Fulfilling the Missing Puzzle Pieces of Arhodomonas sp. Seminole by DNA Sequencing, PCR Distribution, and Gel Electrophoresis

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GRP# 10
Section# 701

ABSTRACT

Arhodomonas sp. Seminole was an aerobic halophilic bacterium that was isolated and enriched from an oil-site in Seminole County here in Oklahoma. Enrichment was performed by starting with a simple soil sample that was placed in a liquid media with high –salt minerals and various aromatic hydrocarbons which grew at room temperature. A small amount would be transferred numerous times to the fresh media that contained the salt. The product of the enrichment was the bacterium of Arhodomonas sp. Seminole: a salt-loving, aromatic hydrocarbon-metabolizing bacterial. This bacterium required salt and grew on a wide variety of carbon sources: lactate was found to be the best carbon source. Since the bacterium was halophilic, salt was a very important requirement in order for this bacterium to prosper. The abilities of Ahrodomonas sp. Seminole were to metabolize benzene, toluene, phenol, 4-hydroxybenzoic (4-HBA), protocatechuis acid (PCA), 12, and phenylacetic acid (PAA) as the sole sources of carbon at a very high salinity. Ahrodomonas was sequenced. However, this led to a problem. Instead of the complete genome of Arhodomonas being one single, contiguous piece of DNA, it was a 750-contig piece of DNA which made the genome incomplete. Our mission was to conclude if our two given contigs were side-by-side on the same chromosome with a small gap in between them by using a reference protein along with a fused contig. From the results, we were able to conclude if our contigs were alongside each other, if there was a gap, and if our fused contig resembled an actual known protein.

INTRODUCTION

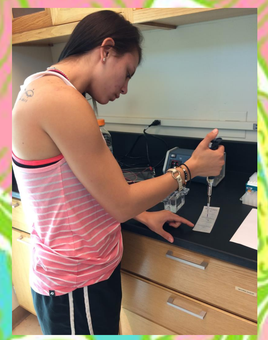
As mentioned in the abstract, Arhodomonas sp. Seminole was enriched from salty, crude-oil impacted soil in Seminole County. Because of this enrichment, Arhodomonas became a halophilic bacterium; in other words, it had a very, very high tolerability of salt. As a result, it became a hydrocarbon-metabolizing bacterial. However, a problem was formed after its genome was sequenced. There were gaps found in the genome. These gaps lead to questions: why were there gaps? How big were the gaps? How could we determine if the contigs were aligned by each other? As a group, we believed that our two given contigs were alongside each other on the same chromosome with a small gap in between them. We extracted several tests and experiments. Through they were easy to perform, the answers were sometimes unclear. After a long tedious semester, we finally found results.

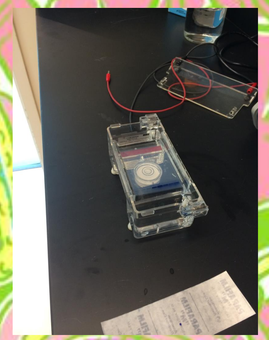
MATERIALS AND METHODS

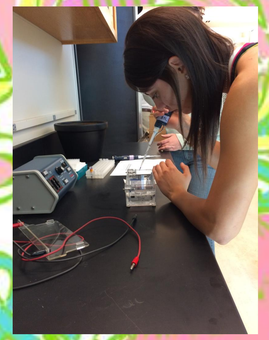
To begin our experiment, we were given two contigs: one possibly being the tail of the contig. Next, we were given a reference protein that allowed us to compare the degree of similarity. All of this was extracted by using by the "Blastx" feature on the NCBI. The next was to find the forward and reverse primers using our fused contigs. Our fused contig contained a line of N's which indicated the point of artificial infusion, and we chose about 200 base pairs in front and behind the N's. We were able to perform this by using the "PrimerQuest" feature on the IDT website. After finding our forward and reverse primers, we performed PCR Distribution. In our designated tube, we added dH2O (this substance had already been provided), tag buffer, all four of the nucleotides, the DNA of Arhodomonas sp Seminole, our forward and reverse primers, and the tag polymerase. We executed this by using a P20 micropipette. Once all of these materials were in the tube, it was placed in a thermo-heater where it experienced a series of heating and cooling. The next thing on our agenda was gel electrophoresis which was used to separate the DNA. We added our PCR to a dark blue dye and inserted it onto an electrophoresis gel. The column on the far left side indicated the base pairs; started at 100 base pairs and continued to increase by 100. The last step in the experiment was to conclude if our fused contig resembled an actual bacterium, with a sequenced genome. To do this, we used the "Blastx" feature on the NCBI website once more. With the help of all of these websites, the professor, and the graduate student, the experiment was easy to produce.

