



***ANTI-QUORUM SENSING ACTIVITIES OF SELECTED SOIL BACTERIA  
AND CHINESE HERBS AND EFFICIENCY OF  
ELECTROTRANSFORMATION BY COSMID PLAFR1 VECTOR***

**LOKE WAI KEONG**

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**By**

**LOKE WAI KEONG**

**Thesis Submitted to the School of Graduate Studies, Universiti  
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Doctor of Philosophy**

**February 2020**

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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**February 2020**

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**Faculty : Agriculture**

*Chromobacterium violaceum* is a pathogenic bacterium to human and distinctly characterized by the production of a purple pigment called violacein. They are commonly found in soil and water in tropical and subtropical areas. From this study, *C. violaceum* was found in 0-5 cm depth from the soil surface in Universiti Putra Malaysia golf course and football field with higher density after rainy day. *Chromobacterium violaceum* also show the characteristics of oligophile, resistant to chlorinated water and antimicrobial activity which allow growing in wider soil and water areas. In both methods (Interaction and Non-interaction), *C. violaceum* reached quorum level produced antibiotics and inhibited all the selected plant growth-promoting rhizobacteria (PGPR) which were (*Azospirillum brasilense* Sp7, *Rhizobium* UPMR1102 and *Bacillus sphaericus* UPMB10) but did not inhibit the selected PGPR in concentration below their quorum level. These incidences indicate the ubiquitous presence of *C. violaceum* in Malaysia soils. It would contaminate in the daily use of soil and has potential threats to agriculture sector. One of the bacteria (*Bacillus subtilis*) randomly isolated from soil was screened based on selective media HiCrome Bacillus Agar. *Bacillus subtilis* showed the capability of resistancy and anti-quorum sensing activity to *C. violaceum* wild type and mutant type CV026 by inhibiting the production of violacein and resistant to antibiotics produced by *C. violaceum*. However, screening the genes of interest by pCC1FOS fosmid vector found that none of the clones contained the characteristics desired from the *B. subtilis* and there were many factors causing the failure of screening in this research. Gene expression from heterologous in new host was facing many limitations and challenges in recent years. Traditional chinese herbs were proven of their anti-quorum sensing activities. Six selected traditional chinese herbs were screened for anti-quorum sensing activity by using *C. violaceum* as biomonitor. Two out of these herbs were found to exhibit anti-quorum sensing properties: *Lycium barbarum* and *Zingiber officinale*. Extracts

from *L. barbarum* has stronger anti-quorum sensing activity than *Z. officinale*. Colonies of *C. violaceum* treated with *L. barbarum* almost fully loss its purple pigment. The loss and lack of purple colour from the colonies of *C. violaceum* indicated that quorum sensing activity was inhibited by the herbal extraction. It was believed that these herbs contained a rich source of compounds to fight or control pathogenic bacteria and potentially a new therapeutic way to reduce the development of antibiotic resistance. In electrotransformation, a large size of cosmid vector was successfully transferred into the selected PGPR and *C. violaceum* by using electroporation. These methods were found to be more efficient by using lower field strength with longer pulse length. The cosmid vector from donor *E. coli* K12 MM294 was also a success by direct transferred to the recipients *A. brasilense* Sp7 and *C. violaceum* by electroduction.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia  
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**AKTIVITI ANTI-PENDERIAAN KUORUM DARI BAKTERIA TANAH DAN  
TUMBUHAN HERBA CINA YANG TERPILIH DAN KECEKAPAN  
ELEKTROTRANSFORMASI OLEH VEKTOR KOSMID PLAFR1**

