Filling the Gaps: Genomic Sequencing of Arhodomonas sp. Seminole

Hannah Wallis, Jessica Ice, Natalie Newman, Dr. Patricia Canaan, Shanell Shoop, Robert Pokoo

Group 23
Section 2

ABSTRACT

Bacteria Arhodomonas sp. Seminole, found in Seminole, Oklahoma, is an aerobic, halophilic bacterium. Arhodomonas was then found to be a salt-loving bacterium, and could metabolize harsh chemicals such as benzene and toluene. Its cells were ruptured to expose and purify the DNA by itself in order to begin examining the makeup of amino acid. After obtaining the DNA, the shotgun effect was used in order to break apart the DNA. This would allow the reconstruction of the pieces in a more manageable structure. As group 23, Dr. Canaan presented us with two segments of DNA, an upstream and downstream contig, which was thought to be adjacent. We then used Blastx, a website to align the two contigs with the related protein sequence. We proceeded to align the fused contig with the protein sequence and found a significantly close match was found. This led to the creation of DNA primers that would allow us to create numerous copies of the joined contig by polymerase chain reaction, or PCR. After formulating the primers, we continued to test whether or not these new DNA segments were the missing bases in our original contigs by agarose gel electrophoresis. Our PCR product did not provide distinct banding indicating inconclusive results. This does not disprove the idea that these contigs could be joined. The alignment blasts done before the gel electrophoresis demonstrates otherwise. Further experimenting is necessary to verify or disprove our hypothesis.

INTRODUCTION

Bacteria Arhodomonas sp. Seminole, found in Seminole, Oklahoma, is an aerobic, halophilic bacterium. Arhodomonas was then found to be a salt-loving bacterium, and could metabolize harsh chemicals such as benzene and toluene. Its cells were ruptured to expose and purify the DNA by itself in order to begin examining the makeup of amino acid. After obtaining the DNA, the shotgun effect was used in order to break apart the DNA. This would allow the reconstruction of the pieces in a more manageable structure. As group 23, Dr. Canaan presented us with two segments of DNA, an upstream and downstream contig, which was thought to be adjacent. We then used Blastx, a website to align the two contigs with the related protein sequence. We proceeded to align the fused contig with the protein sequence and found a significantly close match was found. This led to the creation of DNA primers that would allow us to create numerous copies of the joined contig by polymerase chain reaction, or PCR. After formulating the primers, we continued to test whether or not these new DNA segments were the missing bases in our original contigs by agarose gel electrophoresis. Our PCR product did not provide distinct banding indicating inconclusive results. This does not disprove the idea that these contigs could be joined. The alignment blasts done before the gel electrophoresis demonstrates otherwise. Further experimenting is necessary to verify or disprove our hypothesis.

Reference:
Dalvi, Sonal, et al. 2012. Proteogenomic elucidation of the initial steps in the benzene degradation pathway of a novel halophile, Arhodomonas sp. strain Rozel, isolated from a hypersaline environment. Appl Environ Microbiol. 78(20):7309-16.

MATERIALS AND METHODS

Materials:

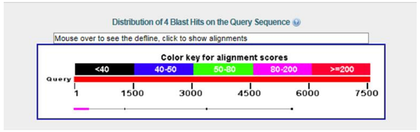
- Small glass tube used for the rupturing of cells
- Glass beads used for the rupturing of cells
- Arhodomonas sp. Seminole DNA fragments
- The sequence for a full length contig, an upstream contig and a downstream contig
- The sequence for a related protein
- http://blast.ncbi.nlm.nih.gov/Blast.cgi used for the aligning of the sequences
- Result from the "blast" of the full length contig and related protein including the Graphic Description, the Alignment and the Description.
- Fused contig sequence for testing
- Results from the "blast" of the fused contig and the related protein including the Graphic Description, the Alignment and the Description
- http://www.idtdna.com/Primerquest/Home/Index
- Appropriate "Assay Set Location for Sequence 1" with the forward and reverse sequences
- P20 micropipeter
- Disposable tips
- Taq Polymerase composed of the following shown in graph
- Cup of ice to place the reagents in
- Thermocycler
- 8 um of PCR sample
- 2 ul of blue, tracking dye
- Micropipeter
- Agarose Gel Electrophoresis cell
- Positive and negative control
- MW standard
- Power source of 80 volts
- Agarose Gel Electrophoresis cell result picture

