Sequencing of GAPDH Gene in Cilantro and Rosemary

Esad Omanovic, Alex Welsch, Sam Graving, Keith Christiansen, Dr. Alina Avanesyan, Dr. Idit Hazan
Grand View University, Des Moines, Iowa

Abstract

GAPDH is an enzyme integral to cellular breakdown of glucose during glycolysis (Fig.1.). Because all cells perform glycolysis for energy, the gene for encoding GAPDH is highly conserved. This study wanted to see if there were in fact any differences in the GAPDH gene between species. PCR was performed on cilantro and rosemary genomes to amplify the GAPDH gene. Genetic sequencing was performed, and the two genes compared to one another.

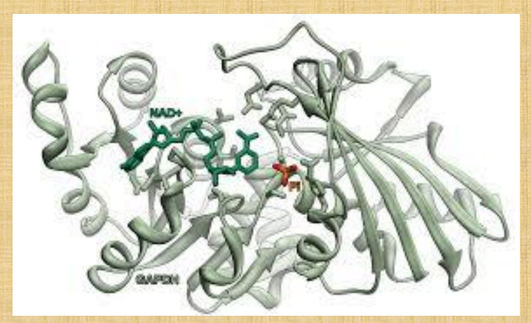

Fig. 1: GAPDH structure.

Introduction

Glycolysis is a multi-step biological pathway that a cell uses to break down glucose molecules to produce energy. The enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is an enzyme involved in the third step of glycolysis, where it is responsible for turning glyceraldehyde-3-phosphate into two 1,3-bisphosphoglycerate molecules. In this study, we wanted to see if there was any differences in the genetic sequence that encodes GADPH between two different plants, cilantro (Fig.2.) and rosemary (Fig.3.). To do this, we purified, isolated, and amplified the GADPH genes of each plant using affinity chromatography and polymerase-chain-reaction (PCR). The DNA was sequenced by a third-party lab, and then compared using Basic Local Alignment Search Tool (BLAST). Because the GAPDH gene is conserved across all living species, we were interested in seeing if there were any differences between species' sequences for this gene, and if differences were present, whether they were large or small.

Fig. 2: Cilantro. As observed in nature.

Fig. 3: Rosemary as observed in nature.

Materials and Methods

To begin this procedure, fresh cilantro and rosemary leaves were picked and ground up using a pestle and mortar (Fig.4.). This was to break the waxy cell walls of the plant cells. Lysis buffer was added to the cells to break open the cell membranes. Ethanol was added to bind the DNA in the cell lysis. The DNA was purified using a DNA purification spin column and centrifugation.

PCR was performed to amplify the GAPDH gene. The PCR product was then nested, and ran again to remove the non-essential DNA sequences.

Materials and Methods

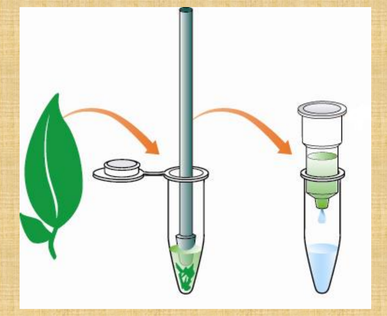

Fig. 4: Mortar and pestle technique used to ground the leaves.

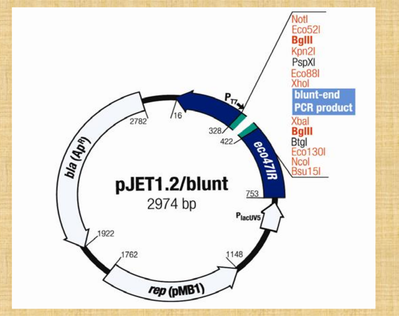

Fig. 5: Vector used to insert DNA into the plasmid.

To store the PCR product, the purified DNA sequences were inserted into a vector plasmid (Fig.5), and transformed into *E. coli* through heat shocking.

To test for the identity of the gene that was taken up by the bacteria, the bacteria were grown in culture overnight, and then lysed using a lysis buffer. The DNA was purified using the same procedure as before. The DNA was then digested using restriction enzymes, and the digestion product was run through gel electrophoresis (Fig.6). Upon identifying the corresponding banding to GAPDH, the DNA was sent to Eurofins Genomics (Eurofins MWG Operon LLC, Louisville, KY) for sequencing.

The sequences were then each put into NCBI BLAST, and compared to one another (Fig.7).

