The effects of viral infection and lysis by cyanophage LPP-1 on the lipid composition of the cyanobacterium *Plectonema boryanum*

by
Tessa Allen

A thesis presented to the Honors College of Middle Tennessee State University in partial fulfillment of the requirements for graduation from the University Honors College

Term *Spring 2022*

Thesis Committee:

Dr. Jeff Leblond, Thesis Director

Dr. Mary Farone, Second Reader

Dr. Stephen Howard, Thesis Committee Chair

The effects of viral infection and lysis by cyanophage LPP-1 on the lipid content of the cyanobacterium *Plectonema boryanum*

by Tessa Allen

APPROVED:

_____

Dr. Jeff Leblond, Thesis Director

Professor, Biology Department

_____

Dr. Mary Farone, Second Reader

Professor, Biology Department

_____

Dr. Stephen Howard, Thesis Committee Chair

Professor, Biology Department

Acknowledgments

I would first like to give thanks to my thesis director, Dr. Leblond, who was open to taking on this project despite it being unconventional and full of unknowns. There were many challenges faced in the completion of this research, but Dr. Leblond was consistently supportive—offering much of his time, effort, and expertise to push this project forward. I appreciate his patience in teaching me the methods and concepts required for the completion of this work, and through the process, I have gained a great respect for the study of both algae and lipids. I would also like to thank my husband and family for their unwavering support which has always given me the confidence to pursue my ideas.

Abstract

The role of host lipids during viral infection is an area of study only recently examined within the last two decades, and existing literature is limited. The few studies that exist have been limited to observations in eukaryotes and heterotrophic prokaryotes. Since cyanophages have been considered as a means for biotic control of cyanobacterial harmful agal blooms, understanding the replication of cyanophages and their effect on host cells is critical to optimizing their utilization. This research focused on establishing the galactolipid profile of cyanobacterium *Plectonema boryanum*, and on the effects of cyanophage LPP-1 on the galactolipid composition of *Plectonema boryanum*. Nine major galactolipids were found and identified as either mono- or digalactosyldiacylglycerol (MDGD or DGDG, respectively) with the dominant regiochemisty exhibited being the C_{18}/C_{16} form. A difference in the relative abundance of 18:3/16:1 MGDG between infected and uninfected cultures of *Plectonema boryanum* was observed; however, further research must be conducted to confirm the significance this observation.

Table of Contents

List of Tables

List of Figures

Introduction

Cyanobacteria and Harmful Algal Blooms

Unlike heterotrophic bacteria species, members of the phylum Cyanobacteria obtain their energy via oxygenic photosynthesis (Schoch et al. 2020). Also known as "blue-green algae," these bacteria are notable for being the first organism and only prokaryotes to have evolved oxygenic photosynthesis, and they are credited for having a primary role in the Great Oxygenation Event leading up to the evolution of eukaryotes (Sánchez-Baracaldo et al., 2022). The evolutionary history of cyanobacteria and eukaryotes is further intertwined by the rise of plastid organelles in plant and algae cells. It is thought that the origin of the modern plastids is due to the endosymbiosis of cyanobacteria in eukaryotes—similar to the evolution of mitochondria (Moore, 2019).

Today, cyanobacteria function as important primary producers with roles in both the carbon and oxygen cycles of their environments, and they are estimated to contribute to around 25% of primary productivity globally (Bullerjahn & Post, 2014). They are considered nearly ubiquitous in photic zones and—while often associated with fresh water sources—can be found in a diverse range of extreme habitats such as marine environments, desert soils, rock, and artic regions (Rajaneesh et al., 2020). Cyanobacteria may exist as free-living cells, symbionts or endosymbionts with eukaryotes ranging from animals, plants, and fungi (Foster & Zehr, 2019).

