FUNCTIONAL CHARACTERIZATION OF OMEGA-3 FATTY ACID DESATURASE (*ω-3 FAD*) GENE INVOLVED IN THE FATTY ACID BIOSYNTHESIS PATHWAY IN *CHLORELLA VULGARIS* (STRAIN UMT-M1)

NORASHIKIN BINTI MD. NOR

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ABSTRACT

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

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Main Supervisor: Associate Professor Cha Thye San, Ph.D.

Co- Supervisor: Professor Aziz bin Ahmad, Ph.D.

School/ Institute: Institute of Marine Biotechnology

A large number of microalgae have been studied in term of lipid class and fatty acids composition as microalgae such as *Chlorella vulgaris* have great potential sources of polyunsaturated fatty acids (PUFAs). Genetic engineering of the fatty acid biosynthesis pathway has been applied to improve PUFAs production of microalgae. Thus, it is essential to identify the genes coding for the key enzymes that contribute to fatty acid synthesis and accumulation. One of the enzyme involved in desaturation is omega-3 fatty acid desaturase (*ω-3 FAD*) enzyme which takes part in conversion of linoleic acid (LA, C18:2) to alpha linolenic acid (ALA, C18:3n3). ALA is the precursor for the synthesis of other essential fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). In this study, the promoter of *ω-3 FAD* gene was successfully isolated from genomic DNA of *C. vulgaris* (strain UMT-M1) using PCR-Genome Walking method. From five GenomeWalker libraries (*Dra*I, *Eco*RV, *Pvu*II, *Sma*I and *Stu*I), *Stu*I managed to produce higher putative fragment with approximately 2.3 kb. Alignment analysis revealed that from the 2.3 kb fragment, only 170 bp sequence contained 100 % of homology regions with the full-length *ω-3 FAD* cDNA sequence isolated from the same species and the remaining sequence of the fragment (2186 bp) upstream from the *ω-3 FAD* putative transcription start site (+1) was undoubtedly a fragment of *ω-3 FAD* gene promoter

(O3D-Pro). Sequence analysis of promoter region using the online PlantCARE and PLACE program demonstrated the presence of potential functional elements in the promoter region such as basic element in eukaryote's promoter gene (TATA-box, CAAT-box and GC-box), several light responsive elements, DNA binding with one finger (Dof), G/A-hibrid/ bZIP, abscisic acid responsive element (ABRE), CAT Box, CGTCA-motif, ERE, GARE-motif, MBS, GC-motif, silencer-element (SBF-1) and AT-rich region as enhancer-element. Other than that, the full-length endogenous *ω-3 FAD* gene was also successfully isolated from genomic DNA of *C. vulgaris* which contain seven exons and six introns. The four vector cassettes, pO3DPro-VF1, pO3DPro-VF2, pO3DPro-VF3 and pO3DPro-VF4 were successfully constructed and mobilized into *C. vulgaris* (strain UMT-M1) using improved *Agrobacterium*-mediated transformation method using cellulase enzyme. Treating cells with cellulase before co-cultivation steps in transformation showed a dramatic increment in transformation efficiency with approximately 82 ± 1.69 % of GUS positive cells as compared to non-cellulase treatment which produce only 21 ± 1.18 % GUS positive cells. Thus, the inclusion of cellulase enzymatic treatment to degrade the *C. vulgaris* cell wall layer successfully aids the simultaneous *Agrobacterium*-mediated transformation. The highest GUS positive cells were observed for pO3DPro-VF3 vector cassette with approximately 88.5 ± 0.52 % as compared to other vector cassettes. After three cycles (90 days) of alternate hygromycin selection, four out of 30 stable transgenic lines harboring the pO3DPro-VF3 vector cassette were randomly selected for verification with PCR technique. The four transgenic lines produced PCR products of specific size for *hpt* gene (687 bp), *gfp-gus*A gene (676 bp), *ω-3 FAD* gene (302 bp) and Hpt-GG fragments (7372 bp). The overexpression of pO3DPro-VF3 vector cassette in transgenic *C. vulgaris* line especially Ch-TL2 successfully increased the total SFAs, total PUFAs, C18:3n3 production and *ω-3 FAD* gene expression in both growth phases (mid exponential and early stationary) and under nitrate-deficient condition as compared to wild-type *C. vulgaris*. The potential transgenic line (Ch-TL2) was further investigated with genome integration analysis and the presence of unknown sequences at both borders of pCAMBIA1304 binary vector confirmed the integration of T-DNA into the genome of *C. vulgaris*. Thus, from this study, it is possible to manipulate genetically the fatty acid biosynthesis pathway by overexpressing the *ω-3 FAD* gene driven by O3D-Pro

endogenous promoter (pO3DPro-VF3 vector cassette) in microalgae or other plants in order to achieve both high lipid and high PUFAs under different stressors for industrial production.