Testing of possible beta lactamase gene within Elizabethkingia miricola via PCR and Heat Shock plasma insertion

Matt Ferrell, Briten Green, Patrick Olfers, Leigh Brown, Dr. Canaan, Dr. Jessica Matts, Shanell Shoop, and Nathanial Torres

GRP# 5
Section# 1

ABSTRACT

In this experiment we took about three months to test which genes created beta lactamase resistance to antibiotics within our subspecies of Elizabethkingia which was miricola. We used many different materials and methods to come up with the conclusion that our gene was one that would create a beta lactamase resistance.

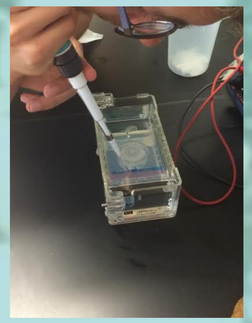
INTRODUCTION

Elizabethkinya is disease causing bacteria that has many sub species including anopheles (found in the gut of mosquitos), meningoseptica (found in nature), and miricola (found in the Mir space station). This species as a whole is resistant to around 20 different antibacterial medications. In this experiment we were focused on the Elizabethkingya miricola. In our experiment we tested for the gene that is coded to resist the antibiotic beta lactamase. Alongside our experiment, there were 25 other experimental groups with us who were testing other suspected genes along with negative and positive controls.

MATERIALS AND METHODS

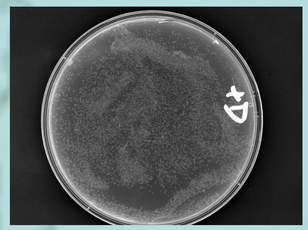
- Bioinformatics database
- Specially ordered forward and reverse primers
- dH2O
- 10x Taq buffer
- 10x dNTP's
- E. Miricola DNA
- Taq Polymerase
- Thermocycler
- Agarose Gel
- Buffer (for electrophoresis)
- Xylene Cyanol FF
- Bromephenal Blue
- Molecular weight standard
- Linear Plasmid Vector
- T4 DNA ligase enzyme
- E.coli
- Agar Plates (kanamysin positive)
- Nitrocefin saturation discs

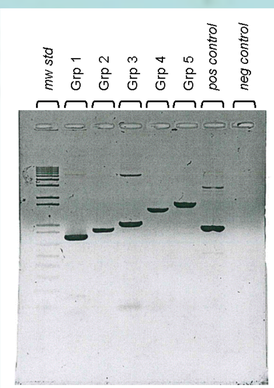
After we identified the specific forward and reverse primers for our DNA sample, we amplified the targeted gene through PCR. Once amplified, we performed gel electrophoresis and compared the PCR product to a series of controls and a molecular weight standard ladder in order to ensure that the cloning was successful. From there we inserted the gene into a designated plasma vector that could be inserted into E.coli cells, and coded for kanamysin resistance. Utilizing the antibiotic resistance provided by the plasmid, we cultured the E.coli on agar plates that would test for the proper indoctrination of the plasmid vector. Finally we were able to use Nitrocefin saturation discs to identify if the gene inserted trough the plasmid vector encoded for a variety of betalactam enzymes.



RESULTS

- ✓Based on the molecular weight standard, our cloned gene sample was approximately 1500 base pares in length.
- ✓The E.coli sample successfully grew on the agar plate containing kanamysin after the heat shock transformation, and approximately 3000 colonies were cultivated.
- ✓Once added to the nitrocefin saturation discs, the reaction produced a slight change in color. The discs were initially yellow, but changed to red, as seen above.





Gel 1

Group	Sample
1	E.men_bla557
2	E.mir_strep257
3	E.mir_bla1019
4	E.mir_bla1527
5	E.mir_bla1534
PCR Pos control	E.mir_chlor174
PCR Neg control	E.mir_chlor174 no primers

DISCUSSION

Once we obtained the results from the round of gel electrophoresis, we were able to compare that approximated base pair length to the base pair length of the gene we had planned to clone. These two values were extremely close in measurement, thus confirming that the PCR was successful. The next evaluation we performed pertained to that of the E. coli plasmid insertion. The ability of the cultured E. coli cells to grow on the kanamysin positive petri dish after treatment confirmed that the heat shock method was indeed successful. At this point, however, we could not be sure that the plasmids we had inserted were the ones that had also bonded to the gene of interest. From there we were able to evaluate the reaction of the nitrocefin discs to our cultured bacteria, which caused the slight color change of said disks. This change confirms that the gene we inserted via the plasmid vector did code for some form of beta lactamase resistance.

REFERENCES

❖ Dr. Patricia Canaan and D2L power points