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

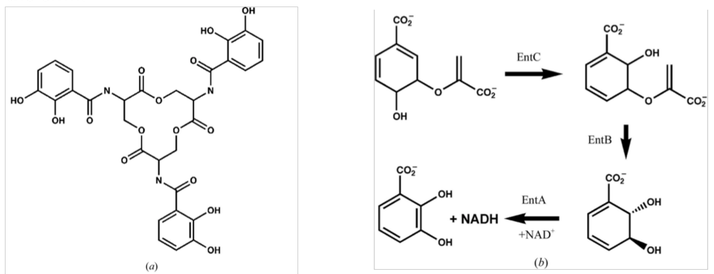
Abstract

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 it's 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.

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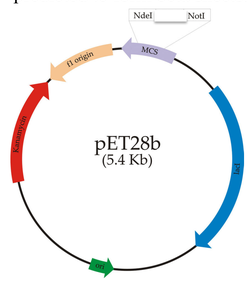
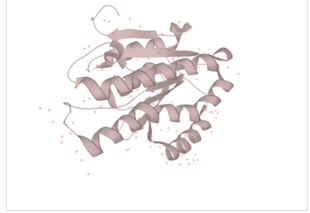
Shown above is the *A. baumannii* EntA protein sequence we are going to crystallize. Each letter represents an amino acid. This allows us to predict the secondary structure of the EntA protein. The prediction suggests a well-folded protein. Red letters are predicted to form alpha helices and blue letters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



Shown above is the pET28b plasmid we are inserting the gene into in order to make many copies of the EntA protein.



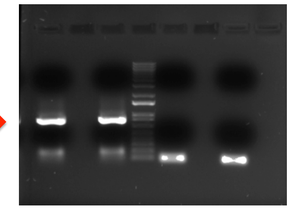
The crystallized protein should look similar to this EntA protein from *E. coil* shown above.

Methods

Obtain Genomic DNA Template → PCR amplify entA genes (Figure 1) → Use an agarose gel to purify genes → Cut genes and circular DNA molecule with restriction enzymes (Figure 2) → Insert genes in circular DNA molecule (Figure 3) → Insert DNA into bacteria (*E. coli*) → Select colonies to confirm correct DNA sequence (Figure 4) → Express genes and isolate protein for crystallization (Next Steps)

Results

Figure 1. The agarose gel with our PCR amplified gene products.



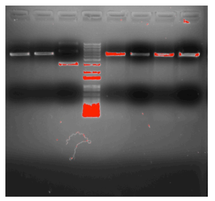
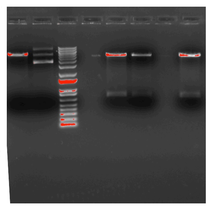
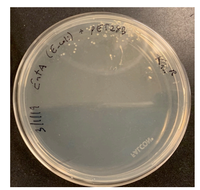


Figure 2. Left: Our circular DNA molecule was successfully cut with restriction enzymes.

Figure 3. Right: Confirmation of EntA gene insertion by fragment analysis





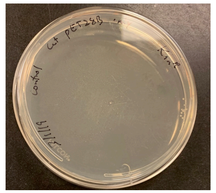
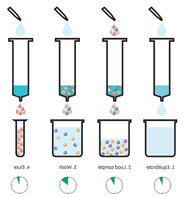
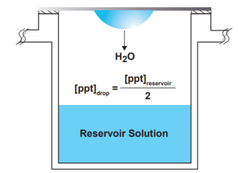


Figure 4. Above Left: the gel plate with our bacteria that have been transformed with our gene. Above Right: Our control plate of bacteria that do not have our gene.

Next Steps



Once our cells have expressed the entA gene and made many copies of the protein, we will purify the protein using a His tag method.



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

Acknowledgements

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References

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