Methods of PCR Amplification and BLASTX Procedures to Determine Nature of Gene Sequence Gap and Function of Protein in Arhodomonus *sp. seminole*

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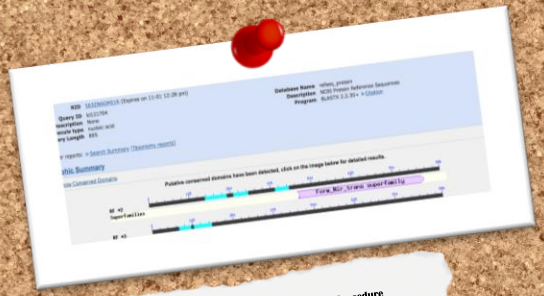
INTRODUCTION

In this experiment we looked at a newly discovered bacterium found on an oil site in Seminole, Oklahoma called Arhodomonas sp. Seminole (Canaan 1). This microorganism is aerobic and survives in environments with high salinity (Dalvi, 2012; Azetsu, 2012). For the purpose of creating a more efficient clean up at oil sites, we looked at an organism that requires carbon and salt that has the ability to metabolize Benzene, Toluene, 4-hydroxybenzoic acid (4-HBA), protocatechuic acid (PCA), 12 and phenylacetic acid (PAA)(Canaan 7). Having the ability to degrade hydrocarbons, could help make cleaning up oil-sites more environmentally friendly, as well as, save money and time. The problem with trying to find an organism that will completely clean up an oil-site, is that there is not much known on this type of bacterium. The purpose of this experiment was to sequence a hypothetical gap in-between two strands of DNA in order to better understand the function of the gene as a whole. In the Biochemistry Freshman Research class, we were each assigned a gap in the DNA sequence, and we were tasked with generating the sequence of a gap of DNA to bring two fragments of DNA together.

ABSTRACT

The goal of this study was to better understand the degradation of hydrocarbons by halophilic organisms. In Seminole, Oklahoma, a bacterium was found, and classified as Arodimonious species Seminole, and was found in the soil at a fracking site (Canaan). This bacterium was found to break down benzene, toluene, phenol, 4-hydroxybenzoic acid (4-HBA) protocatechuic acid (PCA), 12 and phenylacetic acid (PAA) with sources of high salinity (Canaan, Powerpoint). In order to better understand the function of a particular gap in the protein, we had to perform a method of DNA Replication named PCR. The PCR Process replicated the DNA millions of times and then we used that cluster of DNA strands to determine the length and complexity of the strand through a process called Electrophoresis. The electrophoresis was cosidered to be unusable due to many factors including a weak PCR product and old photo equipment. The mistakes learned can be used to improve the experiment in the future.



Protein size domains from BLASTX Procedure

Buffers and DNA from PCR Procedure

Electrophoreses Gel and Inconclusive Band

SuperFamily Sceenshot of related bacterium and gene

PCR AMPLIFICATION METHODS

PCR or the Polymerase Chain Reaction is a biochemical technique used to enzymatically amplify a specific, desired piece of DNA by several orders of magnitude, generating millions of copies of a particular DNA sequence for further study and analysis. PCR relies on the use of Taq DNA Polymerase (enzyme originally isolated from the bacterium Thermos aquaticus) and a dedicated instrument called a ThermoCycler to synthesize the DNA Copies in a small test tube.

Taq Polymerase has several requirements; template DNA (source of DNA containing the desired region of DNA), forward and reverse primers flaking the region of interest, dNTP's or DNA nucleotide building blocks used to make the PCR products and a buffer with Magnesium and the correct pH for optimum activity.

PROCEDURE

Using a 20 microliter pipet and disposable tips use the following reagents to provide the proper tools for proper DNA replication.Keep all Reagents on Ice.Do not re-use tips.Double Check the reagent tubes – Make sure you have everything you need in advance.Seventy microliters of dH20 has been predispensed into your PCR tube already.In this order, pipet the according fluids into the PCR Tube.

Add 10 microliters of 10X Taq buffer.
Add 10 microliters of 10X dNTP's (all 4)
Add 5 microliters of Arb. Sp. Seminole gDNA (55ng/microliter)
Add 2 microliters of F-Primer.
Add 2 microliters of R-Primer.
Add 1 microliter of Taq polymerase.

The total volume should add up to 100 microliters of mixed solution. Take mixed solution and run through DNA Electrophoresis to obtain bands to analyze.

BLASTX PROCEDURE AND DESIGNING PCR PRIMER

On the NCBI website, go to the primer design and copy and paste the given fused contig sequence into the query box

Select Reference protein from the menu, and press blast

After blast is completed, chose a protein that is 200 base pairs from either end of the unknown gap

Canaan, Patricia. "BIOC 1990-Asignment." Message to the class. 30 Oct, 2014. Email.

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DISCUSSION

We expected to bring two fragments of the DNA sequence together through PCR sequencing, using a predicted forward and reverse PCR primer. We had reason to believe that the two fragments came together based on references to other, related proteins, found in similar bacterium. Our PCR product failed, which produced an unsuccessful result in mapping the DNA between our two contig sequences. The results came back inconclusive, and not what we predicted because of several possible reasons. When we were running our fused contig through Blast-X, an error could have occurred in choosing our PCR primers. There could have also been error in preparing the PCR solution while mixing small amounts of the reagents. To retest our gap, we would have to run another Blast-X and choose another PCR primer sequence. Along with our forward and reverse sequences, we were given a fused contig sequence, which predicted the protein of our unknown sequence. We would want further explore the possible protein obtained from the fused contig sequence, and identify the function of our gap.

RESULTS

We did not obtain a PCR Product, but we don't know specifically were the results went wrong. There are many places for error though. The major error that occurred in this experiment is choosing a PCR primer, because the base pair range was much bigger than what was considered a good amount (200-250 bases)

However, running the BLASTX procedure revealed some curious results for the bacterium in question. The BLASTX concluded that the gene is 87% probable to pertain to Formate-Nitrate Transportation. The Prokaryotic amino acid is located within the cell membrane of the bacterium and uses energy coupling mechanisms to transport HCO2– (formate) and NO2- (nitrate) coupled with a H+ symport through the membrane and to relative organelles. The contig's genomic structure length is roughly 895 bases long with a flank missing in the middle. Given by the SuperFamily screenshot to the left.

REFERENCES

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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." Applied and Environmental Microbiology 78.20 (2012): 7309-316. NCBI. Web.