BMC Bioinformatics

Poster presentation

Theoretical and quantitative aspects of iron acquisition by a bovine isolate of *Pasteurella multocida* A:3

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from BioSysBio: Bioinformatics and Systems Biology Conference
Edinburgh, UK, 14–15 July 2005

Published: 21 September 2005

BMC Bioinformatics 2005, **6**(Suppl 3):P20

Current epidemiological data (VIDA UK) relating to bovine pneumonic pasteurellosis, a disease of welfare and economic significance worldwide, indicate that *Pasteurella multocida* serotype A has overtaken *Mannheimia haemolytica* as the leading cause of disease. Iron acquisition mechanisms *in P. multocida*, as with the majority of bacteria, are an essential element for survival and proliferation and a major virulence factor, although the detailed processes involved are not defined. A mathematical approach described here has constructed a reaction network representing iron acquisition from host transferrin, suggested target genes for quantitative RT-PCR (qRT-PCR) and provided data for initial theoretical modelling. Primers were designed for qRT-PCR study of 8 iron acquisition genes: *fur*, *tbpA*, *tonB*, *exbB*, *exbD*, *fbpA*, *fbpB* and *fbpC* and applied to the analysis of total RNA extracted from *P. multocida* A:3 grown *in vitro* in iron replete and iron restricted conditions in a 4 x 1L benchtop fermenter (B. Braun Biotech); 3 vessels iron restricted (αα-dipyridyl), 1 control. Growth rates were measured from hourly sequential samples by estimated and live viable counts. Results for qRT-PCR revealed differences in expression of iron restricted outer membrane protein (IROMP) genes in iron replete and iron restricted conditions. In replete conditions, all genes showed a similar transcription pattern with a steady increase from 0–4 hours, followed by a plateau from 4–8 hours. Under iron restriction, all genes with the exception of *fur* exhibited a faster initial phase of transcription from 0–2 hours followed by a drop at 2 hours and a subsequent recovery at 6–8 hours. The transcription pattern for *fur* showed an initial drop from 0–2 hours followed by a steady increase in expression tending to plateau between 6–8 hours. The differing transcription pattern of *fur* is expected due to its role as a transcriptional repressor of IROMP expression. Surprisingly, bacterial growth rate was not suppressed by iron restriction using 200 μM αα-dipyridyl, suggesting that IROMP expression under these conditions was sufficient to provide the necessary iron. The subsequent drop in transcription may represent a period wherein acquired iron is metabolised after which a recovery in transcription is triggered by the need to replenish intracellular iron levels. Results suggest that the initial period of growth from 0–3 hours should be studied in greater depth.