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By

YOW WENG KIT

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

March 2012

THIS THESIS IS DEDICATED

TO

MY BELOVED FATHER AND MOTHER,

AND

MY SISTER

THANK YOU

FOR ALL THE SUPPORTS

YOU GAVE TO ME

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

MITOCHONDRIAL DNA POLYMORPHISM IN SEVEN CATTLE BREEDS IN MALAYSIA

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March 2012

Chair: Professor Jothi Malar Panandam, PhD

Faculty: Agriculture

Although the Kedah Kelantan (KK) is the indigenous cattle of Malaysia, a number of cattle breeds have been introduced into the country to enhance the local beef and dairy cattle industry. The cattle breeds in Malaysia comprise of *Bos indicus* types (present in tropical countries), *Bos taur*us types (indigenous to the European countries but is also found in Africa and Asia), and the hybrids of these two types of cattle. There are not many publications available on the origin of Asian cattle. There is a lack of studies on the molecular variation within and among the cattle breeds in Malaysia as well as the genetic relationship among these breeds. In addition, the phylogenetic relationship of cattle breeds in Asia, especially using mitochondrial DNA (mtDNA), has not been widely conducted. No study has been conducted on mtDNA polymorphism of Kedah Kelantan (KK), the indigenous cattle breed of Malaysia.

Mitochondria are cytoplasmic organelle that contain DNA. MtDNA has many special features which has made it a popular marker to determine evolutionary and phylogenetic relationships. It has been used in studies to identify the genetic relationships between and within species of mammals. Displacement loop (D-loop) is the highly variable and most rapidly evolving region in the mtDNA. Variation observed in this region is frequently used for phylogenetic analysis of related breeds within species. The mtDNA cytochrome *b* (cyt *b*) is significantly conserved and, therefore, suitable for phylogenetic relationship and forensic investigations. It is suitable for between species comparison. Mitochondrial NADH dehydrogenase 5 (ND5) gene is another highly conserved genes and is commonly used for species identification.

With the above in mind, this study was conducted to evaluate the mtDNA polymorphism in seven cattle breeds in Malaysia, namely KK, Brahman, Brakmas, Brangus, Charoke, Droughtmaster and Jersey at four regions of the mtDNA, which are the D-loop, cyt *b* and ND5 regions.

Blood samples were randomly collected from 30 animals of each breed except for Brangus (n=17). DNA was extracted and quantified using a spectrophotometer. The D-loop, cyt *b* and two ND5 regions were amplified using specific primers. These regions were digested with nine, five and two restriction endonucleases (RE), respectively, generating 16 polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) loci. DNA cloning was performed on two samples of KK to obtain the nucleotide sequence for each of the four regions of mtDNA. The sequence information was compared with that for *B. indicus* and *B. taurus* which were available in the National Center for Biotechnology Information (NCBI) database.

The total percentage of polymorphism for the loci was 50%. The ND5 locus recorded 100% polymorphism. Polymorphism at the D-loop locus was 44.44% and for the cyt *b* locus it was 40%. The *Dde*I, *Eco*NI and *Mbo*I RFLP patterns for D-loop, the *Msp*I and *Msp*R9I RFLP patterns for cyt *b*, and the *Hind*III and *Tas*I RFLP patterns for ND5 loci were clearly able to distinguish between the *B. indicus* and *B. taurus* cattle breeds. Thus, these make them useful markers to detect variation between species. The dendogram generated using the PCR-RFLP mtDNA data showed two clusters for the seven cattle breeds. The KK, Brakmas, Charoke and Droughtmaster breeds were grouped together while the other cluster comprised of Brahman, Brangus and Jersey breeds. Alignment of the KK nucleotide sequences with those of *B. indicus* and *B. taurus* for the D-loop, cyt *b* and ND5/*Hind*III regions showed KK to be more similar to *B. indicus* than to *B. taurus*. However, comparison of the ND5/*Tas*I region, showed KK to be similar to *B. taurus* instead of *B. indicus*.

More regions of the mtDNA should be investigated and more RE should be used in order to determine the reliability of the result. Sequencing of more samples and from all breeds for the various regions would provide more information on the genetic structures and relationships among the cattle breeds.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

POLIMORFISMA DNA MITOKONDRIA DALAM TUJUH BAKA LEMBU DI MALAYSIA

Oleh

YOW WENG KIT

Mac 2012

Pengerusi: Profesor Jothi Malar Panandam, PhD

Fakulti: Pertanian

Walaupun Kedah Kelantan (KK) adalah lembu asli Malaysia, beberapa baka lembu telah diperkenalkan ke negara ini untuk meningkatkan industri lembu pedaging dan tenusu tempatan. Baka lembu di Malaysia terdiri daripada jenis *Bos indicus* (sedia ada di negara-negara tropika), jenis *Bos taurus* (asli kepada negara-negara Eropah tetapi juga didapati di Afrika dan Asia), dan hibrid daripada kedua-dua jenis lembu. Tidak banyak penerbitan yang boleh diperolehi pada asal usul lembu Asia. Terdapat kekurangan kajian tentang variasi molekul di dalam dan di antara baka lembu di Malaysia serta hubungan genetik antara baka. Di samping itu, hubungan filogenetik ternakan lembu di Asia, terutamanya menggunakan DNA mitokondria (mtDNA), tidak dijalankan secara meluas. Tiada kajian yang dijalankan ke atas polimorfisma mtDNA Kedah Kelantan (KK), baka lembu asli Malaysia.

