MOLECULAR CLONING OF

TWO VIRULENCE GENES FROM

Agrobacterium tumefaciens

By

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A project report submitted to Department of Biological Science

Faculty of Science

Universiti Tunku Abdul Rahman

In partial fulfillment of the requirements for the degree of

Bachelor of Science (HONS) Biotechnology

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ABSTRACT

MOLECULAR CLONING OF TWO VIRULENCE GENES FROM *Agrobacterium tumefaciens*

GOH HUI TING

Agrobacterium tumefaciens causes tumour formation, known as crown gall, in plants. This is due to the ability of the bacterium in transferring a portion, known as T-DNA, from the tumour-inducing (Ti) plasmid, to the host cell nucleus. Hence, *A. tumefaciens* has been widely used as a genetic transformation tool. The virulence (*vir*) genes encode the Vir proteins, which are responsible for the translocation of the T-DNA. VirE2 and VirD2 proteins are involved in the cleavage of T-DNA from the Ti plasmid and translocation of the T-DNA-protein complex to the host cell nucleus. Therefore, the VirE2 and VirD2 proteins play key roles in determining the efficiency of the genetic transformation process. The main objective of this study is to separately clone *vir*D2 and a non-translational fusion *vir*D2 and *vir*E2 genes into an expression vector pING2, thereby increasing the efficiency of the *Agrobacterium*-mediated transformation by increasing the expression of the key virulence genes. The *vir*D2 gene was successfully subcloned from the cloning vector into the *Agrobacterium* expression vector pING2. The *vir*E2-*vir*D2 fusion gene construct contains an intervening ribosomal-binding site

(RBS), to facilitate efficient translation of *vir*D2. For generating the fusion gene construct, first, the two *vir* genes were separately amplified by polymerase chain reaction (PCR). The RBS sequence in the PCR-amplified *vir*D2 was generated by including the RBS sequence in the 5' primer. A complementary sequence was inserted to the 3' end of *vir*E2 and 5' end of *vir*D2 to facilitate the fusion of PCR-amplified *vir*E2 and *vir*D2 fragments. The Gibson assembly method produced the desired fusion gene construct DNA fragment, but the yield was insufficient for further cloning experiment. This fusion fragment requires further amplification. For future studies, the expression vector pING2 carrying *vir*E2, *vir*D2 and *vir*E2-*vir*D2 will be transferred into *A. tumefaciens,* where their effects on *Agrobacterium*-mediated transformation efficiency can be evaluated.

ACKNOWLEDGEMENTS

There were several difficulties faced while I was doing this project. However, I received the help from many people. First of all, I would like to express my gratitude to my project supervisor, Dr. Wong Hann Ling for his patience, guidance and encouragement thoughout this project. I would not able to complete my project without his advice. Furthermore, I would also like to convey my appreciation towards Mr. Toh Wai Keat from postgraduate lab for his efforts to help me in solving problems. Last but not least, I would like to thank all my lab mates who always offered me with their help whenever they could. I enjoyed the company of my lab mates and appreciate the great time we have been together.

DECLARATION

I hereby declare that the project is based on my original work except for quotations and citations which have been duty acknowledged. I also declare that it has not been previously or oncurrently submitted for any other degree at UTAR or other institutions.

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GOH HUI TING

APPROVAL SHEET

This project report entitled **"MOLECULAR CLONING OF TWO VIRULENCE GENES FROM *Agrobacterium tumefaciens*"** was prepared by GOH HUI TING and submitted as partial fulfillment of the requirements for the degree of Bachelor of Science (HONS) Biotechnology at Universiti Tunku Abdul Rahman.

Approved by:

_____

(Assoc. Prof. Dr. WONG HANN LING) Date:……..

Supervisor

Department of Biological Science

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FACULTY OF SCIENCE

UNIVERSITI TUNKU ABDUL RAHMAN

Date: _____

PERMISSION SHEET

It is hereby certified that **GOH HUI TING** (ID: **11ADB04063**) has completed this final year project entitled "MOLECULAR CLONING OF TWO VIRULENCE GENES FROM *Agrobacterium tumefaciens*" supervised by Assoc. Prof. Dr. Wong Hann Ling from the Department of Biological Science, Faculty of Science.

I hereby give permission to the University to upload the softcopy of my final year project in pdf format into the UTAR Institutional Repository, which may be made accessible to the UTAR community and public.

Yours truly,

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(GOH HUI TING)

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