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A Study of Methods for Detecting Gene Expression of the Sterol Biosynthetic Pathway in the Microalgae Strain Heterosigma akashiwo

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Purpose

Biotechnological applications for various microalgae strains are beginning to bloom. There is interest in these microbes for their production of useful compounds such as phytosterols and lipids which can be produced in mass culture for large scale manufacture in the food, pharmaceutical, and biofuel industries (Romano *et al*., 2016). To this end, we are using the gold-brown microalgae, *Heterosigma akashiwo* (CCMP1680), a known producer of phytosterols, to test a molecular method for quick and easy measurements of production of the phytosterol compound sitosterol, which has potential as an anti-inflammatory agent. (Giner *et al*., 2008).

Figure 1.

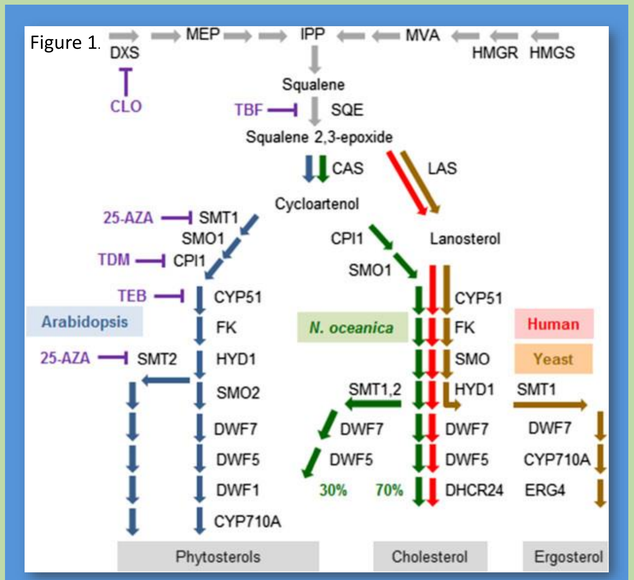
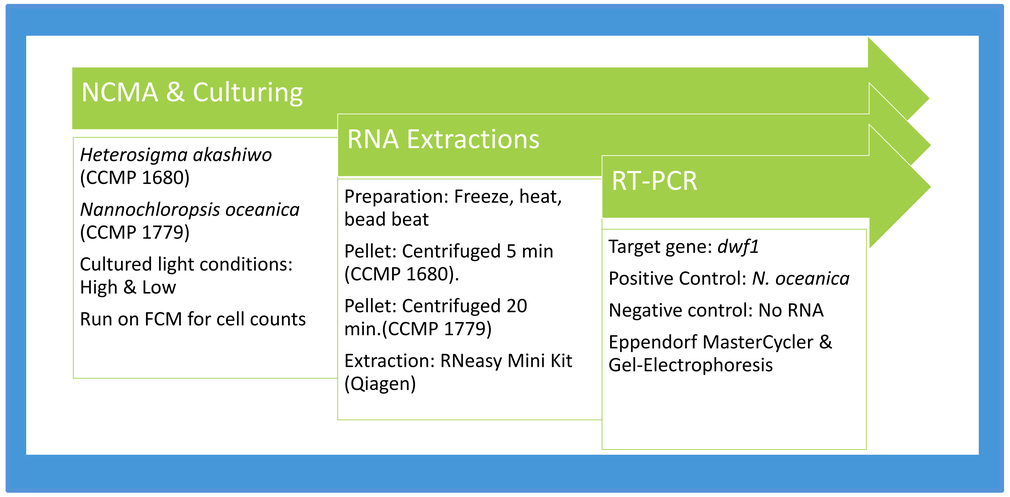


Figure 1. The DWF1 gene encodes the enzyme for the last step in the biosynthetic pathway of isofucosterol to sitosterol, and is our focus for determining the growth conditions for optimal sterol producing capacity of *H. akashiwo* (Lu et al. 2014).

