The Completion of the Genomic Sequence of *Arhodomonas sp. Seminole* Supported by the Use of Polymerase Chain Reaction

GRP#8
Section
#001 & #701

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ABSTRACT

Arhodomonas sp. Seminole is a halophilic bacterium that was found in the crude-oil filled soil in Seminole, OK. After using enrichment to obtain pure culture of the bacteria, the researchers were left with a major question. What DNA sequence completed the incomplete genomic sequence? In order to answer this question, using the BLASTX program, we identified the sequence gap and used the given related protein sequences through PrimerQuest and determined a forward and reverse primer sequence. Using a physical version of our primers, we mixed them along with several other ingredients into a mixture that allowed PCR to be performed. We put this mixture into a gel to have Agarose Gel Electrophoresis performed to create a product. After PCR was performed, the mixture we created failed in producing a PCR product. So, in conclusion, the failure in producing a PCR product could mean a variety of things including: something was performed incorrectly in the experiment, the PCR primers were designed were not compatible, or the related protein sequence we were given was not a proper match.

INTRODUCTION

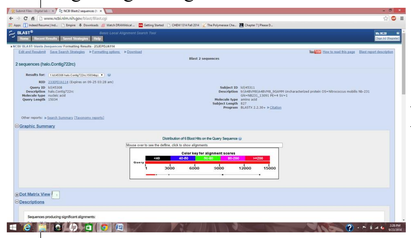
Arhodomonas sp. Seminole is a strain of *Arhodomonas* bacterium extracted from the oil-rich soil of Seminole, Oklahoma. *Arhodomonas* is a halophillic and halotolerant bacteria (1,2), meaning it survives and thrive well in a high-salt area. Our purpose for this experiment emerged from a previous experiment performed by Dr. Patricia Canaan and her fellow colleagues. After studying this extracted sample of bacteria, they were left with a question: What related protein sequences is the missing piece to fill in the gaps in the contigs of the bacteria? This was the thing we did not know about this specific strain of *Arohodomonas* which was what filled in these gaps. The hypothesis was simple: Does our related protein sequence produce a DNA strain that could potentially fill in the missing part between our upstream and downstream contig? As you will see, we went through a long process in order to come up with an answer to this question.

MATERIALS AND METHODS

In the beginning of the semester, our group was given our own individual Forward and reversed Contig, Fused Contig, and protein sequences in order identify and examine the sequence gap of the genomic sequence for the bacterium arhodomonas seminole.
In order to find the forward and reverse primers, we went to the website PrimerQuest in order to figure out the gap for PCR (Polymerase Chain Reaction). From there, we needed to determine which was the most centered in order to for to be a good fit for the PCR. Next, we used the website www.ncbi.nlm.nih.gov in order to use the BlastX feature. We used BlastX to find the percentage of how much of our genomic DNA matches with the bacterium, for both Contig 722rc (amino acid coordinate: 453-805) and Contig 750rc. Since we wanted to find out region of interest (the region of missing DNA where Ns were substituted in just to fill in the gap), we thus conducted an experiment with Agarose Gel Electrophoresis. In this experiment, we used a P20 micropipeter with disposable tips, in order to add the reagents (dH2O, 10X Taq buffer, 10X dNTP's (all 4), Arh. Sp. Seminole gDNA (58ng), F-primer, R-Primer, Taq Polymerase) to a tube provided for amplifying PCR of out group's predicted sequence gap and then put the test tube into a thermocycler for PCR analysis. We then used blastx again to see if there was any protein that was most similar to our given protein, find that it was the hypothetical protein arhodomonas aquaeolei.

