# Discovering the Genomic Sequence of Arhodomonas sp. Seminole by Polymerase Chain Reaction and Gel Electrophoresis

GRP# 3
Section# 001

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## ABSTRACT

Our goal was to discover a portion of the genomic sequence of Arhodomonas sp Seminole, which is important due to its ability to break down dangerous hydrocarbons in very salty soils. We did this by PCR to replicate the desired sequence and gel electrophoresis to separate it. Our results were unsuccessful in determining the results we desired as the PCR failed to produce a result, likely, due to a variety of reasons. According to our BlastX analyses, the sequence in question would likely have coded for the protein 3-ketoacyl-CoA thiolase, which is necessary in the breakdown of glucose during respiration. Despite the current failure, we believe that the current gap is correctly aligned and that rerunning the PCR with different primers then a product should be made.

## INTRODUCTION

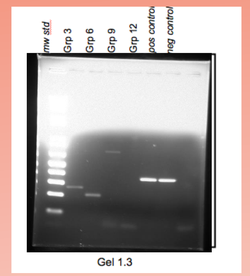
The project bacteria's name is Arhodomonas sp Seminole. It is an aerobic, halophillic bacterium enriched from salty, crude-oil impacted sites in Seminole, OK. Halophilic bacteria have become of particular interest due to their ability to breakdown dangerous hydrocarbons (Van Hamme, 2003). Arhodomonas sp Seminole is of particular interest since it was derived from salty environment of Oklahoma and it can breakdown various polluntants such as; benzene, toluene, phenol, 4-hydroxybenzoic (4-HBA), protocatechuic acid, and phenylacetic acid (Dalvi, 2012). Our primary goal was to complete the genomic sequence of Arhodomonas sp Seminole which has already been partially sequenced and been shown to have the proper DNA code for enzymes that breakdown hydrocarbons (Dalvi et al, 2012) which is helpful to maintain a safe environment. Our hypothesis is halo.Contig553's tail joins to halo.Contig140's head if a small gap in the amino acid sequences is filled because analysis through BLASTX indicates that they are likely joined together.

## MATERIALS AND METHODS

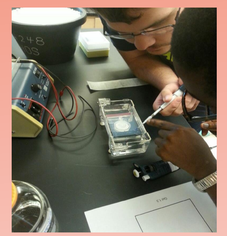
The initial steps were preformed by Dr. Caanan. She obtained the samples, ruptured the cells, purified the DNA by organic extractions, concentrated the precipitation, and prepared DNA for analysis. Obtained contig 1 and contig 2 and fused contig data from Dr. Caanan. Obtained related protein from Dr. Caanan. Using the related protein as a reference, we used blastx to make a comparison between contigs and the protein sequence. This information is used to determine if there is a similarity between the head and tail of the contig in an attempt to bridge the gap. By using the fused contig and Primer Quest, we were able to develop primers to be used in the upcoming steps. Submitted primers to Dr. Caanan, who then ordered the primers from Integrated DNA Technologies. By use of a p20 micropipeter with disposable tips we added the following reagents to a tube; 70 microliters of deionized water, 10 microliters of Taq buffer, 10 microliters dNTP's, 5 microliters of Arhodomonas sp. Seminole DNA at a concentration of 58 nanograms per microliter, 2 microliters of forward primer, 2 microliters of reverse primer, and 1 microliter of Taq polymerase. Technicians performed PCR by using a thermocycler to synthesis the DNA. After the final cycle of PCR is finished, 8 microliters of PCR mixed was placed on a wax sheet. 2 microliters of tracking dye was then added. Load product into designated well of 1% argarose gel (in 1x TAE buffer). Our well was the second from the left in gel 1.3. TA's loaded molecular weight standard, positive and negative controls and then ran at 80 volts submerged 1X TAE buffer for 1-2 hours until the fastest dye is almost to the end of the gel. Entered fused contig into blastx on ncbi.nml.nih.gov website. All similar sequences were shown, for us to research to better understand the possible protein that results from our fused contig. Results were reviewed and recorded.

