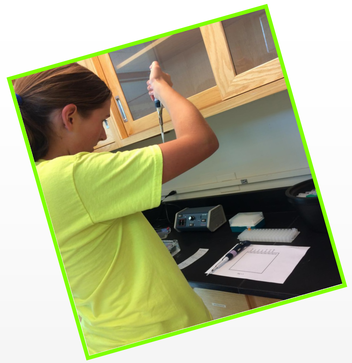
Determining An Unknown Genomic Sequence of Arhodomonas sp. Seminole Through Polymerase Chain Reaction



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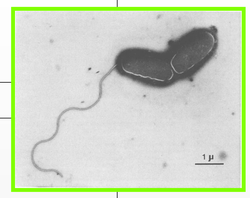


GRP 4
Section 001

ABSTRACT

Our central focus is to see if halo.Contig697rc with an alignment at the head joins with halo.Contig478 with an alignment at the tail. From our initial analysis using BLASTX, we showed possibility of the gap of the amino acid to be filled. We used BLASTX in order to determine the alignment of the given fused contigs and the related protein. Primer Quest allowed us to use the fused contig in order to develop forward and reverse primers to be used in PCR. The gel showed that further testing needed to be done due to no definite sequence being found. We used BLASTX of the genomic DNA flanking region by using the fused contig sequence in order to determine a reference protein. No domains were detected. Dr. Canaan ran our PCR a second time, and we were able to utilize ClustalW2 for multiple sequencing alignments to find our final alignment results. Results were limited to the forward primer, so further testing is required.

INTRODUCTION

Arhodomonas sp. Seminole was isolated from a crude oil-site in Seminole Co, Oklahoma. According to Dr. Patricia Canaan, this aerobic, halophilic bacterium grows on a wide variety of carbon sources and requires a considerable amount of salt to thrive. The department of Microbiology & Molecular Genetics at Oklahoma State took special interest in halophilic organisms for their ability to degrade hydrocarbons. Therefore, Arhodomonas sp. Seminole was a key interest regarding the research project. As the OSU Department of Microbiology explains, derived from the salty, crude oil impacted sites in Oklahoma, this extremely halophilic bacteria is able to degrade benzene, toluene, phenol, 4-hydroxybenzoic acid (4-HBA), protocatechuic acid (PCA), and phenylacetic acid (PAA) as the sole sources of carbon at high salinity. The central focus was to complete the genomic sequence of Arhodomonas sp. Seminole. The studies of sequencing that were done prior to our research, established the initial steps of the benzene degradation pathway in halophiles (Acton, p. 156). Therefore, our hypothesis is halo.Contig697rc with an alignment at the head joins with halo.Contig478 with an alignment at the tail. From our initial analysis using BLASTX, we show possibility of the gap of the amino acids to be filled.

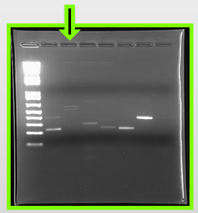
MATERIALS AND METHODS

We first obtained contig 1, contig 2, the fused contig, and the related protein from Dr. Canaan after she prepared the extracted DNA for analysis. Using the related protein for referral, we used BLASTX in order to determine the alignment of the given fused contigs and the related protein. This information was used to better determine the similarities of the contigs in order to sequence the gap. Primer Quest allowed us to use the fused contig in order to develop the forward and reverse primers that would be used in the upcoming PCR. Dr. Canaan then sent off an order for the primers from Integrated DNA Technologies. Next, we added the following reagents into a test tube by the use of a p20 micropipeter with disposable tips: 70 microliters of deionized water, 10 microliters of Taq buffer, 10 microliters dNTP's, 5 microliters of Ahrodomodous sp. Seminole DNA at a concentration of 58 nano grams per microliter, 2 microliters of forward primer, 2 microliters of reverse primer, and 1 microliter of Taq polymerase. This solution was then sent to the lab for the PCR procedure using a thermocycler. Following the last cycle, 8 microliters of PCR product and 2 microliters of tracking dye was then added and placed on a wax sheet. We loaded the product into a well for the gel electrophoresis procedure of 1% argarose gel (in 1x TAE buffer). The molecular weight standard and positive and negative controls were added in their designated wells, and then the gel was ran at 80 volts submerged 1X TAE buffer for 1-2 hours until the fastest dye was almost to the end of the gel. (Gel results showed that further testing must be done, no definite sequence was found.) The next step included running a BLASTX of the genomic DNA flanking region by using the fused contig sequence in order to determine a reference protein. (Though we found only hypothetical proteins, and no domains were detected.) Dr. Canaan and her crew ran our PCR a second time, and we were able to utilize ClustalW2 for multiple sequencing alignments to find our final alignment results. (Limited results found for only one forward primer, more testing required.)

