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FAKULTI SAINS DAN TEKNOLOGI
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA
2006

ELECTROTRANSFORMATION OF *AGROBACTERIUM TUMEFACIENS* WITH p35S-AP AND pCAMBIA 1304 CONSTRUCTS

By

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PENGAKUAN DAN PENGESAHAN LAPORAN PROJEK PENYELIDIKAN I DAN II

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk: ELECTROTRANSFORMATION OF *AGROBACTERIUM TUMEFACIENS* WITH P35S-AP AND PCAMBIA 1304 CONSTRUCTS oleh Tan Lay Kim, no. matrik: UK 7863 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, Kolej Universiti Sains dan Teknologi Malaysia.

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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	Lurie Bertani
M	Molar
$MgCl_2$	Magnesium Chloride
mL	Mililiter
µg	Microgram
µL	Microliter
OD	Optical Density
TAE	Tris-Acetate-EDTA
YEM	Yeast-Extract Mannitol

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ABSTRACT

Agrobacterium-mediated gene transfer is an important genetic transformation tool that has been used in plant genetic engineering to generate a wide variety of fertile transgenic plants. The p35S-AP construct carries antisense palmitoyl-ACP thioesterase cDNA driven by CaMV 35S promoter while the pCAMBIA 1304 construct carries genes encoding β-glucuronidase (*uidA*) and green fluorescent protein (*gfp*). Both constructs were successfully electroporated into wild type *A. tumefaciens* strain LBA 4404 using electroporation apparatus, MicroPulser. In order to confirm the insertion of the constructs into wild-type *A. tumefaciens*, plasmid DNAs were extracted from five randomly selected putative single colonies of transformant Agro-35SAP and Agro-1304. Agarose gel (1%) electrophoresis of the extracted plasmids showed the presence of both constructs from all the five putative single colonies of Agro-35SAP and Agro-1304. This result indicates the plasmids had been successfully transformed into *A. tumefaciens*. Polymerase Chain Reaction (PCR) techniques was utilized to further confirm the successfully transformation of p35S-AP construct into *A. tumefaciens*. Primer combination of PTE-VF1 and PTE-VR2 successfully amplified the 617 bp of antisense palmitoyl-ACP thioesterase cDNA from the five extracted plasmid DNAs from Agro-35SAP. The transformant of Agro-35SAP and Agro-1304 will be further used to transform *Chlorella* sp. in the future study.

ELECTROTRANSFORMASI *AGROBACTERIUM TUMEFACIENS* MENGGUNAKAN KONSTRUK p35S-AP DAN pCAMBIA 1304

ABSTRAK

Transformasi genetik perantaraan *Agrobacterium tumefaciens* merupakan satu kaedah kejuruteraan genetik tumbuhan yang penting dalam penghasilan pelbagai tumbuhan transgenik yang subur. Konstruk p35S-AP membawa antisense cDNA palmitol-ACP thioesterease yang dikawal oleh promoter CaMV 35S manakala konstruk pCAMBIA 1304 membawa gen-gen yang mengkodkan β-glucuronidase (*uidA*) dan green fluorescent protein (*gfp*). Kedua-dua konstruk ini berjaya dielektroporasi ke dalam *A. tumefaciens* strain LBA 4404 jenis liar dengan menggunakan peralatan elektroporasi, MicroPulser. Plasmid DNA berjaya diekstrak daripada lima koloni bakteria yang dipilih secara rawak daripada Agro-35SAP dan Agro-1304. Elektroforesis (1%) agarose gel plasmid DNA yang diekstrak menunjukkan kehadiran kedua-dua konstruk dalam semua koloni yang dipilih. Keputusan ini menunjukkan konstruk p35S-AP dan pCAMBIA 1304 telah ditransfomasi ke dalam *A. tumefaciens*. Kaedah Rantai Bertindakbalas Polimerase (PCR) berjaya mengesahkan kehadiran serpihan yang bersaiz 617 bp antisense palmitol-ACP thioesterase dalam plasmid p35S-AP yang berjaya diekstrak dari kelima-lima koloni Agro-35SAP dengan menggunakan kombinasi primer PTE-VF1 dan PTE-VR2. Transformant Agro-35SAP dan Agro-1304 akan digunakan untuk transformasi *Chlorella* sp. dalam kajian yang selanjutnya.