EFFECT OF CHROMOBACTERIUM VIOLACEUM AND QUORUM SENSING ON THE GROWTH OF GREEN BEAN (VIGNA RADIATA) AND PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR)

LOKE WAI KEONG

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LOKE WAI KEONG

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By

LOKE WAI KEONG

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

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By

LOKE WAI KEONG

December 2011

Chair: Associate Professor Halimi Mohd Saud, PhD

Faculty: Agriculture

Chromobacterium violaceum is a Gram-negative facultative anaerobic bacteria and pathogenic to human with high fatality rate. C. violaceum inhabits in soil and water in the tropical and subtropical regions of the world. The tropical climate in Malaysia offers a suitable environment for the growth of C. violaceum and it is believed that it is highly distributed in the soil and water in Malaysia. Soil is important in agriculture and the soil surface is most often in contact with people during their daily activities. However, the understanding of C. violaceum distribution has not been fully revealed especially in local soils and the effects on agriculture are still unknown. The production of antibiotics and hydrogen cyanide by C. violaceum was suspected as a possible compound that to give a negative impact on crops and beneficial microbes present in the soil. The main objective of this study was to determine the effect of Chromobacterium violaceum on plant-microbe interaction and quorum sensing mechanism. In this study, C. violaceum was isolated from 0-5 cm depth of the soil covered with grass surface in Universiti Putra Malaysia golf course and football
field. We found the density of *C. violaceum* in 5 g soil were higher after a raining period about $1 \times 10^7$ c.f.u. in golf course and $7 \times 10^7$ c.f.u. in football field compared to usual non-rainy day where only $9 \times 10^4$ c.f.u. in golf course and $6 \times 10^5$ c.f.u. in football field. The *C. violaceum* also showed the oligophile characteristic that allowed them to grow in a wider range of soil and water areas. *C. violaceum* which reached quorum level inhibited the growth of green bean seedling as much as 86.5% for the shoot length and 92.1% for the root length. However inhibition was reduced to 37.5% for the shoot and 17.5% for root if the quorum level of *C. violaceum* was not reached under aseptic environment. Furthermore sterilized inoculant (killed) which has not reached quorum level did not affect the growth of green bean seedlings. These indicated that quorum sensing in *Chromobacterium violaceum* was a factor that determines its inhibitory effect on seedling growth. *C. violaceum* also have the same effect on PGPR where *Chromobacterium violaceum* that reached quorum level kill the selected PGPR; *Azospirillum brasilense* Sp7 (Gram negative), *Rhizobium* UPMR1102 (Gram negative) and *Bacillus sphaericus* UPMB10 (Gram positive). This study also showed that catabolite repression occurred in *C. violaceum* and the operon to controlling the genes for production of purple pigment and other antibiotics were catabolite-sensitive when the operon was not maximally expressed. The information about catabolite sensitivity in pathogenic *C. violaceum* can be a very useful tool in future research.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**KESAN CHROMOBACTERIUM VIOLACEUM DAN PENDERIAAN KUORUM KEATAS TUMBESARAN KACANG HIJAU (VIGNA RADIATA) DAN RIZOBAKTERIA PENGGALAK TUMBESARAN (PGPR)**

