Journal of the Arkansas Academy of Science

Volume 77 Article 4

2023

Determination of Fatty Acid Concentrations in Various Strains of Freshwater Green Picoplankton Algae (Class Eustigmatophyceae)

P. Ashcraft
University of Arkansas at Monticello, Monticello, AR

R. Jacks
University of Arkansas at Monticello, Monticello, AR

B. Martinez
University of Arkansas at Monticello, Monticello, AR

J. Rodriguez
University of Arkansas at Monticello, Monticello, AR

A.B. Burns
University of Arkansas at Monticello, Monticello, AR

See next page for additional authors

Recommended Citation

Ashcraft, P.; Jacks, R.; Martinez, B.; Rodriguez, J.; Burns, A.B.; Gurnsey, A.; Taylor, L.; Teague, A.; Totty, J.; Fox, V.L.; and Williams, A. (2023) "Determination of Fatty Acid Concentrations in Various Strains of Freshwater Green Picoplankton Algae (Class Eustigmatophyceae)," *Journal of the Arkansas Academy of Science*: Vol. 77, Article 4.
https://doi.org/10.54119/jaas.2023.77103
Available at: https://scholarworks.uark.edu/jaas/vol77/iss1/4

Determination of Fatty Acid Concentrations in Various Strains of Freshwater Green Picoplankton Algae (Class Eustigmatophyceae)

Authors

P. Ashcraft, R. Jacks, B. Martinez, J. Rodriguez, A.B. Burns, A. Gurnsey, L. Taylor, A. Teague, J. Totty, V.L. Fox, and A. Williams

Determination of Fatty Acid Concentrations in Various Strains of Freshwater Green Picoplankton Algae (Class Eustigmatophyceae)

P. Ashcraft, R. Jacks, B. Martinez, J. Rodriguez, A.B. Burns, A. Gurnsey, L. Taylor, A. Teague, J. Totty, V.L. Fox, A. Williams*

Department of Mathematical and Natural Sciences, University of Arkansas at Monticello, Monticello, AR 71655

*Correspondence: williamsa@uamont.edu

Running title: Determine of Fatty Acid Concentrations in Picoplankton Algae

Abstract

Algae are of scientific and commercial interest due to their ease of culture and high fatty acid content. The extracted fatty acids from these phytoplankton may potentially be used as an additional test for phylogenetic classification of new algal strains (Tonon *et al.* 2002), as well as in a supplement for human consumption and producing next-generation biofuels. Of interest is the fatty acid content contained within various algal isolates within the class Eustigmatophyceae. Algal strains were collected and isolated from locations in Lake Chicot in Arkansas, Tower Pond and Lake Itasca at Itasca State Park in Minnesota, and Thayer Lake in the upper peninsula of Michigan (Fawley *et al*. 2007, Prior *et al*. 2009). The strains collected were then subjected to a 5-step process for lipid preparation: lypholization, lipid extraction, filtration, esterification, and methyl ester extraction. The fatty acid methyl ester extracts were analyzed using GC-MS. After qualitative determination of fatty acids by mass spectrometry, relative quantities of the fatty acids were determined by peak integration, and tricosanoic acid (C23:0) was used as a standard to determine absolute quantities. Preliminary results show differences between algal strains of Eustigmatophyceae via relative fatty acid concentration.

Introduction

Fatty acids have been of interest to scientists for many years. Of particular interest for such applications are the algae in the class Eustigmatophycaea. Eustigmatophyceae itself is a class of algae that has a greenish color because while it contains chlorophyll a, it lacks chlorophyll c and fucoxanthin. The class Eustigmatophycea strains isolated in this investigation contain a brownish lipid body called a pyrenoid that produces and stores high concentrations of fatty acids. These lipid bodies have been observed without any additional preservation. When strains of Eustigmatophyaceae contain these characteristic lipid bodies, fatty acid composition is a more efficient, precise and useful tool for determining strains isolated from field algal samples.

Investigations are being pursued to determine whether mass cultures of these algae isolates are efficient in producing high amounts of fatty acids needed for various applications. Fatty acids have been used to compare and identify different algal cultures (Lang *et al*. 2011). Biochemical data from Eustigmatophyceae isolate profiles may be used to compare new isolates to those previously studied, as fatty acids produced may be specific to each strain regarding the amount and type. As the fatty acids are extracted from new isolates the data collected may be used to help place each strain in its respective phylogeny. Fatty acid profiles could thus be used as an additional tool for algal classification within Eustigmatophyceae. Based on the strain and the quantity of fatty acids produced, fatty acid content could also determine applicability of the strain (Fahl *et al*. 1993, Mourente *et al*. 1990, Volkman *et al*. 1989, Zhang *et al*. 2003, Zittelli *et al*. 1999). Strains that produce large amounts of fatty acids of interest may be mass cultured and undergo mass fatty acid extraction. Because algae are easily cultured, this type of sustainable energy could be used to make many scientific advancements. Using nutrients for human consumption, such as omega-3 fatty acids in primary health care, sustainable oxygen sources in space, and uses in sustainable energy, such as biofuel, are applications for fatty acids from algae.