Methods:

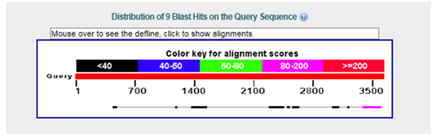
- Samples from Arhodomonas sp. Seminole were taken for DNA extraction and sequenced which contained 750 fragments of broken Arhodomonas sp. Seminole DNA.
- We were give the full length contigs, upstream and downstream, and aligned the full contig with the related protein using http://blast.ncbi.nlm.nih.gov/Blast.cgi . The results were described based on position of alignment to the protein and how similar they were to the protein.
- We align the related protein and the fused contig sequence and made a hypothesis on whether the contigs were adjacent to each other based on the alignment with the protein. We also formed a hypothesis on whether there was a gap or overlapping.
- Using the website, http://www.idtdna.com/Primerquest/Home/Index, a trimmed version of our fused contig, around 400 bases, was used to create a primer. The best primer was chosen on length and the placement in relation to the gap.
- To create the PCR product to copy our DNA and test our hypothesis, the following substances, shown in the results section, were added with a P20 micropipeter into a small test tube. The product is put into a thermocycler that completes the PCR process.
- The process of Agarose Gel Electrophoresis is started. The 8 um of PCR product is added to 2 ul of the blue, tracking dye using the micropipeter. Then, this product is added into the assigned cell in the gel. 80 volts of electricity is ran through the gel with positive and negative controls for 1-2 hours. Our results were inconclusive because of the absence of banding.
- Using the http://blast.ncbi.nlm.nih.gov/Blast.cgi website, we blasted our fused contig with all known bacteria in databases. We chose a similar protein and researched it to see how close they related to our sequence and Arhodomonas.

RESULTS

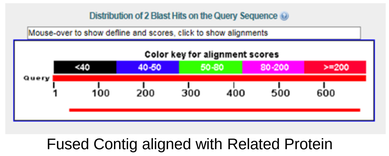
Dr. Canaan set up a way to check our gels of PCR for bands. In the positive control Dr. Canaan put in a mixture that she knew would work, and for the negative control she put in a mixture that she knew wouldn't work. Approximately 50% of our classes PCR's banded, while 50% did not band. Ours (Group 23) was among the 50% that did not come back with a clear band. Thus our hypothesis was not supported. There are many reasons that could provide for an error when banding the PCR making the Agarose Gel Electrophoresis fail. There is a possibility that our region that the primer had to fill was too great. We can only synthesize a certain length of DNA. If it is too large, we cannot perform a DNA synthesis. Our gene also might not have been made to fit between the contigs we were given. In order to fix this we could pick a new region so that we can design a new primer for a DNA sequence that is accurate. We do not believe that either of these reasons applied to the PCR we were given. I think this is due to the fact that when I put the micropipeter into the well the solution spread causing me to think that I broke the well. I was informed by the TA that this was okay, but I believe the well was broken. A broken well could have been the cause for our primer to come back negative.



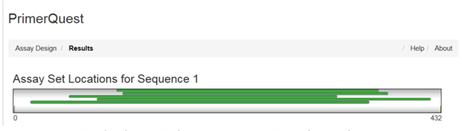
Upstream Contig aligned with Related Protein



Downstream Contig aligned with Related Protein



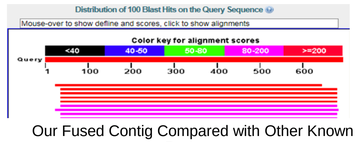
Fused Contig aligned with Related Protein