While cyanobacteria are important for their essential functions as primary producers, they may often become a nuisance or even detrimental to their ecosystems and humans. Harmful algal blooms caused by eukaryotic algae (HABs) and cyanobacteria

(cyanoHABs) have been increasing over the last few decades (Anderson et al., 2008; Pal et al., 2020). This is primarily attributed to the anthropogenic eutrophication of water supplies, and the increase of average temperatures associated with climate change (Burkholder, 2009; Abeysiriwardena et al., 2018). Concerns over the increase in HABs and cyanoHABs are mostly directed at the toxins produced by certain species during blooms. Toxins known to be produced by cyanobacteria (cyanotoxins) include microcystins, saxitoxin, anatoxin, ciguatoxin, lipopolysaccharide endotoxins, cylindrospermopsin, and nodularins (Abeysiriwardena et al., 2018; Anderson et al., 2008). These toxins can pose a serious threat to both aquatic environments and human health (Abeysiriwardena et al., 2018; O'Neil et al., 2012). Notably, some members of the *Oscillatoriaceae* family were found to produce these (Burkholder, 2009; Lefler et al., 2020).

Attempts to manage the increase in cyanoHABs have usually involved the use of mechanical water disruption, chemical water treatments, and algicides (Burkholder, 2009). Due to the mixed efficacy and potential for negative side effects, alternative methods of mitigating cyanoHABs have been explored. This includes the use of biotic control measures such as cyanophages (Pal et al., 2020; Wurtsbaugh et al., 2019)

Cyanophage

Cyanophages are classified as bacteriophage which infect members of the Cyanobacteria phylum (Schoch et al. 2020). Morphologically, cyanophages resemble and possess a similar range of appearances as bacteriophages with many identified cyanophages falling into the families of Myoviridae, Podoviridae, and Siphoviridae (Lefkowitz et al., 2018). Cyanophages have been poorly studied

compared to bacteriophages infecting heterotrophic bacteria, but this may be explained by their more recent discovery. Virus LPP-1 was the first discovered cyanophage and was isolated by Safferman & Morris in 1963. LPP-1 is the most well studied cyanophage; however, compared to well known bacteriophage, such as T-4 or Lambda, little is understood about the replication of LPP-1 in its host, *Plectonema boryanum*. The majority of what has been establish on the host-pathogen relationship between LPP-1 and *P. boryanum* is from literature produced in the 1960-70s (Safferman & Morris, 1963, 1964; Padan & Shilo, 1970), and there is much need to pursue current studies of LPP-1 and other cyanophage utilizing modern techniques.

The Role of Lipids in Viral Replication

As the field of lipidomics has expanded within the last decades, interest in the role of cellular lipids during viral infections has increased (Heaton & Randall, 2011; Züllig & Köfeler, 2020). It is well understood that certain viruses use the cell membrane of their hosts to create capsid envelopes, but recent studies have suggested that viruses may target the use of host lipids more directly. Studies of the West Nile Virus (WNV) have demonstrated the upregulation of *de novo* cholesterol synthesis in Vero cells induced by WNV infection (Mackenzie et al., 2007). In *Escherichia coli,* it was observed that infection by bacteriophage T-4 resulted in the increased biosynthesis of specific phospholipids while decreasing others (Kutter et al., 2018). The modulation of host lipids and recruit of lipid synthases to viral replication centers have also been observed with Epstein-Barr virus and Dengue virus (Heaton & Randall, 2011). This research

background forms the rational for examining the possible effects of viral infection on the lipid composition of *Plectonema boryanum.*

Lipids of Cyanobacteria

The primary source of cellular lipids in cyanobacteria are from the thylakoid membrane (Hölzl & Dörmann, 2007; Zepke et al., 1978). Used for oxygenic photosynthesis, the thylakoid is composed approximately of ~60% protein and ~40% lipid by weight (Awai, 2016). The lipid portion is made up of galactolipids and most commonly monogalactosyldiacylglycerol (MDGD) and digalactosyldiacylglycerol (DGDG) (Gray et al., 2009; Konopka, & Brock, 1978; Murata & Wada, 1995). Other galactolipids found less commonly are sulfoquinovosyldiacylglycerol (SQDG) and trigalactosyldiacylglycero (TGDG) (De Loura et al., 1987; Murata & Wada, 1995). Phospholipids, such as phosphatidylglycerol, have also been found in cyanobacteria (De Loura et al., 1987; Zepke et al., 1978). Since there have been no studies on the changes to cyanobacterial lipid composition due to viral infection, there are no data on changes in galactolipids in cells induced by viral infection.

