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MALINA JOSOH

THE EFFECT OF PLANT HORMONES ON OIL ACCUMULATION, FATTY ACID COMPOSITIONS AND EXPRESSION LEVELS OF FATTY ACID BIOSYNTHETIC GENES IN THE MARINE MICROALGA, *Chlorella vulgaris* (UMT-MM)

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Lihat Sebelah

The effect of plant hormones on oil accumulation, fatty acid compositions and expression levels of fatty acid biosynthetic genes in the marine microalga, *Chlorella vulgaris* (UMT-M1)

Malinna Jusoh

Thesis Submitted in Fulfilment of the Requirement for the
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DEDICATION

Dedicated to my family and friends for making me to be who I am today and supporting me all the way. I hope that this achievement will complete the dreams that all of you had for me all those many years ago.

and

To all who have a passion for science without barriers

ABSTRACT

Abstract of thesis presented to the Senate of Universiti Malaysia Terengganu in fulfilment of the requirement for the degree of Doctor of Philosophy

THE EFFECTS OF PLANT HORMONES ON OIL ACCUMULATION, FATTY ACID COMPOSITIONS AND EXPRESSION LEVELS OF FATTY ACID BIOSYNTHETIC GENES IN THE MARINE MICROALGA, *Chlorella vulgaris* (UMT-M1)

MALINNA JUSOH

MAY 2014

Chairperson : Prof. Madya Dr. Noraznawati binti Ismail

Member : Prof. Madya Dr. Yeo Chew Chieng

: Prof. Dr. Tengku Sifzizul bin Tengku Muhammad

School : Fundamental Sciences

Microalgae lipids and oils are potential candidates for renewable-biofuels and nutritional purposes. It has long been known that many microalgae species accumulate substantial amount of lipids and oils under environmental stresses, presumably by channelling the photosynthesis product originally allocated to support cell division and growth to the production of energy-rich storing molecules. However, low growth rates under these adverse conditions account for the decreases in overall biomass productivity which in turn penalised the net production efficiency of microalgae-based oils. This study attempts at maximizing the production of oils in *Chlorella vulgaris* (strain UMT-M1) through introduction of selected plant hormones. In this study, auxin (IAA), gibberellin (GA_3) and jasmonic acid (JA) were exogenously supplied to the microalga,

C. vulgaris (strain UMT-M1) cultures at early stationary growth phase and left to grow until late stationary growth phase. Among the three hormones studied, only GA_3 and JA promoted microalgal growth with increment between 42 and 52 % of cell density relative to the control while IAA had no stimulatory effect on microalgal growth. Besides, IAA and JA increased the total oil production of microalgal cells by 40 and 50 %, respectively. Further analysis of fatty acid compositions showed that all three hormones induced the increment of saturated fatty acids notably C16:0 (palmitic acid) and C18:0 (stearic acid) while inhibited the production of polyunsaturated fatty acids of C18:2 (linoleic acid) and C18:3n3 (α-linolenic acid). In order to investigate the influence of fatty acid regulation by these hormones, the expression levels of four fatty acid biosynthetic genes were quantified by real-time PCR. Results showed that elevated levels of saturated fatty acids are consistent with high expression of ß-ketoacyl-[acyl carrier protein] synthase I (*KAS I*), a gene responsible for *de novo* palmitic acid biosynthesis. Low expression of omega-6 (*ω-6*) fatty acid desaturase (*FAD*) and omega-3 (*ω-3*) *FAD* genes was in agreement with the low production of linoleic and α-linolenic acids. However, increment of stearoyl-ACP desaturase (*SAD*) gene expression upon hormones induction did not correspond to C18:1 (oleic acid) production suggesting that another limiting step might control the fatty acids biosynthesis pathway. Taken together, the results indicate that exogenous application of plant hormones not only induced transient increment of microalgal oil but also modified the fatty acids composition. These changes were correlated with the levels of gene expression and could be utilized in cultivation to facilitate the commercial mass production of microalgae lipids through manipulation of fatty acid biosynthetic genes regulation.

