Abstract:
Spider silk has many applications including human health (sutures, vaccine encapsulations, i.e.) and military usage (underwater Velcro-type fasteners). In order to test these applications, we need large quantities of spider silk protein to use in our experiments. One way to make spider silk is by using *Escherichia coli* (*E. Coli*) to produce the protein. *E. Coli* has many advantages: it can be produced in larger amounts, it is inexpensive to grow, and it can be easily transported. We usually start with a culture mass weighing 4 kg but we are scaling up to produce even greater amounts of product. The process of making spider silk from *E. Coli* is a continuous research experiment because we are always asking ourselves questions, for example: how can we make more product? Or how can we make the product cleaner (only spider silk protein left). 

Methods:
- First, the *E. Coli* is cultured under controlled conditions using the BL22434 strain of *E. Coli*. This strain is not harmful to humans and, at the time *E. Coli* production was started, it was the best strain to work with.
- To the pelleted bacteria add water and buffer. The buffer is a mixture of Sarkosyl, NaCl, Tris, and Lysozome. We use these buffers to maintain the pH of the solution so we can work with a stable solution.
- Next, sonicate the *E. Coli*, applying sound energy to shear the DNA and decrease viscosity, and to help in the process of lysis.
- The sample is then heated to 70-80 °F for 45 minutes. This removes many of the *E. Coli* proteins while leaving the spider silk protein in solution.
- We centrifuge to remove contaminants.
- Then we add ammonium sulfate to the supernatant to precipitate the spider silk protein.
- We then collect the pellet and pour out supernatant.
- Finally, we wash the pellet with Isopropyl alcohol (IPA) to remove contaminants. It also makes the spider silk protein insoluble so it is not lost in the washing.

Results:
The figure below represents the gel we took after we cleaned the spider silk protein after each step. First is the MW ladder (ctl). We then took samples of lysis (ls), heat (ht), supernatant 1 (s1), pellet 1 (p1), after ammonium added (am), of the foam (fm), supernatant 2 (s2), and of pellet 2 (p2). Changes: We have been trying to get better results by changing different parts of the process. We have tried: changing different parts of the buffer so that we can get a solution that is stable and allows us to precipitate out the maximum amount of spider silk protein. Changing how much bacteria we start with so we can try to make the most synthetic spider silk, when we do so, there are still changes that need to be made to make the greater amount more synthetic. Also, how much liquid we add; which changes the viscosity of the solution. We have also changed the conductivity of the final product, the rate of the centrifuges, and tried different types of centrifuges to see if slowing down the centrifuge will take more contaminants out and leave behind a cleaner solution of the spider silk protein. Lastly, the amount of time we heat so see if heating for more time or less time makes a difference on the amount of *E. Coli* broken up and leave the spider silk protein still intact.

Introduction:
*Escherichia coli* is one way to make spider silk protein, but not the only way. We also get spider silk protein from goats, Alfalfa, and Silkworm. The reasons we are using *E. Coli* is because it can be produced in large amounts and it is relatively inexpensive. Spider silk has a wide variety of applications. It can be used to improve human health (through sutures for doctors and dentists) to military usage.

Conclusion and Future Plans:
Spider silk has a wide variety of applications if it can be produced in large amounts. Through *E. Coli*, we believe that we can achieve this. We will continue to refine not only the purification process but also the culture conditions.

Authors:
Dr. Randolph V. Lewis
Dr. Justin Jones
Todd Brown