Methods

Plectonema boryanum *Culture Growth Conditions*

Plectonema boryanum UTEX B 594 culture was obtained from the Culture Collection of Algae at the University of Texas at Austin. Six cultures were grown to logarithmic phase in 1 liter of Modified WC medium (Guillard, 1975). For optimal growth, all cultures were grown at 28 ℃ on a 14/10-hour light/dark cycle at approximately 50 µmol photons/$m^2 \cdot s^1$ (Cannon et al., 1976; Ivanov et al., 2017; Konopka & Brock, 1978).

LPP-1 Rehydration and Lysate Collection

Freeze-dried LPP-1 ATCC 18200-B1™ was obtained from the American Type Culture Collection and was rehydrated using 1 mL Chu No. 10 media (Safferman & Morris 1964). To produce lysates, 2 mL of *P. boryanum* culture were inoculated with 10 µL of LPP-1 suspension then combined with 3 mL molten MWC 1.5% agar medium (~45 ℃) after 15 min (Howard Hughes Medical Institute [HHMI], 2018; Safferman & Morris, 1963). The molten agar suspension was poured over Petri dishes containing 20 mL of solidified MWC 1.5% agar medium, and plates were incubated at 28 ℃ for 72 hours (Safferman & Morris, 1963). After plaque formation, 8 mL of MWC medium was pipetted onto to agar surface of a webbed plate and allowed to sit for 20 min (HHMI, 2018). The lysate was collected by passage through a 0.2 µm nylon filter and stored at 4 ℃. The titers of collected lysates were calculated in pfu/mL using the formula outlined by the HHMI *Phage Discovery Guide* (2018).

Inoculating and Harvesting Plectonema boryanum *Cultures*

Three of the six *P. boryanum* logarithmic phase cultures were inoculated with 5 mL of LPP-1 lysate with a titer of $3.0x10^5$ pfu/mL. After 72-hours, all six cultures were harvested via Büchner funnels onto glass microfiber filters. Lipids were extracted from the filters following a modified version of the Bligh and Dyer method used by Leblond and Chapman (2000). Extraction yielded between 8.6-17.7 mg of total lipid per culture.

Lipid Fractionation

Column chromatography using 1 g of activated Unisil silica was used to separate lipid classes into fractions based on polarity as described by Leblond and Chapman (2000). The solvents used and their corresponding lipid classes are listed by increasing polarity as follows: Fraction 1, sterol esters) 12 mL methylene chloride; Fraction 2, free sterols, free fatty acids, and tri- and diacylglycerols) 15 mL 0.05% acetic acid and 5% acetone in methylene chloride; Fraction 3, monoacylglycerols) 10 mL 20% acetone in methylene chloride; Fraction 4, galactolipids) 45 mL acetone; Fraction 5, phospholipids) 0.1% glacial acetic acid in 15 mL methanol.

Galactolipid Preparation and Positive-ion Electrospray/Mass Spectrometry Analysis

After evaporation under nitrogen, Fraction 4 lipids were prepared by dissolving in 50 µL of a solvent mixture containing methanol, chloroform, and 50 mM sodium acetate as outlined by Welti et al. (2002). The addition of this solvent resulted in positive sodium adducts (Na^+) which can be detected using positive-ion electrospray/mass spectrometry (ESI/MS). To scan the sample using ESI/MS, 5 µL of Fraction 4 sodium adducts were

directly injected into a Thermo Finnigan LCQ Deca XP ion trap mass spectrometer with a methylene chloride carrier solvent (0.5 mL min^{-1}) and a scanning range of 100-2000 amu (Gray et al., 2009). The ESI mass spectra obtained were used to identify the major galactolipids present and calculate their relative abundance to one another to account for the contribution of 13C isotopes to the peak heights (Leblond et al., 2010).

Each major galactolipid was further characterized by using positive-ion electrospray/mass spectrometry/mass spectrometry (ESI/MS/MS). The spectra obtained from using ESI/MS/MS on specific masses showed the original intact galactolipid mass along with two associated fragments resulting from cleavage of the galactolipid fatty acid chains (Figure 2). The differences in mass between the intact lipid and the fatty acid chains were used to determine the length and number of double bonds in the cleaved fatty acid. The regiochemistry describing the position of the fatty acid chains on the glycerol backbone as *sn*-1 or *sn*-2 was determined by comparing the relative abundance of each fatty acid fragment as done by Gray et al. (2009) which was based on the methods of (Guella et al., 2003).

