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Fat and foul foal fiends

The role of fatty acid and cholesterol catabolism in the pathogenesis of *Rhodococcus equi*

VAC reporter C Marr

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What is R equi?

- *R. equi* causes “rattles” - a potentially fatal respiratory disease in foals.
- It is particularly prevalent on foal breeding farms.
- In severe cases infection with *R. equi* as a foal subsequently impairs athletic ability in the adult.
- *R. equi* is closely related to the human respiratory pathogen *Mycobacterium tuberculosis* (Mtb). Mtb has been extensively studied and finding out which genes the two organisms share may be a useful short-cut to understanding R equi’s metabolism
- This project builds on previous work, funded by HBLB in which R equi’s genetic code was defined. Go to http://www.hblb.org.uk/documents/blog/Prj%20712%20Vazquez-Boland%20FINAL.pdf to find out more.

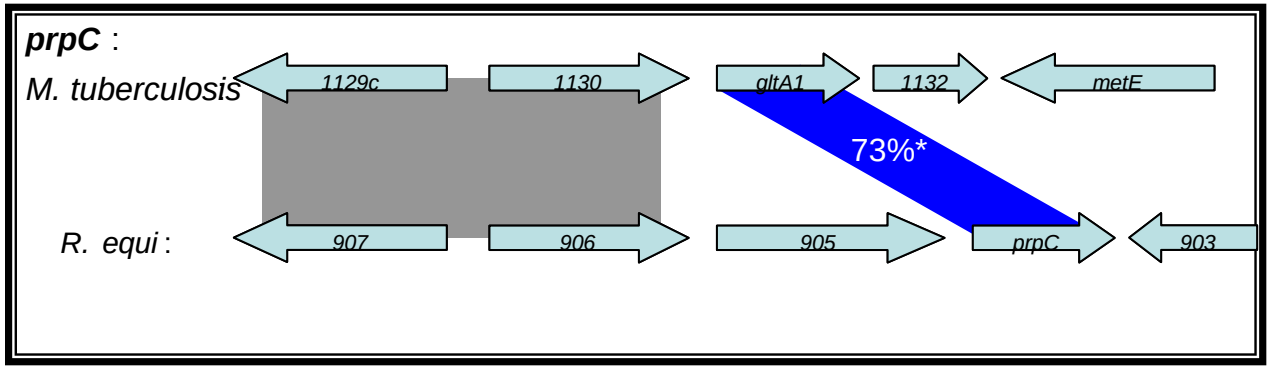
How does R equi survive and multiply within the horse and cause disease?

- Both *R. equi* and Mtb survive within alveolar macrophages which are cells involved in immunity within the lungs.
- Mtb uses fats and cholesterol as a carbon source during infection and the genes that control this process have been defined.
- The *R. equi* genome has recently been mapped. By comparing genes found in both *R. equi* and Mtb, we may learn more about similarities in the way both organisms are able to cause disease.
- If we can learn more about how R equi survives, we might in future use genetic engineering find a way to disable strains of R equi to use in a vaccine to protect foals from disease.

