Comparative genomic analysis of GAPDH from Mint and Thyme

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MINT, THYME, and GAPDH

GAPDH is an enzyme responsible for the sixth step in glycolysis, consuming energy to phosphorylate glyceraldehyde-3-phosphate. GADPH is found in all plants making it easy to assume that this enzyme plays an important role in plant evolution.

The goal to researching this enzyme is to be able to comparatively analyze its evolutionary significance. By amplifying this specific gene in *Mentha spicata L* (Mint) and *Thymus vulgaris L* (Thyme), the similarities or differences between nucleotide sequencing will hopefully provide the predicted evidence supporting the evolutionary significance of GAPDH. These nucleotide sequences will then be compared with other known sequences for further support.

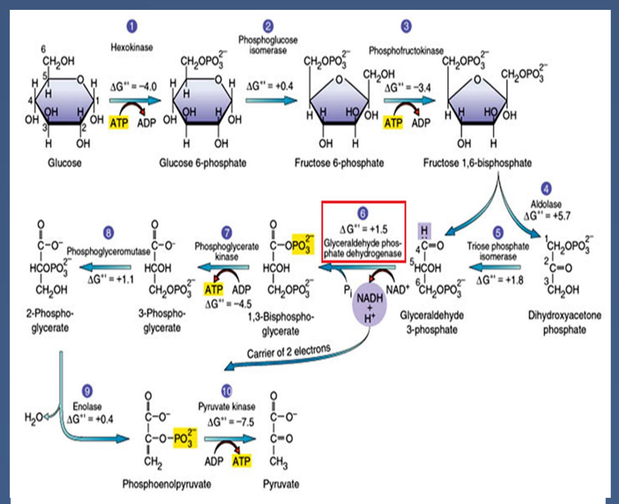

Figure 1: The role of GAPDH in the Glycolysis Cycle.

Mentha spicata L, or spearmint, part of the Lamiaceae family, is a dicot plant and is native to Europe and Asia, yet has a wide range of habitats world-wide. *Thymus vulgaris L*, also part of the Lamiaceae family, is native to the rocky regions of the Mediterranean, but also inhabits much of the dry, temperate regions of the world (Daniel, 2006). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is the enzyme that adds a phosphate group to the 3 carbon molecule in the sixth step of glycolysis (Fig. 1.). GAPDH sequences from mustard and from tobacco show that the photosynthetic enzyme is closer in similarities to bacteria than to the glycotic enzyme within the same nucleus . These differences found enhance the probability of GAPDH having evolved differently in different plant populations.

OBJECTIVE

Our objective was to develop skills in biotechnology and biochemistry while working with comparative genomics with Mint and Thyme. We focused on the GAPDH gene and its amplification, looking for either the similarities or differences between the band sizes of the DNA isolated from the two plants.

Materials and Methods

Plants were purchased from local stores. Total DNA was Isolated. GAPDH gene was amplified with degerate primers and then with nested primers. The PCR product was then cleaned and cloned into pJet1.2 vector for sequencing. See Figure 2 for all steps.

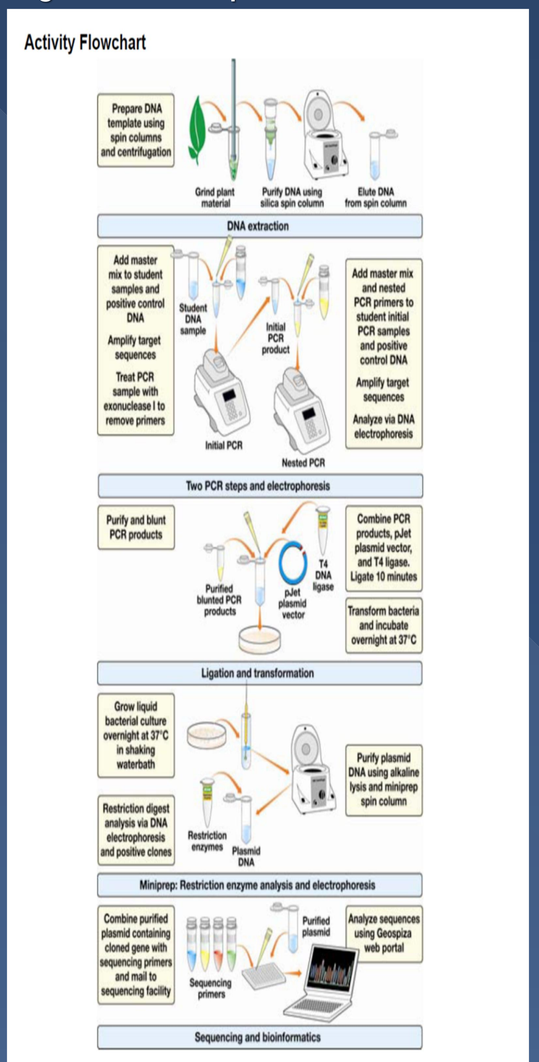

Figure 2: Flow chart of the procedure followed this basic guide for isolating the genomic DNA all the way through restriction digestion as designated in the figure.

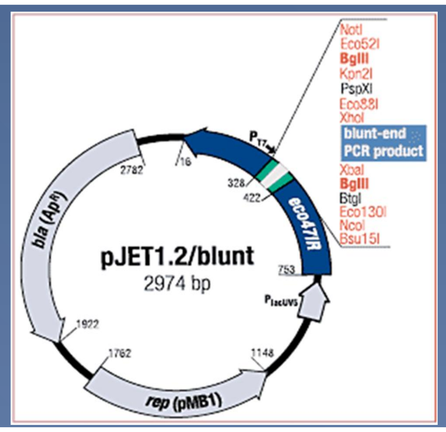

Figure 3: The blunted PCR product was inserted into pJET1.2, a cloning vector which contains a Bgl II restriction enzyme recognition site on both sides of where the blunted PCR product was inserted.