Mitokondria merupakan organel sitoplasmik yang mengandungi DNA. MtDNA mempunyai banyak ciri-ciri istimewa yang menjadikan ia penanda yang popular untuk menentukan hubungan evolusi dan filogenetik. Ia telah digunakan dalam kajian untuk mengenal pasti hubungan genetik antara dan dalam spesies mamalia. *Displacement loop* (*D-loop*) merupakan bahagian yang mempunyai perubahan tinggi dan yang paling pesat berkembang dalam mtDNA. Perubahan yang diperhatikan di bahagian ini sering digunakan untuk analisis filogenetik baka yang berkaitan dalam spesies. MtDNA *cytochrome b* (*cyt b*) adalah dipelihara dengan ketara dan, oleh itu, sesuai untuk hubungan filogenetik dan siasatan forensik. Ia adalah sesuai untuk perbandingan antara spesies. Gen *mitochondrial dehydrogenase NADH 5* (*ND5*) merupakan satu lagi gen yang sangat dipelihara dan biasanya digunakan untuk mengenal pasti spesies.

Dengan di atas dalam fikiran, kajian ini telah dijalankan untuk menilai polimorfisma mtDNA dalam tujuh baka lembu di Malaysia, iaitu KK, Brahman, Brakmas, Brangus, Charoke, Droughtmaster dan Jersey di empat bahagian mtDNA, yang ada di bahagian *D-loop* , cyt *b* dan *ND5*.

Darah diambil secara rawak daripada 30 haiwan setiap baka kecuali Brangus (n = 17). Sampel DNA diekstrak dan kuantitinya diperiksa mengguna spektrofotometer. *D-loop*, cyt *b* dan dua bahagian *ND5* diamplifikasi mengguna primer khusus. Bahagian-bahagian ini telah dicerna dengan sembilan, lima dan dua *restriction endonuclease* (RE), masing-masing menjana 16 lokus *polymerase chain reaction-restriction fragment length polymorphism* (PCR-RFLP). DNA pengklonan telah dilakukan ke atas dua sample KK untuk mendapatkan urutan nukleotida bagi setiap empat bahagian mtDNA. Maklumat urutan dibandingkan dengan *B. indicus* dan *B. taurus* yang terdapat dalam pangkalan data *National Center for Biotechnology Information* (*NCBI*).

Jumlah peratusan polimorfisma untuk lokus adalah 50%. Lokus *ND5* mencatat 100% polimorfisma. Polimorfisma pada lokus *D-loop* adalah 44.44% dan bagi lokus *cyt b* ia adalah 40%. Corak RFLP *Dde*I, *Eco*NI dan *Mbo*I untuk *D-loop*, corak RFLP *Msp*I dan *Msp*R9I untuk *cyt b*, dan corak RFLP *Hind*III dan *Tas*I untuk lokus *ND5* dengan jelasnya dapat membezakan antara baka lembu *B. indicus* dan *B. taurus*. Oleh itu, ini menjadikan mereka petanda berguna untuk mengesan variasi di antara spesies. Dendogram yang dijana mengguna data mtDNA PCR-RFLP menunjukkan dua kelompok tujuh baka lembu tersebut. Baka KK, Brakmas, Charoke dan Droughtmaster dikumpulkan bersama manakala kelompok yang lain terdiri daripada baka Brahman, Brangus dan Jersey. Penjajaran jujukan nukleotida KK dengan *B. indicus* dan *B. taurus* untuk bahagian *D-loop*, *cyt b* dan *ND5/Hind*III menunjukkan KK akan lebih serupa dengan *B. indicus* berbanding dengan *B. taurus*. Walau bagaimanapun, perbandingan di bahagian *ND5/Tas*I, menunjukkan KK sama dengan *B. taurus* dan bukannya *B. indicus*.

Lebih banyak bahagian-bahagian mtDNA harus disiasat dan lebih RE harus digunakan untuk menentukan kebolehpercayaan keputusan. *Sequencing* sampel yang lebih banyak dan dari semua baka bagi pelbagai bahagian akan memberi maklumat yang lebih mengenai struktur genetik dan hubungan antara baka lembu.

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I certify that a Thesis Examination Committee has met on 1st March 2012 to conduct the final examination of Yow Weng Kit on his thesis entitled “Mitochondrial DNA Polymorphism in Seven Cattle Breeds in Malaysia” in accordance with the Universities and University College 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.

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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:

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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.

YOW WENG KIT

Date: 1 March 2012

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