Oleh

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*Chromobacterium violaceum* adalah bakteria patogenik kepada manusia dengan ciri-ciri penghasilan pigmen ungu yang dipanggil violacein. Ia berhabitat di dalam tanah dan air yang banyak dijumpai kawasan tropika dan sub-tropika. Dalam kajian ini, *C. violaceum* telah berjaya diasingkan dari kedalaman 0-5 cm dari permukaan tanah padang golf dan bola sepak Universiti Putra Malaysia. Ia juga menunjukkan ciri-ciri seperti oligofil, rintangan terhadap air klorin dan aktiviti antimikrob membenarkannya tumbuh di kawasan tanah dan air yang lebih luas. Dalam kedua-dua kaedah (Interaksi dan Bukan interaksi), *C. violaceum* yang mencapai tahap kuorum menghasilkan antibiotik dan merencatkan semua rhizobakteria penggalak tumbesaran tumbuhan (PGPR) terpilih iaitu (*Azospirillum brasilense* Sp7, *Rhizobium* UPMR1102 and *Bacillus sphaericus* UPMB10) tetapi tidak merencatkannya apabila konsentrasi dibawah tahap kuorumnya. Ini menunjukkan kewujudan *C. violaceum* di tanah Malaysia. Ia akan mencemarkan penggunaan tanah kita setiap hari dan berpotensi mengancam sektor pertanian. Salah satu bakteria (*Bacillus subtilis*) dapat diasingkan secara rawak dari tanah melalui kaedah selektif media HiCrome Bacillus Agar. *Bacillus subtilis* menunjukkan keupayaan daya tahan dan aktiviti anti-penderiaan kuorum kepada *C. violaceum* jenis liar dan jenis mutan CV026 melalui perencatan produksi violacein dan rintangan terhadap antibiotik yg dihasilkan oleh *C. violaceum*. Walaubagaimanapun, saringan gen keperluan melalui vektor fosmid pCC1FOS didapati tiada klon mengandungi ciri-ciri yang diperlukan dari *B. subtilis* dan ia didapati banyak factor yang menyebabkan kegagalan saringan di dalam kajian ini. Gen ekspresi dari hos baru menghadapi banyak had dan cabaran dalam beberapa tahun kebelakangan ini. Herba tradisional cina terbukti mempunyai aktiviti anti-penderiaan kuorum. Enam herba tradisional cina terpilih diperiksa untuk aktiviti anti-penderiaan kuorum dengan menggunakan *C. violaceum* sebagai biomonitor. Dua daripada enam herba tradisional cina menunjukkan ciri anti-

penderiaan kuorum iaitu *Lycium barbarum* dan *Zingiber officinale*. Pengekstrakan dari *L. barbarum* mempunyai aktiviti anti-penderiaan kuorum yang lebih kuat daripada *Z. officinale*. Koloni dari biomonitor *C. violaceum* dirawat dengan *L. barbarum* hampir sepenuhnya kehilangan pigmen ungunya. Kehilangan dan kekurangan warna ungu dari koloni *C. violaceum* menunjukkan aktiviti penderiaan kuorum dihalang oleh pengambilan ekstrak herba. Ia dipercayai mengandungi sumber yang kaya dengan sebatian untuk melawan atau mengawal bakteria patogen dan berpotensi sebagai cara terapi baru untuk mengurangkan perkembangan rintangan antibiotik. Dalam elektrotransformasi, vektor kosmid yang bersaiz besar berjaya dipindahkan ke dalam PGPR yang terpilih dan *C. violaceum* dengan menggunakan kaedah elektroporasi. Kaedah ini didapati mempunyai kecekapan yang lebih baik dengan menggunakan kekuatan medan yang lebih rendah dengan kepanjangan pulse elektrik yang lebih panjang. Vektor kosmid dari penderma *Escherichia coli* K12 MM294 juga berjaya secara terus memindah kepada penerima *Azospirillum brasilense* Sp7 dan *C. violaceum* oleh keadah elektroduksi.

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I certify that a Thesis Examination Committee has met on 18 February 2020 to conduct the final examination of Loke Wai Keong on his thesis entitled “Anti-Quorum Sensing Activities of Selected Soil Bacteria and Chinese Herbs and Efficiency of Electrotransformation by Cosmid pLAFR1 Vector” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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## LIST OF ABBREVIATIONS

UPM	Universiti Putra Malaysia
%	Percentage
°C	Degree Celsius
µm	Micrometer
mm	Milimeter
cm	Centimeter
g	Gram
µl	Microliter
ml	Mililiter
ms	Milisecond
kb	Kilobase
µF	Microfarad
Ω	Ohm
kV	Kilovolt
N	Normality
No.	Number
H <sub>2</sub> SO <sub>4</sub>	Sulfuric acid
NaOH	Sodium hydroxide

## CHAPTER 1

### INTRODUCTION

#### 1.1 General

*Chromobacterium violaceum* is a Gram-negative pathogenic bacterium which can be found in most of the tropical country around the world mainly in stagnant water and soil (McGowan and Steinberg, 1995; Kothari *et al.*, 2017; Marcia *et al.*, 2017; Donny *et al.*, 2018). The purple coloured *C. violaceum* was due to the production of violacein which is an antibiotic that is active against amoebae and trypanosomes (Forbes, 2002; Duran *et al.*, 2007; Duran *et al.*, 2016). Other antibiotics produced by *C. violaceum* were show on Table 1.1.