In order to determine fatty acid contents within these algae strains, faculty and students at the University of Arkansas at Monticello have collected and cultured Eustigmatophyceae strains (Fawley *et al*.

2007, Prior *et al*. 2009). The various algal strains studied were isolated cultures determined through DNA analysis to be genetically associated with one another. Additional biochemical testing is needed to determine differences because between strains, as there is a high diversity of fatty acids (Hibberd *et al*. 1971, Prior *et al*. 2009). In this study, some strains of Eustigmatophyceae were analyzed to determine variation in fatty acid expression between different strains based on their relative concentrations of fatty acids.

Methods and Materials

Fatty acid content and concentration were analyzed for algae samples of interest. All algae strains used were previously collected and cultured by faculty at the University of Arkansas at Monticello (Fawley *et al*. 2007, Prior *et al*. 2009). Once samples were collected, methods from several different sources were used, which accounts for the overlap in standard extraction protocols (Bigogno *et al*. 2002, Caramujo *et al*. 2008, Krientz *et al*. 2006).

The samples tested were named based on collection location, date, and when plated, which well and in which they were located. The naming system is based on the location and environment in which the algae were found. The strains of Eustigmatophyceae analyzed were Bog D 8/9, Tow 8/18, and Tow 8/18 T-8d. As such two were collected from Tower Lake, both on 8/18, with the BogD sample collected in the Czech Republic (Fawley *et al*. 2007, Prior *et al*. 2009).

Algal isolates were then collected from the Fawley lab and centrifuged in a 50.0 ml tube. After decanting excess cultured media, 5.0 ml of culture were retained for each sample. The concentrated algae samples were centrifuged again at 13000 rpm for 10 min and excess media was decanted leaving an algal pellet. These algal pellets were placed in a freezer for at least 24 hours. The samples were then placed in a Vacufuge Plus (with vacuum attached) for 2 hours. The samples were mixed with 5.0 ml of a chloroform/methanol (2:1 v/v) solution, then placed in an ultrasonic device for 90 minutes for lipid extraction. Once the sample was broken down, it was filtered and washed with an additional 5.0 ml of the chloroform/methanol solution. It was then allowed to react via Fisher Esterification by adding 5.0 ml of methanol/sulfuric acid (95:5 v/v) and heating in a hot water bath at 80℃ for 4 hours. Fatty acid methyl esters (FAME) were extracted with hexane after esterification. A 1.0 ml sample of extracted hexane solution was transferred to a stoppered vial and analyzed by GC-MS.

The fatty acid methyl esters were analyzed by a Varian 450-GC, using a Varian FactorFour VF-5ms Capillary Column (30m x 0.25 mm ID, 0.25 µM film thickness), with a 20:1 split. The injector was held at 240 ℃, while the oven was started at 50 ℃, held for 3 min, ramped to 300 ℃ at 10 C/min and held at 300 ℃ for 5 min. The transfer line to the MS was held at 250 ℃. The MS used was a Varian 320-MS, a triple quadrupole mass spectrometer with EI source, with the source at 200 ℃. The samples were scanned from a m/z of 35-600. Samples were injected using a CTC Analytics CombiPal autosampler, injecting 1 µL of sample at a time.

GC-MS results were then placed into an Excel spreadsheet and the results were converted from counts to relative mass percentages using the molar mass of each fatty acid.

GC-MS results were analyzed using the Related-Samples Friedman's Two-way Analysis of Variance by Rank using the SAS software suite. This test was used to compare all the relative percentages of fatty acids from new and old observations in each individual algae strain. Post Hoc (Pairwise Comparison) tests were used to determine the significant differences between pairs of fatty acids in each strain. A Bonferroni adjustment was included in the Post Hoc test to avoid type 1 error. Graphs showing the node of the pairwise comparison table were created to show this relationship. Specifically, these pairwise comparisons are showing how close each of the relative masses is to one another.

Results

The relative percentages of 6 selected fatty acids for Bog D 8/9, Tow 8/18, and Tow 8/18 T-8d are shown in Figures 1-3.

