Can Elizabethkingia Miricola Resist Antibotic Resistance?

Testing Elizabethkingia Miricola's Resistance to Antiboitics

GRP#08
Section01

Dr. Patricia Canaan, Kaysten Goins, Kristy Johnson, Vivienne Hasenbeck

ABSTRACT

Many types of bacteria live in our body, on our body and everywhere around us. Some of these bacteria are beneficial and some are harmful. Harmful bacteria that invade the human body are treated with antibiotics. Some types of bacteria have immunity to these aforementioned antibiotics (New World Encyclopedia). We are interested in the antibiotic resistaning abilities of a specific bacteria by the name of Elizabethkingia miracola. We hypothesized that this bacteria would be resistant to a specific group of antibiotics because it has structures similar to that of other bacteria that are resistant to the same group of antibiotics. To test this, we made multiple copies of E.miricola and then exposed it to beta lactam antibiotics. Our results supported our hypothesis that the E.miricola bacteria would be resistant to b-lactam antibiotics.

INTRODUCTION

Our investigation will answer the question, "is E.miricola resistant to b-lactam antibiotics?". A contributing factor to the antibiotic resistance of bacteria is the type of enzymes they produce. When a bacteria produce a type of enzyme that destroys a certain type of an antibiotic, it is resistant to that type of antibiotic. In our study we are testing E.miricola's resistance to b-lactam antibiotics. B-lactam antibiotics are a wide range of antibiotics that include penicillins, cephamycins, and many others. Certain bacteria have a gene which creates the beta-lactamase enzyme which destroys the efficacy of these antibiotics (Bradford 2015). Elizabethkingia miricola is a type of bacteria that have the genes which create the beta-lactamase enzyme and our experiment was to test the hypothesis, solidify the information which suggests that bacteria with the beta-lactamase gene will be able to cleave b-lactam and destroy the antibiotics, and to prove or disprove wether or not E.miricola is resistant to beta-lactam antibiotics.

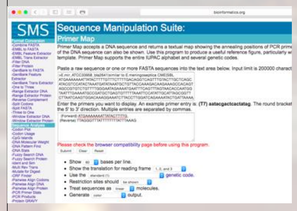
MATERIALS AND METHODS

- P20 micropipette & disposable tips
- PCR primer, Taq polymerase, R-primer, Taq buffer, gDNA, F-primer, R-primer & dNTP's
- Gel electrophoresis & dye
- E-coli cells, ice, heat shock-water bath 42 C, & incubator
- Testing plates & black marker
- Kanamycin broth, clone, & cube

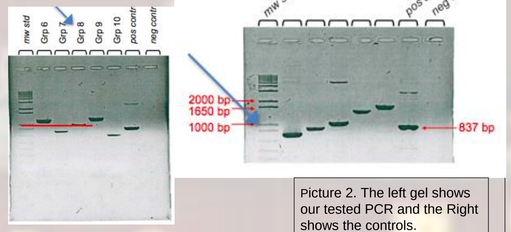
To begin we had to design primers based off of the given DNA sequences so that they could be ordered. Using the P20 micropipette and disposable tips we mixed the PCR primer(70), Taq buffer(10), dNTP's(10), gDNA(5), F-primer(2), R-primer(2), and Taq polymerase. This mixture was used in the amplification of the PCR primers. We then used the PCR DNA and the gel electrophoresis to check the number of base pairs. We added the dye to ensure that we would see the DNA in the electrophoresis gel. Next we we determined if the PCR actually happened based on the electro freesias and do ligation of the PCR product to plasmid vectors for modeling clones. We compared to the positive control, ours was close so the PCR did happen. The next step we did was take the prepared E-coli cells and the ligations from our last step and proceed with the heat shock. We mixed E-coli cells(20) & ligation mixture(2), then placed it on ice for 30 mins., heat shocked the mixture for 30 secs. at 42 C, added recovery broth(200), let incubate for an hour, then placed them in plates and let incubate overnight. The next week we then received the plates compared to positive and negative controls and counted the colonies that had grown. The clones were screened for beta-lactamase activity. Finally we tested the beta-lactamase clones to see if they are true beta-lactamase bacteria, the tablet did turn red as you can see in picture 4, so the results were positive.

RESULTS

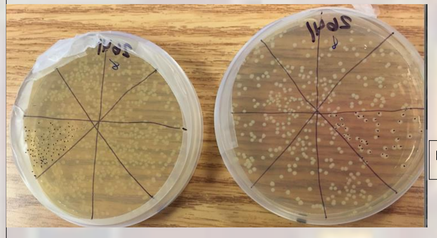
- To begin the experiment we used the first 21 base pairs, from our the provided sequence, stopping at a G base pair, and used the last 26 base pairs ending on a C to maintain the primer. Once the base pairs were chosen we got the reverse sequence and paired them. To ensure our sequence were correct we used the website pictured in **Picture 11.**
- Following the making of the primers we performed PCR, and to check to see that PCR happened we place the PCR in a gel electrophoresis and compared our results to the predicted base pair number (894). **Picture 2** shows our results and how we compared them.
- After we test the PCR we did heat shock transformation of E. coli. The heat opened the pores of the membrane for the vector to enter the cell. We tested the transformation to see which ones are Kanamycin resistance. To test this we compared the agar gels to a positive and negative control. The plates shown in Table 3 are the plates group 8 made.
- For the final part of the experiment, a lab technician used a pre-made Kanamycin broth to add 20 micro liters to the clone. After 30 minutes we check the beta lactamases clones for a color change. Our results (pictured in table 4) showed that our gene was transformed correctly and is sensitive to hydrolysis by all know beta-lactamases produced by bacteria.



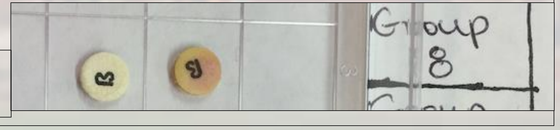
Picture 1. Shows the site used to check our primers.



Picture 2. The left gel shows our tested PCR and the Right shows the controls.



Picture 3: These are the agar gels for group 8.



Picture 4: This is the result of our clone after the Kanamycin broth had set for 30 minutes.

Discussion

- After the electrophoresis gel test we were able to determine that a clone had been formed, and finally with the transformation plate we knew the correct gene had been cloned and it was the desired product to continue testing antibiotic resistance.
- With the sequenced DNA further testing can be done on the antibiotic resistant cells such as identifying enzymes and traits.
- The clone we developed a was E.mir_ATCC33958_bla2641.
- With the findings extended research can be completed that could possibly uncover ways to treat illnesses that are antibiotic resistance.

REFERENCES

- "Bacteria." - *New World Encyclopedia*. N.p., n.d. Web. 01 Nov. 2015.
- Bradford, Patricia A. "Extended-Spectrum β-Lactamases in the 21st Century: Characterization, Epidemiology, and Detection of This Important Resistance Threat." *Clinical Microbiology Reviews*. American Society for Microbiology, n.d. Web. 01 Nov. 2015.
- Canaan, Patricia. "The Experiment". August 24, 2015. PowerpointPresentation.
- Shaikh S, Fatima J, Shakil S,Rizvi SMD and Kamal MA. Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. *Saudi Journal of Biological Sciences* 2015; **22**: 90–101