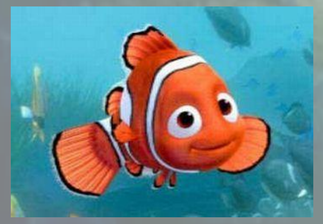

FINDING (N)EMO:

Sequencing the track of n's between fused contigs and the DNA sequence

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GRP# 18
Section#2

ABSTRACT

We were presented with the bacterium Arhodomonas sp. Seminole, the full DNA sequence of which is currently unknown. Our job was to track the DNA of a missing contig by utilizing database resources, such as BLASTX, and the technique of PCR amplification. Our group had a positive forward PCR product, which led us to the conclusion that two fragments of the original sequence and forward primer had aligned.

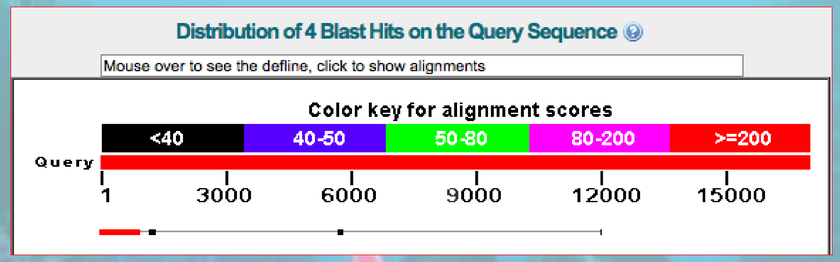
INTRODUCTION

Arhodomonas sp. Seminole is a halophilic bacterium obtained from an oil production facility in Seminole, OK (Dalvi 2012). We are specifically interested in finding the DNA sequence of this bacterium, which is presently unknown. The bacterium has been sequenced, but 8-10% of the genes are broken into pieces known as contigs. Therefore, there are several gap regions in which the genomic sequence is unknown. Our experiment involved finding the DNA sequence of a specific gap region of Arhodomonas sp. Seminole in order to piece together genes of the bacterium to better help recognize its structure.

MATERIALS AND METHODS

In order to retrieve the DNA sequence of the gap region, we used PCR amplification. PCR, also known as the Polymerase Chain Reaction, is a technique that amplifies specific pieces of DNA. The procedure required the use of *Taq DNA polymerase* (an enzyme) and a thermocycler to synthesize DNA copies in a test tube. We began the process by combining the following reagents: dH20, Taq buffer, dNTP, DNA of Arhrodomonas sp. Seminole, the f-primer, r-primer, and the *Taq* polymerase using a P20 micropipeter. We then implemented the solution into the thermocycler to receive a PCR product. After that process was complete, we learned that our forward primer created a product, while our reverse primer did not. Finally, we used the BLASTX program to compare the new DNA sequence obtained from the forward primer with original fused contig sequence.

RESULTS



Before we started the experiment, we used BLASTX to compare our original contig sequence with a related protein sequence. Through that, we found that the fused contig is continuous on the head.

The image to the left is the gel image that contains our group's PCR product. As our gel produced a visible band, we had a positive PCR product.

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----ATCCCGAGCTCGTGGT-C-----------GGATCGACGAGGCGGTCTACCCCGGA       43
TGTTATCCCGAGCTCGTGGGTCGGCTCATCGCGNNNATCGACGAGGCGGTCTAC-CCGGA      839
    ***************  *             ****************** *****

GACGGCCCAGGC-GTTTACGGT--CGCCATGCTACTCCGAGCGCGGCATCCTCTA-----      95
GACGGCCCAGGCCGTTTACGGTCTCGCCATGCTACT-CGAGCGCGGCATCCTCTACGCGC      898
************ *********  ************ ******************
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Through PCR Amplification, we discovered that our forward primer did work, while our reverse primer did not.

DISCUSSION

The goal of our experiment was to determine the DNA structure of a gap sequence found in Arhodomonas sp. Seminole. Fortunately our experiment provided us with a positive result in the forward primer, which allowed corresponding base pairs to be added to create the DNA strand for the missing contig.

Besides finding the DNA of the gap sequence, the original hypothesis desired to know if the new proteins would stabilize the environment and habitat of the bacteria. We were able to determine that by correlating the new protein back to what was predicted at the gap. We utilized the program, BLASTX, by blasting the new DNA sequence to the original fused contigs. This program allowed us to determine if there was a gap. Our results concluded that the two fragments had aligned, and therefore, supported the hypothesis.

We are happy with our result, but it is not completely ideal. In the future, we would recommend redesigning the forward and reverse primers in the hopes of receiving a positive result for the reverse primer sequence.

REFERENCES

• Dalvi S, Azetsu S, Patrauchan MA, Aktas DF, Fathepure BZ. 2012. 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 Environmental Microbiology. 78(20): 7309-7316.