The pairwise comparisons are shown in Figures 4-5. In the case of Figure 4, the lines shown between C18:1 and C16:1 is much thicker than between C16:1 and C18:2. The thicker line is showing that these concentrations are different to a significant degree.

During the analysis of three different strains, the profiles of two strains were found to be significantly different in their fatty acid distributions. After completing the Related-Samples Friedman's Two-way Analysis of Variance by Rank, two strains showed a statistically significant difference between fatty acids. Tow 8/18 T-8d showed significant differences in the distributions of C14:0, C16:1, C16:0, C18:2, C18:1, C18:0, C20:4, C20:5, and C22:0, ($p = 0.000$), as shown

https://scholarworks.uark.edu/jaas/vol77/iss1/4
DOI: 10.54119/jaas.2023.77103

in Figure 4. Bog D 8/9 also showed significant differences in the distributions of C:14:0, C16:1,

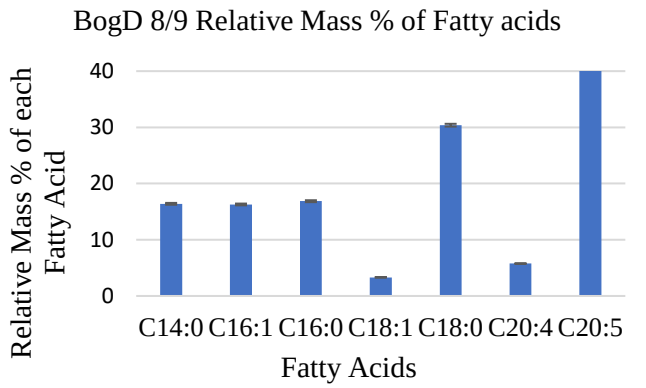

Figure 1. Relative mass percentages of fatty acids in BogD 8/9 sample. Error bars show 1 standard deviation from the mean.

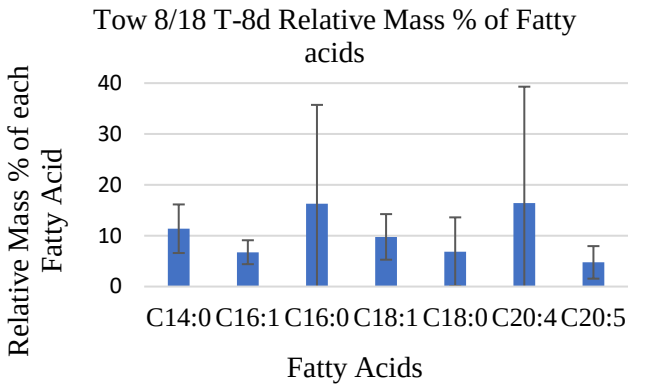

Figure 2. Relative percentages of fatty acids in Tow 8/18T-8d sample. Error bars show 1 standard deviation from the mean.

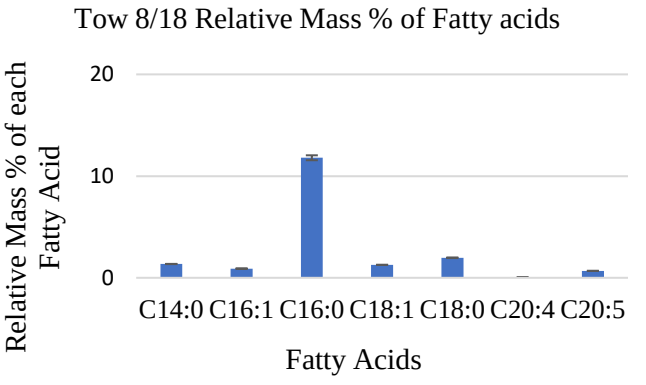

Figure 3. Relative percentages of fatty acids in Tow 8/18 sample. Error bars show 1 standard deviation from the mean.

C16:0, C18:2, C18:1, and C20:4, (p = 0.007, as shown in Figure 5.

To further analyze these differences in each strain, each fatty acid was compared to another using a Post Hoc test. In Tow 8/18 T-8d, there were significant differences between C20:4 and C16:0 (p = 0.002), C22:0 and C16:0 (p = 0.006), and C18:1 and C16:0 (p = 0.030), as seen in Figure 4 and Table 1. In Bog D 8/9, there were significant differences between C18:2 and C16:1 (p = 0.025), as seen in Figure 5 and Table 2.

Table 1. Tow 8/18 T-8d Statistical variance parameters for a Related-Samples Friedman's Two-Way Analysis of Variance by Ranks Summary. See methods for details.

