Exploring the possibility of Arhodomonas sp. Seminole Bacteria as a source of sustainable consumption of harmful oil products using Gel Electrophoresis and PCR Products.

GRP#17
Section#002

Cody Duttry, Mariah Hooper, Devin Hoover, Caleb Wilson

ABSTRACT

In regions where oilrigs are used, the resulting oil product and its high salinity causes massive environmental damage. The objective of our experiment is to obtain bacteria that can thrive in extremely salty areas and that consume oil runoff, turning it into organic waste. A species of Arhodomonas bacterium was found to be living in such an area in Seminole. After reproducing and studying the genomic code of the bacteria using a method of photocopying called PCR, The experimenters found that thiolase is a fatty acid protein produced by these bacteria that allow it to stand these high levels of salt.

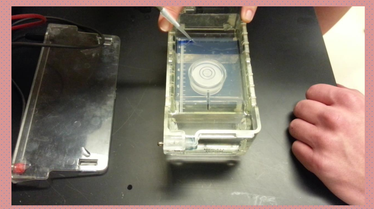
INTRODUCTION

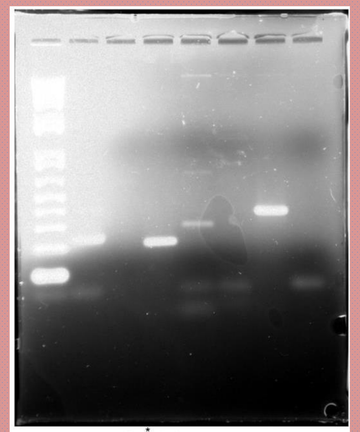
Arhodomonas sp. Seminole is an aerobic, halophilic bacterium able to degrade benzene, toluene, phenol, 4-hydroxybenzoic acid, protocatechuic acid, and phenylacetic acid. This bacteria was extracted from hypersaline crude-oil-impacted soil in Seminole County, Oklahoma. It is of the genus Arhodomonas and in the Gammaproteobacteria class. Currently we have 750 pieces in the genomic sequence of Arhodomonas sp. Seminole. About 8-10% of the genes are broken between two pieces, leaving "gaps" in the sequence. Our goal is to generate a novel DNA sequence of the "gap". By doing so we will be able to bring two larger pieces in the DNA sequence together (Davis, Nicholson, etc.).

MATERIALS AND METHODS

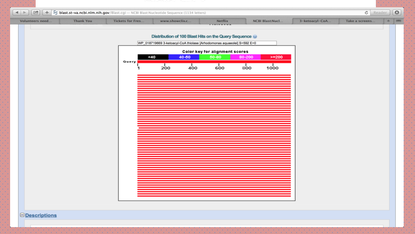
- The group 17 experimenters began with the Arhodomonas Bacterium enriched from the Seminole Company's salty, crude-oil-impacted soil in Oklahoma.
- The desired Arhodomonas bacteria was singled out and cultured a few times to ensure the sample was purely Arhodomonas.
- The cells were ruptured, the DNA was purified using organic extractions, and it was concentrated by precipitation.
- To analyze the DNA and, more specifically, sequence the genome, the "shotgun" approach was utilized. This method of genome sequencing involves cloning of small fragments of the DNA and the ends of different gene codes to make a continuous sequence.
- Group 17 was then given a region of the bacteria's DNA that contained a gap in order to bring the two larger pieces together and fill in the missing DNA sequence data.
- PCR was used to enzymatically amplify the region by generating millions of copies of the specific DNA sequence.
- After synthesizing the DNA copies in a small test tube using a thermocycler, the experimenters mixed Taq plymerase (enzyme), a thermocycler, our template DNA (our region of interest), dNRP's (DNA building blocks), forward and reverse primers, and a buffer into the test tube.
- 8um of the mixture was then pipetted into a well of 1% agarose gel with 2ul of tracking dye.
- The gel, submerged in buffer, was run at 80volts, with a positive and negative end.
- Results were analyzed and recorded.

RESULTS





Gel 2.1



The Group 17 experimenters did not end up getting a successful result. As shown in Gel 2.1, our group (the third well, or column, from the left) did not have any visible DNA bands. This is most likely due to a fault in our PCR, seeing as there was not any holes in the agarose gel and the control well was successful. This means that the electromagnetic pull, the buffer, and the gel itself were in working condition. Therefore, either our PCR mixture had some sort of issue with it, or our two contig segments do not actually connect. However, something we did find was a specific protein that was strongly related to the genomic code of the bacteria. As can be seen in the image from the blast website, there is nearly a 100% match of the DNA from our Arhodomonas bacteria to the protein thiolase. Thiolases are enzymes that have key roles in biochemical pathways, including the oxidation pathway of fatty acid degradation. One particular form of thiolase that was prevalent is called 3-ketoacyl-CoA thiolase and is found in the bacteria known as *Arhodomonas aquaeolei*. One of the primary function of this protein in bacterial cells is for regulation of the cell itself through polyhydroxyalkanoate pathways of metabolism (Kessler, Witholt). The functions of this protein could be a huge reason why Arhodomonas sp. Seminole is able to survive and even thrive in harsh, salty environments.

DISCUSSION

PCR is an appropriate tool to study the possibility of eliminating the hazards of oil spills in the environment because DNA microarray analysis can reveal the source of a biological issue and often a foundation for a solution. Because PCR is the primary method of amplifying sections of DNA when there isn't enough to analyze, it is crucial for criminal forensic teams, archeologists, doctors, and chemists alike. For this experiment, PCR was very necessary to try to find the missing and to analyze the full DNA. Because we were unsuccessful, it is quite possible that our contigs did not match up, meaning that we received two segments that do not have any missing genes between them. Perhaps we did not include enough of the code for each segment and, therefore, the genetic information filling in the gap was unable to be identified. Regardless, much more research should be done on this bacteria and any other bacteria later found with similar salt-loving characteristics. The environment that is directly or indirectly affected by oilrigs should also be further studied. Once a significant amount of research has gone into the bacteria and the environments in which they thrive, hopefully a mechanism can be made that uses the enzymatic proteins of the bacteria to turn the harmful substances in the oil into combustible organic material. Of course, that is the goal of this experiment and the future research ahead in this entire project.

REFERENCES

- "Genomics: Spruce Shotgun Sequencing." Nature 498.7452 (2013): 8. CSUS. Sacramento State. Web. 11 Nov. 2014.
- Davis, A., C. Nicholson, BA Roe, P. Canaan, SD Hartson, and BZ Fathepure. "Arhodomonas Sp. Strain Seminole and Its Genetic Potential To Degrade Aromatic Compounds under High-Salinity Conditions." PubMed. American Society for Microbiology, 22 Aug. 2014. Web. 14 Nov. 2014.
- Kessler, Brigit, and Bernard Witholt. "Download PDFs." Factors Involved in the Regulatory Network of Polyhydroxyalkanoate Metabolism. Journal of Biotechnology, 30 Mar. 2001. Web. 15 Nov. 2014.
- Antler, Christine. "Polymerase Chain Reaction." SCQ. N.p., Aug. 2003. Web. 15 Nov. 2014.