mRNA Knockdown of Acyl-CoA Dehydrogenase in *Chlamydomonas reinhardtii*

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Abstract:

Chlamydomonas reinhardtii is a species of algae that is of interest due to its ability to efficiently produce triacyl glycerides (TAGs) from fatty acids. Triacyl glycerides are then able to be broken down into glycerol and biodiesel fuel. However, there is an alternative pathway in which fatty acids can be broken down into. The β-oxidation pathway breaks down fatty acids into acetyl CoA which is incorporated into the Electron Transport Chain. We are looking to manipulate this β-oxidation pathway in order to maximize the amount of TAGs synthesized from fatty acids. The hope is to find a simple and efficient way to produce large amounts of biodiesel fuel from *C. reinhardtii*. Using RT-qPCR, a standard curve was generated showing the mRNA levels of each of the genes of interest involved in the β-oxidation pathway. Future goals of the research project involve using miRNA to knockdown each of the genes involved in the β-oxidation pathway. RT-qPCR will then be used to measure the success of each of the miRNA knockdowns of each target gene. Following successful knockdown of the target genes, thin layer chromatography (TLC) will be used to analyze the amount of triacyl glycerides present in each cell. These accumulated triacyl glycerides could then be extracted and used for biodiesel fuel synthesis. A transgenic strain of this *C. reinhardtii* could then be used as a "green" alternative for biodiesel fuel production.

Background:

Chlamydomonas reinhardtii utilizes the β-oxidation pathway to degrade fatty acids. This occurs through a series of enzyme-catalyzed reactions in which two carbons are removed in each step of the pathway. The result of the pathway is the release of free energy (Voet 2008).

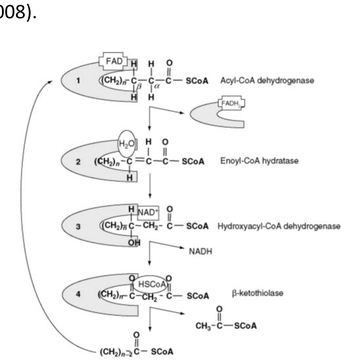

Figure 1. β-oxidation pathway of fatty acids including enzymes involved. (Kumari 2018)

MicroRNAs (miRNA) can be used to cause the degradation of protein of interest, by designing an miRNA that is complementary to the target sequence (Molnar 2009). miRNA can be used in this experiment to knock down different proteins involved in the β-oxidation pathway.

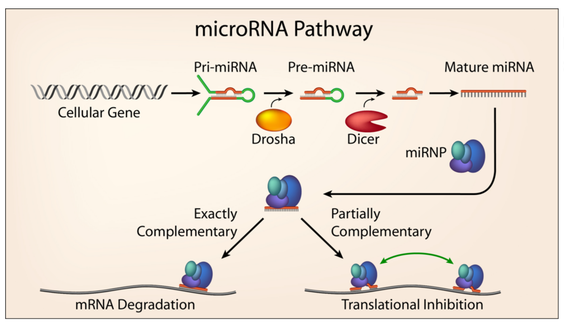

Figure 2. MicroRNA pathway leading to mRNA degradation. (Sharp)

qRT-PCR is used to determine specific mRNA levels in a sample. mRNA is then made into cDNA using reverse transcription. Primers can then bind to specific regions of the cDNA to amplify certain sequences. SYBR Green binds to cDNA. The amount of cDNA for a specific sequence can be determined by the number of cycles needed to reach the calculated threshold concentration based on the standard curve.

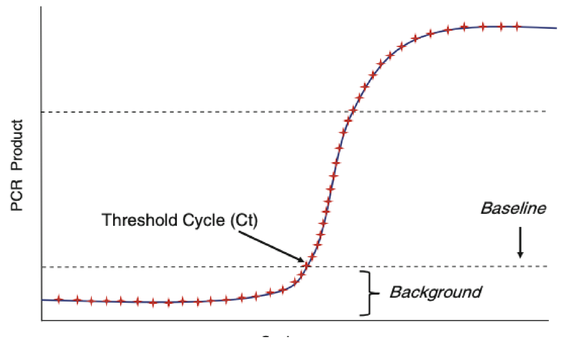

Figure 3. qRT-PCR quantification of mRNA levels (Isidoro-Garcia 2016).

Materials and Methods:

RNA Isolation

RNA from the 125 stressed and 1010 stressed samples of *Chlamydomonas reinhardtii* was isolated using the Direct-zol RNA MiniPrep Plus kit and its concentration was determined using a Qubit Fluorometer (Ferguson 2019).