Results

Galactolipids of Plectonema boryanum

Galactolipids, such as MGDG and DGDG, are characterized by the carbon-chain length, location and number of double bonds, and the regiochemistry of their fatty acid (acyl) chains. The regiochemistry of the acyl chains are designated as *sn*-1 or *sn*-2. This refers to the attachment of the acyl chain to either carbon 1 or 2 of the glycerol backbone. The notation for MGDG and DGDG reflects these structural components, such that, 18:2/16:1 MGDG describes a MGDG molecule having an 18 carbon *sn*-1 acyl chain with 2 double bonds and a 16 carbon *sn*-2 acyl chain with 1 double bond (Figure 1).

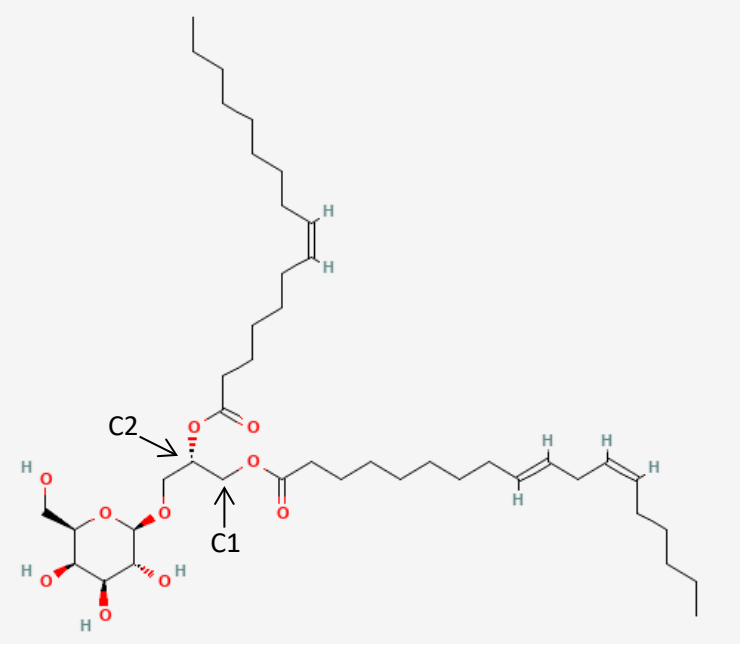

Figure 1. Structure of 18:2/16:1 MGDG. Arrows designate carbons 1 and 2 of the glycerol backbone corresponding to *sn*-1 and *sn*-2 acyl chain positions (National Center for Biotechnology Information, 2022).

Nine different galactolipids, identified as either digalactosyldiacylglycerol (DGDG) or monogalactosyldiacylglycerol (MGDG), were present in Fraction 4. Within the uninfected control group, 18:3/16:1 MGDG (*m/z* 773) (Figure 2) had the highest relative abundance with a mean of 35.1% and standard deviation (SD) of 1.012. The next three highest a were 18:2/16:1 MGDG (*m/z* 775) with a mean relative abundance of 14.3% (SD 1.320), 18:3/16:0 MGDG (*m/z* 775) with a mean relative abundance of 13.2% (SD 1.266), and 18:2/16:0 MGDG (*m/z* 777) with a mean relative abundance of 12.7% (SD 1.328). The five remaining galactolipids composed less than 10% of the relative galactolipid abundance distribution. Within the LPP-1 inoculated group, 18:3/16:1 MGDG (*m/z* 773) also had the highest relative abundance with a mean of 26.9%. The remainder of the LPP-1 inoculated group lipid composition closely following the same distribution as the control group (Table 1.)

Table 1. Galactolipids identified in *Plectonema boryanum* from the uninfected control and LPP-1 infected cultures with % relative abundance based on ESI/MS and ESI/MS/MS spectra peak intensity data.

