

Complete Genome Sequence Analysis of *Pasteurella multocida* Subspecies *multocida* Serotype A Strain PMTB2.1

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Abstract : *Pasteurella multocida* (PM) is an important veterinary opportunistic pathogen particularly associated with septicemic pasteurellosis, pneumonic pasteurellosis and hemorrhagic septicemia in cattle and buffaloes. *P. multocida* serotype A has been reported to cause fatal pneumonia and septicemia. *Pasteurella multocida* subspecies *multocida* of serotype A Malaysian isolate PMTB2.1 was first isolated from buffaloes died of septicemia. In this study, the genome of *P. multocida* strain PMTB2.1 was sequenced using third-generation sequencing technology, PacBio RS2 system and analyzed bioinformatically via de novo analysis followed by in-depth analysis based on comparative genomics. Bioinformatics analysis based on de novo assembly of PacBio raw reads generated 3 contigs followed by gap filling of aligned contigs with PCR sequencing, generated a single contiguous circular chromosome with a genomic size of 2,315,138 bp and a GC content of approximately 40.32% (Accession number CP007205). The PMTB2.1 genome comprised of 2,176 protein-coding sequences, 6 rRNA operons and 56 tRNA and 4 ncRNAs sequences. The comparative genome sequence analysis of PMTB2.1 with nine complete genomes which include *Actinobacillus pleuropneumoniae*, *Haemophilus parasuis*, *Escherichia coli* and five *P. multocida* complete genome sequences including, PM70, PM36950, PMHN06, PM3480, PMHB01 and PMTB2.1 was carried out based on OrthoMCL analysis and Venn diagram. The analysis showed that 282 CDs (13%) are unique to PMTB2.1 and 1,125 CDs with orthologs in all. This reflects overall close relationship of these bacteria and supports the classification in the Gamma subdivision of the Proteobacteria. In addition, genomic distance analysis among all nine genomes indicated that PMTB2.1 is closely related with other five *Pasteurella* species with genomic distance less than 0.13. Synteny analysis shows subtle differences in genetic structures among different *P. multocida* indicating the dynamics of frequent gene transfer events among different *P. multocida* strains. However, PM3480 and PM70 exhibited exceptionally large structural variation since they were swine and chicken isolates. Furthermore, genomic structure of PMTB2.1 is more resembling that of PM36950 with a genomic size difference of approximately 34,380 kb (smaller than PM36950) and strain-specific Integrative and Conjugative Elements (ICE) which was found only in PM36950 is absent in PMTB2.1. Meanwhile, two intact prophages sequences of approximately 62 kb were found to be present only in PMTB2.1. One of phage is similar to transposable phage SfMu. The phylogenomic tree was constructed and rooted with *E. coli*, *A. pleuropneumoniae* and *H. parasuis* based on OrthoMCL analysis. The genomes of *P. multocida* strain PMTB2.1 were clustered with bovine isolates of *P. multocida* strain PM36950 and PMHB01 and were separated from avian isolate PM70 and swine isolates PM3480 and PMHN06 and are distant from *Actinobacillus* and *Haemophilus*. Previous studies based on Single Nucleotide Polymorphism (SNPs) and Multilocus Sequence Typing (MLST) unable to show a clear phylogenetic relatedness between *Pasteurella multocida* and the different host. In conclusion, this study has provided insight on the genomic structure of PMTB2.1 in terms of potential genes that can function as virulence factors for future study in elucidating the mechanisms behind the ability of the bacteria in causing diseases in susceptible animals.

Keywords : comparative genomics, DNA sequencing, phage, phylogenomics

Conference Title : ICACCM 2018 : International Conference on Animal Cytogenetics and Comparative Mapping

Conference Location : Dublin, Ireland

Conference Dates : December 20-21, 2018