Reverse Transcription and Endpoint PCR

Reverse transcription of mRNA was performed using the SuperScript IV First-Strand cDNA Synthesis Kit. Primers that were specific for the Acyl-CoA Dehydrogenase cDNA were used in an endpoint PCR reaction. This endpoint PCR amplified the Acyl-CoA Dehydrogenase cDNA from the samples. Gel electrophoresis of the endpoint PCR amplification products was performed to determine the purity and concentration of the Acyl-CoA Dehydrogenase cDNA (Ferguson 2019).

Endpoint PCR product purification and qPCR

The product from the endpoint PCR was cleaned and concentrated and the cDNA concentration was quantified using a Qubit Fluorometer. Serial dilutions of the cDNA were made and the Acyl-CoA Dehydrogenase primers were mixed with the solutions. qPCR was run and a standard curve was generated (Ferguson 2019).

Dilution	100%	1:10	1:100	1:1,000	1:10,000	1:100,000	1:1,000,000	1:10,000,000
Purified PCR	6 µL	5 µL of 100%	5 µL of 1:10	5 µL of 1:100	5 µL of 1:1,000	5 µL of 1:10,000	5 µL of 1:100,000	5 µL of 1:1,000,000
Water	0 µL	45 µL	45 µL	45 µL	45 µL	45 µL	45 µL	45 µL
Tube #	1	2	3	4	5	6	7	8

Figure 4. Serial dilutions for standard curve generation (Ferguson 2019).

Results and Discussion:

After running endpoint PCR to amplify the Acyl-CoA Dehydrogenase cDNA, the 1:10 dilution was not amplified. The 1:1 dilution did amplify, however the band was very faint, indicating a poor amplification.

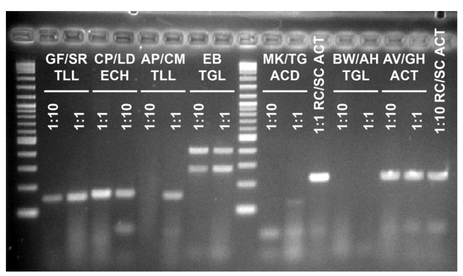

Figure 5. Agarose gel electrophoresis results. Lanes 1 and 10 are hyper ladder. Lanes 11 and 12 are from the Acyl-CoA Dehydrogenase amplification (Ferguson 2019).

After qRT-PCR amplification of the serial dilutions for Acyl-CoA Dehydrogenase controls, a standard curve was generated which excludes on outlier serial dilution. This produced a much better standard curve with an R^2 value of 0.999, almost perfect (Ferguson 2019).

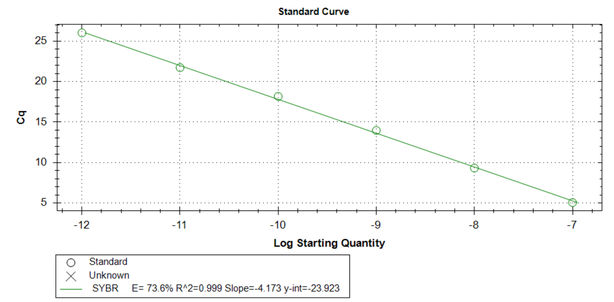

Figure 6. Standard curve of the Acyl-CoA Dehydrogenase serial dilutions qRT-PCR.

A melt curve was generated to indicate the level of non-specific binding. The multiple peaks indicate some degree of non-specific primer binding, although this level of non-specific binding is acceptable (Ferguson 2019).

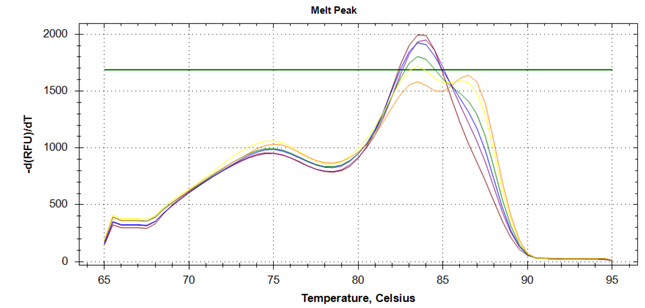

Figure 7. Melting curve of the Acyl-CoA Dehydrogenase serial dilutions qRT-PCR.

In the future, this standard curve can be used determine the success of miRNA knockdown of the Acyl-CoA Dehydrogenase gene in the β-oxidation pathway as well as the other genes encoding necessary enzymes.
Thin layer chromatography can then be used to measure the triacyl glyceride levels in each sample.

References:

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