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

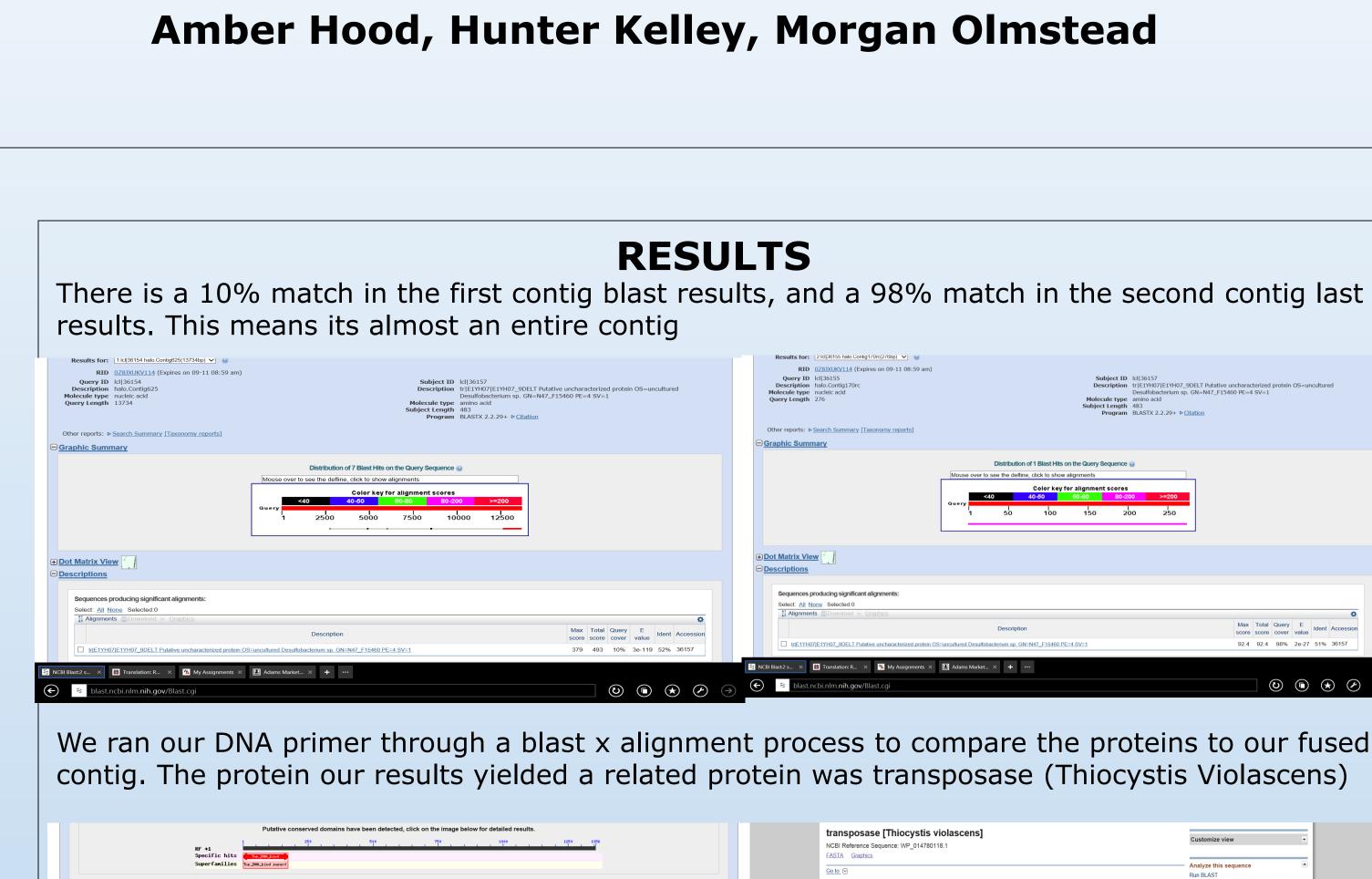
EVERYTHING WE USED NCBI website: blast x Computer Course material and sample papers PCR The 100 µl PCR mix contained ~50 ng of DNA, $0.6 \,\mu\text{M}$ of each primer, 200 µM of PCR nucleotide mix (Fisher Bioreagents, Fisher Scientific, Pittsburgh, PA),1.75 mM MgCl2, 2.5 units Taq polymerase in Buffer 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