**Table 1.1: Production of antibiotics by *Chromobacterium violaceum***

Antibiotic	Active against
Aerocyanidine	Gram-positive bacteria
3,6-dihydroxyin doxazene	Gram-negative bacteria
6-hydroxy-3-oxo-1,2-benzisoxazolin	Gram-negative bacteria
Aerocavin	Gram-negative and Gram-positive bacteria

(Source: Nelson and Carlos, 2001)

*Chromobacterium violaceum* also produce hydrogen cyanide (HCN) which was reported negatively affect the plant growth and development (Lambers, 1980; Alstrom *et al.*, 1989; Schippers *et al.*, 1990). The production of antibiotics and HCN from *C. violaceum* was related to the quorum sensing system. Quorum sensing is a bacteria communication system using signal molecules and receptors to control the gene expression (Miller and Bassler, 2001; Lee *et al.*, 2013). This system also controls virulence factors, motility, biofilm formation and toxin production in most pathogenic bacteria including *C. violaceum* (Fuqua and Greenberg, 1998; De Kievit and Iglewski, 2000; Donabedian, 2003).

## 1.2 Statement of problem

The tropical weather in Malaysia offers a good environment for the growth of *C. violaceum*. The bacterium is commonly found in Malaysia soil. Soil is often contacted by people during their daily activities and also important in agriculture. However, the understanding of pathogenicity caused by *C. violaceum* was not fully discovered and the effects to the agriculture are still unknown especially to beneficial microbes and plantation crop. The quorum sensing that controlled the virulence factors and biofilm formation also causing danger to human livings where more than 80% of bacterial infections were caused by the formation of biofilm and also increase the resistance ability of bacteria against antibiotics after biofilm development (Costerton *et al.*, 1999; Kothari *et al.*, 2017).

Some of the characteristics found in soil-borne bacteria had the potential to overcome the problems caused by quorum sensing (Lee *et al.*, 2002; Dong *et al.*, 2004). These characters would be very useful if able to apply, transfer and combine into a single bacteria strain. Genetic engineering is considered one of the most promising ways to combine few useful characters from different strains of bacteria by inserted few interest genes into a single bacterium (Babu-Khan *et al.*, 1995; Amarger, 2002). However, screening of the potential bacteria, genes of interest and efficient transformation methods were needed to increase the probability of success in developing genetically-modified bacteria.

## 1.3 Objectives of the study

The overall objective of this study was about to gain an understanding on anti-quorum sensing activities against *C. violaceum* and as a beginning plan for developing a genetically-modified bacteria through isolation, characterization and screening followed by genetic transformation that is related to anti-quorum sensing.

The specific objectives are:

1. To detect *Chromobacterium violaceum* from soil and determine the effects of *C. violaceum* on Gram-negative and Gram-positive bacteria from selected Plant Growth-Promoting Rhizobacteria (PGPR).
  - *C. violaceum* was chosen in this study because of the easy identification purple pigment on the colonies and antibiotics that able to kill both Gram-negative and Gram-positive bacteria which were controlled by quorum sensing system. This objective not only can determine the effectiveness of *C. violaceum* on selected Plant Growth-Promoting Rhizobacteria (PGPR) but those selected PGPR sensitive to *C. violaceum* can be used as a control for anti-QS and resistant ability screening in next objective.
2. To evaluate anti-QS and resistant ability against *C. violaceum* from soil bacteria.

- The bacteria showed anti-QS activity and resistant to *C. violaceum* have a great potential for developing genetically-modified bacteria by isolating the genes of interest that related to the abilities.
3. To detect the genes of interest related to inhibition of violacein production and resistant ability against *C. violaceum* from selected soil bacteria by using fosmid vector pCC1FOS.
    - Gene of interest is the most important source for developing genetically-modified bacteria. It is a must to isolate for genetic transformation and future research.
  4. To investigate the effectiveness of electrotransformation and electroproduction on selected (PGPR) and *C. violaceum* by using cosmid vector pLAFR1.
    - Parameters of electroporation like electric field strength (kV/cm) and pulse length (ms) very important to achieve optimum level during genetic transformation. The gene of interest can be transform into the new host efficiently.
  5. To detect anti-QS activity against *C. violaceum* from selected chinese herbs.
    - Besides bacteria, herbs were reported to have anti-QS activities with potential interest for future study.



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