Characterization of a Key *Acinetobacter baumannii* Iron Scavenging Protein

Ciara Phares & Dr. Michael Rowse

Div. Science, Indiana University-Purdue University Columbus, 4601 Central Ave., Columbus, IN, 47203, USA

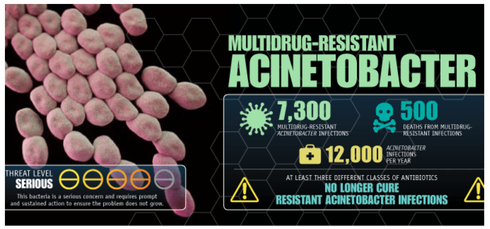
IUPUC OFFICE OF STUDENT RESEARCH
INDIANA UNIVERSITY-PURDUE UNIVERSITY COLUMBUS

Abstract

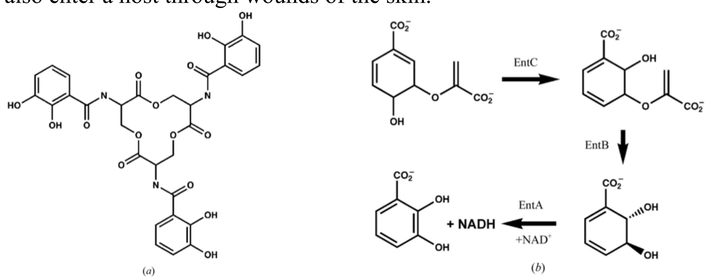
Bacterial pathogens are becoming increasingly resistant to antibiotics. Soldiers in conflict zones and immunocompromised hospital patients are the most susceptible to antibiotic resistant infections. One of the ways to fight this is to continually develop new antibiotics.

Our research is focused on characterizing the *A. baumannii* EntA protein that catalyzes a key step of the synthesis of an iron scavenging molecule. The *E. coli* analog allowed us to compare our results to a well characterized similar protein and served as a control for our experiments. Few factors are known that allow *A. baumannii* to persist in and infect the human body other than its iron-scavenging pathway that allows it to obtain iron in the iron- limiting environment of the human body. *A. baumannii* cannot produce its own iron, and if an antibiotic could be made to disrupt this pathway, *A. baumannii* infections can be controlled. This project will provide a greater understanding of *A. baumannii's* ability to scavenge iron and to assess the potential of targeting the EntA protein with antibiotics.

Introduction



A. baumannii is a bacterium that is naturally resistant to many antibiotics and is difficult to control due to its ubiquity in moist environments such as soil and surface water. This organism can also persist on dry, abiotic substances such as medical equipment, catheters, implants and the dry biotic surface of our skin. *A. baumanii* is involved in an estimated 2-4% of hospital acquired infections and often can directly cause the death of infected individuals (Penwell, 2012). This pathogen targets moist tissues such as the mucous membranes and can also enter a host through wounds of the skin.



Above left: The complete iron scavenging siderophore named enterobactin produced by *E. coli*. Above right: Details of the reaction catalyzed by EntA required for production of enterobactin.

```
1...*...10....*...20....*...30....*...40....*...50....*...60....*...70....*...80....*...90....*..100
IAWVTGVNQGMGAEIMQRLIAQHIYVVGFDLSTNNIVESDRYEVHQCDVRDAVQISSLCQKLLKTSPPDYFINTAGVLHLDEHDTLPEDAWLQTFEVNTF

....*..110....*..120....*..130....*..140....*..150....*..160....*..170....*..180....*..190....*..200
APFYFLKHLSPYFREKRNGNIVMVSSNSAHVPRMKMAAYGASKAALTSFSKTVGLELAEYGIRVNIVSPGSTATPMLRQLWKDASGEKQTIHGNLAQYKV

....*..210....*..220....*..230....*..240..
GIPLQKIALTEDIAHAVMFLISDQASHITMHDLVIDGGATLG
```

Shown above is the *A. baumannii* EntA protein sequence we are going to crystallize. Each letter represents an amino acid. This allows us to predict the secondary structure of the EntA protein. The prediction suggests a well-folded protein. Red letters are predicted to form alpha helices and blue letters are predicted to form beta sheets.

