What's Behind Gene Number 2658

An analysis of the suspected β-lactamase gene bla 2658 for β-lactamase activity.

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ABSTRACT

Elizabethkingia miricola is a gram negative, rod shaped bacteria strain. It was first isolated from the air conditioning condensation on board the Russian space station Mir. The bacteria's phenotype showed a resistance to a wide variety of β-lactam antibiotics. After sequencing the DNA of the bacteria, computers were able to pinpoint what could be a potential gene for β-lactamase. Through isolation and amplification, we were able to transfer the gene through a vector to *E. coli*. Then the *E. coli* was placed onto a nitrocefin capsule, which changed colors to indicate that gene does create a true β-lactamase protein, which cleaves the β-lactam ring located in β-lactam antibiotics.

INTRODUCTION

Certain strains of bacteria are resistant to antibiotics through the production of β-lactamase. There are four different ways that a bacteria can be resistant to antibiotics: producing a β-lactamase, which in this study hydrolyzes the β-lactam ring located in the β-lactam antibiotic, changing the active site of PBPs, decreasing permeability in the cell wall, and producing an efflux pump which exports the β-lactamase from the interior of the cell (Drawz et al.). A related species, *E. meningoseptica,* is classified as an emerging nosocomial pathogen, as a patient became infected my meningitis after a hospital procedure (Tak et al.). *Elizabethkingia* genera include many suspected β-lactamase genes, are resistant to the action of antimicrobials, and contain phenotypes that are antimicrobial resistant. The strand tested here was found on the Mir space station and causes human disease *E. miricola* is different from other species in the genus, as is hydrolyzes urea (Matyi et al.). The overall goal of the experiment is to determine if gene bla 2658 located in *Elizabethkingia miricola* is antibiotic resistant. If the gene is a manufacturer of a β-lactamase, the disk will change from a light yellow color to red. Our part of the lab was to clone the putative antibiotic resistant gene, and determine if the gene we cloned is a β-lactamase.

MATERIALS AND METHODS

We started by finding our start primer and a compliment to our end primer in order to recreate our strand of DNA. Next, we used a P20 micropipette to add the reagents to the water. The reagents that we added to the water were 10x Taq buffer, 10x dNTP's, *E. miricola* gDNA, F-primer and Taq polymerase. The next procedure in lab was placing 2 µL of tracking dye on parafilm and adding the 8 µL of PCR to the 2 µL of tracking dye then mixing the two together. The PCR primer was then loaded into an agarose gel using a micropipette. Next in lab, using a p20 micropipette and disposable tips, the reagents were added into the tube provided for PCR amplification. First, 2 microliters of 10X Ligase buffer were added, followed by 2 microliters of Linear Plasmid Vector, 4 microliters of the PCR product, and 1 microliter of T4 DNA Ligase Enzyme to 11 microliters of dH20. From this we were able to successfully transition the 10X Ligase buffer, Linear Plasmid Vector, PCR product, and T4 DNA Ligase Enzyme into the dH2O. In the next lab and 20 µL of E. coli, 2 µL of ligation mixture were added. The solution was mixed and placed on ice for 30 minutes and then heat shocked for 30 seconds in a 42ºC water bath. The content was then returned to the ice for 3 minutes before being treated by 200 µL of recovery broth at room temperature. The solutions were incubated and added to kanamycin agar plates. The next step we took was splitting each plate into eight parts and then counting the number of colonies in each section and then multiplied that number by eight, repeating that for both dishes. Lastly, we tested to see if our β-lactamase is in fact a true β-lactamase. By putting 20 ml of bacteria culture on a nitrocefin disk test plate, we are able to test gene production of β-lactamase through color change on the disk.

RESULTS

The test used to expose β-lactamase was a nitrocefin assay, which will change colors in the presence of a β-lactamase. When the positive control was applied to the nitrocefin disks, it created a strong positive response, which was signaled by turning red in color. When both the negative control and the empty vector were placed on the nitrocefin disks, both created a negative response, which was signaled by not changing colors (Fig. 1). The *E. coli* containing the gene bla 2658 was applied to the nitrocefin disk and created a yellowish color with a small red dot (Fig. 2) that showed a positive response to β-lactamase, although it was weak.

Fig. 1: The positive control group (top) created a red affect that should be recorded as a strong reaction. The negative control group, which contained a gene that was streptomycin resistant, did not appear to change in color when compared to the unused disk (bottom left). The group with the empty vector also exhibited a no change in color.

Fig. 2: *E. coli* was applied to the disk on the right, which exhibited a weak change in color compared to the disk that had nothing applied to it (left).

DISCUSSION

The resulting color created by the nitrocefin disk allows us to interpret that there is a high probability that the gene bla 2658 is a true β-lactamase. The nitrocefin disk mimics the β-lactam antibiotic as it contains a β-lactam ring that causes the change in color that is detected. The β-lactam ring is hydrolyzed, congruent to the mechanism that inhibits the activity of a β-lactam antibiotic (Dai et al.). This discovery helps to confirm to genes responsible for the high resistance exhibited by *Elizabethkingia miricola.* To date, only 3 genes have been proven to be a true β-lactamase in any of the three species of *Elizabethkingia,* and all three of those genes were located in the chromosomal DNA of *E. meningoseptica* (González, et al.). Further research may now be conducted on the β-lactamase found in this study to deduce which gene is actually the gene responsible for antibiotic resistance to each drug, and whether each have a similar signaling mechanism or each has an independent signaling mechanism.

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