Filling in a gap for the DNA of Arhodomonas sp. Strain Seminole

GRP 1
Section 1

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ABSTRACT

Arhodomonas is a bacterium that was found in crude oil contaminated soil. The problem was that when first sequenced, only segment contigs were found. Our group then was given two contigs and we hypothesized that the two pieces will fit together with a gap in between them and the gap could be sequenced by finding a related gene for the contigs. To determine if the contigs go together we used a fused contig sequence in a blast program compared to our two segments and our results were positive for a gap. Then we ran a blast program to determine what the protein was to fill the gap, which we discovered to be 1-aminocyclopropane-1-carboxylate deaminase.

INTRODUCTION

We were working with a bacteria, Arhodomonas, and specifically the strain known as Seminole. This bacteria was found in a salty soil near an oil well site which had been impacted by contamination of crude oil. The bacteria has been found to be able to metabolize some of the important organic compounds within crude oil, as well as be able to live in a salty environment. Because of this, this bacterium is important because it can be put into use to clean up crude oil which would otherwise be an environmental toxin. The gap of knowledge was that the genome sequenced was unable to be pieced together. The focus of our research done was to "fill" one of the many gaps and to connect together two contigs given to us with a fitting gene By filling this gap, two pieces of known contigs in the DNA sequence can be put together in an effort to have the entire sequencing of the Arhodomonas genome. Although our research is an essential part to sequencing the genome, much more research remains to be done.

MATERIALS AND METHODS

We downloaded the contigs for both 520rc and 451rc given to us by Dr. Canaan, along with the Related Protein Sequence. We then used the ncbi.org blast on the contigs to see if there are any matches. We used the Integrated DNA Technology website to find our primers. We copied our fused contigs onto the website and found the forward and reverse primers. Dr. Canaan took these found primers and ordered them for us. After the primers were sent to us, we used the PCR procedure and gel electrophoresis to test the primers. Using a P20 micropipeter, we transferred reagents to a tube along with our primers. 70µL of dH2O, 10µL of 10X Tag Buffer, 10µL of 10X dNTP's, 5 µL of Arh. sp. Seminole gDNA, 2 µL of F-primer, 2 µL of R-primer, and 1 µL of *Taq* polymerase were all placed in one tube. We took the micropipeter and placed the contents in the tube onto the DNA gel electrophoresis by filling in the appropriate well, after mixing the PCR mix with the 2 microliters of tracking dye. Through this process, we should be able to see the DNA sizes from largest to smallest starting from top to bottom. We got our results back and found out that there was a filling in one of the gaps. Through this information, we used the Blast procedure to find our protein that matched our DNA sequence.

RESULTS

Here is our gel electrophoresis results. As can be seen, our group's lane is the first, and the bar selected shows our PCR product (Fig 1).

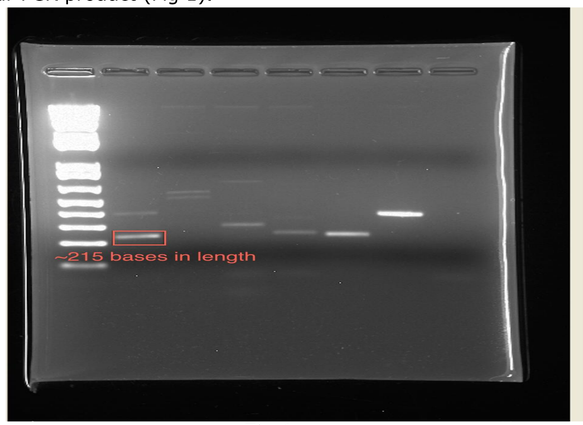

Fig. 1

The PCR was a large portion of our project, and once we confirmed we have a product (Fig. 1). We then were able to use the blast program to decide the protein it coded for, 1-aminocyclopropane 1-carboxylate deaminase (Fig. 2).

Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenPept Graphics

Description	Max score	Total score	Query cover	E value	Ident	Accession
1-aminocyclopropane-1-carboxylate deaminase [Pseudomonas sp. GM55]	375	564	75%	3e-151	80%	WP_008016613.1
1-aminocyclopropane-1-carboxylate deaminase [Pseudomonas sp. GM79]	377	562	75%	8e-151	81%	WP_008074175.1
1-aminocyclopropane-1-carboxylate deaminase [Pseudomonas sp. GM102]	375	561	75%	2e-150	80%	WP_007905793.1
1-aminocyclopropane-1-carboxylate deaminase [Pseudomonas sp. GM18]	369	556	75%	5e-149	79%	WP_007939746.1

Fig. 2

>1F
TGGCCCGGTTTAATCCACCGACAGCCGTCTACGACCTGGGTCCGGCAACATTCTCTGAGTCGCGTCATGGGCGCGAACGTGGAGCTGG
TGGACGAGGGTTTCGACATCGGCATCCGCGAAAGCTGGCGGCGCGCCATCGAGGACGTGAAGGCGCAAGGTGGA

Fig. 3

Once we discovered there was a positive PCR product (fig. 1) which contained the segment to fill in the gap, we sent the product into the OSU core lab to get it sequenced received only the forward sequence data (from 5' to 3') (fig. 3).

DISCUSSION

Our results showed excitingly positive information bridging the gap in the Arhodomonas genomic sequence. We had received a positive result for our blast alignments and sequencing. We were successful in many aspects of our research. For example, we know our primer designs worked due to receiving positive results from electrophoresis. Because of all this we had been able to create a forward sequence, one which binds from 5' to 3' for the full genomic sequence. Also important is that we had showed a potential for a complete bridging of the two fused contigs, although only creating a forward sequence. This was an impressive feat because we had little understanding of the research process and thus we had learned a great deal about PCR and realistic applications of microbiology. We did have some experimental issues such as that we only found a forward sequence and the proposed backward sequence did not work. The backward sequence is the one which binds from the 3' to the 5' prime ends. Because of this, we have only arguably found half of this one gap. The reason for this is unclear and as explained to us by Dr. Canaan, is simply a natural part of Science.

In our strain of Arhodomonas, it is useful for us to learn more because of its impact for cleaning crude oil contamination. This remains an important environmental issue because the contaminants of crude oil are often deadly to wildlife. The organism is also important and useful to us because it is able to live in very salty environments which would normally kill other organisms. The future of this bacteria could possibly be for an industrial use to save companies the money otherwise spent on expensive techniques for cleaning. Once we know more about this bacterium and understand how to manipulate it and the environmental factors of it, this can be used industrially. However, this project is only for the genomic sequence and thus future research on this organism would involve learning more about what it does on a macroscopic scale.

REFERENCES

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