MOLECULAR CHARACTERISATION AND ASSESSMENT OF FATTY ACID BIOSYNTHESIS PATHWAY IN TRANSGENIC *CHLORELLA VULGARIS* TRANSFORMED WITH DISRUPTED OMEGA-3 DESATURASE GENE VECTOR CASSETTE

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For my families for nursing me with affections, love and encouragements on the road of success and honor in my life

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Microalgae have emerged as one of the potential candidate for oil production due to its capability to accumulate more than 20 % of oil content in its cell dry weight. The utilization of microalgae for oil production has been studied intensively in recent years, focusing mostly on manipulating the culture conditions to obtain maximum oil yield and desired fatty acid composition for specific downstream applications. However, there are still limited studies to improve oil-producing strains via genetic engineering and elucidate the regulation of fatty acid biosynthesis pathway at the genomic level. Therefore, this study demonstrated the genetic modification of *Chlorella vulgaris* by targeting the omega-3 desaturase gene, the translated product of which is responsible for the desaturation of linoleic acid (C18:2) to α-linolenic acid (C18:3n3). A copy of the disrupted omega-3 fatty acid desaturase (*ω-3 FAD*) gene vector cassette was transformed into the *C. vulgaris* UMT-M1 genome through *Agrobacterium*-mediated transformation and the transgene effect on fatty acid

biosynthesis pathway was assessed. As the outcome, two stable transgenic lines (C28 and C30) with active expression of hygromycin resistance gene (*hpt*II) and the *mgfp5:gusA* reporter gene (translational fusion of modified green fluorescent protein gene and beta-glucuronidase gene) were successfully recovered for further characterization. The molecular analysis of both transgenic lines revealed that the expression of endogenous *ω-3 FAD* gene was temporarily suppressed at the early stage of sub-culture and subsequently reactivated after about six months of alternate sub-culturing. Furthermore, the *ω-3 FAD* expression in both transgenic lines was upregulated more than 3-fold without notable changes in C18:3n3 composition (5.5 - 5.9 % of total oil content) when cultured under nitrate-deficient medium, after about one year of alternate sub-culturing. However, a significant shift in fatty acid saturation profile towards the production of higher C16:0 coupled with a reduction in C18:1 proportion was observed in the transgenic lines. Data from this and other studies suggest that maintaining a small pool of C18:3n3 is vital to the viability of this microalga species. In addition, PCR walking confirmed the entire T-DNA region and vector backbone were randomly integrated into the host genome possibly at the left-flank (LF) sequence likely through microhomology-dependent integration model. It was postulated that post-transcriptional gene silencing (PTGS) and feedback regulation pathways could be the regulatory mechanisms involved in the observed phenomenon of temporary suppression and subsequent reactivation of the endogenous *ω-3 FAD* gene in transgenic lines. These findings provide significant insights in understanding the regulation of fatty acid biosynthesis pathway in microalgae, the knowledge of which will help in future genetic engineering of fatty acid biosynthesis pathway in microalgae. Moreover, the increment of the accumulation of saturated fatty

acids in transgenic microalgae obtained from this study could serve as alternative for biodiesel production.

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PENCIRIAN DAN PENILAIAN MOLEKUL TAPAK JALAN BIOSINTESIS ASID LEMAK DALAM TRANSGENIK *CHLORELLA VULGARIS* YANG DITRANSFORM DENGAN GEN OMEGA-3 DESATURASE KASET VEKTOR

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Mikroalgae merupakan salah satu organisma yang berpotensi dalam penghasilan minyak kerana berkemampuan dalam pengumpulan kandungan minyak yang melebihi 20 % daripada keberatan sel keringnya. Sejak kebelakangan ini, kegunaan mikroalga dalam penghasilan minyak telah dikaji secara intensif dan lebih menumpukan kepada manipulasi keadaaan kultur, supaya dapat mencapai penghasilan minyak maksimum dan komposisi asid lemak yang dikehendaki untuk penggunaan bidang industri tertentu. Bagaimanapun, hanya terdapat bilangan kajian yang terhad menumpukan penambahbaikan strain yang menghasilkan minyak dengan kaedah kejuruteraan genetik, serta menjelaskan proses pengawalaturan dalam laluan biosintesis asik lemak pada peringkat genomik. Demikian, kajian ini berdemonstrasikan modifikasi genetik *C. vulgaris* dengan menyasarkan omega-3 desaturas yang bertanggungjawab dalam pertukaran asid linoleik (C18:2) ke asid α-linolenik (C18:3n3). Satu salinan gene

omega-3 asid lemak desaturase yang tergendala telah diselitkan ke dalam genom *C. vulgaris* dan kesan transgen atas laluan biosintesis asik lemak telah dinilai. Hasilnya, dua mikroalga transgenik yang stabil (C28 and C30) dengan aktif pengekspresan gen penyeleksi hygromycin (*hpt*II) dan gen fusion (*mgfp5:gusA)* yang terdiri daripada gene protein berpendar hijau dan gen beta-glucuronidase telah berjaya diperolehi untuk penyifatan selanjutnya. Analisis secara molekul ke atas kedua-dua transgenik mendedahkan bahawa pengekspresan endogenus *ω-3 FAD* gen dalam transgenik telah disekat sementara pada subkultur peringkat awal dan diaktifkan semula selepas lebih kurang enam bulan subkultur berselang. Tambahan pula, pengekspresan *ω-3 FAD* gen dalam kedua-dua transgenik didapati meningkat sebanyak tiga kali berganda tanpa sebarang perubahan ketara dalam komposisi C18:3n3 (5.5 - 5.9 % daripada jumlan kandungan minyak) apabila dikulturkan dalam keadaan kekurangan nitrat, selepas lebih kurang setahun subkultur berselang. Namun begitu, terdapat satu anjakan berkesan di dalam profil tepuan asid lemak bercenderung kepada peningkatan penghasilan C16:0, berserta dengan pengurangan dalam penghasilan C18:1 dalam kedua-dua transgenik. Data sebelum dan terkini menunjukkan bahawa mengekalkan kandungan C18:3n3 pada paras tertentu adalah penting untuk kebolehhidupan spesis mikroalga ini. Di samping itu, experiment PCR-berjalan mengesahkan bahawa kesemua kawasan T-DNA dan tulang belakang vektor telah diselit bersama secara rawak ke dalam genom hos, berkemungkinan di urutan left-flank (LF) melalui microhomology-dependent model. Hasil kajian ini mempostulatkan bahawa mekanisme post-transkripsi gen silencing (PTGS) dan suap balik dalam pengawalaturan laluan mungkin terlibat dalam fenomenon sekatan sementara dan pengaktifan semula endogenus *ω-3 FAD* gen dalam transgenic, seperti yang diperolehi dalam kajian ini. Hasil kajian ini membekalkan wawasan dalam memahami proses

pengawalaturan dalam laluan biosintesis asid lemak di dalam mikroalga, dengan secara tidak langsung menyumbang kepada penambahbaikan dalam kejuruteraan genetik laluan biosintesis asid lemak dalam mikroalga di masa depan. Tambahan, peningkatan dalam penghasilan asid lemak tepu oleh mikroalga transgenik yang diperolehi dalam kajian ini juga boleh digunakan sebagai alternatif dalam penghasilan biodiesel.