RESULTS

The PCR 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 for 30 sec, extension at 72° C for 1 min another 10 min at 72° C for the final extension.

Gel Electrophoresis Results



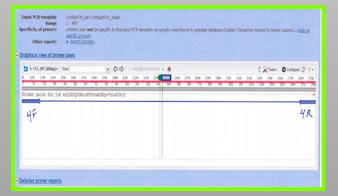
After PCR, the copied DNA can be examined by gel electrophoresis. The process is like DNA "fingerprinting." The image to the left shows our gel product. As you can see, the PCR product failed to show results. Failed PCR products can be caused by numerous reasons including a significant difference in the related gene regarding the sequence we are investigating, using the wrong primer selection, faulty reagents, errors in pipetting due to the small quantities required, and possible denatured/bad DNA or taq polymerase samples.

After further examination of why our PCR product may have failed, more testing was completed by Dr. Canaan. She was able to find helpful alignment results that would further progress the crucial DNA sequencing needed for Arhodomonas sp. Seminole.

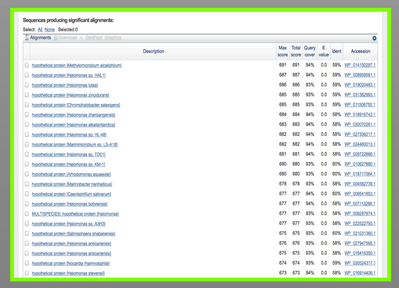
In order to finalize our results, we used ClustalW2 multiple sequence alignment in order to determine our final alignment. Our results did not give a definite answer about the full alignment. Though, our limited results showed an alignment of the forward primer only. Therefore, we need to run additional testing in order to acquire more information about the gap. Our results may be restricted, but the fact that we showed some distinction of the gap is helpful for further research.

Our expected results of an entire genomic sequence were not fully met, though our partial results are supportive in order to find the remaining alignment for the reverse primer.

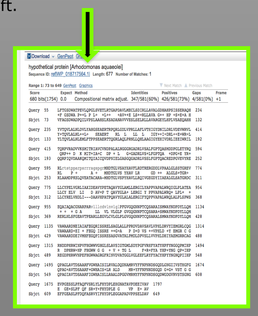
Finalized alignment results showing only the forward primer.



Using BLASTX of the fused contig sequence, we were able to examine the genomic DNA flanking the gap region in order to determine a possible reference protein. In both reliable databases we searched, the sequence shows no specific alignments and that no domains have been detected. All results were that of which a "hypothetical protein." Therefore, we know that this protein is being searched for by many others but has yet to be discovered. We do however see that one possibility with a 73% positive to be hypothetical protein [Arhodomonas Aquaeolei] as shown bottom left.



Reference protein results after examining the genomic DNA flanking region.



DISCUSSION

The hypothesis was that, if the two PCR primers that we found aligned with the related protein, then the sequence would replace a gap in the genome sequence of Arhodomonas sp. Seminole. In our experiment we extracted DNA from Arhodomonas sp. Seminole and drafted genomic sequences to "fill in the blanks" of the DNA. We did this to determine what was missing and put it together.

Our data partially supported our hypothesis in that we found a working forward primer, while we did not find a reverse primer. The PCR and gel electrophoresis gave us a hypothetical reference protein of what could fill in the sequence gap. We had probable data to begin with so there was a moderate chance that our experiment would work. It is possible that the reason our experiment only partially worked was because there may have been slight contamination during the process of transferring all of the different reagents from their test tubes and into a single test tube to undergo PCR. There are many ways that contamination could have occurred like, the tips could have been touched to something other than the pipette and the test tube contents, not all of each reagents could have gone into the test tube correctly, or air bubbles could possibly affect how all of the reagents came together.

Further research into the genomic sequence gap of Arhodomonas sp. Seminole could gain much more information about what exactly lies within the gap. If we continued our experiment and performed PCR again with gel electrophoresis we might be able to determine a reverse primer to go along with our forward primer and could then better determine how to find the sequence in the genomic gap.

In conclusion, though we did not get a complete sequence to support our hypothesis, we did receive an alignment for the forward primer which is promising for further experimentation to complete the entire sequence gap.

REFERENCES

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