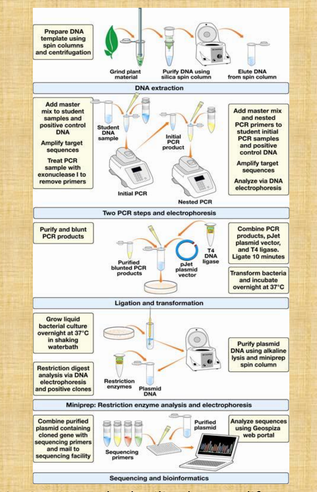

Fig. 6: PCR method utilized to amplify gene of nucleotide sequence of interest

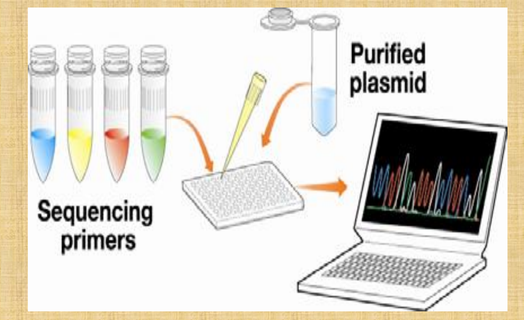

Fig. 7: Analysis of nucleotides obtained from PCR

Results

Three different contig sequences were obtained and blast was ran. We ended up excising the exons out of each contiq and a blast was ran to find the specific gene. For our rosemary 1_3 contiq we had 624 exon bases. For the rosemary 2 contiq, we had 638 exon bases. For our cilantro 2 contiq we had 127 exon bases. These samples were then ran using NCBI blastx program to analyze the specific gene of interest. Cilantro DNA was analyzed using Blast technique and the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene was found to have a coverage of 25.2% in *Cynara cardunculus var. scolymus* species (Fig.8). This was read in a forward direction. The rosemary GAPDH gene had much better coverage in other species. One of the samples provided 94.7% coverage in the reverse relative orientation. 591 bases were aligned between the *Tecoma stans* species (Fig.9) with the one sample of rosemary. The other rosemary sample had a 90.3% coverage when compared with the *Cornus florida* (Fig.10)and was read in a forward direction. It had a coverage of 576 aligned bases between the two species.

Results

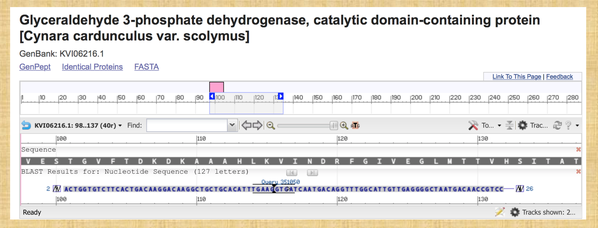

Fig. 8: *Cynara cardunculus var. scolymus* gene analysis of the GAPDH gene. Image on the right is how the plant looks in its natural environment

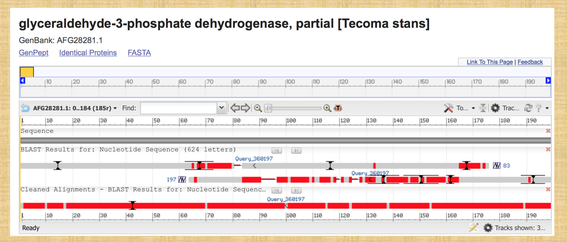

Fig. 9: *Tecoma stans* gene analysis of the GAPDH gene utilizing NCBI blast. Image on the right is the plant in its natural environment

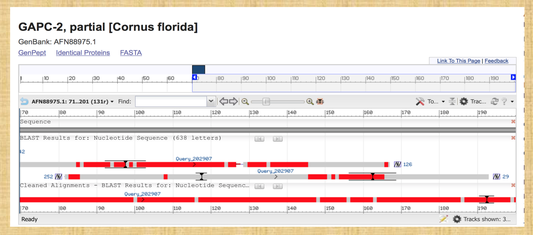

Fig. 10: *Cornus florida* gene analysis of the GAPC gene utilizing NCBI Blast. Pictured is *Cornus florida* in its natural environment

Conclusion

All the sequences obtained were unique in a manner that the GAPDH gene has not been sequenced previously for either cilantro or rosemary. Due to the sequencing being better for the rosemary in comparison to the cilantro, we will submit these sequences to GenBank for access to other scientists to conduct further research if necessary.

Discussion

The Cilantro DNA did have a coverage of 25.2% relative to *Cynara cardunculus var. scolymus* species. This is low when we compare the coverage of one of the Rosemary GAPDH sequence which had a coverage of 90.3% and the other Rosemary GAPDH gene which had a coverage of 94.7% relative to its species of comparison. These drastically different coverage values may be the result of the fact that we used more samples of Rosemary DNA than Cilantro DNA. The greater coverage of the Rosemary plant reflects the disproportionate use of Rosemary over the Cilantro plant. Regardless of the coverage all three samples, the two Rosemary samples and the single Cilantro sample did seem to have at least part of the GAPDH gene in the exons we ran through the BLAST software. The Rosemary samples may provide us with a more accurate understanding of the structure of the GAPDH gene since we had samples that went in both the forward and reverse directions. The GAPDH gene fragment identified in the Cilantro sample was only oriented in the forward direction. This lack of a reverse orientation for Cilantro also lowers the accuracy of the GAPDH gene for that plant.

References:

Tom Hall. (1997-2017). BioEdit [Computer software]. Retrieved from http://www.mbio.ncsu.edu/BioEdit/bioedit.html
Bio-Rad. (2008). Biotechnology Explorer Cloning and Sequencing Explorer Series Curriculum Manual. Hercules, CA: Bio-Rad Laboratories Inc.

Internet sources, image credits:

http://www.hhmi.org/biointeractive/search?text=sequence&sort_by=search_api_relevance&redirect=1 ; http://www.bio-rad.com/en-us/category/cloning-sequencing-explorer-series ;
National Center for Biotechnology Information (NCBI)[Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988]. Available from: https://www.ncbi.nlm.nih.gov/ ;
Image retrieved from: http://www.henryhartley.com/?p=10203 ; Image retrieved from: https://commons.wikimedia.org/wiki/File:Tecoma_stans_%27Gold_Star%27.jpg
Image retrieved from: http://www.alamy.com/stock-photo-seen-here-in-flower-the-globe-artichoke-cynara-cardunculus-var-scolymus-94996103.html