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IMPROVEMENT OF PASTEURELLA MULTOCIDA SEROTYPE B:2 DRAFT GENOME SEQUENCE AND ANALYSIS OF ITS GENOME STRUCTURE AND FUNCTION

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By

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Chairman: Abdul Rahman Bin Omar, PhD

Faculty: Institute of Bioscience

Pasteurella multocida serotype B:2 and E:2 are the main causative agents of hemorrhagic septicaemia (HS) in ruminants. Hemorrhagic septicemia is an acute, fatal and septicemic disease of economic importance in tropical regions of the world especially in Asian and African countries. To date, the available vaccines are unable to induce long term immunity and require individual injection which is not feasible since animals normally live in large wild herds or kept semi-wild by the farmers. The molecular basis for pathogenesis of HS caused by *P. multocida* during natural infections is still vague. Thus, there is an imminent need for whole genome sequencing of HS causing strain as the first step towards understanding of the pathogenicity of *P. multocida* in causing HS in infected ruminants. Hence, in previous study, the genome of *P. multocida* serotype B:2 strain PMTB isolated from a dead buffalo during an outbreak of HS in Kelantan, Malaysia, year 2003 was sequenced using Illumina Genome Analyzer. Approximately 7.2 million single end

reads with data size 250 MB and an average length of 35 bp was generated, providing ~100 fold genome coverage. *De novo* assembly using software Velvet produced scaffold with 2191094 bp in length and 89 genome gaps. In this study, Polymerase Chain Reaction (PCR) sequencing approach was used and successfully closed 80 genome gaps with 9 unresolved gaps. The 9 remaining unresolved gaps were due to the large length of the gaps, repeat sequence related or missasemblies of contigs. The predicted total length of the remaining unresolved gap was about 215 k bp, which is impossible to amplify in PCR assay even by using pfu polymerase that specialize in long range PCR. The misassembled contigs and repeat sequence related gaps caused no amplification in PCR assay or the sequences obtained were unable to map to the targeted region. The improved scaffold with 2203419 bp in length with G + C content of 40.46 % was submitted to Prokaryotic Genome Annotation Pipeline (PGAAP) provided by National Center for Biotechnology Information (NCBI) for gene prediction and annotation. The genome was predicted to contain 2021 Coding DNA Sequences (CDSs), 51 tRNA genes and 10 rRNA genes. The *toxA* gene that encodes dermonecrotic *Pasteurella multocida* toxin (PMT) which was frequently associated with serogroup D was absent in the strain PMTB genome. The absence of *toxA* in strain PMTB was validated by PCR assay detection. In addition, integrative conjugative element of *Pasteurella multocida*, ICE*Pmu*1 which was detected in *P. multocida* strain 36950 (serogroup A) was found absent in the genome of strain PMTB via sequence similarity search. HTH-type transcriptional regulator for conjugative element sulfamethoxazole/trimethoprim (SXT), another type of integrative conjugative element that originally derived from *Vibrio cholera* was predicted present in strain PMTB. Further analysis at the locus of predicted SXT element in strain PMTB showed that a large chunk of DNA fragment in the middle

part that supposedly harbored antibiotic resistance genes was missing due to unresolved genome sequence gap. However, 12 genes that play important roles in transposase, regulatory and conjugative transfer located in the left and right ends of the element were present in the SXT genome of strain PMTB. Antibiotic susceptibility test (AST) was performed to determine the function of antibiotic resistance genes carrying by SXT element by using disk diffusion method. The AST results showed that strain PMTB resistant to streptomycin (inhibition zone diameter=10.54 mm) only. Strain PMTB was susceptible to chloramphenicol (inhibition zone diameter=25.56 mm), gentamycin (inhibition zone diameter=15.77 mm) and SXT (inhibition zone diameter= 24.31 mm). In addition, strain PMTB was shown that intermediate sensitivity to kanamycin (inhibition zone diameter=17.85 mm) and erythromycin (inhibition zone diameter=18.49 mm). Findings from this study provide valuable information for future studies in the identification of virulence-associated genes to elucidate the pathogenicity of *P. multocida* strain PMTB.

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

MEMPERTINGKATKAN DRAF JUJUKAN GENOM PASTEURELLA MULTOCIDA SEROTIP B:2 STRAIN PMTB SERTA ANALISIS STRUKTUR DAN FUNGSI GENOM

Oleh

YAP HUAN YONG

Julai 2013

Pengerusi: Abdul Rahman Bin Omar, PhD

Fakulti: Institut Biosains

Pasteurella multocida serotip B:2 dan E:2 merupakan agen utama yang menyebabkan hawar berdarah (HS) dalam ruminan. HS ialah sejenis penyakit akut, membawa maut dan septisemia yang amat penting dari segi ekonomi di kawasan tropika di dunia terutamanya di negara Asia dan Afrika. Setakat ini, vaksin yang sedia ada tidak dapat mengaruh keimunan jangka panjang dan memerlukan suntikan individu yang dianggap menyusahkan kerana haiwan ini biasanya hidup dalam kumpulan besar atau yang dipelihara secara separa liar oleh penternak. Asas patogenesiti HS yang disebabkan oleh jangkitan semula jadi *Pasteurella multocida* masih lagi kurang difahami. Oleh itu, jujukan keseluruhan genom strain bakteria yang menyebabkan HS amat diperlukan secepat mungkin sebagai langkah pertama menuju ke arah pemahaman terhadap kepatogenan penyakit HS dalam ruminan yang dijangkiti oleh *P. multocida*. Oleh itu, dalam kajian sebelum ini, genom *P. multocida* serotip B:2 strain PMTB yang dipencilkan daripada bangkai kerbau semasa wabak