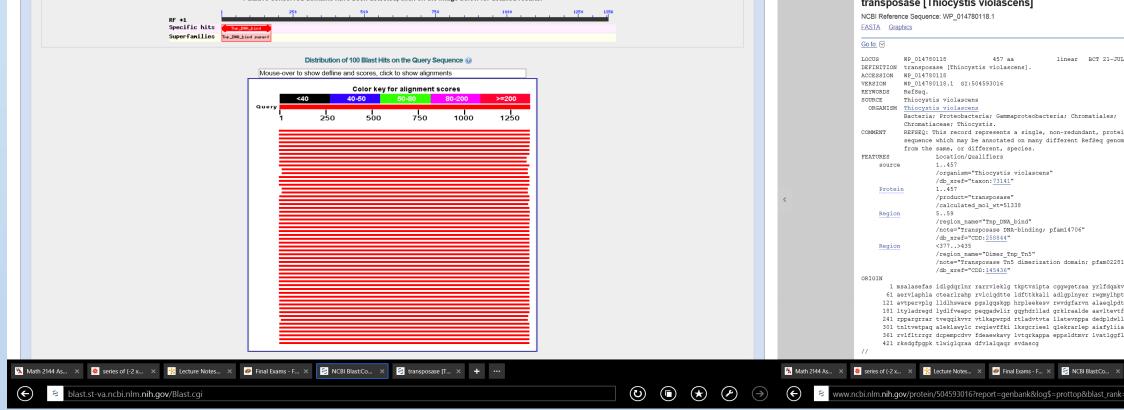
Methods

base pairs were.

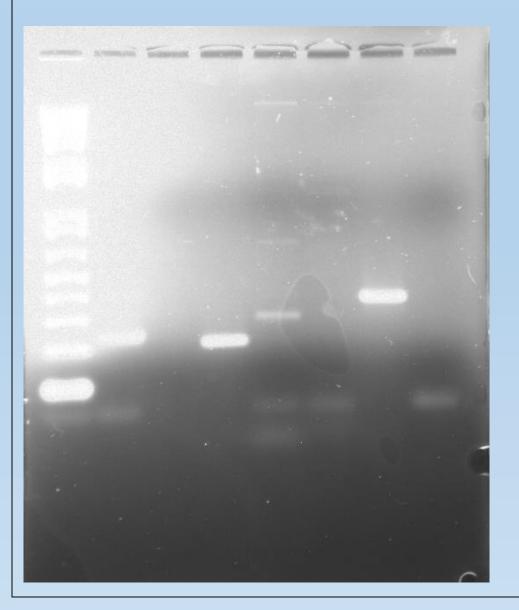
1 Studied Arhodomonas in class 2 Examined the given contigs with the blastx 3 Took the provided contigs and used around 150 bases from the tail of the first and the head of the second in the Primer Ouest website to produce primers for the PCR 4 Hypothesized that there would be a PCR product, therefore, the missing ba 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 Tag 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 fina 7 Added the dye to the PCR product with the micropipeter 8 Ran electrophore 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

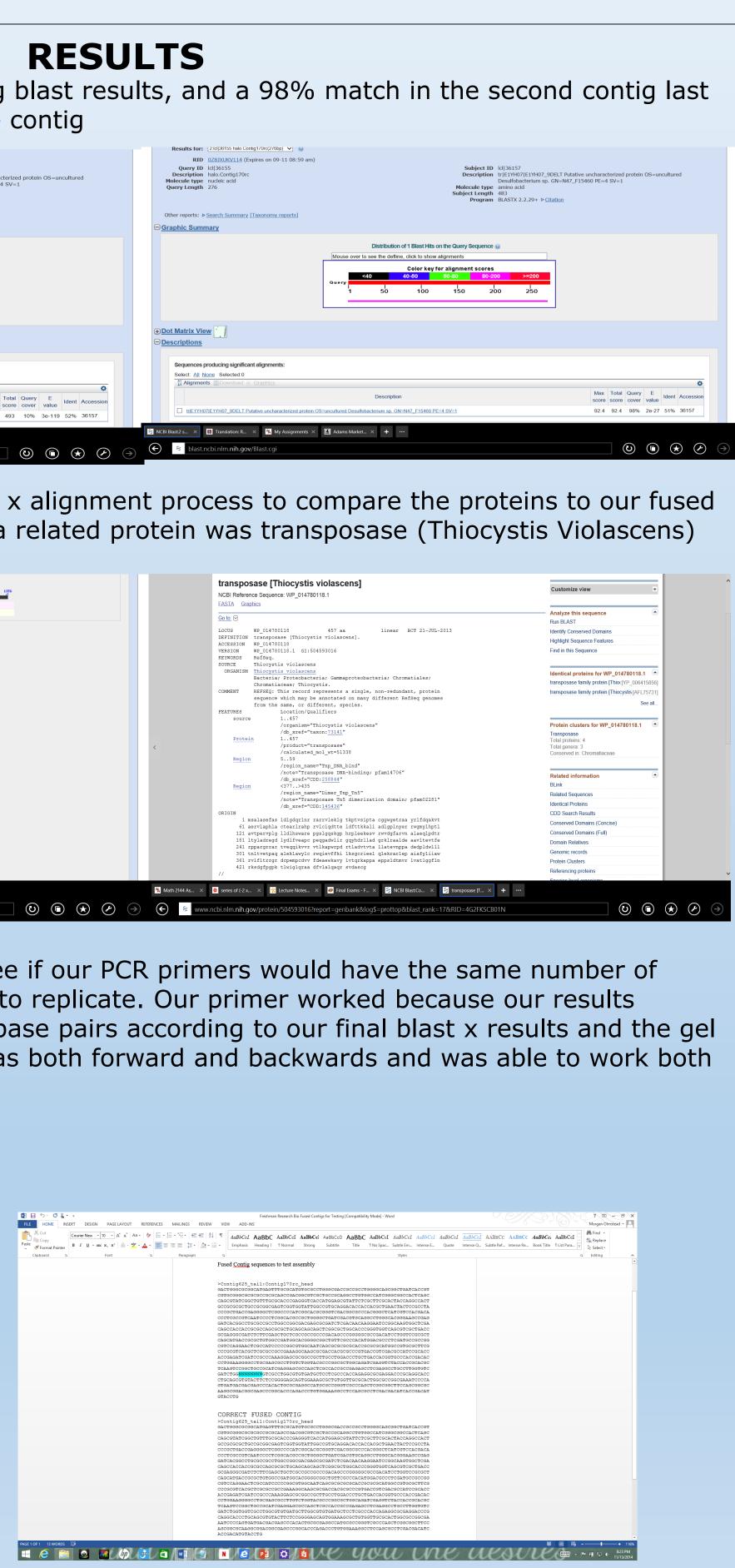
Genomic DNA Sequencing of Arhodomonas sp. Seminole