Galactolipid	Mass[1]	Uninfected Control [2]Mean Relative Abundance (%)	Uninfected Control [3]Standard Deviation	LPP-1 Infected [2]Mean Relative Abundance (%)	LPP-1 Infected [3]Standard Deviation
16:1/16:1 MGDG	749	4.3	1.976	4.3	0.557
18:3/16:1 MGDG	773	35.1	1.012	26.9	0.764
18:2/16:1 MGDG	775	14.4	1.320	16.9	0.416
18:3/16:0 MGDG	775	13.2	1.266	15.6	0.416
18:2/16:0 MGDG	777	12.7	1.328	15.4	3.676
18:3/16:1 DGDG	935	9.1	2.858	8.5	0.557
18:2/16:1 DGDG	937	4	0.557	4.2	1.311
18:3/16:0 DGDG	937	2.9	0.400	3.0	0.907
18:2/16:0 DGDG	939	4.3	0.058	5.1	0.551

[1]Mass rounded to nearest odd number. [2]Mean relative abundance rounded to nearest tenth. [3]Standard deviations rounded to nearest hundredth.

Comparison of Galactolipid Relative Abundance Between Control and LPP-1 Inoculated Groups

Only one galactolipid, 18:3/16:1 MGDG, presented a notable difference in the mean relative abundance between the control and LPP-1 inoculated groups. The difference was approximately 8.2% with the control group having a 35.1% mean relative abundance and the LPP-1 inoculated group having a 26.9% mean relative abundance. A two-sample t-test (H_1 $\mu 1 \neq \mu 2$) was used to evaluate the statistical significance of this

difference, and it yielded a t-score of 11.2, a p-value of 5.3108251×10^{-4} ($\alpha = 0.05$), and 3.7 degrees of freedom (Table 2.).

Table 2. Results from two-sample t-test (H_1 $\mu 1 \neq \mu 2$) for each identified galactolipid.

Lipid	t-score	p-value	degrees of freedom
16:1/16:1 MGDG	0.0281271975	0.9798009716	2.315686033
18:3/16:1 MGDG	11.20499296	5.3108251×10^{-4}	3.720934702
18:2/16:1 MGDG	3.211121976	0.0667751167	2.39381247
18:3/16:0 MGDG	3.118666189	0.069578205	2.427436823
18:2/16:0 MGDG	1.181850231	0.3369530231	2.513337384
18:3/16:1 DGDG	0.3568732136	0.7531773541	2.151556586
18:2/16:1 DGDG	0.2431322695	0.8251937888	2.698248486
18:3/16:0 DGDG	0.2328890039	0.8320946477	2.74904081
18:2/16:0 DGDG	2.293658555	0.1460739209	2.043950737

Discussion

Galactolipid Comparison of Plectonema boryanum to other Cyanobacteria

The thylakoid membrane is considered highly conserved in cyanobacteria, and accordingly, the galactolipid composition of the thylakoid is believed to remain relatively consistent among cyanobacteria species (Hölzl & Dörmann, 2007; Awai, 2016). While no lipid analyses have been conducted for other species within the genus *Plectonema,* the results of this research may be compared to other members of the same family, *Oscillatoriaceae*. Almost all the MGDG and DGDG acyl chains are the C_{18}/ C_{16} form (with the exception of 16:1/16:1 MGDG). This was found to be true for other members of *Oscillatoriaceae,* including *Oscillatoria splendida* and certain *Pseudanabaena* spp. (Zepke et al., 1978). Outside of *Oscillatoriaceae,* the dominance of C_{18} at the *sn*-1 position and C_{16} at the *sn*-2 position is considered to be the most prevalent configuration of galactolipids in cyanobacteria (Awai, 2016; Hölzl & Dörmann, 2007). This is thought to be due to the conserved selective preference of monoglucosyldiacylglycerol (MGlcDG) synthase for the C_{18}/ C_{16} configuration (Awai, 2016; Hölzl & Dörmann, 2007; Zepke et al., 2004).

The eight C_{18}/ C_{16} forms of MGDG and DGDG had an even distribution of saturated and unsaturated fatty acid chains. Of the four C_{16} MGDG fatty acids, half had fully saturated fatty acids (16:0) and half had a single double bond (16:1). The C_{18} MGDG unsaturated fatty acids were similarly split evenly between two (18:2) or three (18:3) double bonds. All four DGDG lipids exhibit the same pattern of saturated and unsaturated fatty acids (Table 1.). The existing data on galactolipid fatty acids in cyanobacteria is limited, but the mirrored distribution of double bonds between MGDG

and DGDG fatty acids is not surprising since MGDG is the precursor for DGDG (Awai, 2016; Hölzl & Dörmann, 2007). Levels of desaturation from zero to four double bonds per fatty acid chain have been described in other cyanobacteria species including *Oscillatoria chalybea* (Murata & Wada, 1995; Zepke et al., 1978).

