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### Materials and methods

#### 2.1 Bacterial strains, media and growth conditions

*Mycobacterium smegmatis* mc<sup>2</sup>155 (Msmeg) was obtained from the Division of Molecular Biology and Human Genetics at Stellenbosch University (South Africa) and maintained as a frozen glycerol stock at –80°C for all experiments done in this project. Liquid cultures of Msmeg were grown in Middlebrook 7H9 medium (Becton Dickinson, Franklin Lakes, NJ, USA) supplemented with 2% glucose, 0.85% sodium chloride (NaCl) and 0.05% Tween 80 (7H9 glucose-salt), and incubated at 37°C in a shaking incubator at 200 rpm. Solid cultures were maintained on 7H10 agar (Becton Dickinson) supplemented with 2% glucose, 0.85% sodium chloride (NaCl) and incubated at 37°C.

Growth curves were performed by inoculating a 1 ml glycerol stock into 50 ml 7H9 glucose-salt and incubated for 12 hours. This starter culture was then used to inoculate a volume of 0.5 ml in liquid culture, which was subsequently incubated for 27 hours, and the growth monitored by transferring 1 ml of each culture into a cuvette and measuring the OD<sub>600nm</sub> at intervals of 3 hours. Cultures with OD<sub>600nm</sub> readings above 1 were diluted appropriately.





































































## References

- Adams, KN, Takaki, K, Connolly, LE, *et al.* (2011). Drug Tolerance in Replicating Mycobacteria Mediated by a Macrophage-Induced Efflux Mechanism. *Cell* 145: 39–53.
- Ahmad, S (2010). Pathogenesis, Immunology, and Diagnosis of Latent *Mycobacterium tuberculosis* Infection. *J Immunol Res* 2011: e814943.
- Amaral, L, Martins, A, Spengler, G, *et al.* (2014). Efflux pumps of Gram-negative bacteria: what they do, how they do it, with what and how to deal with them. *Exp Pharmacol Drug Discov* 4: 168.
- Andrews, JM (2001). Determination of minimum inhibitory concentrations. *J Antimicrob Chemother* 48: 5–16.
- Andries, K, Verhasselt, P, Guillemont, J, *et al.* (2005). A diarylquinoline drug active on the ATP synthase of *Mycobacterium tuberculosis*. *Science* 307: 223–7.
- Ansorge, WJ (2009). Next-generation DNA sequencing techniques. *New Biotechnol* 25: 195–203.
- Balasubramanian, V, Solapure, S, Iyer, H, *et al.* (2014). Bactericidal activity and mechanism of action of AZD5847, a novel oxazolidinone for treatment of tuberculosis. *Antimicrob Agents Chemother* 58: 495–502.
- Balganesh, M, Dinesh, N, Sharma, S, *et al.* (2012). Efflux Pumps of *Mycobacterium tuberculosis* Play a Significant Role in Antituberculosis Activity of Potential Drug Candidates. *Antimicrob Agents Chemother* 56: 2643–51.

