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. Sequences producing significant alignment Query 1258 LGGFQRRADGEPGTQTIMKGLQRLDDIT 1344 LGGF RK+DG PG +TLM GLQR+ D Sbjct 418 LGGFLNRKSD3FPGPKTLMIGLQRIPDFV 446 Select: All None Selected:0 Max Total Query E score score cover value 458 513 99% 1e-161 51% 8769 P to 944 softward Softward P to 044 softward P Alignments Download v Graphics Sort by: E value tr[E1YH07]E1YH07_9DELT Putative uncharacterized protein OS=uncultured Desulfobacterium sp. GN=N47_F15460 PE=4 SV=1 Sequence ID: ICI|8769 Length: 483 Number of Matches: Score Expect Method Identities Positives Gaps Fri 458 bits(1179) 1e-161 Compositional matrix adjust. 230/452(51%) 310/452(68%) 6/452(1%) +1 ry 1078 XVARKVMLLAHQREDPOAPCSYLFSGEQWKALMLERGAGEISSDDDEPFLEEARKW +ANKV+L + R+ P+ PC V F +NKA++L T P+ P+ L+ R V ct 357 ILANKVLFIKRGRDCPENPCDVYFDEARKAYVLFUGRKAPFA--EPFSLDFWYRUX

DISCUSSION

ery 1258 LGGFQRREADGEPGTQTLMKGLQRLDDIT 1344 LGGF RE+DG PG +TLM GLQR D vjct 415 LGGFLNRESDGFPGPKTLMIGLQRAADFV 443

 Range 1: 16 to 457 Gui/Ngi Gradics
 West Mitch & Previous Match

 Score
 Expect Method
 Identifies
 Positives
 Caps
 Fram

 308 bits/788)
 7e-96
 Compositional matrix adjust.
 189/453(42%)
 262/453(57%)
 14/453(3%)
 +1

Convoluted ~ GenPlect Graphics
transposase [Nitrolancea hollandica]
Sequence ID: <u>refWP_000481337.1]</u> Length: 470 Number of Matches: 1

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**

Query 541 GTRVVSVADREGDLFEL-LABARQPGGADILVRAQHDRAVADGTGRLFAHMDALDARGVQ T VSV DEE D++EL + A +P GA +LVRA+ +R + D G L+ HH ++ARAGFQ 5bjct 198 DTARVSVGNERADIYELBMATAFERGARLLVRAQGVRLTDRGQeHMBENMGSINAAGIQ

Query 718 ELAIPRGNORARTARMAVRFARVTLAPFKGKRDHAFVTVUAIRTTEIDPFKGRPLAWT E+ IPR+G + AR A++ VRF+ TL PFK K++ P+ + A++ E++ P+ L W Shiet 258 RHFPGKRMARIAGLEVFRSTETIEFKSKGLEDERIKMVGLEVNTERDALBKM

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>.

GRP#19 Section#002

Gene - associated gene details Identical Proteins - Proteins identical to the Related Information