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Schou, Kirstine Klitgaard; Rundsten, Carsten Friis; Boye, Mette

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Intra-species variation in *Actinobacillus pleuropneumoniae* – transcriptional response to iron limitation in serotypes with different virulence potential

Klitgaard, K¹, Friis C.² & M. Boye¹

¹National Veterinary Institute, Technical University of Denmark, Bülowsvej 27, DK-1790, Copenhagen, Denmark.

²National Food Institute, Technical University of Denmark, Kemitortvet, building 204, DK-2800 Kgs. Lyngby, Denmark.

Background: Comparative analysis of gene expression among serotypes within a species may provide valuable information of important differences in related genomes. For the pig lung pathogen *Actinobacillus pleuropneumoniae* (Ap), 15 serotypes with a considerable variation in virulence potential have been identified^{8, 5, 6, 7}. This difference is only partly explained by the difference in RTX toxin genes in their genomes^{4, 1}. Iron acquisition *in vivo* is an important bacterial function during infection. In this study, gene expression in response to iron restriction *in vitro* in six Ap serotypes of variable virulence was studied, applying a NimbleGen microarray targeting the genomes of all the included serotypes.

Results: In total, 45 and 67 genes were significantly ($p < 0.0001$) up- or down-regulated, respectively, in response to iron limitation. 12 of these genes also displayed significant serotype related response to iron limitation including three co-regulated, putative haemoglobin-haptoglobin binding proteins which have recently been described in Ap³ and share homology with the HmbR haemoglobin receptor of *Neisseria meningitidis* (Nm), which contributes to Nm survival in rats⁹. Except for the moderately virulent serotype 6, the expression of this gene cluster was at the highest in the most virulent serotypes, 1 and 5.

Conclusion: Comparative analysis of gene expression among 6 different serotypes of Ap identified a common set of genes involved in iron regulation. The results support previous observations concerning the identification of new potential iron acquisition systems in Ap^{3, 2}, showing that this bacterium has evolved several strategies for scavenging the limited iron resources of the host. The conjugated effect of iron-depletion and serotype proved to be modest, indicating at least *in vitro* that serotypes of both medium and high virulence are reacting almost identical to iron restriction. One notable exception, however, is the haemoglobin-haptoglobin binding gene cluster, which merits further investigation.

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