Parameter	Value used
Total N	23
Test Statistic	32.581
Degree of Freedom	8
Asymptotic Sig.(2-sided test)	0.000

Table 2. BogD 8/9 Statistical variance parameters for a Related-Samples Friedman's Two-Way Analysis of Variance by Ranks Summary. See methods for details.

Parameter	Value used
Total N	9
Test Statistic	15.794
Degree of Freedom	5
Asymptotic Sig. (2-sided test)	0.007

Discussion

Algae of class Eustigmatophyceae have a wide variety of physical characteristics, and their relative quantities of fatty acids are no different. The various isolates shown in this study appear to have noticeable differences in these quantities that may allow it to be used as an additional form of characterization for future work. By comparing the percentages of fatty acids within one strain, significant differences can be seen and used to create a profile for each individual strain to identify them in the future.

Before broad statements can be made regarding the use of fatty acid profiles to help characterize these strains of algae, more samples will need to be tested, and in large enough quantities to ensure statistical relevance.

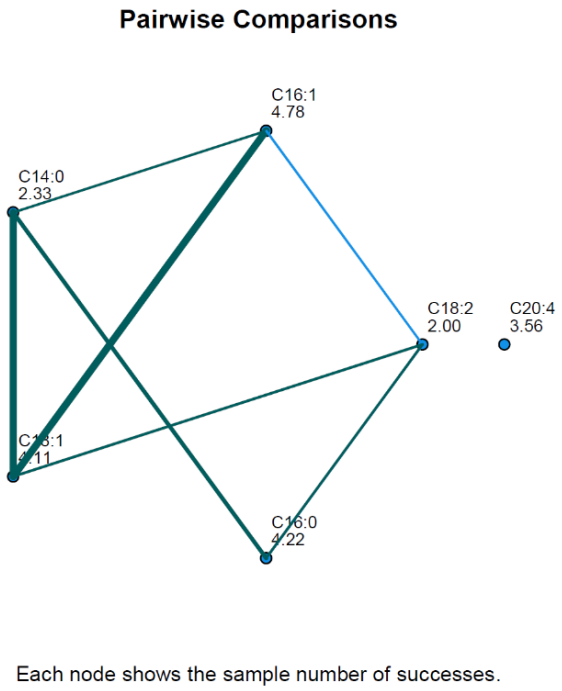

Figure 4. Tow 8/18 T-8d Pairwise Comparison Node showing significance of differences between concentrations of fatty acids. Interpretation of the graph shown in the Results section.

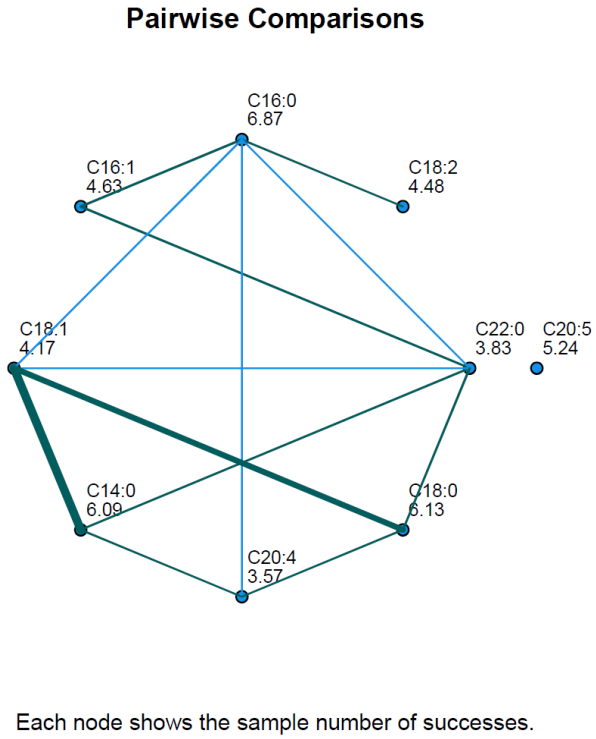

Figure 5. Bog D 8/9 Pairwise Comparison Node showing significance of differences between concentrations of fatty acids. Interpretation of the graph shown in the Results section.

While many strains have been tested up to this point, growth speeds have made sample sizes small for most strains. In addition, due to this slow turnover, reproducibility due to student changes must be considered as well.

One other major issue that needs to be addressed is genetic changes over isolate lifetime. While initial strain cultures were tested were tested with genetic analysis (Fawley *et al*. 2007, Prior *et al*. 2009), these tests should be repeated to see if they have changed over time.

