ELECTROPORATION OF *Chlorella* sp. WITH pBSS-AP LINEARIZED CONSTRUCT

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ELECTROPORATION OF *Chlorella sp.* WITH p35S-AP LINEARIZED CONSTRUCT

By

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Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk: **ELECTROPORATION OF** *Chlorella sp.* **WITH 35S-AP LINEARIZED CONSTRUCT** oleh **NORBAITI BINTI MOHD ISA,** no. matrik: **UK10375** telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Biologi sebagai memenuhi sebahagian daripada keperluan memperolehi Ijazah Sarjana Muda Sains (Sains Biologi), Fakulti Sains dan Teknologi, Universiti Malaysia Terengganu.

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LIST OF ABBREVIATIONS

ACP	Acyl Carrier Protein
AP	Antisense Palmitoyl-ACP Thioesterase
bp	Base Pair
cDNA	Complementary Deoxyribonucleic Acid
CoA	Coenzyme A
DNA	Deoxyribonucleic Acid
dNTP	Deoxyribonucleic Triphosphate
EDTA	Ethylene Diamide Tetra-Acetate
g	Gram
L	Liter
LB	Luria Bertani
M	Molar
$MgCl_2$	Magnesium Chloride
mL	Mililiter
µg	Microgram
µL	Microliter
OD	Optical Density
TAE	Tris-Acetate-EDTA

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ABSTRACT

Electroporation has been used in plants and microalgae genetic engineering to produce beneficial transgenic plants and microalgae. Genetic transformation by electroporation increase the level of polyunsaturated fatty acids in *Chlorella* sp. such as ω-3 C18:3 and ω-6 C18:2. The objectives of this study are to transform *Chlorella* sp. with p35S-AP construct by electroporatian and to select for the putative recombinant chlorella cells. The extracted plasmid DNA of p35S-AP was verified using PCR technique. In this step, three types of primer combination were used as follows: 35S-F/35S-R, PTE-VF1/PTE-VR2 and 35S-F/PTE-VF1. The 35S-F/35S-R primer combination successfully amplified the CaMV 35S promoter with the size of 326 bp. The combination of PTE-VF1/PTE-VR2 primer successfully amplified the antisense palmitoyl-ACP thioesterase gene fragment with the size of 617 bp while 35S-F/PTE-VF1 successfully amplified the CaMV 35S promoter and antisense palmitoyl-ACP thioesterase gene fragment with the size of 934 bp. The DNA plasmid was then digested with *Eco*R1 restriction enzyme to produce linear plasmid followed by DNA plasmid purification. For the determination of suitable voltage for electroporation of *Chlorella* sp. with p35S-AP plasmid, six parameters were used. They are Sc2, Ec1, ShS, Agr, Ec2 and Ec3. The result showed that Agr programme (2.2kV) was suitable for electroporation of *Chlorella* sp. with p35S-AP construct. The culture of transformed *Chlorella* sp. cells on BBM agar plate with 10μg/mL hygromycin showed positive growth. *Chlorella* sp. was successfully transformed with p35S-AP linear plasmid by electroporation. The putative recombinant *Chlorella* sp. was randomly selected and transferred from primary BBM 10 μg/ml hygromycin plate to the new BBM hygromycin (10 μg/ml) grid plate. The transgenic chlorella will be used in the further genetic transformation study.

ELEKTROPORASI *CHLORELLA* SP. DENGAN KONSTRAK SELARI p35S-AP

ABSTRAK

Elektroporasi telah digunakan dalam kejuruteraan tumbuhan dan mikroalga untuk menghasilkan tumbuhan dan mikroalga transgenik yang berfaedah. Penukaran genetik meningkatkan tahap kandungan asid lemak tak tepu di dalam *Chlorella* sp. seperti ω-3 C18:3 and ω-6 C18:2. Objektif kajian ini adalah untuk menukar *Chlorella* sp. bersama konstrak selari p35S-AP dengan kaedah elektroporasi dan juga untuk memilih sel chlorella yang dijangka rekombinan. Plasmid DNA p35S-AP yang diekstrak telah disahkan dengan menggunakan kaedah PCR. Dalam langkah ini, tiga jenis kombinasi primer telah digunakan seperti berikut: 35S-F/35S-R, PTE-VF1/PTE-VR2 dan 35S-F/PTE-VF1. Kombinasi primer 35S-F/35S-R berjaya mengamplifikasi promoter 35S CaMV dengan saiz 326 bp. Kombinasi primer PTE-VF1/PTE-VR2 mengamplifikasi serpihaan gen antisense palmitoyl-ACP cDNA dengan saiz 617 bp manakala VF1/PTE-VR2 berjaya mengamplifikasi promoter 35S CaMV dan serpihaan gen antisense palmitoyl-ACP cDNA bersaiz 934 bp. Plasmid DNA p35S-AP kemudiannya dicernakan dengan menggunakan enzim *Eco*R1 bagi mendapatkan plasmid DNA yang selari dan ini diikuti oleh pembersihan plasmid DNA. Bagi menentukan voltage yang sesuai bagi elektroporasi *Chlorella* sp. bersama bersama konstrak p35S-AP, enam parameter telah digunakan. Ia adalah Sc2, Ec1, ShS, Agr, Ec2 and Ec3. Keputusan menunjukkan bahawa Agr (2.2 kV) adalah sesuai bagi elektroporasi *Chlorella* sp. bersama konstrak p35S-AP. Kultur chlorella yang telah ditukar pada pinggan agar BBM dengan 10μg/mL hygromycin menunjukkan pertumbuhan positif. *Chlorella* sp. berjaya ditukar bersama plasmid selari p35S-AP menggunakan elektroporasi. *Chlorella* sp. yang dijangka rekombinan telah dipilih secara rawak dan dipindahkan dari pinggan agar BBM 10 μg/ml hygromycin ke pinggan agar BBM hygromycin (10μg/ml) yang baru. Chlorella yang telah diubah genetiknya ini akan digunakan dalam kajian transfromasi genetik seterusnya