What the Frack is in the Gap?: Genome Sequencing of Arhodomonas Sp. Seminole

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ABSTRACT

Fracking taints soil by continually saturating it in crude oil and salt water. It is necessary to find a better way to clean up this soil to protect the environment. To begin, bacterium was isolated from the soil. Once a pure species of the bacterium, Arhodomonas sp. Seminole, was isolated,DNA was extracted. Using laboratory techniques such as PCR amplification and eventually gel electrophoresis in combination with DNA and RNA sequencing technology online we were able fill in the gap of the unknown genome and find what genes Arhodomonas sp. Seminole has in order to continue research.

INTRODUCTION

Biochemistry is a science full of research. On our first day of class we were given our first section of DNA and asked to do all these things that most of us had not even heard of. The section of DNA was from Arhodomonas sp.Seminole, an aerobic, halophilic bacterium enriched from salty, crude-oil-impacted soil in Seminole Co, OK. It can metabolize benzene, toluene, phenol, 4-hydroxybenzoic acid (4-HBA), protocatechuic acid (PCA), 12 and phenylacetic acid (PAA) as the sole sources of carbon at high salinity. However, this section had gaps in the DNA sequence. In an effort to try and sequence these unknown gaps, we took contigs from the bacteria and did PCR. Using a fused contig, we predicted the sequence of the missing gap. Then, to test the predicted sequence we used gel electrophoresis. With this gap filled, we can gain more information on the DNA and figure out better ways to further its benefits for our environment.

MATERIALS AND METHODS

Materials

-2 Full length contigs from Arhodomonas sp. Seminole
-1 Fused contig for sequencing
-1 related protein
-A P20 micropipeter with disposable tips
-1 test tube containing 70µl of dH_2O and PCR
-1 test tube containing at least 10µl of 10X *Taq* buffer
-1 test tube containing at least 10µl of 10X dNTP's (all 4)
-1 test tube containing at least 5µl Ard. Sp. Seminole gDNA (58ng/µl)
-1 test tube containing at least 2µl of F-Primer
-1 test tube containing at least 2µl of R-primer
-1 test tube containing at least 1µl of *Taq* polymerase
-Gel electrophoresis machine
-Thermocycler

Methods

-Predicting the Sequence Gap

1. Get a sample of 2 full length contigs, a fused contig, and a related protein for testing
2. Plug these into BlastX to find the missing sequences
3. Record the Predicted Sequence Gap

-PCR Amplification of Predicted Sequence Gap

1. Obtain your reagents and keep them on ice
2. Using a P20 micropipeter and disposable tips add 10µL of 10x Taq buffer to the 70µL of dH_2O
3. Dispose of the tip on the micropipeter and put a new one on.
4. Continue using the micropipeter and add 10µL of 10x dNTP's(all 4), 5µL Ard. Sp. Seminole gDNA(58ng/µL), 2µL of F-Primer, 2µL of R-Primer, and 1µL of Taq polymerase. Replacing the disposable tip for every reagent.
5. Using the micropipeter and a new tip again, transfer the 100 µL of PCR into the thermocycler machine.
6. Take the resulting amplified DNA and run in gel electrophoresis
7. Record the results

(The amplification was preformed with the program which consists of an initial denaturation at 94℃ for 30 seconds, annealing at 55℃ for 30 seconds, extension at 72℃for 1 minute and another 10 minutes at 72℃ for the final extension.

RESULTS

Following preparation, we ran PCR so that we would have adequate DNA for gel electrophoresis. Our gel electrophoresis results were very positive. Our gel is 3rd from the top, directly beneath the positive control. See Image 1.

Using the results produced by the gel electrophoresis we analyzed the forward and reverse contigs and found exact overlap for the majority of the length further proving our hypothesis. See Image 2

After completing the experiment, we entered our full length contig into blastx on the NCBI website. From this we found what protein our DNA sequence of Arhodomonas sp. Seminole likely codes for. We found that our gene codes for Glutamine Synthetase. Glutamine Synthetase is an enzyme that aids with the breakdown of nitrogen. This occurs with the catalysis of glutamate and ammonia to form glutamine. This a nitrate reduction process combined with amino acid degradation, and photorespiration. The "good match" is typically considered to be over 40%. Because the database found multiple matches to Glutamine Synthetase, some up to 70% we are fairly confident that this is a reasonable match. See Image 3.

While our results were positive, experimental error could have occurred in a when pipetting to prep for PCR amplification, or pipetting during the gel electrophoresis process because both of these steps required very careful laboratory technique.

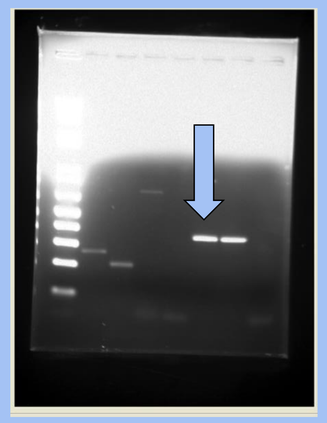

Image 1: Gel Electrophoresis

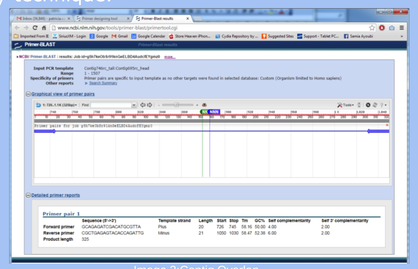

Image 2:Contig Overlap

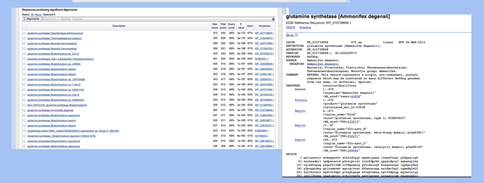

Image 3: Analyzed Gene

DISCUSSION

The results of our experiment told us the DNA sequence of the missing gap of Arhodomonas sp. Seminole bacteria. Based off of our research we were able to figure out what protein our sequence of bacteria coded for and were able to fill in the gap. The code we were able to transcribe was the protein Glutamine Synthetase. We used blastx, clustal, and several other websites to give us our results.

After four months of preparing our data and using many different tools and systems we were able to synthesize the DNA given to us. We are confident in our findings that they are exact and also well documented. The bacteria is selected for over 40% for a "good match." The databases we used found the traces and matches of our protein Glutamine Synthetase.

Our research has taught us many different lessons. First of all it taught us how to work as a team. The experiment required each of us to perform certain tasks that we have never done before. Another lesson it taught us was how important research truly is. Research is responsible for all the findings that we have before us today. Without it we would not know anything at all.

We have spent the remaining time (after the experiment was over) preparing our data to be documented on this posterboard. We are more than pleased with our findings. The Arhodomonas sp. Seminole bacteria has a played a huge role in further developing new interests in the field of research.

The significance in our research is that bacteria like Arhodomonas sp. Seminole can tolerate the salty, oil rich soils. By studying the DNA of these microorganisms, a way to clean up the tainted soil of fracking sites may be found. For this reason, our research has significant ecological value.

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