## RESULTS

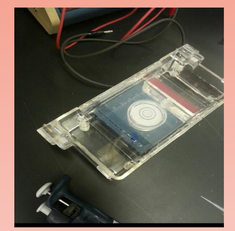
The PCR amplification was performed with the program which consists of an initial denaturation at 94° C for 30 sec, 30 cycles of denaturation at 94° C for 30 sec, annealing at (51° C, 53° C54° C or 55° C) for 30 sec, extension at 72° C for 1 min another 10 min at 72° C for the final extension. After PCR was finished, our gel electrophoresis did not demonstrate a result, as indicated by the following image.



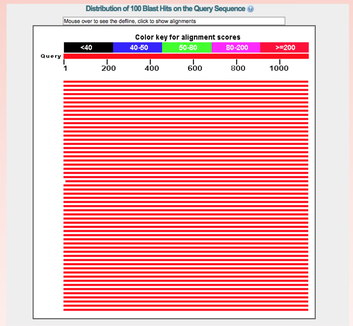

Our Electrophoresis gel



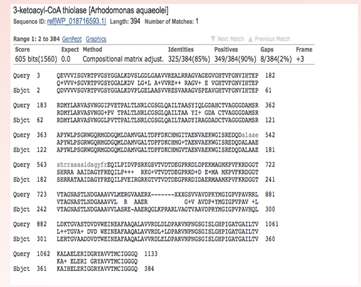
Loading the PCR products into the gel well.



Gel after our well was loaded.




As indicated by the blastX results to the left, our fused Contig sequence is most likely codes for a 3-ketoacyl-CoA thiolase. It had a 85% identity match to the nucleotide code for the protein. This is a very high percent match. In fact, each protein that was found similar to the fused contig sequence by use of blastX was a code for 3-ketoacyl-CoA thiolase from differing bacteria including other species of Arhodomonas.

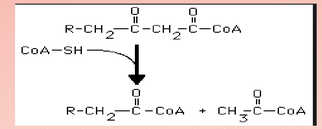



There are a variety of reasons why our PCR failed. The failure may have been a result of our related gene being too different from the sequence we are investigated since it is from a different species. Also, our PCR primers may have been in bad locations. Errors in pipetting may have occurred due to the extremely small quantities we were dealing with. The Taq polymerase may have been bad or denatured. Our hypothesizied target length may have been too short so the actual result could have been to large to run through the gel. The DNA, because it was gathered in the summer, could have degraded and denatured making it useless for PCR. The thermocycler conditions may have not been ideal. The bacterium has a high GC (guanine-cytosine) content which interferes with PCR.

## DISCUSSION

As indicated by the lack of results by our gel electrophoresis , we cannot confirm that the two contigs align. This however does not mean it is impossible that the two contigs align, any number of errors as discussed previously on the results section could have occurred and changed the results we received. Due to previous analysis by blastX, we are led to believe that they do in fact, align. If the experiment was rerun using different forward and reverse primers, there is a chance the PCR would succeed.

If the PCR were to occur as we predicted the likely protein that our sequence would have coded for would most likely have been 3-ketoacyl-CoA thiolase, which catalyzes the below reaction. This reaction splits 3-ketoacyl-CoA into acetyl-CoA and acyl-CoA.




(Baggott)

Since the sequence that the protein codes for participates in this metabolic reaction, it makes the sequence extremely necessary. The reaction breaks down 3-ketoacyl-CoA in order to to continue the metabolic process within the cell and, thus, allows it to make greater amounts of ATP for continued functioning.

Further research could be conducted to finish the genomic sequence of Ahrodomonas. PCR can be unpredictable. If the entire experiment was repeated, following the exact same procedure, results could turn out very differently. Thus, if the experiment was rerun using even the same primers then a result may be made.

In conclusion, Arhodomonas is a very important bacteria with its ability to break down hydro-carbons in high salinity environments. Further research should be done to to examine the gaps in the genomic sequence so we can better understand its value and how it enhances the Earth. Thus, although it is not certain that this particular gap codes for 3-ketoacyl-CoA thiolase, it is relatively possible that it does, and further research is necessary for this gap in order to prevent future issues in working with this bacteria for modifying its genetic sequence.

## REFERENCES

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