Synthetic Biology Approach For A New Tuberculosis Treatment

David Pohlman

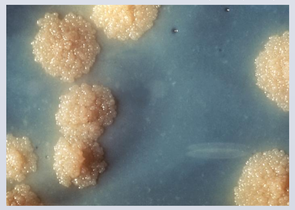
Advisors: Dr. David Westenberg and Dr. Katie Shannon

Abstract

Tuberculosis is caused by a *Mycobacteria tuberculosis* infection that can arise from ingesting or inhaling *M. tuberculosis* tubercle bacilli. The bacteria then take residence within the body, mainly the lungs, causing painful lesions and possibly death. The body's natural defense to *M. tuberculosis* infections is to ingest the cells by means of endocytes, but all Mycobacterium produce a waxy coating made up of free mycolic fatty acids and the endocytes cannot breakdown the bacteria. I propose to use synthetic biology to create a hybrid protein of the *Clostridium cellulovorans'* cellulosome and the peroxisomal multi -functional protein 2 to be able to breakdown extracellular free fatty acids that will allow anti – TB drugs and endocytes to stop the growth or kill the infectious bacteria. This system will implemented into *E. coli* and further characterization and analysis will be done with those cells.

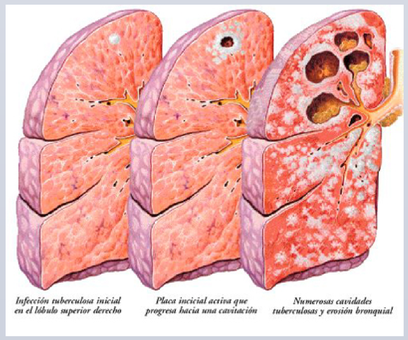
Background

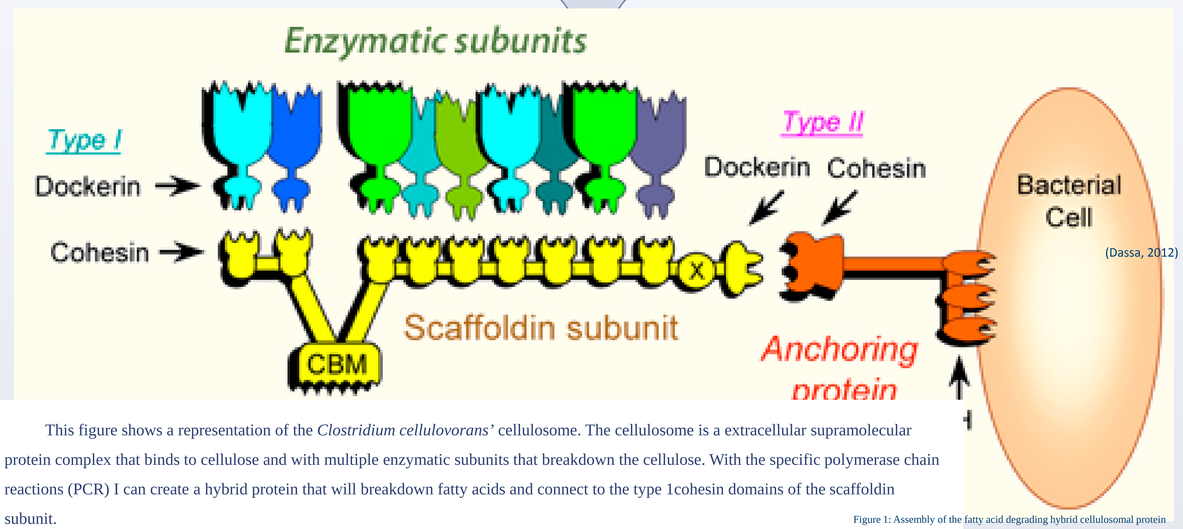
(Kubica, 1976)



With the advancement of modern medicine Tuberculosis (shown to the right) infections still cause many deaths around the world. This increasing number of deaths can be attributed to the emergence of muti- and extreme drug resistant *M. tuberculosis* strains in large numbers around the world. In recent years total drug resistant (TDR) *M. tuberculosis* has been discovered in multiple countries (5). India has had the most recent discovery of TDR *M. tuberculosis* within the past 1-2 years, following cases documented in Italy in 2007 and Iran in 2009 (5).

Typical treatment of TB takes 6-9 months with a rigorous amount of four different anti-TB antibiotics. With this long treatment time comes the opportunity for patients to miss or stop treatment allowing the resistant strains of *M. tuberculosis* to grow in higher numbers and cause more complications. The treatment for TB is so long due to the bacteria producing a waxy coating consisting of mycolic acids, a complicated fatty acid. This waxy coating causes problems due to the anti-TB drugs being water soluble; not allowing the drugs to get to the cells.





