Elucidating the Mechanism of ADEP Resistance in *Streptomyces coelicolor*

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BACKGROUND

Drug Resistance in *Mycobacterium tuberculosis*

- *Mycobacterium tuberculosis*, the causative agent of tuberculosis, is a pathogen that affects more than one third of the world's population.

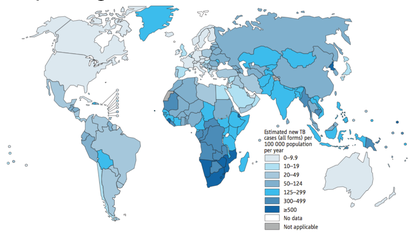

Figure 1: Estimated TB Incidence Rates, 2012[1]

- Many of the drugs previously effective against the bacterium are no longer useful due to increasing antibiotic resistance.
- New antibacterial agents are thus needed to combat infection

Synthetic Cyclic Acyldepsipeptides (ADEPs) are a powerful new way to attack drug resistant strains

- Acyldepsipeptides (ADEPs) cause unregulated protein degradation in bacteria by targeting the ClpP peptidase.

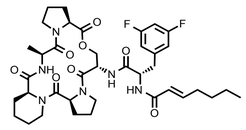

Figure 2: ADEP 1A

- ADEPs have been shown to be more effective in *M. tuberculosis* when co-administered with efflux pump inhibitors[2], implicating a mechanism of efflux for resistance

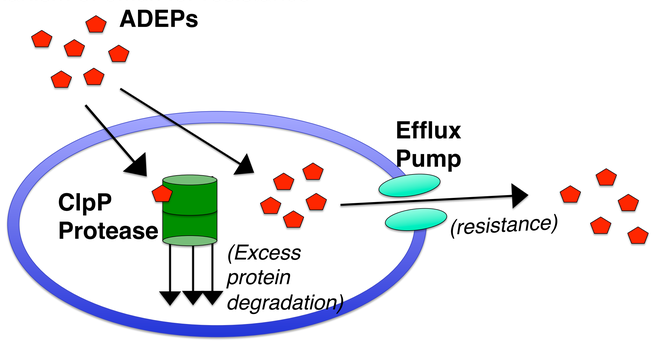

Figure 3: A proposed mechanism of ADEP resistance in *M. tuberculosis* and *Streptomyces coelicolor*

Streptomyces coelicolor is a model organism for *M. tuberculosis*

- The efflux pump responsible for ADEP resistance in *M. tuberculosis* has yet to be characterized
- Because *Streptomyces coelicolor* is non-pathogenic, easily genetically manipulated, and grows faster than *M. tuberculosis,* it is and ideal model organism for laboratory experiments.

The Identification of the Efflux Pump Responsible for ADEP Resistance in *Streptomyces coelicolor*

- Bioinformatic analyses have implicated that an ABC-type transporter is involved in ADEP resistance[3]
- *sco1719* and *sco1720* are two genes in *S. coelicolor* that encode a promising ABC transporter candidate for the extrusion of ADEPs
- *sco1718* is hypothesized to encode its regulator, likely a repressor of the pump

Figure 4: The genetic locus thought to encode the efflux pump responsible for ADEP resistance and its regulator

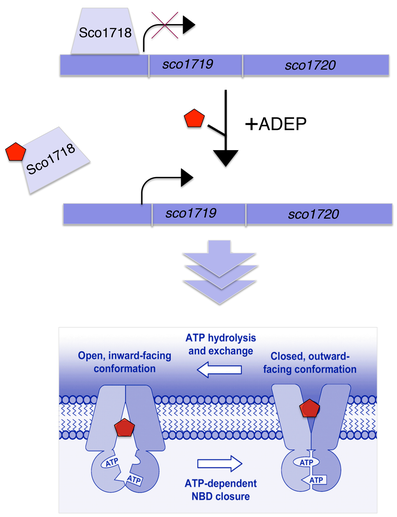

Figure 5: Predicted mechanism of efflux regulation

EXPERIMENTAL DESIGN

Generation of *S. coelicolor* Strains Lacking either *sco1718* or *sco1719-20*[4]

