BACTH Assay

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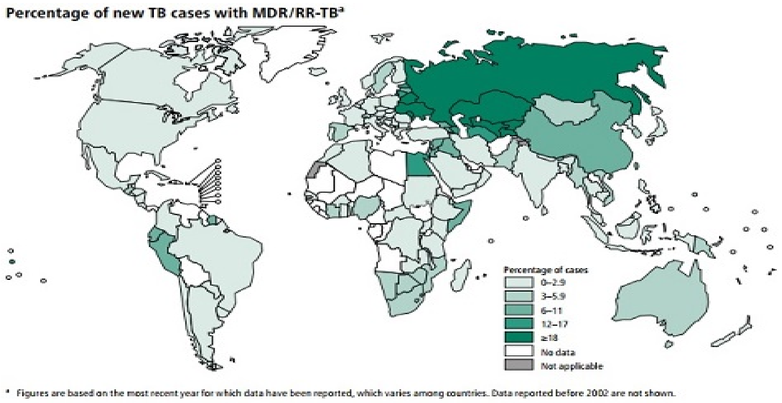
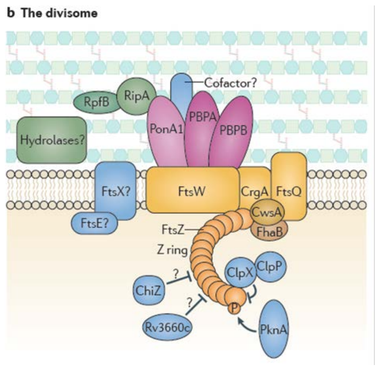
ABSTRACT

Drug resistant tuberculosis (TB) is a disease affecting many around the world. In 2016, drug resistant TB accounted for 240,00 deaths.



Due to TB's resistance to common antibiotics, new ones are needed to halt the spread of this disease. Along with several researchers, we studied several different proteins (CrgA and CwsA) in the divisome of TB to see if there is an interaction between the proteins that could possibly be interrupted in order to dsable the cell from dividing and thus continuing the infection.

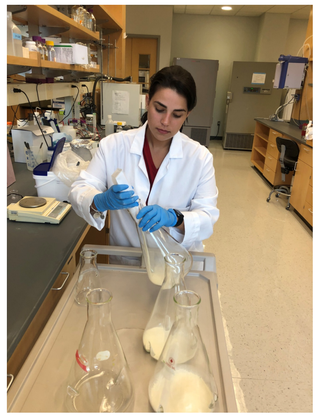
The figure to the right shows what we know to be the divisome complex of drug resistant TB. Proteins in this complex could make ideal drug target sites because this complex controls whether or not the cell divides.



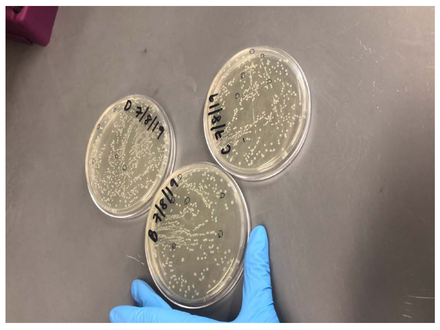
"The bacterial adenylate cyclase two-hybrid system (BACTH) is a genetic approach used to test protein interactions in vivo in E. coli. This system takes advantage of the two catalytic domains of Bordetella pertussis adenylate cyclase (CyaA) toxin, which can be fused separately to proteins of interest. If the proteins of interest interact, then the adenylate cyclase domains will be brought in close proximity to each other, reconstituting cyclic AMP (cAMP) production. Interacting proteins can be both qualitatively and quantitatively assessed by the expression of chromosomal genes of the E. coli lac or mal operon, which are positively regulated by cAMP production. Because cAMP is diffusible, the proteins of interest do not need to interact near the transcriptional machinery. Consequently, both cytosolic and membrane protein-protein interactions can be tested. The BACTH system has recently been modified to be compatible with Gateway® recombinational cloning, BACTHGW". After completing this experiment several times, we found that there was a potential interaction, but statistically the evidence was not strong. There was no statistical difference in our averages.

BACTH Assay process summary:
1. Grow overnight culture
2. Protein transformations
3. Protein Expressions
4. Assay of beta-galactose activity
5. Enzymatic reaction
6. Measure final absorbance (AB)

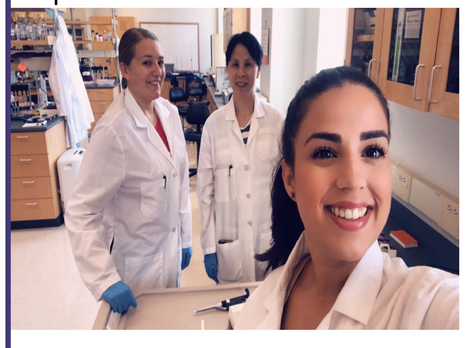
1.Overnight cultures are used to grow cells of many types that can be used to grow and express proteins. Luria-Bertani broth is used as a medium for this growth and as food for the cells. We worked with E. coli cells which thrive in LB broth.



2. Protein Transformations allow researchers to isolate certain constructs and focus on certain proteins, in essence "transforming" a certain part of the connections made by the competent cells used. We used this process to insert proteins CrgA and CwsA into the cells.



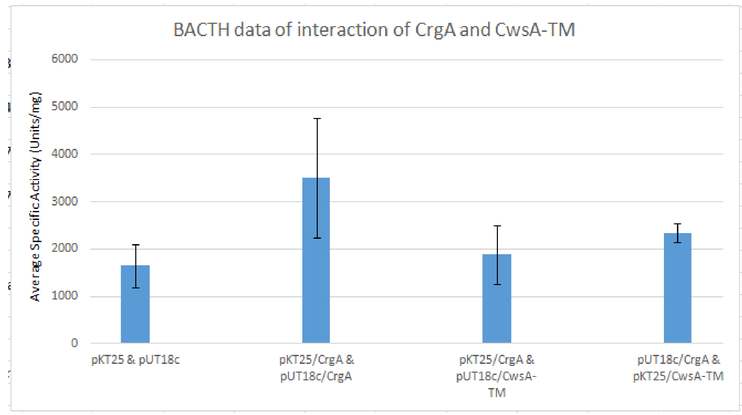
3. Protein Expressions let researchers to see if our constructs (proteins) bound to the cell and that the cell is still living! This step was used to find colonies that had protein interactions. The blue colonies in the picture showed protein expression.



4. An assay of beta-galactose activity was used to determine how many cells were in each sample. From here, the cells were lysed so that only the proteins could be measured in the next step, not the whole cell.

5. The enzymatic reaction was used to facilitate the interaction between proteins CrgA and CwsA. Two buffers were added, the solution vortexed, then left to sit in a hot bath (28 degrees Celcius) for 5 minutes. Sodium carbonate (Na_2CO_3) was added to stop the reaction. This should turn the solution yellow.

6. Measure final AB (RESULTS!)



After centrifuging the microtubes with the protein solution, the supernatent was pulled off and the AB was measured. The raw data indicated that there were some interactions between CrgA and CwsA. After running an ANOVA test, we found that there was no statistical difference in our averages.

Acknowledgements:
Dr. Tim Cross
Dr. Huajun Qin
Dr. Rongfu Zhang
Dr. Sean Smrt
Yiseul Shin
Marco Marotta
Joshua Hancock

This research is funded by the National Science Foundation award number DMR1644779.

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