Examination of a DNA Gap in Arhodomonas sp. Seminole Using PCR and DNA Sequencing

GRP#12
Section# 001

Robert Devor, Joshua Duke, Linda Le, Marina Alvarado

ABSTRACT

The objective of the experiment was to attempt to fill in an unknown gap in the DNA of Arhodomonas sp. Seminole. We examined two known DNA contigs from the bacterium for potential alignment with a fused sequence of the two. Potential alignment was observed so we compared the fused sequence with a related protein sequence to design primers for PCR. Possible primers were designed to attempt PCR sequencing of the gap area. This was followed by agarose gel electrophoresis which was unsuccessful.

INTRODUCTION

Arhodomonas sp. Seminole is an aerobic, halophillic bacterium that was enriched from salty, crude-oil-impacted soil in Seminole Country, Oklahoma. The full genome has not yet been discovered; there are numerous unknown gaps in the genetic information; this experiment was conducted to attempt to fill in one such gap. The experiment that we performed involved the examination of two nucleotide sequences that are believed to be separated by a gap, a fusion of the two, and a potentially related protein sequence. The goal was to perform a successful PCR experiment and examine the product.

MATERIALS AND METHODS

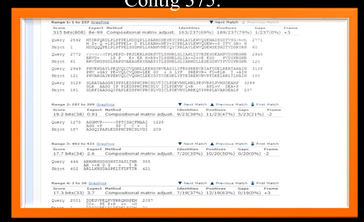
We were provided with two full length contigs that were possibly separated by a single gap in the DNA, a related protein sequence from a similar supposedly similar section of DNA from another organism, and a fusion of the two contigs. The separate contigs and protein were examined on the blast website for potential alignment. The fused sequence was then plugged into the website to design primers for PCR. The forward primer was CAAGTGCCCAAGGTGCT
and the reverse primer was TCGTCTTCCACCGAGACTT. PCR was performed, followed by agarose gel electrophoresis. The PCR occurred in a one-hundred microliter solution consisting of 70 microliters of distilled water, 10 microliters of Taq buffer, 10 microliters of nucleotides, 5 microliters of DNA, 2 microliters of each primer, and 1 microliter of Taq polymerase. The product was placed in the gel. The overall result was unsuccessful, which was followed by attempting PCR at different temperature parameters: 51, 53, 55, nad 55 degrees celcius. We performed a final blast to search the NCBI database for any proteins that matched the fused contig sequence.

RESULTS

The experiment began with two contigs (contig 375 and contig 615rc), a potential fusion of the contigs, and a related protein sequence. These were compared to the related protein sequence, and showed alignment at opposite ends, fueling the hypothesis that they may be related. Contig 615rc exhibited alignment between it's head and the tail of the protein sequence, while contig 375 exhibited alignment near it's tail and the head of the protein sequence.

Contig 615rc:

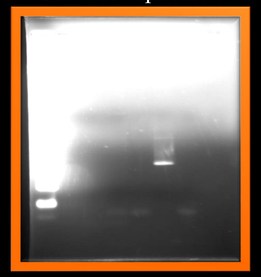
Contig 375:



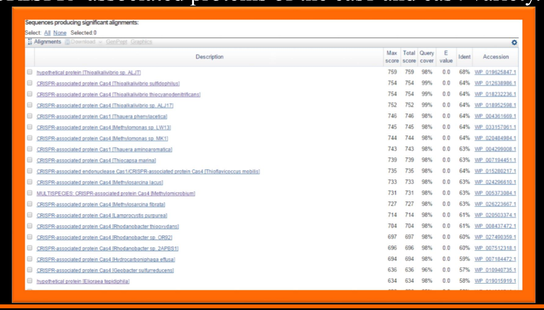
Examination of the fused contig sequence as it exhibited heavy alignment with the protein sequence as well, as shown in the blast results here:

Notable query section: subjects 1-1750 and subjects

Gel Electrophoresis



Although the overall results were unsuccessful, we performed a single blast of the fused contigs to search for any additional related proteins. The majority of matches came from CRISPR- associated proteins of the cas1 and cas4 variety.



DISCUSSION

Our experiment failed to sequence the gap between our two contigs. However, a majority of the data that was collected did support the hypothesis that a genetic sequence exists that can bridge the gap between our contigs. The alignment with the contigs and protein, as the heavily related results from an examination of the fused contig sequence supports the relationship of the two sequences. When we preformed the final blast, to identify potentially related proteins, a majority of matches were either hypothetical proteins or CRISPR-associated proteins of the cas1 and cas4 varieties. The CRIPR-associated are related to the adaptive immune system of organisms; they provide protect organisms from infectious agents such as viruses and conjugative plasmids. Cas 1 proteins are believed to be universally present in all organisms that posses CRISPR-associated proteins. Based on the agreement between these potentially related proteins and our specimen it appears likely that they may be a small gap of genetic information between the two contigs However we found no information that would suggest they are related to Arhodomonas sp. Seminole.. The source of error is not immediately determinable, but perhaps the most likely source was human error in setting up the PCR or agarose gel electrophoresis, or perhaps poor primers were chosen for the experiment.

REFERENCES

•"Basic Local Alignment Search Tool." *Blastx: Search Protein Databases Using a Translated Nucleotide Query*. N.p., n.d. Web. 04 Nov. 2014.

Dalvi, S., Nicholson, C., Najar, F., Roe, B. A., Canaan, P., Hartson, S. D., & Fathepure,
•B. Z. (2014). Arhodomonas sp. Strain Seminole and Its Genetic Potential To Degrade Aromatic Compounds under High-Salinity Conditions. Applied & Environmental Microbiology, 80(21), 6664-6676. doi:10.1128/AEM.01509-14

•Deltcheva, Elitza, Krzysztof Chylinski, Cynthia M. Sharma, Karine Gonzales, Yanjie Chao, Zaid A. Pirzada, Maria R. Eckert, Jörg Vogel, and Emmanuelle Charpentier. "Abstract." National Center for Biotechnology Information. U.S. National Library of Medicine, 31 Mar. 2011. Web. 9 Nov. 2014.