Methods

Obtain Genomic DNA Template → PCR amplify entA genes → Use an agarose gel to purify genes → Cut genes and circular DNA molecule with restriction enzymes → Insert genes in circular DNA molecule to make a recombinant plasmid (figure 1a) → Insert plasmid into bacteria (*E. coli*) (figure 1b) → Express entA gene to make many copies of the EntA protein (figure 2) → Purify the protein using a His-tag method (figure 3) → Deduce the concentration of protein produced

Results

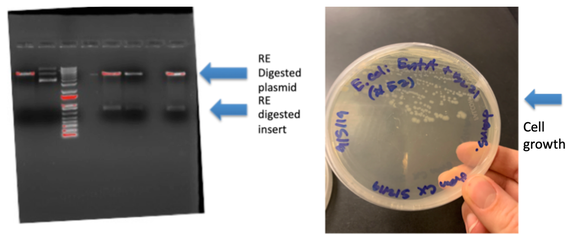


Figure 1 (a & b): After ensuring that we had successfully ligated the entA gene with the plasmid by separating the fragments using restriction enzymes and an agarose gel (figure 1a), we transformed the recombinant plasmid into E. *coli* cells (figure 1b).

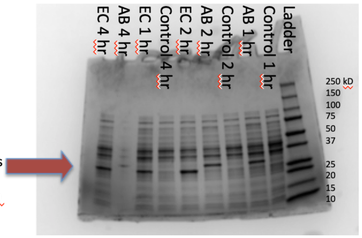


Figure 2: After growing transformed E. *coli* cells with the recombinant plasmid, we induced the cells for different time lengths to make many copies of the EntA protein. Using a protein gel we ensured the size of the protein and determined that a four hour induction time point produced the most protein.

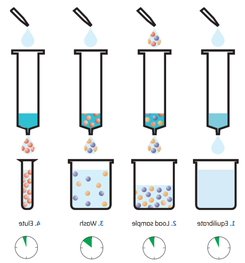


Figure 3: Once our cells expressed the entA gene and made many copies of the protein, we purified the protein using a His tag method shown above.

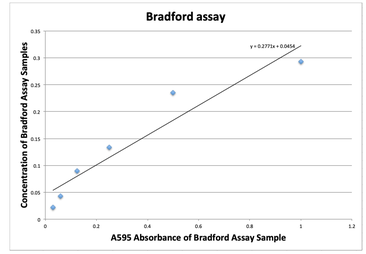
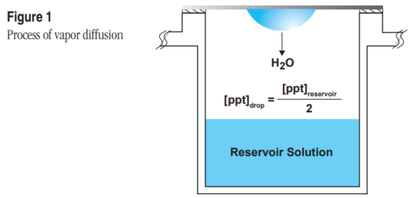


Figure 4: After purification of the EntA protein, we deduced the concentration using spectrophotometry and a Bradford assay. This Excel graph line provided us with the estimate of being able to produce 13.5 milligrams EntA per 1 liter of E. *coli* cells.

Next Steps



Crystal growth of our purified proteins will be conducted via the hanging drop method.

Acknowledgements

This study was funded by 2017 and 2019 IUPUC Office of Student Research Grants. David Cool allowed us to use biology lab equipment throughout the research.

References

Penwell, W. F., Arivett, B. A., & Actis, L. A. (2012). The *Acinetobacter baumannii* entA gene located outside the acinetobactin cluster is critical for siderophore production, iron acquisition and virulence. PloS one, 7(5), e36493.

"Healthcare- associated infections (HAI): Diseases and Organisms: *Acinetobacter* in Healthcare Settings." U.S. Department of Health and Human Services: Centers For Disease Control and Prevention, 24, Nov. 2010. Web.

"UniProtKB-P15047 (ENTA_ECOLI)." Uniprotconsortium, 13, Feb. 2019. Web.

"Prediction of Protein Crystallibility." XtalPred-RF, 2, June 2016. Web.

Hanging Drop Vapor Diffusions Crystallization: Crystal Growth 101. *Hampton Research Corp.*, 2017. Web.

Gravity-flow Purification Using His GraviTrap and His Gravitrap Kit. Affinity Chromatography Vol. 2: Tagged Proteins, GE Healthcare, 2016.