

IDENTIFICATION OF *AGROBACTERIUM*
TUMEFACIENS WITH β -55S-AB AND
 β -CAMPT-18 ANTISERUMS

DEVI LIAWATI

FAKULTI SAINS DAN TEKNOLOGI
MOLTA UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA
2006

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

By

Tan Lay Kim

Research Report submitted in partial fulfillment of
the requirements for the degree of
Bachelor of Science (Biological Sciences)

Department of Biological Sciences
Faculty of Science and Technology
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA
2006

This Project should be cited as:

Tan, L. K. 2006. Electrotransformation of *Agrobacterium tumefaciens* with p35S-AP and pCAMBIA 1304 constructs. Undergraduate thesis, Bachelor of Science in Biological Sciences, Faculty of Science and Technology, Kolej Universiti Sains dan Teknologi Malaysia, Terengganu. 64 p.

No part of this project report may be produced by any mechanical, photographic, or electronic process, or in the form of phonographic recording, nor may it be stored in a retrieval system, transmitted, or otherwise copied for public or private use, without written permission from the author and supervisor(s) of this project.



**JABATAN SAINS BIOLOGI
FAKULTI SAINS DAN TEKNOLOGI
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA**

**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 pembedaan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Biologi sebagai memenuhi sebahagian daripada keperluan memperoleh ijazah Sarjana Muda Sains (Sains Biologi), Fakulti Sains dan Teknologi, Kolej Universiti Sains dan Teknologi Malaysia.

Disahkan oleh:

.....
Penyelia Utama

Nama: Dr. Cha Thye San

Cop Rasmi: **DR. CHA THYE SAN**
Pensyarah
Jabatan Sains Biologi
Fakulti Sains dan Teknologi
Kolej Universiti Sains dan Teknologi Malaysia
(KUSTEM)
21030 Kuala Terengganu.

Tarikh: 7/5/2006

.....
Penyelia Kedua (jika ada)

Nama:

Cop Rasmi

Tarikh:


.....
Ketua Jabatan Sains Biologi

Nama: Prof. Madya Dr. Nakisah Mat Amin

Cop Rasmi: **PROF. MADYA DR. NAKISAH BT. MAT AMIN**
Ketua
Jabatan Sains Biologi
Fakulti Sains dan Teknologi
Kolej Universiti Sains dan Teknologi Malaysia
(KUSTEM)
21030 Kuala Terengganu.

Tarikh: 7/5/2006

ACKNOWLEDGEMENT

First and foremost, I would like to give my complete gratitude to my God for granting me wisdom and understanding. With His blessings, I was granted with spiritual strength to fully accomplish my thesis. By His grace, my project was able to accomplish in time.

I would like to give my sincere thankfulness to my supervisor, Dr. Cha Thye San. Dr. Cha was patient and professionalism enough to help me to make the right decision when my project started to run. His scientific excitement inspired and encouraged me to involve in this molecular biology field.

I would like to thank my family members for their moral and financial supports. With their full supports, I have been able to travel to Kuala Terengganu and study at higher academic level. I sincerely thank my friends and lecturers for their helps in giving ideas and moral supports.

Indeed, I would like to express my gratitude to Biological Science Department and KUSTEM for the permission to use the lab and all the facilities in order to accomplish my project.

Last but not least, I would like to thank all the lab assistances for their guidance and cooperation during the laboratory work.

CONTENTS

	Page
ACKNOWLEDGEMENTS	ii
LIST OF TABLES	vi
LIST OF FIGURES	vii
LIST OF ABBREVIATIONS	viii
LIST OF APPENDICES	ix
ABSTRACT	x
ABSTRAK	xi
CHAPTER 1 INTRODUCTION	
1.1 Introduction	1
1.2 Importance of Study	2
1.3 Objectives of Study	3
CHAPTER 2 LITERATURE REVIEW	
2.1 Introduction to <i>Agrobacterium tumefaciens</i>	4
2.2 <i>Agrobacterium</i> -Plant Cell Interactions	8
2.3 Gene Transfer	10
2.3.1 Gene Transfer in Bacteria	10
2.3.2 Gene Transfer in Plants	10
2.4 Electrotransformation	12
2.5 <i>Agrobacterium</i> -mediated Gene Transfer In Microalgae	14
2.6 Fatty Acid Synthesis in Plants	14