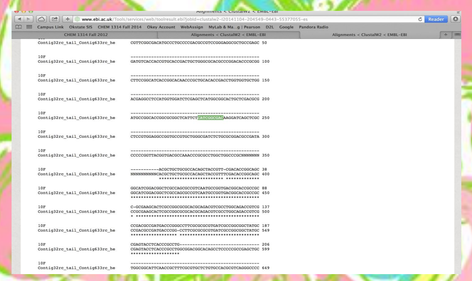
RESULTS

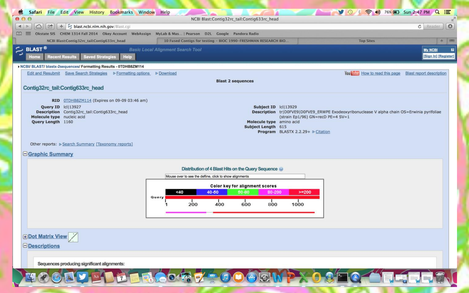
Compared to other groups, we received faint results from the gel electrophoresis. Since we did have a faint result instead of a complete failed result, our PCR was ran once more; this was done to see if we could get better results. Many things could have cause our experiment to go wrong: the equipment, human error, any of the substances added to make the PCR. As a whole class, the results were very, very good. This was the first time that this was being performed. So , getting four to five great results was very successful. One of the last parts to the experiment was to discover if our given fused contig resembled on actual known bacterium that has its genome sequenced. As a result, we found that our fused contig resembled the species of chromohalobacter selexigens. In relation to Arhodomonas sp. Seminole, this bacterium also has a very high tolerability of salt, making this bacterium halophilic. What can this bacterium specifically do for Arhodomonas sp. Seminole? Chromohalobacter selexigens could potentially help Arhodomonas exist in environments outside its comfort zone as well as provide it with the nutrients it potentially need. Our first test failed and so it was run again. The new test resulted positively. The amplification was performed with the program which consists of an initial denaturation at 94˚ C for 30 seconds, 30 cycles of denaturation at 94˚ C for 30 seconds, annealing at 51˚ C for 30 seconds, extension at 72˚ C for one minute another 10 minute at 72˚ C for the final extension.

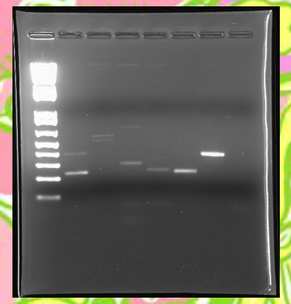












DISCUSSION

We were given a sequence by Dr. Canaan. With this sequence and our fused contig we copied it into the ClustalW2 website. This gave us an alignment of our fused contigs. It showed us that our PCR experiment did not work. The ClustalW2, if achieved would have showed us what the gap was and what protein it was. Our forward primer overlapped our track of N's; however, our reverse primer did not. This did not help in our results. All in all our experiment failed, for future research if we wanted to perfect this experiment, we could try new primers and keep running and rerunning this experiment. We know the gap exists; however, we were unable to discover what specific gene fills that specific gap.

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