HS yang berlaku di Kelantan, Malaysia pada tahun 2003 telah dijujuk dengan menggunakan Illumina Genome Analyzer. Sejumlah kira-kira 7.2 juta bacaan satu hujung yang mempunyai saiz data 250 MB dan purata kepanjangan 35 bp telah dijana. Ini telah menyediakan ~100 lipat liputan genom. Perhimpunan secara *de novo* dengan menggunakan perisian Velvet telah menghasilkan kerangka bersaiz 2191094 bp dan mempunyai 89 jurang genom. Dalam kajian ini, pendekatan secara jujukan PCR telah diambil dan berjaya menutup 80 jurang genom, tinggalkan 9 jurang tidak diselesaikan. Sembilan jurang yang belum diselesaikan adalah berkaitan dengan kepanjangan jurang bersaiz terlalu besar, jujukan berulangan ataupun kontig salah dipasang. Jumlah panjang yang diramalkan bagi jurang yang belum diselesaikan adalah 215 kb yang mustahil dapat diganda dalam PCR, walaupun dengan menggunakan polimerase pfu yang khas untuk PCR jarak jauh. Kontig-kontig yang salah dipasang dan jurang jujukan berulang yang menyebabkan ketiadaan penggandaan dalam asai PCR ataupun jujukan yang didapati tidak dapat dikesan pada kedudukan jujukan yang disasarkan. Kerangka yang telah ditingkatkan mempunyai 2203419 bp dan kandungan G + C sebanyak 40.46 % telah dihantarkan ke Prokaryotic Genome Annotation Pipeline (PGAAP) yang disediakan oleh pihak National Centre for Biotechnology Information (NCBI) untuk ramalan gen dan anotasi. Genom diramalkan mengandungi 2021 Coding DNA Sequences (CDSs), 51 gen tRNA dan 10 gen rRNA. Gen *toxA* yang mengekod toksin dermonecrotic *Pasteurella multocida* (PMT) yang selalu dikaitkan dengan serogroup D didapati tidak wujud di genom strain PMTB. Ketidakhadiran gen *toxA* di strain PMTB telah disahkan dengan cara pengesanan PCR. Sebagai tambahan, elemen konjugasi integratif *Pasteurella multocida*, ICE*Pmu*1 yang dikesan dalam genom *P. multocida* strain 36950 (serogroup A) juga tidak dapat dikesan di genome strain PMTB secara

pencarian persamaan jujukan. Pengawal atur transkripsi jenis HTH bagi elemen konjugasi SXT, satu lagi jenis elemen konjugasi integratif yang secara aslinya didapati dari *Vibrio cholera* telah diramalkan wujud dalam genom strain PMTB. Analisis secara mendalam tentang lokus elemen SXT yang diramalkan menunjukkan sebahagian besar serpihan DNA di tengah-tengah yang sepatutnya membawa gen rintangan antibiotik telah hilang disebabkan jurang jujukan genom yang belum diselesaikan. Walau bagaimanapun, 12 gen yang memainkan peranan penting dalam tranposase, pengawalseliaan dan pemindahan secara konjugasi didapati berada di hujung kiri dan kanan elemen hadir pada genom SXT strain PMTB. Ujian kepekaan antibiotik (AST) telah dilaksanakan untuk menentukan fungsi gen rintangan antibiotik yang dibawa oleh elemen SXT dengan meggunakan kaedah cakera penyebaran. Keputusan AST menunjukkan strain PMTB hanya rintang kepada streptomisin (diameter zon perencatan=10.54 mm). Strain PMTB adalah rentan kepada chloramphenicol (diameter zon perencatan =25.56 mm), gentamisin (diameter zon perencatan =15.77 mm) dan SXT (diameter zon perencatan = 24.31 mm) Tambahan pula, strain PMTB menunjukkan kerentanan perantaraan kepada kanamisin (diameter zon perencatan =17.85 mm) dan erythromisin (diameter zon perencatan =18.49 mm), Penemuan kajian ini menyediakan informasi yang berharga dan informasi untuk kajian pada masa hadapan dalam menentukan gen yang berkaitan dengan kepatogenan *P. multocida* strain PMTB.

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I certify that a Thesis Examination Committee has met on **3 July 2013** to conduct the final examination of Yap Huan Yong on her thesis entitled **"Improvement of *Pasteurella multocida* serotype B:2 draft genome sequence and analysis of its genome structure and function"** 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.**

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This thesis was submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirements for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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DECLARATION

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

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YAP HUAN YONG

Date: 3 July 2013

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