Providing an Experimental Negative Control to Enable the Determining of Antibiotic Resistant Genes in Elizabethkingia miricola

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ABSTRACT

Beta-lactamase genes cause bacteria to be resistant to beta-lactam antibiotics(1). Our goal was to provide a negative control for experiments conducted to determine if genes from *Elizabethkingia miricola* were beta-lactam antibiotic resistant. We did this by testing a gene for a putative inhibitor of streptomycin rather than beta-lactam. By amplifying the gene, ligating it into plasmids, executing molecular cloning, and screening transformed cells, we successfully provided a negative control by which the other groups could compare their results and determine beta-lactam resistance.

INTRODUCTION

For every human cell in our bodies, there exists roughly ten bacterial cells. Some of these bacteria, such as *Elizabethkingia miricola*, are resistant to antibiotics. Bacteria can resist antibiotics in multiple ways, including pumping the antibiotic out of the cell, destroying the antibiotic, and "dodging" the antibiotic(3). Previous research has deduced that there are at least 12 supposed beta-lactamase genes in *E. miricola*(1). We were given a gene from *E. miricola* that has a putative resistance to streptomycin rather than beta-lactam. Before this experiment, we expected that our group's gene would not produce beta-lactamase proteins because our gene should not encode beta-lactamase genes. Therefore, the other groups would be able to compare their experimental results to our negative control to determine whether or not they had found beta-lactam antibiotic resistant genes in *E. miricola*.

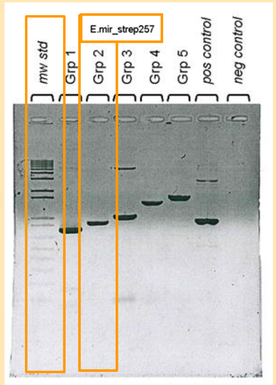
MATERIALS AND METHODS

We first copied our assigned gene from *Elizabethkingia miricola* using PCR Amplification. PCR required the following reagents: *E. miricola* gDNA, forward and reverse primers, PCR nucleotide mix, magnesium chloride (buffer), and Taq polymerase. These reagents were placed in a thermocycler for 30 cycles under conditions necessary for Taq polymerase activity. We confirmed that amplification worked by staining our PCR product and separating it by size with agarose gel electrophoresis. Next, we ligated our PCR product into plasmid vectors by adding the following: dH2O, 10X Ligase buffer (containing Mg and ATP), and T4 DNA Ligase enzyme. Then, these plasmid vectors were inserted into *E. coli* bacteria by inducing heat shock in a 42°C water bath, returning samples to ice, and then nurturing the bacteria in recovery broth at room temperature. Samples were incubated for an hour and later spread onto two kanamycin agar plates. Transformants on these plates were counted, removed, and inoculated in broth. Then plasmids were isolated and examined. Agarose gel electrophoresis was used once again to screen for clones. Lastly, the following qualitative colorimetric assay was conducted using nitrocefin saturated disks. Our samples of *E. coli* bacteria were grown in liquid culture, and 20 mL of culture was added to a nitrocefin disk. This disk was examined to determine if any color change had occurred.

RESULTS

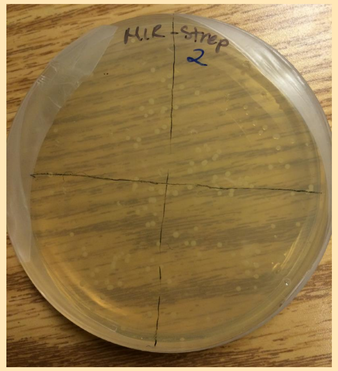
After PCR Amplification, our results from agarose gel electrophoresis showed that PCR worked and that our sample now contained many copies of the original DNA. Our DNA segment contained 867 bases in its original sequence. The molecular weight standard in the first agarose gel well matched this, showing a rough estimate of 850 bases. Our electrophoresis result was also in the majority, without any detectable outlying results.

Pictured left: Drew transferred the Group 2 sample from PCR into well #3 of the agarose gel using a p20 micropipette.

