Elizabethkingia miricola Antibiotic Resistance with Beta-lactamase Gene

Group 6
Section 1

Jessyca Naegele, Ashton Budaus, Austin Jester, Dr. Patricia Canaan, Dr. Jessica Matts,, Shanell Shoop, Nathanial Torres

ABSTRACT

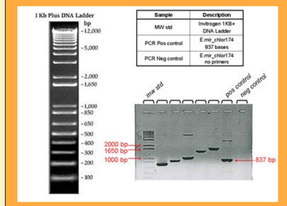
B-lactamase is a known gene that is resistant to B-lactams due to its ability to disrupt the bond between the four members of the B-lactam ring (Poole 2004). We researched a certain gene sequence within Elizabethkingia miricola to screen for B-lactamase activity. After creating primers and cloning our gene, we grew them in kanamycin broth. Then screened them for B-lactamase by saturating disks with nitrocefin and observed the color change. Compared to the control disk, our gene had a slight B-lactamase activity.

INTRODUCTION

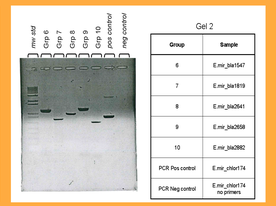
B-lactams are the most widely used antibiotic for treatment of infectious diseases of gram-negative and gram-positive infections. They consist of over 50% of the drugs on the market right now; these include penicillins, cephalosporins, carbapenems, monobactams, and penicillin-cephalosporin hybrids (Poole 2004). Penicillin is characterized by a B-lactam ring which is very unstable. When faced with staph infections, penicillin will mimic the structure of the bacteria in order to destroy the bacteria from the inside (Tymoczko 2010). Some gram-negative infections are becoming resistant to B-lactams by producing B-lactamases. The B-lactamases disrupt the B-lactam rings on the antibiotics and therefore are not affected by the drug. The most common gram-negative diseases that are B-lactam resistant are *H. influenza* and *Neisseria* (Poole 2004). Another gram-negative bacteria that is resistant to B-lactams is *Elizabethkingia miricola*. In this study, we were interested in cloning a specific gene of *E. miricola* to determine if it produces B-lactamase and therefore is antibiotic resistant.

RESULTS

Electrophoresis will show you how long your DNA stand is by stimulating it with an electric current. We expected ours to be around 1,164 bases long and saw that our fragment was about 1,200 bases long which is a positive result. After heat shock and ligation, we counted 88 colonies on our agar plates, without further testing we do not know whether we have B-lactamase or just a vector. After growing cells in a Kanamycin broth, we added the solution to nitrocefin disks and watched for discoloration. Our sample showed a slight discoloration when added to the disk.



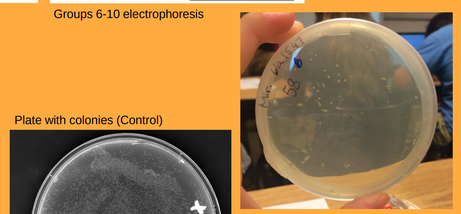
Sample electrophoresis gel



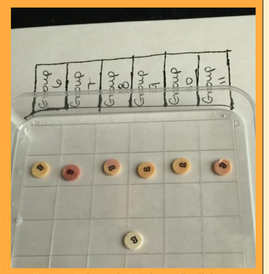
Groups 6-10 electrophoresis

Plate with no colonies (Control)

Plate with colonies (Control)



