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Jessica C. Mayhew  
*University of Southern Maine*

Marcia Ackerman  
*University of Southern Maine*

Mike Lomas  
*Bigelow Laboratory for Ocean Sciences*

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

Jessica C. Mayhew\textsuperscript{1} | Marcia Ackerman\textsuperscript{1} | Mike Lomas\textsuperscript{2} | Lisa Moore\textsuperscript{3} |
\textsuperscript{1}University of Southern Maine, \textsuperscript{2}Bigelow Laboratory for Ocean Sciences

Purpose

Biotechnological applications for various microalgal 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 microalga, Heterosigma akashiwo (CCMP1680), a known producer of phytosterols, to test a molecular method for quick and easy measurements of the production of the phytosterol compound sitosterol, which has potential as an anti-inflammatory agent. (Giner et al., 2008).

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 dwf\textsuperscript{1} gene expression levels, and therefore sitosterol levels.

Results: Growth Rates

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. akawisho.

Results: Molecular Assessment

Appropriate RNA extraction methods (Lu et al., 2010), primers for detection of expression of the dwf\textsuperscript{1} gene (Lu et al., 2014), and PCR methods (One-Step RT-PCR) were identified and carried out for both phytoplankton strains. However, no dwf\textsuperscript{1} band was observed (results not shown). So, we then checked whether or not the dwf\textsuperscript{1} 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 dwf\textsuperscript{1} and 18S primers). There was no amplification for H.akashiwo with the dwf\textsuperscript{1} primers, however there was for the 18S primers.

Discussion & Future Work

We developed the initial steps towards devising a more cost efficient method for gene expression studies of dwf\textsuperscript{1} gene encoding sitosterol. A protocol has been laid out for the RNA extraction and RT-PCR steps. However, either the dwf\textsuperscript{1} gene is not present in H.akawisho or the dwf\textsuperscript{1} 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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