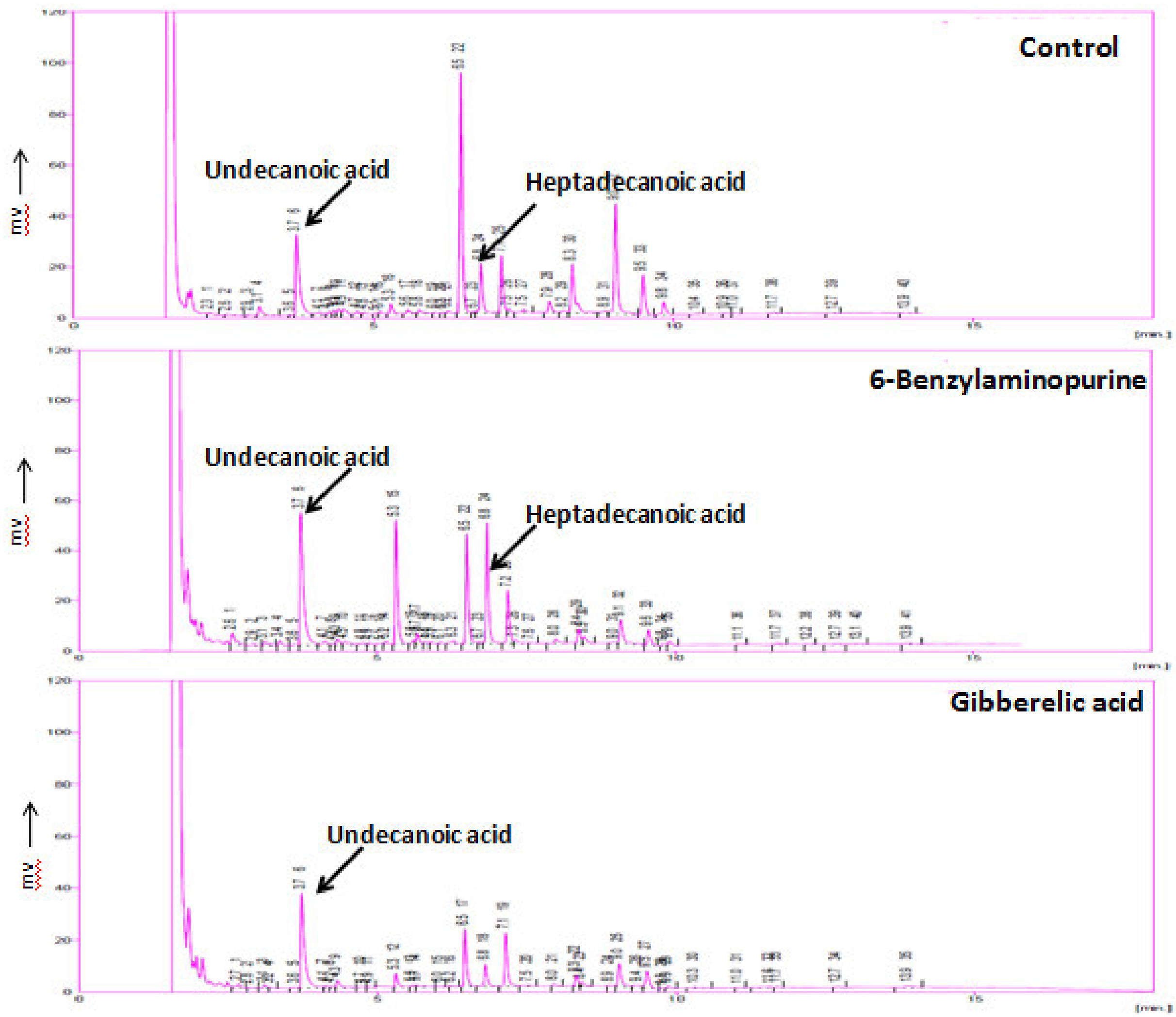
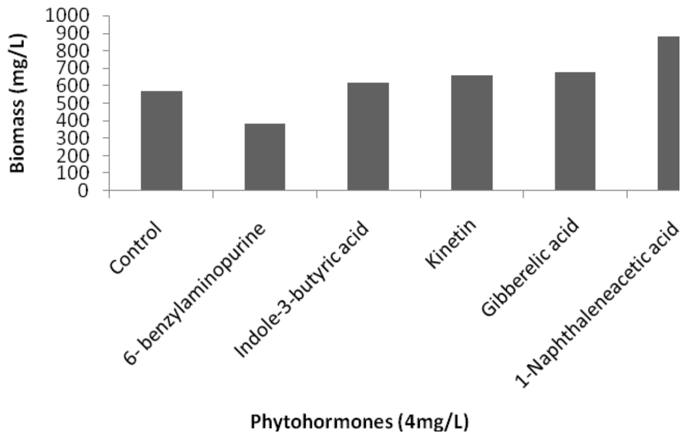


Figure 2
Comparision of gas chromatograms showing the enhanced heptadecanoic acid and undecanoic acid peak areas of spirulina platensis treated with 6- benzylaminopurine and gibberelic acid over that of control.

Plant growth hormones also showed varying effect on biomass yield. Treatment with 1-Naphthaleneacetic acid (NAA) has resulted in highest biomass production with 1.55 fold increase over control. Unlike other plant growth hormones 6- benzylaminopurine showed a decreased biomass production by 1.46 fold. The effect of various phytohormones on biomass productivity is shown in Fig.3. Thus phytohormones can used for enhancing the biomass yields in *Spirulina platensis.*



Graph 1
Showing the effect of phytohormones on biomass productivity of Spirulina platensis

CONCLUSION

Phytohormones thus used in the study have shown some positive effect on enhancing the production of certain free fatty acids in *Spirulina platensis*. Addition of certain phytohormones like IBA, kinetin, GA and NAA free fatty acids also resulted in increasing the biomass productivity in microalgae.Thus plant growth hormones are going to act as an interesting field of research for studing their effect on various commercially important metabolites in both micro and macrolagae.

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CONFLICTS OF INTEREST

Conflict of interests declared none.

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