2.7	p35S-AP and pCAMBIA 1304 Constructs	17
-----	-------------------------------------	----

CHAPTER 3 METHODOLOGY

3.1	Materials	22
3.1.1	Source of Samples	22
3.1.2	Chemicals	22
3.1.3	Enzymes	23
3.1.4	Antibiotics	23
3.1.5	Apparatus	23
3.2	Methodology	24
3.2.1	<i>Agrobacterium tumefaciens</i> culture	24
3.2.2	Plasmid DNA Extractions of p35S-AP and pCAMBIA 1304 from <i>Escherichia coli</i> Using Alkaline Lysis Miniprep	24
3.2.3	Electroporation of <i>Agrobacterium tumefaciens</i> with p35S-AP and pCAMBIA 1304 Constructs	25
3.2.3a	Preparation of Electrocompetent Cells	25
3.2.3b	Electroporation	26
3.2.4	Plasmid DNA Extraction of Putative Transformed Agro-35SAP and Agro-1304	27
3.2.5	Screening for Putative Transformed Agro-35SAP with PCR Techniques	27

CHAPTER 4 RESULTS

4.1	<i>Agrobacterium tumefaciens</i> Culture	29
-----	--	----

4.2	Plasmid DNA Extractions of p35S-AP and pCAMBIA 1304 from <i>E. coli</i>	31
4.3	Electrotransformation of <i>Agrobacterium tumefaciens</i> with p35-AP and pCAMBIA 1304	35
4.4	Plasmid Extraction of Agro-35S and Agro-1304-Alkaline Lyses Miniprep	38
4.5	Screening for positive transformant of Agro-35SAP with PCR tool	42
CHAPTER 5 DISCUSSION		44
CHAPTER 6 CONCLUSION		50
REFERENCES		51
APPENDICES		55
A.	Culturing Mediums	56
B.	Buffer Solution	62
C.	Plasmid DNA's Map	63
CURRICULUM VITAE		64

LIST OF TABLES

Table		Page
2.1	The Taxonomy of <i>Agrobacterium tumefaciens</i>	7
4.1	The Purity and Quantity of Extracted Plasmid DNA from <i>E. coli</i> containing p35S-AP and pCAMBIA 1304 Constructs	33
4.2	The Purity and Quantity of Extracted Plasmid DNA from Agro-35SAP and Agro-1304 Cultures	40

LIST OF FIGURES

Figure		Page
2.1	Basic Steps in <i>Agrobacterium</i> -Plant Cells Interactions	9
2.2	Fatty Acid Biosynthesis Pathway (Ohlrogge, 1994)	16
2.3	The T-DNA Map of p35S-AP (Cha, 2001)	18
2.4	The T-DNA Map of Pcambia 1304 (Kumar <i>et al.</i> , 2004)	20
4.1	<i>Agrobacterium tumefaciens</i> Cultures	30
4.2	<i>Escherichia coli</i> Cultures	32
4.3	Agarose Gel (1%) Electrophoresis of Extracted Plasmid DNAs from <i>E. coli</i> containing p35S-AP and pCAMBIA 1304 Constructs	34
4.4	Primary YEM-agar plates containing Agro-35SAP and Agro-1304 colonies	36
4.5	Grid YEM-agar plates containing Agro-35SAP and Agro-1304 colonies	37
4.6	Agro-35SAP and Agro-1304 Cultures in YEM-liquid medium	39
4.7	Agarose Gel (1%) Electrophoresis of Extracted Plasmid DNAs from Agro-35SAP and Agro-1304 Cultures	41
4.8	Agarose Gel (1.2%) Electrophoresis shows the presence of plasmid p35S-AP in Agro-35SAP obtained from PCR reaction	43
5.1	Primer combination of PTE-VF1 and PTE-VR2 to amplify antisense palmitoyl-ACP thioesterase	48

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

LIST OF APPENDICES

	Page
A.1 LB Medium (Luria-Bertani Medium)	58
A.2 YEM Medium (Yeast-Extract Medium)	59
A.3 LB Kanamycin Agar Medium	60
A.4 YEM Rifampicin Agar Medium	61
A.5 YEM Kanamycin Agar Medium	62
A.6 SOC Medium	63
B.1 TAE Buffer	64
C.1 Plasmid DNA's Map	65

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* MENGUNAKAN 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 thioesterase 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 ditransformasi 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.