RESULTS and DISCUSSION

We isolated genomic, specifically chloroplastic, DNA from Mint and Thyme by lysing the cells and using the spin fracture technique to spin down the remaining cell debris. Leaving the cell debris alone, we used the supernatant to run an initial PCR containing degenerate primers to amplify the gene coding for the enzyme, GAPDH. We then ran a 1% agarose gel which showed the success of DNA isolation and the success of the primers attaching to target DNA. (Fig. 4). This gel not only showed the positive results, but that Thyme and Mint, have similar band sizes, yet are both smaller than the Arabidopsis PCR DNA.

After the first PCR, to get rid of all the primers that didn't attach to target DNA , we used the enzyme exonuclease I, which catalyzes the hydrolysis of single stranded DNA. This enzyme was inactivated by heat before proceeding to the second PCR so it didn't effect the nested PCR primers. After inactivating exonuclease I, we performed a second PCR with nested primers to ensure the target DNA was the only thing amplified. The next step was to purify the PCR product using a BIO RAD DNA purification kit to remove all the extra, unwanted primers, buffer solutions, and enzymes. After we purified the DNA we checked the DNA content with 1% agarose gel electrophoresis (Fig. 5). Then we did blunting and ligation into the cloning vector, pJET1.2 (Fig. 3.). We then transformed the chimeric vector into E. coli cells by heat shock. After the cells had been transformed we grew them on LB plates containing ampicillin to select for the bacteria that contained our recombinant DNA. We took three of the transformed colonies from each plant and inoculated overnight cultures to preform a plasmid purification. We then did restriction digestion with the Bgl II enzyme overnight and tested the final DNA content with 1% agarose gel electrophoresis (Fig. 6.).

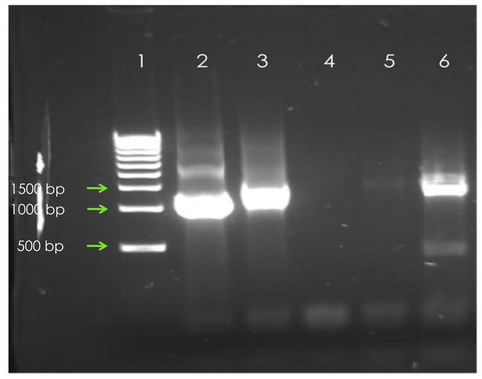

Figure 4: Initial PCR with degenerate primers.Lane 1: 500 bp marker DNA. Lane 2: pGap control for PCR. Lane 3: Arabidopsis gDNA. Lane 4: Sterile Water. Lane 5: Mint gDNA. Lane 6: Thyme gDNA.

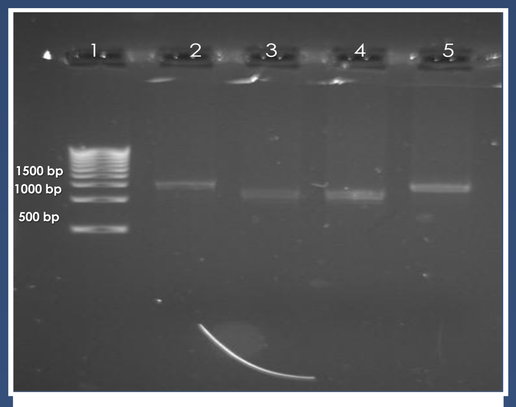

Figure 5: Purified PCR product from Nested PCR.
Lane 1: 500 bp Marker DNA. Lane 2: Mint DNA 1µL. Lane 3: Thyme DNA 1 µL. Lane 4: Thyme DNA 2 µL. Lane 5: Mint DNA 1µL.

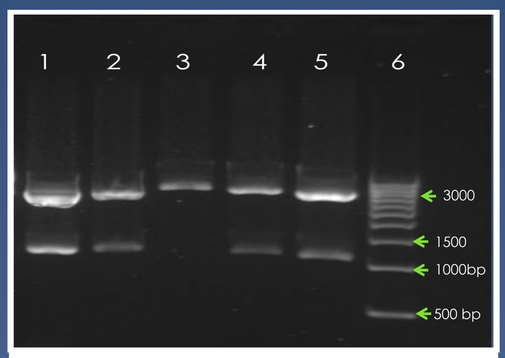

Figure 6: Restriction Digestion with Bgl II Lane 1: Mint colony 1. Lane 2: Mint colony 2. Lane 3: Mint colony 3. Lane 4: Thyme colony 1. Lane 5: Thyme colony 2. Lane 6: 500 bp Marker DNA.

CONCLUSION

We took the target section of the GAPDH gene and amplified it for closer examination. Results seem to indicate evolutionary similarities between Mint and Thyme based on the similar band sizes. This conclusion was a predicted one because of their shared family. To further our research we are sequencing the gene to get an even better idea of how similar these two plants' evolutions are.

REFERENCES

1. Daniel, M. (January 2006). Medicinal Plants: Chemistry and Properties. Science Publishers.
2. Cerff, W. (1987) Endosymbiotic Origin and Codon Bias of the Nuclear Gene for Chloroplast GAPDH from Maize. J Mol Evol 26:320-328.

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