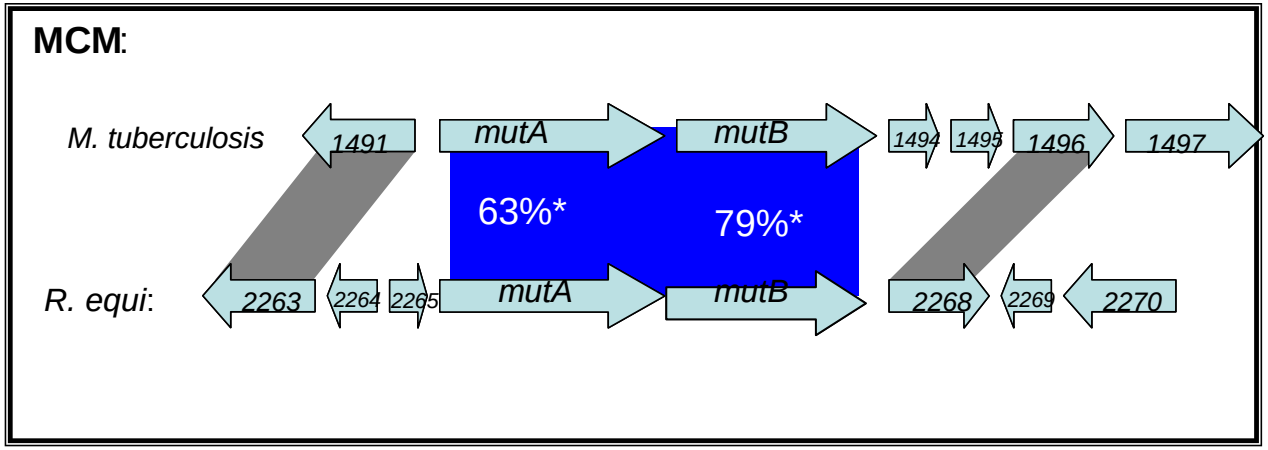
Aims of this study

- Identify genes involved in fat and cholesterol metabolism in *R. equi*
- In the laboratory, create mutant strains of *R. equi* which form which specific genes have been deleted.
- Investigate whether the missing genes affect growth of *R. equi* in isolated cells and in laboratory animals.

Results: we found both R. equi and Mtb had several genes involved in growth on fats.

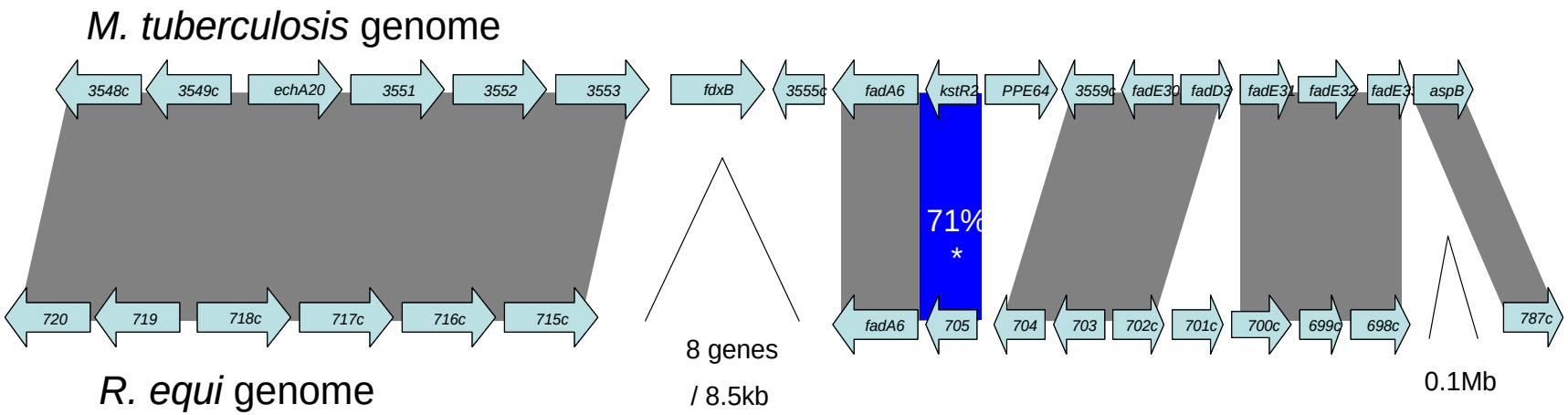


prpC is a gene which codes for an enzyme involved in growth on fats in Mtb. This gene is also in the *R. equi* genome with 73% identity (blue shading)



mutA/mutB are genes coding for an enzyme also involved in growth on fats in Mtb. These genes are also in the *R. equi* genome with 63% and 79% identity, respectively (blue shading)

We also found similarities between *R. equi* and Mtb in genes involved in cholesterol catabolism



kstR2 is a gene coding for a transcriptional regulator involved in growth on cholesterol in Mtb. This gene is also in the *R. equi* genome with 71% identity (blue shading). In Mtb this regulator controls genes involved in cholesterol catabolism and these genes are also present in *R. equi* (grey shading).

