

New vaccine development

Recombinant production of three *Mycobacterium tuberculosis* proteins in submerged cultures of *E. coli*

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Mycobacterium tuberculosis (Mtb) causes death of 2-3 million people annually and is considered one of the most successful intracellular pathogens to persist inside the host macrophage. The fluctuation in effectiveness of the actual vaccine and treatments makes clear the need to look for new alternatives for active immunization and drugs. Recombinant production of Mtb extracellular proteins is an excellent alternative for the development of new treatments. In this work, three major secretory proteins that have been gaining importance for their immunogenicity (CFP-10, 10 kDa; ESAT-6, 6 kDa and APA, 45-47 kDa), were overexpressed using the same cell line; *E. coli* Rosetta (DE3)/PET15b, in the form of inclusion bodies. The expression systems were optimized individually with respect to culture media, inducer concentration and time of induction in shake flasks cultures. This was then scale up to 1.0 L bioreactor cultures controlling pH, temperature and dissolved oxygen tension. The over-production of each protein was measured by gel densitometry and confirmed by western blot analysis. This high cells specific product yields ease the further downstream processing steps and improved product recovery by His-tag protein purification protocols. Finally, using this methodology we can obtain large quantities of proteins that otherwise the production of these are complex, costly and risky because the highly pathogenicity of Mtb.