Genomic DNA Sequencing of Arhodomonas sp. Seminole

GRP#19
Section#002

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ABSTRACT

The objective of the experiment was to determine the missing amino acids in the DNA sequence for Arhodomonas. We were looking to fill in the gap between two contigs by using forward and reverse primers in order to fill the entire genome sequence. DNA was extracted from the bacteria; primers were designed for the PCR procedure. PCR produced millions of copies of the DNA for examination. Once the PCR was complete, the missing pieces of the DNA sequence were determined. The PCR product was examined via Agarose Gel Electrophoresis by the bands produced in the gel. Once the missing base pairs were determined, the bacteria was characterized by gene according to its phenotype. By finding the base pairs in all of the gaps in the sequence, the genome will be complete. A complete genome yields the ability for the degradation of hydrocarbons in the oil-filled waste to be understood.

INTRODUCTION

Previous studies have found that Arhodomonas is an aerobic, halophilic bacterium found in salty, crued-oil-impacted areas in Seminole County, Oklahoma. It degrades hydrocarbons such as benzene, toluene, and phenol (Dalvi, 2014) This experiment was designed to complete the DNA sequence in order to characterize each gene in Arhodomonas. This complete genomic DNA allows the understanding of the degradation of hydrocarbons by Arhodomonas. Questions about which genes specifically are involved in the degradation process are still being addressed. To fill in the gap between two contigs we used the forward and reverse primers in order to fill the entire genome sequence one segment at a time by the PCR process. Once the missing segment of DNA was figured out then the phenotype could be determined to see if it was a part of the degradation of hydrocarbons. We found what we believe to be the missing sequence of base pairs that codes for a specific protein in the DNA sequence. We also found what we believe to be a related protein to which the base pairs code for.

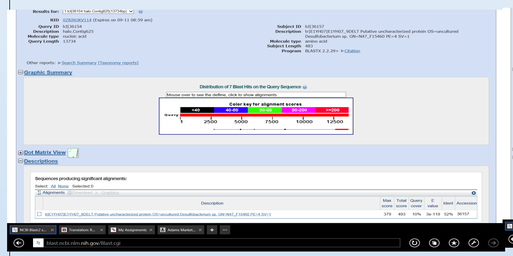
MATERIALS AND METHODS

Materials
EVERYTHING WE USED
NCBI website: blast x
Computer
Course material and sample papers
PCR The 100 µl PCR mix contained
~50 ng of DNA,
0.6 µM of each primer,
200 µM of PCR nucleotide mix
(Fisher Bioreagents, Fisher Scientific,
Pittsburgh, PA),1.75 mM MgCl2,
2.5 units Taq polymerase in Buffer A
M1865, Promega Chemicals,
Madison, WI).
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 53°C for 30
sec, extension at 72°C for 1 min
another 10 min at 72°C for the final
extension.
Thermal cycler
Agarose gel, electrophoresis tray, dye
Micropipeter

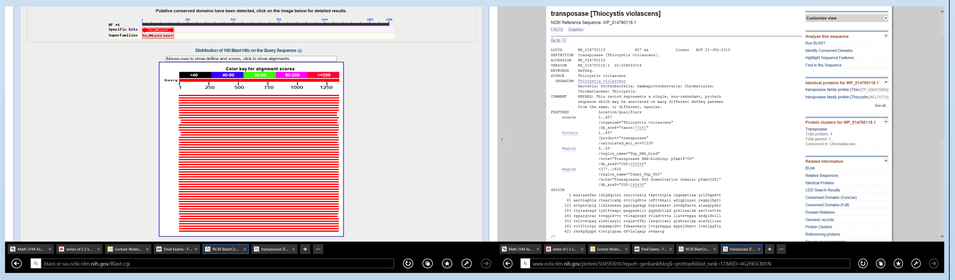
Methods
1 Studied Arhodomonas in class
2 Examined the given contigs with the blastx
website.
3 Took the provided contigs and used around
150 bases from the tail of the first and the
head of the second in the Primer Quest
website to produce primers for the PCR
reaction.
4 Hypothesized that there would be a
PCR product, therefore, the missing base
pairs would be found.
5 Put the materials for the PCR into
the tube. The 100 µl PCR mix contained
~50 ng of DNA,
0.6 µM of each primer,
200 µM of PCR nucleotide mix
(Fisher Bioreagents, Fisher Scientific,
Pittsburgh, PA),1.75 mM MgCl2,
2.5 units Taq polymerase in Buffer A
M1865, Promega Chemicals,
Madison, WI).
6 Ran PCR. 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 53°C for 30
sec, extension at 72°C for 1 min
another 10 min at 72°C for the final
extension.
7 Added the dye to the PCR product with
the micropipeter.
8 Ran electrophoresis
9 Examined the bands as a product
to determine if PCR worked (If it didn't work then
the gap didn't fill and there would be no bands)
10 Determined the hypothesis was correct
11 Found related proteins using blastx
12 Ran clustal test to find what the missing
base pairs were.