In previous studies, gas chromatography/mass spectrometry (GC/MS) has been the standard method to quantify microalgal lipid and sterol content (Ahmed *et al.,* 2015); however, it is quite expensive. The primary goal of this initial project was to ascertain the practicality of the reverse-transcriptase polymerase chain reaction (RT-PCR) method and gel-electrophoresis for establishing *dwf1* gene expression levels, and therefore sitosterol levels.

Framework



Results: Growth Rates

Figure 2.

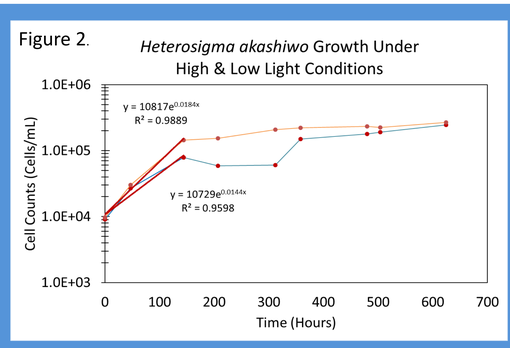


Figure 2 & 3.
Growth curve data was completed for both *H. akashiwo* and positive control *Nannochloropsis oceanica* (CCMP 1779) using Flow Cytometry. The condition of light was included as a possible variable to alter the growth rate over time. Growth rates increased slightly under high light conditions for *N.oceanica* and decreased for *H. akashiwo.*

Figure 3.

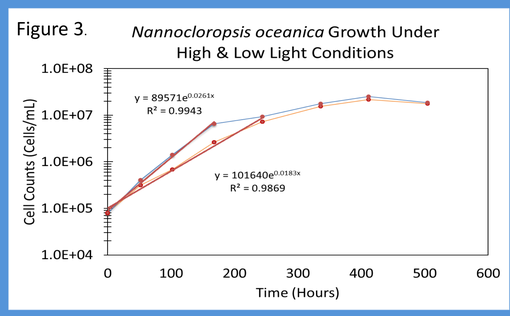


Figure 4.

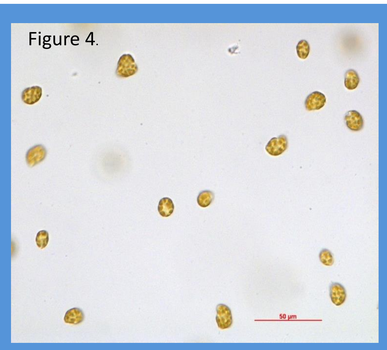
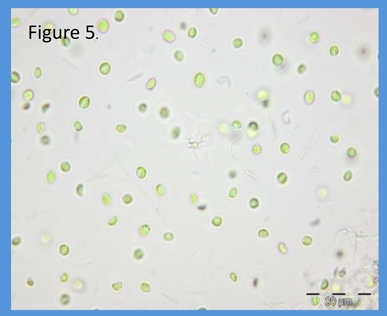


Figure 4 & 5.
Above is an image of *H.akashiwo* (Scale Bar=50 µm).
Below is an image of *N.oceanica* (Scale Bar=30 µm)

Figure 5.



Results: Molecular Assessment

Appropriate RNA extraction methods (Lu *et al.,* 2010), primers for detection of expression of the *dwf1* gene (Lu *et al.,* 2014), and PCR methods (One-Step RT-PCR) were identified and carried out for both phytoplankton strains. However, no *dwf1* band was observed (results not shown). So, we then checked whether or not the *dwf1* gene would show up in the genome of *H.akashiwo*. Figure 6 shows PCR results from gel electrophoresis with bands for both positive controls (*N. oceanica* amplified with *dwf1* and 18S primers). There was no amplification for *H.akashiwo* with the *dwf1* primers, however there was for the 18S primers.

Figure 6.

Discussion & Future Work

We developed the initial steps towards devising a more cost efficient method for gene expression studies of dwf1 gene encoding sitosterol. A protocol has been laid out for the RNA extraction and RT-PCR steps. However, either the *dwf1* gene is not present in *H.akawisho* or the *dwf1* primers used were not appropriate for *H.akawisho*. Thus, in order to move this project forward, more work will need to be done to identify primers that work.

References

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