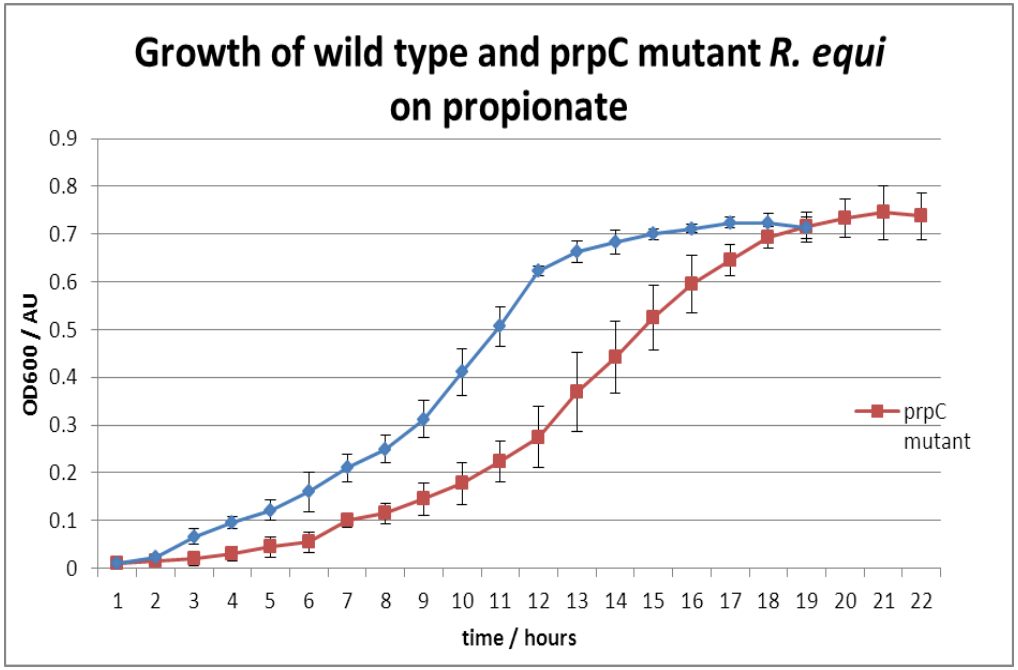
Can we delete these genes in *R. equi?*

- We were successful in deleting (Δ) *kstR2* and *prpC* from the R equi genome

- We were not successful in deleting *mutA/B* from the genome of *R. equi*

- We used a strategy that had been published but was found to be inefficient. This observation was echoed in the rest of the *R. equi* community and there is a need to develop better techniques for this purpose.

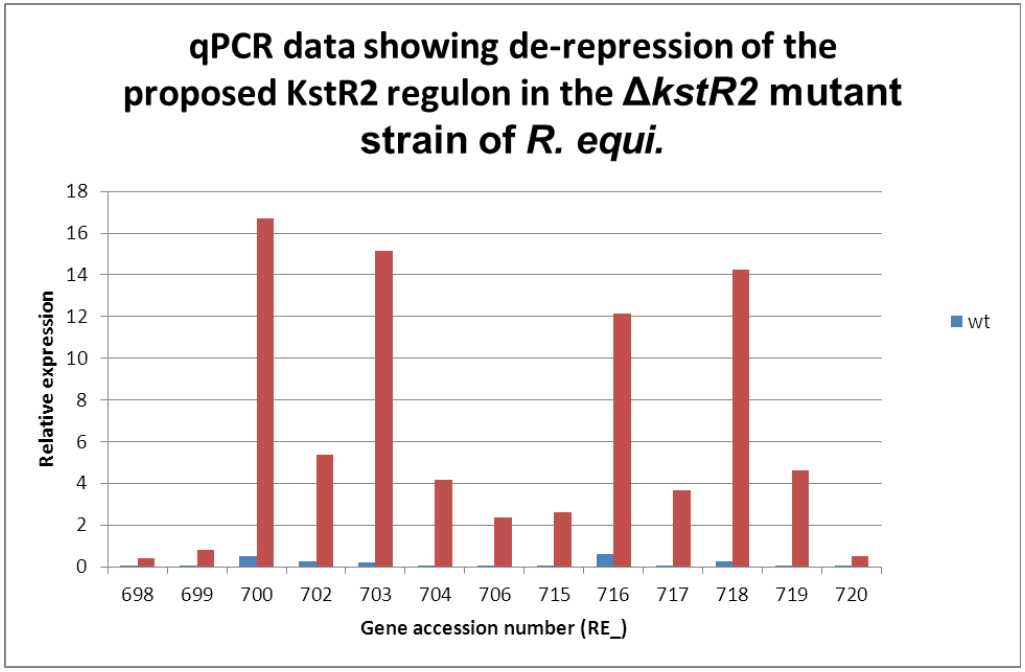
What happened when we deleted *prpC?*



Δ*prpC*

Propionate is a fatty acid. Deletion of *prpC* in *R.equi* causes a growth defect on propionate containing media.

What happened when we deleted kstR2?



Δ*kstR2*

Deletion of the gene *kstR2* causes an increase in expression of 13 genes. These genes are involved in cholesterol catabolism.

Conclusions

- *R. equi* and Mtb are very similar pathogens both genetically and pathogenically.
- The results presented here show that the metabolic pathways that use fatty acids and cholesterol are present and functional in both bacterial species.
- The current mutagenesis procedure in *R. equi* is very inefficient and new molecular tools need to be improved.
- The strains generated in this project can be used for further testing and possibly un future, vaccine development to prevent this important disease of foals.