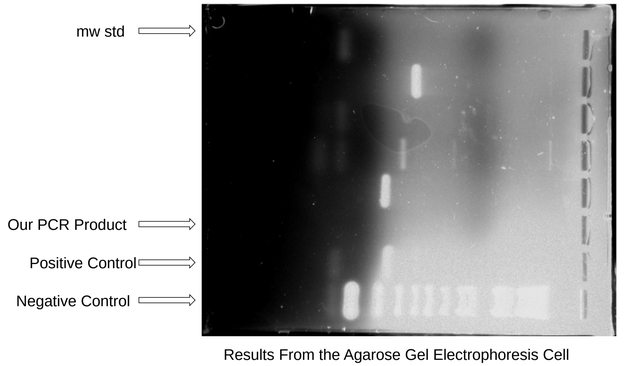
Designing a Primer For Our Fused Contig

Reagent	Volume µL
dH_2O(provided)	70
10X Taq buffer	10
10X dNTP's (all 4)	10
Arh. Sp. Seminole gDNA (58 ng/µL)	5
F-primer	2
R-primer	2
Taq polymerase	1

Components of Taq Polymerase



Our Fused Contig Compared with Other Known Proteins



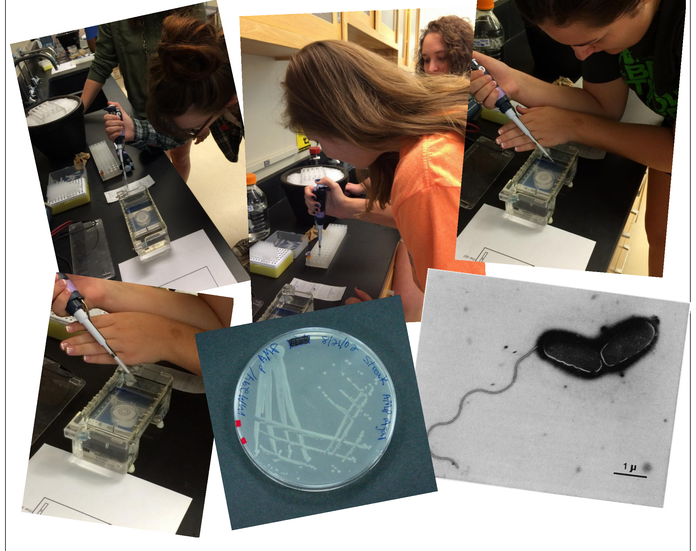
Results From the Agarose Gel Electrophoresis Cell

PCR Conditions Tried

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°C, 54°C or 55°C) for 30 sec, extension at 72°C for 1 min another 10 min at 72°C for the final extension.

DISCUSSION

Based on the results of the Agarose Gel Electrophoresis process, we obtain inconclusive results from our PCR product. Our PRC product did not result in a clear band that indicated similarities. The inconclusive results could have been error in the assembly of our Taq Polymerase solution. This is the most plausible area of error. With such small amounts used, mis-pipetting would result in the absence or wrong amount of certain reagents. The absence of reagents would result in the absence of banding and inconclusive results. Another reason for the inconclusive results could be problems with the primer and where the primer relates to. There could be a primer that is more effective on our strand of DNA. The last place of error could be the DNA not cooperating like PCR requires it to. If the DNA is tangled, the primers are unable to attach at the correct sites and allow the DNA to be replicated. The inconclusive result does not disprove our hypothesis. It is unlikely that our contigs are not adjecnt, because the results from our multiple "blasts" show that the two contigs are very similar to the given protein and is similar to other Arhodomonas related proteins. Due to the high alignment rates in the "blasts," there is some correlation between the two contigs, indicating they should align. In order for our hypothesis to be accepted, our experiment would need to be repeated and new primers should be considered. At the chance of a second experiment, the same procedure would be done and hopefully, without error to verify or disprove our hypothesis.



REFERENCES

- "Basic Local Alignment Search Tool." BLAST:. National Library of Medicine, n.d. Web. 1 Sept. 2014. <http://blast.ncbi.nlm.nih.gov/Blast.cgi?CMD=Web&PAGE_TYPE=BlastHome>.
- "PrimerQuest." IDT. Integrated DNA Technologies, n.d. Web. 1 Sept. 2014. <http://www.idtdna.com/Primerquest/Home/Index>.
- Dalvi, Sonal, et al. 2012. Proteogenomic elucidation of the initial steps in the benzene degradation pathway of a novel halophile, Arhodomonas sp. strain Rozel, isolated from a hypersaline environment. Appl Environ Microbiol. 78(20):7309-16.
- The MetaCyc database of metabolic pathways and enzymes and the BioCyc collection of Pathway/Genome Databases", Nucleic Acids Research 42:D459-D471 2014.