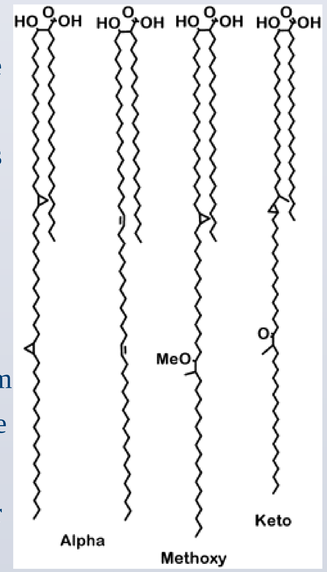
(Dassa, 2012)

This figure shows a representation of the *Clostridium cellulovorans'* cellulosome. The cellulosome is a extracellular supramolecular protein complex that binds to cellulose and with multiple enzymatic subunits that breakdown the cellulose. With the specific polymerase chain reactions (PCR) I can create a hybrid protein that will breakdown fatty acids and connect to the type 1cohesin domains of the scaffoldin subunit.

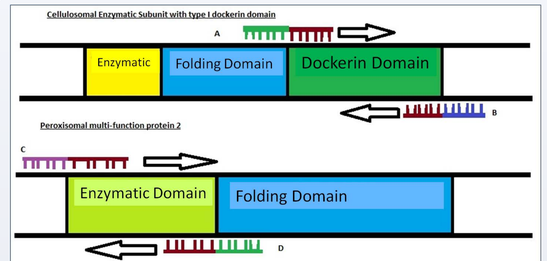
Figure 1: Assembly of the fatty acid degrading hybrid cellulosomal protein

Experimental Design

I propose to create a system that will breakdown the free mycolic acids that are produced by the *M. tuberculosis.* With the mycolic acids broken down the tuberculosis cells will susceptible to anti-TB drugs and the body's own immune system. The function of this system will not depend on the *E. coli* cell's own metabolism but rather just the exposure to the free mycolic acids. This is due to the hybridization of the cellulosomal dockerin domains of the enzymatic subunits and the peroxisomal multi-function protein 2 enzyme.



(Langford, Penkov, Derrington & Gundlach, 2011)



Hybridization of the dockerin domain of the cellulosomal enzymatic subunits and fatty acid degradation enzymatic domain

Future Steps

- A feedback system that responds to the degradation of mycolic acids
  - A system that is suppressed by the presence of mycolic acids
  - Once turned on it will make the cell produce a newly discovered peptide that inhibits *M. tuberculosis* growth called lariatins
    - A lot more research will have to go into this step to make it work properly
- Implementation of "Mycolic Acidosome" and feedback systems into a highly engineered non-pathogenic bacteria
  - This bacteria will have multiple fail-safes to have a strict control of its growth and life span

References

1. Cohn, D. L., Bustreo, F., & Raviglione, M. C. (1997). Drug-resistant tuberculosis: Review of the worldwide situation and the who/iuatld global
2. Kubica, G. (Photographer). (1976). *This is a close-up of a mycobacterium tuberculosis culture revealing this organism's colonial morphology*. [Print Photo]. Retrieved from
3. Kock, R. (2003, December 9). *Tuberculosis.* Retrieved from http://www.nobelprize.org/educational/medicine/tuberculosis/readmore.html?downloadURL=true&loId=BCCBBF73-1B0D-42EA-B590-FF3EA8AA5FDF
4. Walker, C. C. (1910). Tuberculosis and the modes of infection. *California State Journal of Medicine*, *8*(4), 124-127.
5. Rowland, K. (2012, January 13). *Totally drug-resistant tb emerges in india*. Retrieved from http://www.nature.com/news/totally-drug-resistant-tb-emerges-in-india-1.9797
6. Dassa, B. (Designer). (2010). *File:cellulosome.jpg.* [Web Graphic]. Retrieved from http://www.cazypedia.org/index.php/File:Cellulosome.jpg
7. Langford, K. W., Penkov, B., Derrington, I. M., & Gundlach, J. H. (2011). Unsupported planar lipid membranes formed from mycolic acids of mycobacterium tuberculosis. *Journal of Lipid Research*, *52*, 272-277.

Sponsors

• Biological Sciences Department
• Chemistry Department
•Chemical Engineering Department
• Student Design and Experiential Learning Center

RESEARCH POSTER PRESENTATION DESIGN © 2012
www.PosterPresentations.com