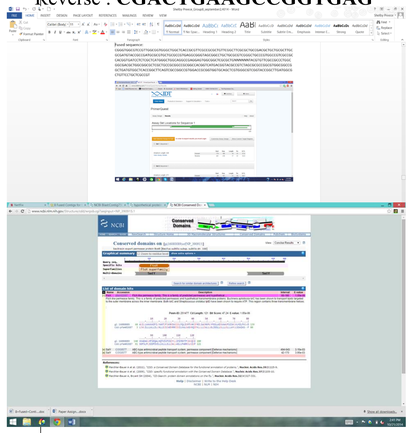
RESULTS

Beginning Contig



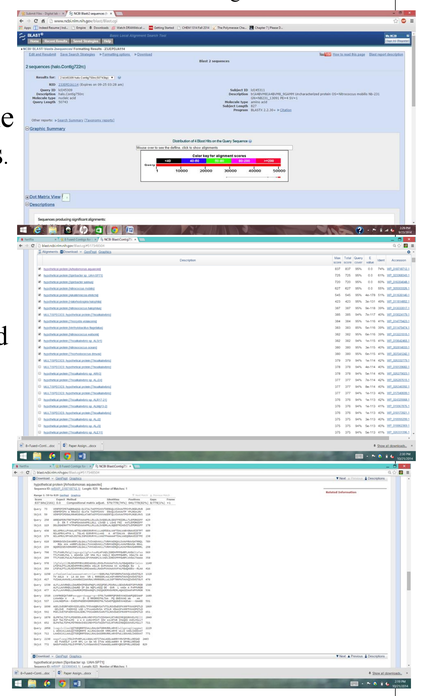
Forward primer: **GCGATGTACCGCCGATG**
Reverse : **CGACTGAAGCCGGTGAG**

Determining the sequence gaps.



Sequence around the gap

End contig



As we attempted to complete the genomic sequence of Arhodomonas sp. Seminole, we found a protein that was similar to our species. We believed this protein sequence would fill the gap of Arhodomonas' genomic sequence. From that protein we found the forward and reverse primers. Then after we completed the PCR set we realized that our hypothesis was in fact incorrect because the protein we found did not in fact complete the genomic DNA of Arhodomonas. Further attempts at trying to get our PCR to work, which was

The 100 µl PCR mix contained
~50 ng of DNA,
0.6 µM of each primer,
200 µM of PCR nucleotide mix (Fisher Bioreagents, Fisher Scientific, Pittsburgh, PA),
1.75 mM MgCl2,
2.5 units Taq polymerase in Buffer A (M1865, Promega Chemicals, Madison, WI).

The amplification was performed with the program which consists of an initial denaturation at 94°C for 30 sec, 30 cycles of denaturation at 94°C for 30 sec, annealing at (51°C, 53°C54°C or 55°C) for 30 sec, extension at 72°C for 1 min another 10 min at 72°C for the final extension.

Making our hypothesis incorrect. The protein we have is called FtsX-like permease family which this is a family of predicted permeases and hypothetical transmembrane proteins. Buchnera aphidicola lolC has been shown to transport lipids targeted to the outer membrane across the inner membrane. Both lolC and Streptococcus cristatus tptD have been shown to require ATP. This region contains three transmembrane helices. Even though our gap was not sequenced, we did realize that our protein was closely relayed to a hypotheitical protein called Arthodomonas Aquaeolei.

DISCUSSION

Discussion:
What we observed was that our gap couldn't be filled and didn't match any real protein, and we did not have a PCR product. They are important because you need a PCR product to fill the gap and it showed that our primers needed to be shifted and changed slightly. There was only a relationship with a hypothetical protein, and that was it. An anomaly was that we did not get a PCR product. For future research I'd suggest trying several different primers, from different areas around the gap.

REFERENCES

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2. A. I. Saralov, B. B. Kuznetsov, E. M. Reutskikh, R. V. Baslerov, A. N. Panteleeva, and N. E. Suzina. 2012. *Arhodomonas recens* sp. nov., a Halophilic Alkane Utilizing Hydrogen Oxidizing Bacterium from the Brines of Flotation Enrichment of Potassium Minerals. Microbiology. Vol. 81, No. 5, pp. 582–588.