
UNIVERSITI PUTRA MALAYSIA

# MOLECULAR CHARACTERIZATION GENOMIC ORGANIZATION AND OF FOWL ADENOVIRUS ISOLATES OF MALAYSIA

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**MASTER OF SCIENCE**
**UNIVERSITI PUTRA MALAYSIA**
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# MOLECULAR CHARACTERIZATION GENOMIC ORGANIZATION AND OF FOWL ADENOVIRUS ISOLATES OF MALAYSIA

**By**

**JULIANA BINTI MOHD AYOB**

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

**January 2014**

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**DEDICATED WITH LOVE AND GRATITUDE**

**TO:**

**MY HUSBAND FAISAL MOHAMAD YUSOFF, MOTHER (FOZIAH ABDUL HAMID), FATHER (MOHD AYOB MAT PIAH) AND SON (IMAN RAYYAN)**

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

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**January 2014**

**Chairperson : Nurulfiza Mat Isa, PhD**
**Faculty : Biotechnology and Biomolecular Sciences**

Fowl adenoviruses (FAdVs) are ubiquitous in all avian species especially in commercial broiler chickens and some of them are associated with diseases such as inclusion body hepatitis (IBH). Previous study on the pathogenicity of Malaysian FAdV isolate that involved in IBH outbreaks was based on serological method, leaving the serotype identification remained unknown. Thus, the aim of this study was to determine and characterize the FAdV of Malaysian isolates and establish the genomic organization of FAdV of UPM04217 isolate.

Five FAdV Malaysian isolates were propagated in specific pathogen free (SPF) embryonated chicken eggs and characterized by the *hexon* based polymerase chain reaction (PCR) method coupled with restriction enzyme analysis (REA), and followed by direct sequencing. Approximately 1.2 kb and 1.3 kb were generated using H1/H2 and H3/H4 primer pairs, respectively. All isolates generated same restriction enzyme patterns that resembled the digestion patterns of the FAdV-8b, strain 764 when H1/H2 and H3/H4 PCR products were digested by *Hae*II and *Hpa*II, respectively. Sequence and phylogenetic analysis based on the loop 1 (L1) region of the *hexon* gene revealed that all Malaysian FAdV isolates that involved in IBH outbreaks were 100% identical and showed highest similarity of 98.1% to FAdV-8b, strain 764. Evolutionary relationship of Malaysian FAdV isolates to FAdV species E was demonstrated by high bootstrap values of 99% in nucleotide sequence by using the distance, maximum parsimony and maximum likelihood methods.

The nucleotide sequence of the whole genome of FAdV-8b UPM04217 isolate was determined using next generation sequencing (NGS) platform and the Sanger sequencing method. The complete genome was found to be 44 059 bp long with 57.9% G+C content and shared 97.5% genome identity with reference FAdV-E genome (HG

isolate). Interestingly, the genome analysis using ORF Finder, Glimmer3 and FGENESV predicted a total of 40 open reading frames (ORFs) compared to FAdV-E HG isolate that possessed 46 ORFs. Gene annotation and comparison analysis using BLASTP, EMBOSS Needle and mVISTA-LAGAN programs showed no additional unique gene and the absence of 3 ORFs were also detected (ORF11A, unique ORF and ORF32). In contrast to FAdV-E HG isolate, ORF22, ORF25 and ORF33 in FAdV UPM04217 isolate were encoded by a single ORF rather than two small ORF. However, the genome organization was similar to other FAdV members.

Correct identification of the serotypes involved in IBH outbreaks and knowledge of the complete FAdV genome sequence of UPM04217 will provide valuable information on the epidemiological study, molecular evolution, vaccination strategies and for future recombinant vector development purposes.