One of our plates



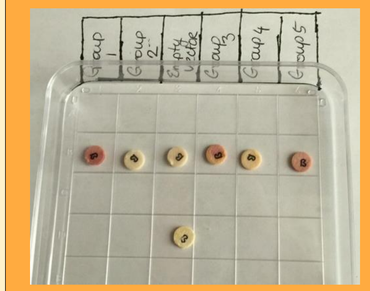
Groups 6 through 11 nitrocefin disks

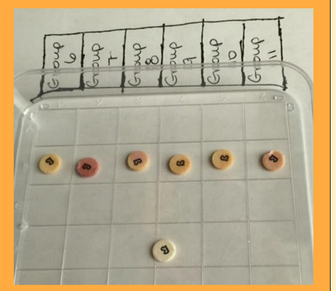
Negative vs. Positive Control

DISCUSSION

Compared to the positive control (group 1) and the negative control (group 2), our nitrocefin disk showed a slight discoloration. Our strand of DNA from *E. miricola* could be slightly antibiotic resistant. However, the other groups in this experiment showed a definite antibiotic resistant; group 7, 11, 3 and 5. These strands produce B-lactamase, which disrupts the B-lactam ring on antibiotics and are resistant to antibiotics that have these rings. Since our strand did not show a prominent change in color on the nitrocefin disk, repeating this experiment would be beneficial for establishing a strong conclusion about resistance. Future research should also explore these specific genes in reactions with B-lactam antibiotics to provide more data for antibiotic resistance. Given that we received positive results throughout the experiment, human error as well as machine error probably did not alter the final result of our experiment. However, we did not produce a lot of cells on our agar plates and this could of affected our final result.

E. miricola contains gene strands that are resistant to B-lactam antibiotics. This information can be useful for other researchers and medicinal scientists as they work toward creating new antibiotics to combat this issue. By knowing the specific strands that are resistant to antibiotics, the research on this bacteria can be narrowed to a more specific field.





MATERIALS AND METHODS

In order to control which gene gets copied, we had to find our forward and reverse primers using the DNA sequence given to us. The forward primer is the first 18-30 bases ending with a C or a G and the reverse primer is the same concept. After creating the primers, we added 10 ul of 10X taq buffer, 10 ul of 10X dNTP's, 5 ul of E. miricola, 2 ul of our forward primer, 2 ul of our reverse primer and 1 ul of taq polymerase to 70 ul of water. Then we loaded them into a thermocycler for PCR. Initial denaturation of PCR is for 30 seconds at 96 C, followed by 30 cycles of denaturation at 94 C for 30 seconds then annealing for 57 C for 30 seconds then extension at 72 C for a minute and a half. Finally, after 30 cycles is extension for 10 minutes at 72 C. After PCR store it at 4 C. Take the PCR product and load it with tracking dye and get it ready for electrophoresis. Pipet out 2 ul of 30% glycerol tracking dye onto parafilm, then 8 ul of PCR onto the same parafilm. Mix it together by piping it in and out of the pipet. Pipet this mix into the well. Also, a positive and negative control was done with electrophoresis. Next step is to set up a ligation mixture with the PCR and a plasmid vector. Add 2 ul of 10X ligase buffer, 2 ul of a linear plasmid vector, 4 ul of PCR product and 1 ul of DNA ligase enzyme to 11 ul of dH20. When adding these reagents together, be sure to add the PCR and the plasmid vector before adding the enzyme to the mix. We used an empty vector as a negative control for this step. Incubate this mixture over night at 16 C and store it ate 4 C. Next we must transform E. coli by heat shock with our ligation reactions. We added 2 ul of our ligation to 20 ul of competent E. coli cells. After dispensing the ligation, flick the mix three times. Place on the ice for 15-45 minutes, then heat shock it for 30 seconds in a water bath at 42 C. Then return it to the ice for 3 minutes. Add 200 ul of recovery broth growth media and incubate it for 1 hour at 37 C. Place the aliquots onto kanamycin agar plates and incubate overnight. Parafilm and transfer the plate to a refrigerator. Grow a few cells in a kanamycin broth and then isolate them, examine the plasmids for clones by agarose gel electrophoresis. An extra piece of DNA should appear on the gel, take these clones and screen them for beta-lactamase activity. Using nitrocefin disks to screen for beta-lactamase genes by seeing if the disk to turns red when bacteria is added. Positive control has a known beta-lactamase resistant gene. The negative control has a gene that is known to not have a beta-lactamase gene. These controls were put under the same methods and procedures.

REFERENCES

Poole, K. 2004. Resistance to B-lactam antibiotics. Cellular and Molecular Life Sciences 61:2200–2223.

Tymoczko, J., J. Berg, and L. Stryer. 2010. Mechanisms and Inhibitors. Pages 98–100 in Biochemistry: A Short Course. W.H. Freeman and CompanyNew York.