RESULTS

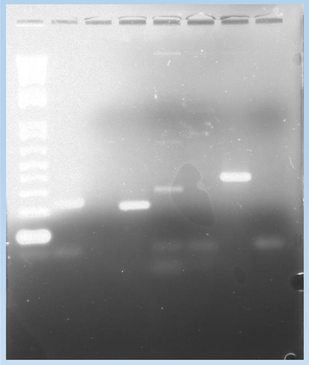
There is a 10% match in the first contig blast results, and a 98% match in the second contig last results. This means its almost an entire contig

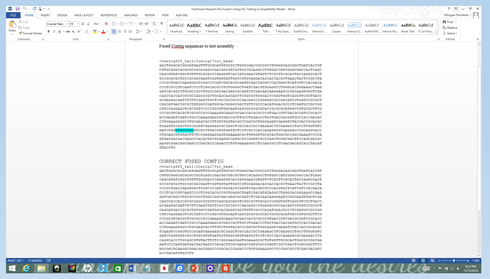


We ran our DNA primer through a blast x alignment process to compare the proteins to our fused contig. The protein our results yielded a related protein was transposase (Thiocystis Violascens)



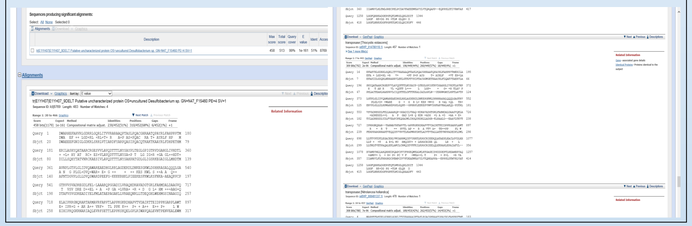
We ran an gel electrophoresis test to see if our PCR primers would have the same number of contigs as the one that we were trying to replicate. Our primer worked because our results yielded the same number, around 200 base pairs according to our final blast x results and the gel results. The PCR primer we acquired was both forward and backwards and was able to work both ways on the gap in the DNA.





RESULTS

The amino acid alignment yielded similar results to that of the transposase.



DISCUSSION

The PCR product was positive, therefore the primers were correct.
We could repeat the experiment with those primers and theoretically yield the same results from PCR. We could use different primers and see how that affects the results.
The next step would be to choose a different contig with a missing sequence and fill in that gap, too.
The experiment was to fill the gaps in the DNA sequence to have a better understanding as to what gene helps breakdown hydrocarbons in crude oil.
We did a PCR, gel electrophoresis, and multiple alignments to determine the missing base pairs needed to determine what protein was being created and used in the degradation process.
Our results were similar to a transposase protein.

REFERENCES

NCBI Website for blastx
Primer Quest website
Course Material

Dalvi, S, S Azetsu, M A. Patrauchan, D F. Aktas, and B Z. Fathepure. "Proteogenomic elucidation of the initial steps in the benzene degradation pathway of a novel halophile, Arhodomonas sp. strain Rozel, isolated from a hypersaline environment." *NCBI*. N.p., 10 Aug. 2012. Web. 13 Nov. 2014. <http://www.ncbi.nlm.nih.gov/pubmed/22885747>.

Dalvi, S, C Nicholson, F Najar, P Canaan, and BA Roe. "Arhodomonas sp. Strain Seminole and Its Genetic Potential To Degrade Aromatic Compounds under High-Salinity Conditions." *NCBI*. N.p., 22 Aug. 2014. Web. 13 Nov. 2014. <http://www.ncbi.nlm.nih.gov/pubmed/25149520>.