ABSTRAK

Abstrak tesis yang dikemukakan kepada Senat Universiti Malaysia Terengganu sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KESAN HORMON TUMBUHAN TERHADAP PENGUMPULAN MINYAK, KOMPOSISI ASID LEMAK DAN TAHAP PENGEKSPRESAN GEN BIOSINTESIS ASID LEMAK DALAM MICROALGA MARIN, *Chlorella vulgaris* (UMT-M1)

MALINNA JUSOH

MEI 2014

Pengerusi : Prof. Madya Dr. Noraznawati binti Ismail

Ahli : Prof. Madya Dr. Yeo Chew Chieng

: Prof. Dr. Tengku Sifzizul bin Tengku Muhammad

Pusat Pengajian : Sains Asas

Minyak dan lipid dari mikroalga merupakan salah satu sumber bagi penghasilan biofuel dan nutrisi. Umum mengetahui bahawa kebanyakan spesies mikroalga dapat menghasilkan kandungan minyak dan lipid yang tinggi di bawah pengaruh tekanan persekitaran, berkemungkinan dengan menukar produk fotosintesis yang pada asalnya diperuntukan untuk pembahagian sel dan tumbesaran kepada molekul penyimpan yang kaya tenaga. Namun, kadar tumbesaran yang rendah di bawah pengaruh tekanan persekitaran ini mengakibatkan penurunan produktiviti secara menyeluruh yang mengurangkan kecekapan pengeluaran bersih minyak berasaskan mikroalga. Kajian ini bertujuan untuk memaksimumkan pengeluaran minyak dari mikroalga *Chlorella vulgaris* (UMT-M1) melalui rawatan hormon tumbuhan. Melalui kajian ini, auksin (IAA), gibberellin (GA_3) dan asid jasmonik (JA) dibekalkan kepada kultur mikoralga *C.*

vulgaris (UMT-M1) ketika fasa awal pertumbuhan pegun dan dibiarkan sehingga fasa akhir pertumbuhan pegun. Di antara ketiga-tiga hormon, hanya GA_3 dan JA yang meransang pertumbuhan mikroalga di antara 42 hingga 52 % pertambahan dari aspek kepadatan sel berbanding kultur kawalan manakala IAA tidak memberi sebarang kesan ransangan terhadap pertumbuhan mikroalga. Selain itu, IAA dan JA masing-masing meningkatkan produktiviti minyak sebanyak 40 dan 50 %. Analisa lanjutan mendapati ketiga-tiga hormon meningkatkan komposisi asid lemak tepu terutamanya C16:0 (asid palmitik) dan C18:0 (asid stearik) sebaliknya mengurangkan pembentukan asid lemak tidak tepu C18:2 (asid linoleik) dan C18:3n3 (asid linolenik). Bagi mengkaji pengaruh hormon ke atas penghasilan asid lemak ini, tahap pengekspresan empat gen yang terlibat dalam biosintesis asid lemak diuji melalui kaedah *real-time PCR*. Paras tinggi asid lemak tepu adalah selaras dengan ekspresi tinggi bagi gen ß-ketoacyl-[acyl carrier protein] synthase I (*KAS I*) yang bertanggungjawab bagi sintesis asid palmitik. Ekspresi yang rendah bagi gen omega-6 dan omega-3 fatty acid desaturase (*ω-6* dan *ω-3 FAD*) adalah selari dengan pengeluaran yang rendah bagi asid linoleik dan linolenik. Walaubagaimanapun, peningkatan ekpresi bagi gen stearoyl-ACP desaturase (*SAD*) ketika dibekalkan dengan hormon adalah tidak selari dengan pengeluaran C18:1 (asid oleik) yang mencadangkan kemungkinan wujudnya langkah pengehadan dalam biosintesis asid lemak. Kesimpulannya, data dari kajian ini mendapati bahawa rawatan hormon tumbuhan bukan sahaja meningkatkan pengeluaran minyak dan komposisi asid lemak, malah boleh digunakan bagi tujuan pengkomersilan minyak berasaskan mikroalga.