Genome Sequencing on Arhodomonas sp. Seminole to Determine Genetic Identity

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Courtney O'Connor, Kenny Wolgamott, John Neisen, Parker Wilson, Dr. Patricia Canaan, Shanell Shoop, Robert Pokoo

ABSTRACT

Arhodomonas sp. Seminole is a member of the genus Arhodomonas in the class Gammaproteobacteria. With each research group receiving a region to sequence, the project's goal was to sequence the genome of the bacterium in order to better understand the species role in the environment and potential benefit in the process of oil field recovery. The first step was to run the contigs through BlastX to determine if there was an alignment. Next, we had to use Primer Quest to determine the forward and reverse primers for the PCR process. After obtaining the primers, we began the PCR process by extracting the DNA from a sample of Sp. Seminole. After copying the DNA, we viewed it by using gel electrophoresis. The PCR happened to have failed for our group. Running a hypothetical fused contig through BlastX yielded outstanding matches with multiple proteins, but the best result was flotillin, a related bacteria Acinetobacter Tandoii with a 77% match to Arhodomonas sp. Seminole.

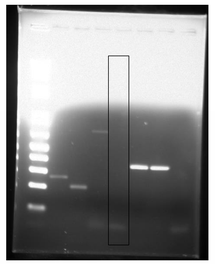
INTRODUCTION

A new bacterium was discovered in Seminole County, OK at a former oil field. The soil where the bacterium was discovered was highly contaminated with crude oil. The bacteria was identified with the name Arhodomonas sp. Seminole. Our objective was to identify the genome sequence of the DNA of Arhodomonas sp. Seminole in order to determine its protein, enzymes, phenotypes, and trait in order to see what aspect of the genomic DNA is survivable in the crude oil.

MATERIALS AND METHODS

We needed a specific bacterium that required a high level of sodium in their environment to survive. We took a bacterium named Arhodomonas sp. Seminole from the soil around a fracking operation in Seminole, Oklahoma. In order to produce a pure sample with our desired traits, we placed that soil sample into a sodium and mineral environment. Then, we transferred that sample to an environment containing normal sodium levels. After repeating this, we were able to obtain the desired sample of bacteria. We then extracted the DNA from this bacterium by using small glass beads and an organic solvent to rupture the bacteria. The DNA produced were fragmented. We were given two DNA strands with a genome sequencing base pair gap. In order to be positive those two strands were a part of a whole, we took the two DNA contigs provided and entered them into the Blastx website. From the results, we concluded that the two fragments were related and connectable. Then, we performed PCR on the two fragments of DNA in order to predict what the missing nitrogen bases are. This is done by imitating the process of DNA replication. For PCR to work we needed two primers; primers are the compliment nitrogen bases connected to either the head or tail end of the contigs. We obtained these primers from Primer Quest. We inserted our estimated fused contig, and the primers were generated. Then we took the forward and reverse primers into a mixture of Arhodomonas sp. Seminole DNA, primers, Nucleotide mix, MgCl2, and taq polymerase. Then, we used a thermocycler four different times at 51, 53, 54, and 55 degrees Celsius in order to induce the PCR reaction. Once the PCR reaction was complete, we examined the product using DNA gel electrophoresis. Gel electrophoresis separates the DNA by size, which allowed us to compare our product with the original DNA from the bacteria.

RESULTS



We ran PCR and viewed the results in a gel electrophoresis. This is a picture of our gels. Our PCR is the one that has the box around it. The DNA band was not able to be seen, either it was too large or the DNA did not work entirely.

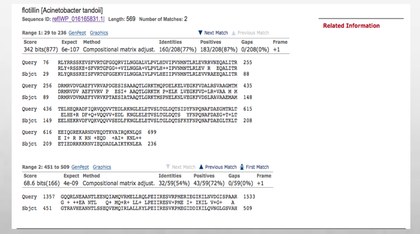
- We ran BlastX to see if there was a related protein to our DNA strand.

•While there were matches to many different hypothetical proteins, the greatest match was to Flotillin.

•Flotillin is found in the bacteria Acinetobacter sp. tandoii. Our strand had a 77% identity to this related protein.

•"Acinetobacter tandoii is a Gram-negative, strictly aerobic bacterium from the genus Acinetobacter isolated from activated sludge" (2).

Our expected result was that we found the correct forward and reverse primers to merge the gap between contig 242 and contig 337, however because PCR failed, we simply don't know if the contigs match. Our data conflicts with our expectations and the estimated predications that the two contigs should align. It agrees with similar experiments, especially on the bacteria Acinetobacter tandoii with the related protein Flotillin. There is a picture of the BlastX report of the similarities between the fused contigs and the bacteria Acinetobacter tandoii.



It is possible that the failed PCR is due to either individual errors or errors with the experiment itself.

• Individual errors could have been from a lack of consistency across the board, as we involved the whole group in running the experiment,

•Experimental errors could be due to something such as supercoiling, or that our ultraviolet reader for the gel electrophoresis is very old and worn down.

DISCUSSION

The meaning of this experiment was to start to connect all of the gaps in the genomic sequence of Arhodomonas sp. Seminole. Once all of the gaps are connected, it is possible to study the bacteria itself to understand what specifically makes it possible for the bacteria to thrive in the salinity of the crude oil spills. This is important because once this trait is studied, it is possible to use Arhodomonas sp. Seminole to help clean up the land after drilling for oil and natural gas, and the land can be reused to benefit society. This would mean that oil and natural gas can potentially be collected without harming the land itself, and that the land that was once just left desolate can now even be used for farming and agriculture.

Our PCR did not work, that could have been due to many different reasons. It could've been due to either individual error or an experimental error. Individual error is likely, because while performing the experiment, everyone got a chance to use the micropipette and due to that, there was a lack of consistency in the experiment. In the future, we would fix that by having one person perform the experiment. Experimental errors are possible, as supercoiling is a common problem in PCR reactions and the DNA strand could have wound upon itself. The ultraviolet machine that is used to read the DNA Gel Electrophoresis plates is old and worn down, and it's possible the DNA band was simply not recorded. There is no simple fix for these problems, as the machine is expensive.

The next step, would be to generate different primers and retest the DNA using PCR and the gel electrophoresis. We still strongly believe that our two contig strands should align. We believe that because the percentage match of identity is so high, that the contigs have to match.

REFERENCES

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