Oleh

LOKE WAI KEONG

Disember 2011

Pengerusi: Profesor Madya Halimi Mohd Saud, PhD

Fakulti: Pertanian

*Chromobacterium violaceum* adalah bacteria Gram-negatif, anaerobik fakultatif dan patogenik kepada manusia dengan kadar kematian yang tinggi. *C. violaceum* hidup di dalam tanah dan air yang ada di kawasan tropika dan sub tropika. Iklim tropika di Malaysia memberikan persekitaran yang sesuai kepada *Chromobacterium violaceum* dan dipercayai banyak terdapat di dalam persekitaran tanah dan air di Malaysia. Tanah adalah penting dalam pertanian dan permukaan tanah juga merupakan bahagian yang paling kerap disentuh oleh orang ramai semasa kegiatan harian mereka. Kefahaman terhadap sebaran *C. violaceum* masih rendah terutamanya dalam tanah tempatan dan kesan terhadap pertanian masih tidak diketahui. Penghasilan antibiotik dan hidrogen sianida oleh *C. violaceum* dipercayai adalah sebatian yang memberi kesan negatif kepada tanaman dan mikrob yang berfaedah di dalam tanah. Objektif utama kajian ini adalah untuk menentukan kesan *C. violaceum* ke atas interaksi tumbuhan-mikrob dan mekanisme penderiaan korum. Dalam kajian ini, *Chromobacterium violaceum* telah diasingkan dari tanah sedalam 0-5 cm dari
permukaan tanah di padang golf dan bola sepak Universiti Putra Malaysia. Kami mendapati ketumpatan C. violaceum dalam 5 g tanah adalah lebih tinggi selepas hujan kira-kira 1 X 10^7 c.f.u. di dalam padang golf dan 7 X 10^7 c.f.u. di padang bola sepak berbanding hari biasa di mana hanya 9 X 10^4 c.f.u. di padang golf dan 6 X 10^5 c.f.u. di padang bola sepak. C. violaceum juga menunjukkan ciri-ciri oligofil yang membolehkan mereka berkembang dalam pelbagai kawasan tanah dan air. Chromobacterium violaceum yang mencapai tahap korum merencatkan pertumbuhan anak benih kacang hijau sebagai sebanyak 86.5% pada panjang batang dan 92.1% pada panjang akar. Walau bagaimanapun, kadar perencatan menurun kepada 37.5% pada pajang batang dan 17.5% pada panjang akar jika tahap korum C. violaceum tidak dicapai di bawah persekitaran aseptik. C. violaceum yang tidak mencapai tahap korum dan disterilkan tidak menjejaskan pertumbuhan benih kacang hijau. Ini menunjukkan bahawa penderiaan korum dalam C. violaceum adalah satu faktor yang menentukan kesan perencatan pada pertumbuhan anak benih. C. violaceum juga mempunyai kesan yang sama pada PGPR di mana C. violaceum yang mencapai tahap korum boleh membunuh PGPR terpilih; Azospirillum brasilense Sp7 (Gram negative), Rhizobium UPMR1102 (Gram negative) and Bacillus sphaericus UPMB10 (Gram positive). Kajian ini juga menunjukkan “catabolite repression” berlaku pada Chromobacterium violaceum dari operon yang mengawal gen yang menghasilkan pigmen ungu dan antibiotik yang lain adalah sensitif-katabolit dimana operon tersebut tidak akan diekspres sepenuhnya. Maklumat kepekaan katabolit Chromobacterium violaceum adalah sangat berguna dalam kajian masa depan.
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I certify that a Thesis Examination Committee has met on 22 December 2011 to conduct the final examination of Loke Wai Keong on his thesis entitled “Effect Of Chromobacterium violaceum and Quorum Sensing on the Growth of Green Bean (Vigna radiata) and Plant Growth-Promoting Rhizobacteria” 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 Master of Science.

Members of the Examination Committee were as follows:

**Mihdzar b Abdul Kadir, PhD**  
Associate Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Chairman)

**Radziah bt Othman, PhD**  
Associate Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Internal Examiner)

**Kamaruzaman b Sijam, PhD**  
Associate Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Internal Examiner)

**Koshy Philip, PhD**  
Associate Professor  
Faculty of Science  
Universiti Malaya  
(External Examiner)

_______________________________  
NORITAH OMAR, PhD  
Associate Professor and Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date: 17 October 2013
This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

**Halimi Mohd Saud, PhD**  
Associate Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Chairman)

**Raha Abdul Rahim, PhD**  
Professor  
Faculty of Biotechnology and Molecular Science  
Universiti Putra Malaysia  
(Member)

---

**BUJANG BIN KIM HUAT, PhD**  
Professor and Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:
DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

LOKE WAI KEONG

Date: 22 December 2011
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