

Title: IDENTIFICATION OF DIFFERENTIALLY EXPRESSED TRANSCRIPTS BY *Pasteurella multocida* IRON-STARVED CONDITIONS BY RNA-seq

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Resume:

Pasteurella multocida (*P. multocida*) is a pathogen agent that causes a variety of diseases such as fowl cholera (chickens and turkeys), haemorrhagic septicemia (bovines) and atrophic rhinitis (swine). Studies on the infection of bacteria and fungi have shown that host diminish availability of iron (Fe), since it is an important growth factor and participates in essential biological processes as oxygen transportation, gene regulation and synthesis DNA. Little is known about the transcriptional responses of bacterial genes during infection, including the effects of Fe deprivation, which is the objective of this study. *P. multocida* isolate 16759 (Pm 16759) was obtained by re-isolation from pneumonic lung fragment of a SPF swine. This isolate was from outbreaks of pneumonia in swine. The isolate was grown on two conditions, one with sufficient levels of Fe and other with Fe deprivation. Total RNA of the two conditions were extracted and sequenced by new generation Ion Torrent platform. Data were analyzed in Ion Reporter™ Software and processed in the Rockhopper® software and 2,885,646 readings were obtained with 96% of alignment with the referential genome (NC_017764.1) and 98.8% accuracy. Gene analysis mapped in the genome of *P. multocida* in two conditions shows 2,652 genes with 90 of these (3.39%) were differentially expressed. It was also observed 89 (3.35%) antisense (asRNA) reads, being 37.08% differentially expressed mainly associated to noraml iron condition. Genes related to iron and carbohydrates metabolism showed higher expression in the condition with Fe as well as asRNAs, suggesting these positive action on the expression of other genes of the metabolic pathways. In low level iron, a Fe absorption system differentially expressed in our study was the Gene high-affinity Fe²⁺/Pb²⁺ permease, regardless of TonB complex showing probable existence of alternative pathways of Fe absorption from transferrin, as described in fungi. The RNA-seq technique proved effective in identifying differentially expressed transcripts by *P. multocida* in iron deprivation conditions providing the basis for pathogenicity studies.

Keywords: Pasteurellosis, Gene expression, Iron limitation, RNA-seq, Ion Torrent.

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