The location of double bonds within a fatty acid can be used to determine its identity. While the fatty acid double bonds were not located in this experiment, the identity of the fatty acids observed in *P. boryanum* can be speculated based on the findings of other studies. In *Oscillatoria splendida*, the 16:0 and 16:1 fatty acids (for all galactolipids) were determined to be palmitic acid and palmitoleic acid respectively, and the 18:2 and 18:3 fatty acids (for all galactolipids) were determined to be linoleic acid and linolenic acid respectively (De Loura et al., 1987).

Lipids other than those found in this research, such as sulfoquinovosyldiacylglycerol (SQDG), trigalactosyldiacylglycero (TGDG), and certain phospholipids, have been found in other species within *Oscillatoriaceae* and in other families. This includes *Oscillatoria splendida*, *Pseudanabaena* sp., *Synechocystis* sp., and *Anabaena* sp. (Awai et al., 2006; De Loura et al., 1987). These lipids were not identified in either Fraction 4 or 5 of our *P. boryanum* lipid extracts.

Galactolipid Comparison of Control Group and LPP-1 Inoculated Group

Outside of 18:3/16:1 MGDG, there were no significant differences observed in the relative lipid abundance between the control and LPP-1 inoculated groups. While the p-value (5.3108251×10^{-4}) and relatively high t-score (11.20499296) suggest there may be a statistical significance in the difference between the relative abundance of 18:3/16:1

MGDG, the small sample size used for each group (n = 3) suggests the results should be interpreted with low confidence. Furthermore, while the role of lipids in viral infection and lipid compositional changes due to infection have been observed in other cell types (Cronan & Wulff, 1969; Heaton & Randall, 2011; Lee & Ahlquist, 2003), this is the first experiment examining the possible effect of viral infection of the lipid composition of cyanobacteria and as a result there is no data available for direct comparison to our own. To support the data collected in this experiment, repetitions, preferably involving a larger sample size, would need to be done in order to increase the statistical power of these results. Another variable to consider when interpreting the data collected from this experiment includes the temperature distribution of within the culture incubators. Exposing cyanobacteria to lower temperatures has been shown to upregulate desaturase resulting in the increase of double bonds present on galactolipid fatty acids (Hölzl & Dörmann, 2007; Murata & Wada, 1995). The incubators used in this experiment have a fan positioned in the back, and it is possible that cultures closer to the fan would experience a lower relative temperature compared to the others. Future experiments should consider the effects of uneven temperature distributions within the culture incubators by distributing the culture flasks evenly to rule this factor out.

Conclusion

The results of this research included the first analysis of the galactolipid profile of a cyanobacterium from the genus *Plectonema,* and the first attempt to observe and quantify the possible lipid composition changes due to viral infection in cyanobacteria. The galactolipid profile of *P. boryanum* was consistent with the findings of other

members within the family *Oscillatoriaceae,* including *Oscillatoria splendida* and certain *Pseudanabaena* spp. except for the absence of a phospholipid group. Additional research and repetitions of this experiment should be conducted to confirm the absence of phospholipids group in *P. boryanum*. Of the nine galactolipids identified, the C_{18}/ C_{16} form of MGDG and DGDG was the most prevalent in *P. boryanum.* This aligns with the consensus that MGlcDG synthase prefers this configuration and with the notion that both the thylakoid membrane and MGlcDG synthase are likely highly conserved among cyanobacteria. The desaturation of the fatty acid chains was also distributed evenly among the C_{18}/ C_{16} forms of MGDG and DGDG. The identities of these fatty acids were not determined in this experiment. In order to identify them, future experiments should consider using gas chromatography/mass spectrometry to locate the positions of the double bonds within the observed fatty acids (De Loura et al., 1987; Leblond & Chapman, 2000).

