Introduction

- *Mycobacterium* is a genus of genetically similar bacteria including various human & animal pathogens: *M. tuberculosis*; the cause of tuberculosis (TB) in humans, *M. bovis*; the cause of TB in cattle; and others such as *M. avium*; causing opportunistic infections in immunocompromised people.
- In 2014 alone, there were 9.6 million *M. tuberculosis* infections worldwide, leading to over 1.5 million deaths, the biggest cause of death by infectious disease worldwide.
- *M. bovis* has been estimated to have cost the British government an estimated £500 million for the past decade due to diagnostics, surveillance and culling of infected cattle. With this figure set to rise to over £1 billion in the next decade if improvements are not made in vaccine discovery/diagnostics.
- Extensive research has been conducted on Mycobacterium to discover new drug targets & vaccine candidates, but many have fallen short in their promise as new and effective drugs or vaccines.
- One particular area that promises is targeting cytochrome P450s (CYPs), enzymes involved in core metabolic pathways in some bacteria such as the Actinomycetales group.
- *M. tuberculosis* and *M. bovis* differ in their number of CYPs, with 20 & 17 respectively, whereas other bacteria have none, such as *E. coli* and others such as *Streptomyces avermitilis* have 33 CYPs.
- Anti-fungal drugs such as azoles have been identified to inhibit Mycobacterial CYPs but their use as effective anti-tuberculosis drugs have yet to be utilised.
- CYPs have been identified as potential targets, but their regulation in *Mycobacterium* is widely unknown, with 8 CYP genes within close proximity to TetR family of transcriptional regulators (TFTRs) genes, a group of transcriptional regulators in high abundance in *Mycobacterium* genomes, but the function of all TFTRs in *M. tuberculosis* is unknown.
- A range of bioinformatic and molecular techniques is discussed in this poster and how they will be used to further understand the role of TFTRs in CYP regulation.

**Identifying TFTRs by bioinformatics:**

- **M. tuberculosis** TFTRs (TFTR-1, TFTR-2, & TFTR-3) have been identified using bioinformatic analyses and their close proximity & likely regulation of CYP genes (Figure 2).
- CYP-associated TFTRs have homologues in *M. bovis*, except Rv1255c in *M. bovis* where a region of difference is present (Figure 2C).
- Predicted binding motifs of the three chosen CYP-associated TFTRs (Rv0135c, Rv0775 & Rv1255c) identified (Figure 3).
- All three TFTRs were successfully cloned into pNC28-Bsa4 vector using ligation independent cloning (Figure 4). With sequencing results showing 100% sequence identity & coverage compared to *M. tuberculosis* TFTR gene sequences.
- Expression of three CYP-associated TFTRs confirmed in Rosetta 2 (DE3) pLysS or in BL21 (DE3) pLYS3 (Figure 5). Likely that addition of numerous rare codons in Rosetta 2 strain helps with expression.
- Currently, expression of CYP-associated TFTRs is being pursued and optimised for efficient expression of Rv0775, with purification being conducted.

**Conclusions**

- **TFTRs** are in high abundance in the *M. tuberculosis* genome, with 11 within close proximity to CYP genes (Figure 1).
- Three TFTRs (Rv0135c, Rv0775 & Rv1255c) chosen based on bioinformatic analyses and their close proximity & likely regulation of CYP genes (Figure 2).
- CYP-associated TFTRs have homologues in *M. bovis*, except Rv1255c in *M. bovis* where a region of difference is present (Figure 2C).
- Predicted binding motifs of the three chosen CYP-associated TFTRs (Rv0135c, Rv0775 & Rv1255c) identified (Figure 3).
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**Methods**

- **Verification of binding motifs** by performing electrophoretic mobility shift assays (EMSA).
- **Confirmation of expression** by Western Blot using anti-his tag antibody.
- **Generation of TFTR deletion mutants in Mycobacterium species** e.g. *M. tuberculosis* and *M. bovis*. Other deletion work will potentially include deletion of named CYPs associated with TFTRs.
- **From deletion mutants:** study expression pattern changes using GFP/RFP reporter gene constructs and RNA-Seq/Microarrays.
- **Phenotypic analyses in deletion mutants including drug sensitivity,** as recent publications have shown that Rv1256c can be inhibited by anti-fungal Azole drugs, drugs already approved for use.
- From this work, it is hoped CYPs & TFTR regulators of *M. tuberculosis* will be further understood and facilitate in the discovery of future drug targets and to further understand mycobacterial metabolism.

**References**