UNIVERSITI PUTRA MALAYSIA

NOVEL ACTINOBACTERIA DISCOVERY AND BIOSYSTEMATICS OF BACTERIA FROM ANTARCTIC SOILS ON BARRIENTOS ISLAND

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By

LEE LEARN HAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

April 2012

Dedicated to my parents

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

NOVEL ACTINOBACTERIA DISCOVERY AND BIOSYSTEMATICS OF BACTERIA FROM ANTARCTIC SOILS ON BARRIENTOS ISLAND

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Chair: Cheah Yoke Kqueen, PhD

Faculty: Medicine and Health Sciences

The Antarctic, one of the most poorly explored areas on Earth has emerged as a prospective region for the discovery of novel bacteria and bioactive metabolites. A total of 17 soil samples were collected from different areas of Barrientos Island to determine the distribution of bacteria in the soil and the potential of soil bacteria (particularly *Actinobacteria*) in producing bioactive secondary metabolites. The Biolog EcoPlate system and RAPD both demonstrated that soils with different types of rookery and characteristics could affect the soil DNA sequences and soil microbial diversity. The PCR-DGGE used to study the diversity of bacteria, especially *Actinobacteria* successfully displayed the diverse types of bacteria found within the soil and also provided estimations of their densities. A total of 96 bacterial strains were isolated using 12 different types of isolation media, 39 strains from phylum *Actinobacteria* and 57 strains from phyum *Proteobacteria*. Tests for bioactive secondary metabolites were performed and a total of 15 and 14 isolates of *Actinobacteria* and *Proteobacteria* were bioactive. The novel strain, 39^T was fully characterized by using the polyphasic

approach and results showed that it have many differences from the other genera in the family *Dermacoccaceae* on the basis of phylogenetic, chemotaxonomic, phenotypic and signature nucleotide pattern of the 16S rRNA gene. Therefore, it is suggested that strain 39^T be classified as a novel genus in the family *Dermacoccaceae*, as *Barrientosiimonas* gen. nov., the type species of which is *Barrientosiimonas humi* gen. nov., sp. nov. It is evident from this study that the soil of Barrientos Island is a rich source of taxonomically diverse bacteria, especially *Actinobacteria*. The wide distribution of these bacteria in the Antarctic implies that they are endemic and therefore the bio-prospecting of soil bacteria from a wide range of geographic locations like the Antarctic regions would be of great benefit.

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

PENEMUAN ACTINOBACTERIA BARU DAN BIOSISTEMATIKS BAKTERIA DARI TANAH ANTARTIK PADA BARRIENTOS ISLAND

Oleh

LEE LEARN HAN

April 2012

Pengerusi: Cheah Yoke Kqueen, PhD

Fakulti: Perubatan dan Sains Kesihatan

Antartika, salah satu kawasan yang paling kurang diterokai di Bumi telah muncul sebagai rantau yang bakal bagi penemuan bakteria novel dan metabolit bioaktif. Sejumlah 17 sampel tanah telah dikutip dari lokasi berbeza di Barrientos Island untuk menentukan pengagihan bakteria di dalam tanah dan potensi bacteria dari tanah (terutamanya *Actinobacteria*) untuk menghasilkan metabolit sekunder bioaktif. Sistem Biolog Ecoplate dan RAPD kedua-dua kaedah menunjukkan bahawa tanah dengan ciri-ciri yang berlainan mungkin memberi kesan urutan DNA dan kepelbagaian mikrob tanah. PCR-DGGE digunakan untuk mengkaji kepelbagaian bakteria dan berjaya memaparkan pelbagai jenis bakteria yang dijumpai dalam tanah dan juga memberikan anggaran terhadap kepadatan mereka. Sejumlah 96 strain bakteria telah diasingkan dengan menggunakan 12 jenis media pengasingan, 39 strain dari divisi *Actinobacteria* dan 57 strain dari divisi *Proteobacteria*. Ujian untuk bioaktif metabolit sekunder telah dijalankan dan sejumlah 15 dan 14 strain strain dari *Actinobacteria* dan *Proteobacteria* adalah bioaktif. Strain baru, 39^T dicirikan dengan menggunakan pendekatan polyphasic

and ia didapati mempunyai banyak perbezaan daripada genus yang lain dalam keluarga *Dermacoccaceae* di atas asas filogenetik, kemotaksonomi, fenotipik dan corak tandatangan nukleotida gen 16S rRNA. Oleh itu, adalah dicadangkan bahawa strain 39^T dikelaskan sebagai genus baru dalam famili *Dermacoccacea*, sebagai *Barrientosiimonas* gen. nov., species jenis yang baru ialah *Barrientosiimonas humi* gen. nov., sp. nov. Kajian ini jelas menunjukkan bahawa tanah Barrientos Island adalah sumber yang kaya dengan pelbagai bakteria, terutama *Actinobacteria*. Pengagihan secara meluas bakteria ini di Antartika membayangkan bahawa mereka adalah sangat endemik, terutamanya di dalam tanah, dan oleh itu bio-prospektif bakteria tanah dari pelbagai lokasi geografi seperti kawasan Antartika akan memberikan manfaat yang besar.

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Thank you very much.

I certify that a Thesis Examination Committee has met on 17 April 2012 to conduct the final examination of Lee Learn Han on his thesis entitled "Novel Actinobacteria Discovery and Biosystematics of Bacteria from Antarctic Soils on Barrientos Island" 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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The thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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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.

LEE LEARN HAN

Date: 17 April 2012

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