Summary

Mannheimia haemolytica and *Pasteurella multocida* belong to the causative agents of bovine respiratory disease complex. This severe pneumonia is the most important respiratory disease in cattle and causes enormous financial losses in the cattle industry. As the vaccines and antibiotics to treat bovine pneumonia are not very efficient in reducing the prevalence of the disease, new pharmaceuticals are needed.

A prerequisite of successfully colonising the host is the ability of pathogenic bacteria to adapt to the paucity of iron. Because of the necessity to acquire host-derived iron, pharmaceuticals that intervene in the uptake of iron or its regulation could help reducing pneumonic pasteurellosis.

In order to understand how *M. haemolytica* and *P. multocida* adapt to the paucity of iron microarray technology was used to analyse the response to iron deficiency in a genome wide manner for both pathogens. Growth under iron limitation was chosen since most bacterial genes involved in iron uptake are only transcribed under iron limitation. In this work the *in vitro* iron regulated genes from *M. haemolytica* were identified for the first time and also the iron regulated genes of a bovine isolate of *P. multocida*. The transcriptional profile of a bovine isolate of *P. multocida* was produced to compare two closely related bacteria that colonize the same habitat, the bovine lung. The microarray was a multi-genome microarray and contains the open reading frames of both *M. haemolytica* and *P. multocida*.

The microarray analysis of *M. haemolytica* grown under iron limitation revealed a total of 129 genes with altered transcription. The largest group of genes with induced transcription contained genes encoding several receptors and transporters. Three quarters of them code for proteins involved in iron uptake from different sources. The largest group of genes with reduced transcription was build by genes encoding iron containing proteins involved in energy metabolism. This result shows that under iron limitation *M. haemolytica* intensifies the transcription of genes encoding proteins for iron uptake, while the transcription of genes coding for iron containing proteins involved in energy metabolism is repressed. This strategy is also observed in other bacteria.

Analysis of the transcriptome of the bovine isolate of *P. multocida* grown under iron limitation revealed 173 genes with altered transcription. The functional classification revealed that the largest group of genes with intensified transcription belonged to a group of genes coding for proteins involved in transport and binding. Two thirds of these encode proteins with functions in iron uptake. The largest group of genes with down regulated transcription was also found to be involved in energy metabolism.

Comparing the transcriptomes of *M. haemolytica* and *P. multocida* more different than common strategies were shown. Only 40 of 1424 homologous genes had the same direction of transcriptional change. Homologous genes with increased transcription (15) coded for a haemoglobin receptor of the outer membrane, the ABC-transport systems FbpABC and YfeABCD and the genes coding for the TonB-ExbBD energy transmitting system. Homologous genes with decreased transcription (25) coded for iron containing proteins mostly involved in energy metabolism under anaerobic conditions. They included the genes coding for the nitrate reductase complex NapABCDFGH, the nitrite reductase complex NrfABCD, and the fumarate reductase complex FrdABCD. An obvious difference between the two bacteria was that in *M. haemolytica* genes coding for the entire transport chain of iron derived from transferrin were induced under iron limitation. In contrast, the genes encoding the transferrin receptor were not detected in the genome of the bovine isolate of *P. multocida*. This bovine isolate of *P. multocida* seems to belong to the 30 % of bovine isolates that possess no transferrin receptor but nevertheless colonise the bovine lung (Ewers *et al.*, 2006). A second obvious finding was that in *P. multocida* the induced transcription of several genes encoding proteins for the uptake of iron from serum sources like haem, haemoglobin and haemoglobin-haptoglobin. *M. haemolytica* on the other hand has fewer genes coding for proteins involved in the utilisation of iron from haem or haemoglobin. Possibly, the many possibilities to use haem as an iron source in *P. multocida* compensates for the deficiency in using transferrin as an iron source.

For some genes of *M. haemolytica* with induced transcription *in vitro* the *in vivo* transcription was tested. Transcription of 11 genes induced under *in vitro* iron depletion was detected using RT-PCR in the RNA derived from *M. haemolytica*-infected lung tissue. For the two haemoglobin receptors HmbR1 and HmbR2 a transcriptional increase as compared to the *hmbR1* and *hmbR2* mRNA levels in the inoculum was detected by quantitative *real time* PCR. The level of induction was comparable to the transcriptional change under iron paucity *in vitro* demonstrating that the iron depleted *in vitro* culture conditions mimicked the situation in the bovine lung.

In order to examine the regulation of iron uptake in *M. haemolytica* several attempts were made to produce a mutant lacking the *fur* gene encoding the main regulator for iron uptake. The attempts were not successful, indicating that *fur* may be essential in *M. haemolytica*. A putative function of *fur* for *M. haemolytica* viability was demonstrated by an antisense approach. *M. haemolytica* carrying a *fur*-antisense-plasmid transcribing *fur* in antisense direction grew significantly slower than the control. This hints at an essential necessity of the

gene *fur* in *M. haemolytica*. Further evidence for this notion was produced by Gioia *et al.* (2007), who were also unsuccessful in producing a Δ-*fur*-mutant in *M. haemolytica*.