Conclusions

Analyzing algae strains has raised many questions regarding fatty acid synthesis and concentration variations between strains. Two Eustigmatophyceae strains have been analyzed with enough data for statistical difference, and fatty acid concentrations were determined in this portion of the research. Using a Freidman's Two-way analysis and Pairwise Comparisons, the percentages of each fatty acid within the individual strain were tested for consistency to build a profile. These profiles may give future researchers the ability to compare these strains of Eustigmatophyceae to new isolates. More data is necessary to perform proper statistical analysis of additional strains in order to properly compare samples across strains.

Acknowledgements

This research was supported/funded by the Arkansas Space Grant Consortium. We thank the High Performance Structural Mass Spectrometry Laboratory, located in Chemistry and Biochemistry, University of Arkansas, Fayetteville, for their gas chromatography-mass spectroscopy work. We would also like to show our gratitude to Marvin Fawley and Karen Fawley for their work in collecting and culturing the algae samples.

Literature Cited

Bigogno C, Khozin-Goldberg I, Boussiba S, Vonshak A and **Cohen, Z**. 2002. Lipid and fatty acid composition of the green oleaginous alga *Parietochloris incise*, the richest plant source of arachidonic acid. Phytochemistry 60:497–503.

https://scholarworks.uark.edu/jaas/vol77/iss1/4
DOI: 10.54119/jaas.2023.77103

Caramujo M, **Boschker H** and **Admiraal, W**. 2008. Fatty acid profiles of algae mark the development and composition of harpacticoid copepods. Freshwater Biology 53:77–90.

Fahl K and **Kattner G**. 1993. Lipid Content and fatty acid composition of algal communities in sea-ice and water from the Weddell Sea (Antarctica). Polar Biology 13:405–409.

Fawley KP and **Fawley MW**. 2007. Observations on the Diversity and Ecology of Freshwater *Nannochloropsis* (Eustigmatophyceae), with Descriptions of New Taxa. Protist 158:325–336.

Hibberd, DJ and **Leedale GF**. 1971. A New Algal Class- The Eustigmatophyceae. Taxon 20:523–525.

Krienitz L, **Takeda H** and **Hepperle D**. 1999. Ultrastructure, cell wall composition, and phylogenetic position of *Pseudodictyosphaerium jirisii* (Chlorococcales, Chlorophyta) including a comparison with other picoplanktonic green algae. Phycologia 32:100–107.

Krienitz L and **Wirth M**. 2006. The high content of polyunsaturated fatty acids in *Nannochloropsis limnetica* (Eustigmatophyceae) and its implication for food web interactions, freshwater aquaculture and biotechnology. Limnologica 36:204–210.

Lang I, Hodac L, Friedl, T and **Feussner I**. 011. Fatty acid profiles and their distribution patterns in microalgae: a comprehensive analysis of more than 2000 strains from the SAG culture collection. BMC Plant Biology 11:124

Lubian LM, **Montero O, Moreno_Garrido I, Huertas IE, Sobrino C, Gonzalez-del Valle M,** and **Pares, G**. 2000. *Nannochloropsis oculata* (Eustigmatophyceae) as source of commercially valuable pigments. Journal of Applied Phycology 12:249–255.

Mourente G, **Lubian, LM** and **Odriozola JM**. 1990. Total fatty acid composition as a taxonomic index of some marine microalgae used as food in marine aquaculture. Hydrobiologia 203:147–154.

Prior SE, **Fawley MW**, and **Fawley KP**. 2009. DNA Sequence Analysis of Freshwater Eustigmatophyceae, a Potential Source of Essential Fatty Acids. Journal of the Arkansas Academy of Science 63:139–144.

Tonon T, Harvey D, Larson TR, Graham IA. 2002. Long chain polyunsaturated fatty acid production and partitioning to triacylglycerols in four microalgae. Phytochemistry 61:15–24.

Volkman JK, Jeffrey SW, **Nichols PD, Rogers GI, Garland CD**. 1989. Fatty acid and lipid composition of 10 species of microalgae used in mariculture. Journal of Experimental Marine Biology Ecology 128:219–240.

Zhang J and **Lui S**. 2003. Fatty Acid Composition Analyses of the DCMU Resistant Mutants of *Nannochloropsis oculata* (Eustigmatophyceae). Journal of Ocean University of Qingdao 2:65–68.

Zittelli GC, **Lavista F**, **Bastianini A**, **Rodolfi L, Vincenzini M,** and **Tredici MR**. 1999. Production of eicosapentaenoic acid by *Nannochloropsis sp*. cultures in outdoor tubular photobioreactors. Journal of Biotechnology 70:299–312.