Abstrak tesis yang dikemukan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Sarjana

**PENCIRIAN MOLEKUL DAN ORGANISASI GENOMIK ADENO UNGGAS ISOLAT MALAYSIA**

Oleh

**JULIANA BINTI MOHD AYOB**

**Januari 2014**

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Virus adeno unggas (FAdVs) merupakan virus yang sentiasa terdapat di dalam semua spesies unggas terutamanya di dalam ayam pedaging komersil dan sebahagian daripada virus ini terlibat di dalam penyakit seperti 'inclusion body hepatitis' (IBH). Kajian terdahulu berkaitan patogenisiti isolat FAdV tempatan yang terlibat di dalam wabak IBH hanyalah berdasarkan kaedah serologi, menjadikan identifikasi serotip masih belum diketahui. Oleh itu, tujuan kajian ini adalah untuk menentukan and mencirikan isolat FAdV Malaysia dan mengkaji organisasi genomik isolat FAdV UPM04217.

Lima isolat FAdV tempatan telah di propagasi didalam telur ayam berembrio bebas penyakit khusus (SPF) dan dicirikan oleh kaedah penjujukan rantaian berulang (PCR), bersama analisis enzim penyekat (REA), diikuti oleh penjujukan terus. Dianggarkan 1.2 kb dan 1.3 kb telah dijana menggunakan pasangan pencetus H1/H2 dan H3/H4. Semua isolate telah menjana corak enzim penyekat yang sama yang menyerupai corak pemotongan FAdV-8b, strain 764 apabila produk H1/H2 and H3/H4, masing-masing dipotong menggunakan *Hae*II dan *Hpa*II. Analisis jujukan dan filogenetik berdasarkan kawanan Loop satu (L1) yang terletak di kawasan gen *hexon* mendedahkan bahawa semua isolat FAdV Malaysia yang terlibat dalam wabak IBH, adalah 100% serupa dan menunjukkan persamaan tertinggi kepada FAdV-8b, strain 746 iaitu sebanyak 98.1%. Hubungan evolusi antara isolat FAdV Malaysia dengan FAdV spesies E ditunjukkan oleh nilai bootstrap yang tinggi iaitu 99% bagi jujukan nukleotida dengan kaedah "distance", "maximum parsimony" dan "maximum likelihood".

Genom lengkap FAdV-8b isolat UPM04217 telah ditentukan menggunakan platform penjujukan generasi seterusnya (NGS) dan penjujukan Sanger. Genom lengkap didapati

sepanjang 44 059 pb dengan 57.9% kandungan G+C dan berkongsi 97.5% identiti genom dengan genom rujukan FAdV-E (isolat HG). Menariknya, analisis genom menggunakan "ORF Finder", "Glimmer3" dan "FGENESV" menjangkakan sebanyak 40 rangka bacaan terbuka (ORF) berbanding genom FAdV-E isolat HG yang mempunyai 46 ORF. Analisis anotasi dan perbandingan gen menggunakan program "BLASTP", "EMBOSS Needle" dan "mVISTA-LAGAN" menunjukkan tiada gen unik tambahan dijumpai dan ketidakhadiran 3 ORF dikesan (ORF11A, ORF unik dan ORF32). Berbeza dengan FAdV-E isolat HG, ORF22, ORF25 dan ORF33 di dalam FAdV isolat UPM04217 dikodkan oleh ORF tunggal bukannya dua ORF kecil. Bagaimanapun, genom organisasi adalah sama dengan ahli FAdV yang lain. Identifikasi yang tepat terhadap serotip yang terlibat dalam wabak IBH and pengetahuan tentang jujukan genom FAdV UPM04217 yang lengkap akan menghasilkan maklumat berharga berkaitan kajian epidemiologikal, evolusi molekul, strategi vaksinasi dan pembangunan vektor rekombinan pada masa hadapan.

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I certify that a Thesis Examination Committee has met on (date) to conduct the final examination of Juliana Mohd Ayob on her thesis entitled "Molecular Characterization And Genomic Organization Of Fowl Adenovirus Isolates Of 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 fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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