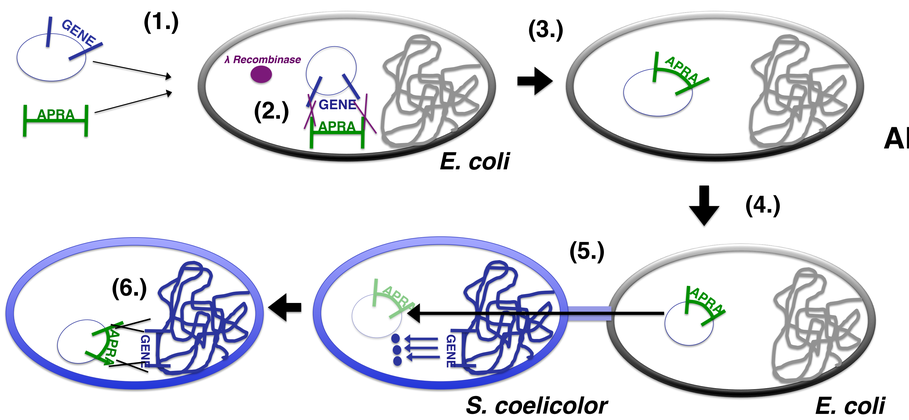

Figure 6: The generation of *Δsco1718::apr* and *Δsco1719-20::apr* null strains

1.) Transformation of *S. coelicolor* cosmid with gene of interest and PCR-amplified *apr* cassette into *E. coli* expressing λ recombinase

2.) Homologous recombination

3.) 30°C→37°C to lose temperature sensitive λ recombinase plasmid

4.) Transformation and amplification in conjugation *E. coli* strain

5.) Conjugation into *S. coelicolor*

6.) Homologous recombination and gene silencing

Creation of *S. coelicolor* Strains Over-expressing Either *sco1718* or *sco1719-20*

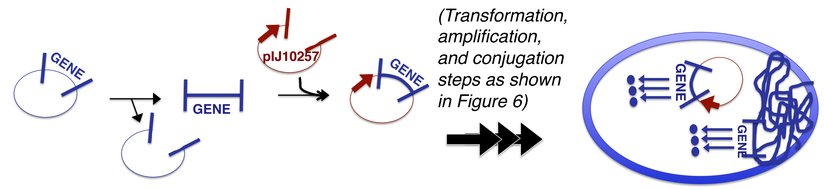

Figure 7: The creation of *sco1718* and *sco1719-20* overexpression strains

RESULTS

Confirmation of Gene Knockouts and Overexpression Constructs via Restriction Analyses

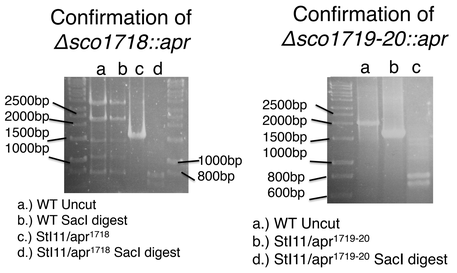

Figure 8: Analytical digests of WT and null strain genomic DNA in the region of gene replacement

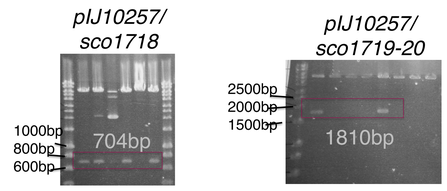

Figure 9: Analytical digests of *sco1718* and *sco1719-20* insertion into overexpression vector pIJ10257

ADEP Susceptibilities of WT and Null Strains of *S. coelicolor*

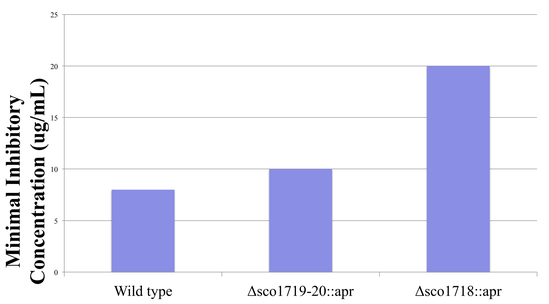

Figure 9: Minimal Inhibitory Concentrations of ADEPs against *S. coelicolor* WT and null strains

FUTURE DIRECTIONS

- MIC testing of overexpression strains
- MIC testing with efflux pump inhibitors
- Further analysis of Sco1718 regulatory role (RT-PCR, electrophoretic mobility shift assays, etc.)
- Testing against other antibiotics

REFERENCES

[1]*Global Tuberculosis Report 2013*. Rep. *World Health Organization (WHO)*. Web. 6 Aug. 2014. <http://apps.who.int/iris/bitstream/10665/91355/1/9789241564656_eng.pdf?ua=1>. **[2]Ollinger, J.; O'Malley, T.; Kesicki, E. A.; Odingo, J.; Parish, T.** 2012. Validation of the essential ClpP Protease in *Mycobacterium tuberculosis* as a novel drug target. Journal of Bacteriology 194(3):663-668. **[3]Gominet, M; Seghezzi, N; Mazodier, P.** 2011. Acyl depepsipeptide (ADEP) resistance in Streptomyces. Microbiology 157(8):2226-34. **[4]Gust, B; Kieser, T; Chater, K.F.** 2002. REDIRECT technology: PCR-targeting system in *Streptomyces coelicolor*