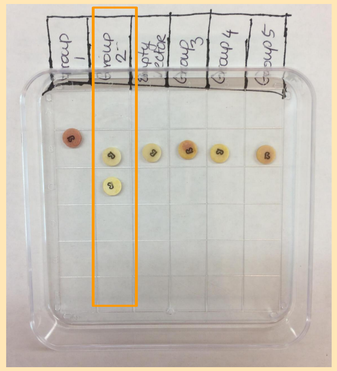


Pictured right: Agarose gel electrophoresis allowed us to confirm PCR amplification. A molecular weight standard in well #1 allowed us to estimate bases in our sample. Because our agarose gel results were in the majority and corresponded closely to the number of base pairs in our DNA segment, we could confirm that PCR worked.

After heat shock transformations of *E.coli* to insert our plasmid vectors, we were able to count colonies on agar plates to determine the numbers of transformants. Our group counted 251 transformed colonies on our two agar plates. Several of these transformants were then grown in a liquid culture for a qualitative colorimetric assay. Because we had a gene for a putative inhibitor of streptomycin, our nitrocefin saturated disk did not change color, remaining a pale yellow. This showed that our genes produced no beta-lactamase enzymes and that our sample was an acceptable negative control. In addition, Group 1's positive control nitrocefin disk did change color from yellow to red. This showed that their genes did produce beta-lactamase enzymes and that their sample was an acceptable positive control. Lastly, the nitrocefin disk with the empty vectors did not change color, demonstrating that the color change to red was dependent on the presence of beta-lactamase encoding genes.



Pictured left: Our plasmid vectors contained a gene for kanamycin resistance, allowing us to identify transformants on our agar plates.



Pictured right: Our nitrocefin saturated disk with bacteria culture applied is shown above a dry disk with no culture applied, demonstrating that our disk did not change color. Therefore, no beta-lactamase enzymes were present.

DISCUSSION

We were assigned a non-beta-lactamase gene from *Elizabethkingia miricola* as a negative control for our class experiment. Other groups would compare their experimental results to our negative control to determine if they had found beta-lactam antibiotic resistant genes. Therefore, it was imperative for the class experiment that our results be negative.

Before we could start this experiment, we first needed to make primers specific for the DNA template we wanted to amplify. We chose two primers (one forward primer and one reverse primer) that had 28 complementary bases. Our next step, a Polymerase Chain Reaction (PCR), enabled us to generate millions of copies of our DNA template strand for further experimentation. We confirmed that PCR resulted in products using agarose gel electrophoresis. Our next step was to set up a "ligation" ("gluing" of DNA strands) to insert our PCR product into plasmid vectors for molecular cloning. We then inserted our plasmid vectors containing the DNA into *E. coli* bacteria by using heat shock to open pores in the *E. coli* membrane. After heat shock, the cells were put on ice to reseal the *E. coli* membrane and to, hopefully, trap DNA inside. After heat shock transformation of *E. coli*, only some cells were "transformed" to pick up our ligated plasmid vectors. We selected for our transformants by spreading samples on agar plates with kanamycin. Because our vectors contained a kanamycin resistance gene, kanamycin resistant colonies on the agar plate could be identified as transformants. Lastly, we conducted a qualitative colorimetric assay to test for beta-lactamase activity in our samples and screen for resistance to any beta-lactam antibiotics(3). We did this by using nitrocefin saturated disks. When intact, nitrocefin is a pale yellow color. When its bonds are hydrolyzed (broken down), it is a red color. Beta-lactamase disables beta-lactam antibiotics by breaking bonds in the beta-lactam structural ring using hydrolysis(2). Therefore, if a sample contains beta-lactamase enzymes, they will hydrolyze bonds in the nitrocefin and be identifiable as beta-lactam resistant. Because our nitrocefin disk remained yellow after our *E. coli* culture was added, we could confirm that our gene produced no beta-lactamase enzymes and was an acceptable negative experimental control.

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(3) Ayoubi-Canaan P. (2015). Lecture notes. [PowerPoint slides]. Retrieved from https://oc.okstate.edu/d2l/le/content/1249097/Home.