Transcriptome analysis of Corynebacterium pseudotuberculosis in an iron deficient environment

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Livestock is one of the fastest growing sectors of the agricultural economy and is boosted by high profitability and technological advances. The sector growth offers development opportunities, poverty reduction and nutritional gains. Nevertheless, one of the limiting factors of this activity is the high prevalence of infectious diseases that affects the flocks, thus reducing viability and exportation, raising costs and reducing profitability. In this context, infections by Corynebacterium pseudotuberculosis are amongst the most relevant and their occurrence is related to high economic impact diseases. "Omics" studies regarding this pathogen have enabled the identification of putative pathogenic islands containing classical virulence elements, including genes involved in iron uptake. For many pathogenic bacteria, iron availability and uptake contributes to a successful host colonization and bacterial survival. Due to its importance and the fact that mammalian host species restrict the iron availability to control bacterial infection, the interference with ironacquisition mechanism can be used as a target for the development of novel antimicrobial treatments as well as a more effective vaccine therapy. Still, although its critical relevance, the mechanism involved in the uptake, virulence and availability of iron in the C. pseudotuberculosis species is poorly understood. Furthermore, large scale RNA sequencing (RNA-seq) technology can be used to expand our comprehension of the functional genomics involved in the infection, resistance and survival of this important pathogen. In this context, the aim of this project is to characterize the RNA response of two strains of Corynebacterium: CpT1 (wildtype) and Cp13 (deficient iron transport binding protein - ciuA mutant), in a low iron environment. In order to achieve a low iron environment, different concentrations of Fe²⁺ chelator, 2,2'-bipyridine (bipyridyl, BIP), were used to analyze bacterial growth in BHI medium. Low, nonfatal iron concentration, was achieved with 250uM of BIP, RNA extraction and purification was done 6 hours after inoculation. RNA-seq is going to be carried out using the Ion Proton platform and analyses will include quality assessment through FASTQC, filtering of high quality transcripts and alignment to a reference genome with the software TopHat v2.1.0. Single aligned reads will be quantified using HTSeq counter and the differential gene expression statistical analysis will be done using the EdgeR/Bioconductor package. Although still under development, the hypothesis is that low environmental iron concentration could trigger a switch in the expression of Corynebacterium pseudotuberculosis genes involved in host colonization and persistence, including those associated with iron acquisition and virulence factors.

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