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Gene Expression Profiling of Iron-Related Genes of Pasteurella multocida Serotype A Strain PMTB2.1

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Abstract : Pasteurella multocida is associated with acute, as well as, chronic infections in avian and bovine such as pasteurellosis and hemorrhagic septicemia (HS) in cattle and buffaloes. Iron is one of the most important nutrients for pathogenic bacteria including Pasteurella and acts as a cofactor or prosthetic group in several essential enzymes and is needed for amino acid, pyrimidine, and DNA biosynthesis. In our recent study, we showed that 2% of Pasteurella multocida serotype A strain PMTB2.1 encode for iron regulating genes (Accession number CP007205.1). Genome sequencing of other Pasteurella multocida serotypes namely PM70 and HB01 also indicated up to 2.5% of the respective genome encode for iron regulating genes, suggesting that Pasteurella multocida genome comprises of multiple systems for iron uptake. Since P. multocida PMTB2.1 has more than 40 CDs out of 2097 CDs (approximately 2%), encode for iron-regulated. The gene expression profiling of four iron-regulating genes namely fbpb, yfea, fece and fur were characterized under iron-restricted environment. The P. multocida strain PMTB2.1 was grown in broth with and without iron chelating agent and samples were collected at different time points. Relative mRNA expression profile of these genes was determined using Taqman probe based real-time PCR assay. The data analysis, normalization with two house-keeping genes and the quantification of fold changes were carried out using Bio-Rad CFX manager software version 3.1. Results of this study reflect that iron reduced environment has significant effect on expression profile of iron regulating genes ($p < 0.05$) when compared to control (normal broth) and all evaluated genes act differently with response to iron reduction in media. The highest relative fold change of fece gene was observed at early stage of treatment indicating that PMTB2.1 may utilize its periplasmic protein at early stage to acquire iron. Furthermore, down-regulation expression of fece with the elevated expression of other genes at later time points suggests that PMTB2.1 control their iron requirements in response to iron availability by down-regulating the expression of iron proteins. Moreover, significantly high relative fold change ($p \leq 0.05$) of fbpb gene is probably associated with the ability of P. multocida to directly use host iron complex such as hem, hemoglobin. In addition, the significant increase ($p \leq 0.05$) in fbpb and yfea expressions also reflects the utilization of multiple iron systems in P. multocida strain PMTB2.1. The findings of this study are very much important as relative scarcity of free iron within hosts creates a major barrier to microbial growth inside host and utilization of outer-membrane proteins system in iron acquisition probably occurred at early stage of infection with P. multocida. In conclusion, the presence and utilization of multiple iron system in P. multocida strain PMTB2.1 revealed the importance of iron in the survival of P. multocida.

Keywords : iron-related genes, real-time PCR, gene expression profiling, fold changes
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