The galactolipid relative abundance comparison between control and LPP-1 inoculated groups suggests there may be a difference in 18:3/16:1 MGDG resulting from viral infection; however, further studies are needed to test the veracity of these findings against possible variables. Since there are no other studies on changes in the lipid content of cyanobacteria post-viral infection, additional research may include repeating the experiment using different species in *Oscillatoriaceae* to compare the lipid profiles.

References

Abeysiriwardena N. M., Gascoigne S., & Anandappa A. (2018). Algal bloom expansion increases cyanotoxin risk in food. *The Yale Journal of Biology and Medicine*, *91*(2), 129-142. Retrieved March 10, 2022, from https://www.ncbi.nlm.nih.gov /pmc/articles/PMC6020737/.

Awai, K. (2016). Thylakoid development and galactolipid synthesis in cyanobacteria. *Subcellular Biochemistry*, *86,* 85–101. https://doi.org/10.1007/978-3-319-25979-6_4

Awai, K., Kakimoto, T., Awai, C., Kaneko, T., Nakamura, Y., Takamiya, K., Wada, H., & Ohta, H. (2006). Comparative genomic analysis revealed a gene for monoglucosyldiacylglycerol synthase, an enzyme for photosynthetic membrane lipid synthesis in cyanobacteria. *Plant Physiology*, *141*(3), 1120–1127. https://doi.org/10.1104/pp.106.082859

Anderson, D. M., Burkholder, J. M., Cochlan, W. P., Glibert, P. M., Gobler, C. J., Heil, C. A., Kudela, R. M., Parsons, M. L., Rensel, J. E., Townsend, D. W., Trainer, V. L., & Vargo, G. A. (2008). Harmful algal blooms and eutrophication: Examining linkages from selected coastal regions of the United States. *Harmful Algae*, *8*(1), 39–53. https://doi.org/10.1016/j.hal.2008.08.017

Burkholder J.M. (2009). Harmful Algal Blooms. In T. Mehner (Eds.), *Encyclopedia of Inland Waters.* (1st ed., pp. 264–285). Academic Press.

Bullerjahn, G. S., & Post, A. F. (2014). Physiology and molecular biology of aquatic cyanobacteria. *Frontiers in Microbiology*, *5*, 359. https://doi.org/10.3389/fmicb.2014.00359

Cronan, J. E., & Wulff, D. L. (1969). A role for phospholipid hydrolysis in the lysis of *Escherichia coli* infected with bacteriophage T4. *Virology*, *38*(2), 241–246. https://doi.org/10.1016/0042-6822(69)90365-1

De Loura, I. C., Dubacq, J. P., & Thomas, J. C. (1987). The effects of nitrogen deficiency on pigments and lipids of cyanobacteria. *Plant Physiology*, *83*(4), 838–843. https://doi.org/10.1104/pp.83.4.838

Foster, R. A., & Zehr, J. P. (2019). Diversity, genomics, and distribution of phytoplankton-cyanobacterium single-cell symbiotic associations. *Annual Review of Microbiology*, *73*, 435–456. https://doi.org/10.1146/annurev-micro-090817-062650

Gray, C. G., Lasiter, A. D., Li, C., & Leblond, J. D. (2009). Mono- and digalactosyldiacylglycerol composition of dinoflagellates. I. Peridinin-containing taxa. *European Journal of Phycology*, *44*(2), 191–197. https://doi.org/10.1080/09670260802419481

Guella, G., Frassanito, R. & Mancini, I. (2003). A new solution for an old problem: The regiochemical distribution of the acyl chains in galactolipids can be established by electrospray ionization tandem mass spectrometry. *Rapid Communications in Mass Spectrometry, 17*, 1982–1994. https://doi.org/10.1002/rcm.1142

Guillard, R. L., (1975). Culture of phytoplankton for feeding marine invertebrates. In W.L Smith. & M.H. Chanley (Eds.), *Culture of Marine Invertebrate Animals*. (pp. 29-60). Springer. https://doi.org/10.1007/978-1-4615-8714-9_3

Heaton, N. S., & Randall, G. (2011). Multifaceted roles for lipids in viral infection. *Trends in Microbiology*, *19*(7), 368–375. https://doi.org/10.1016/j.tim.2011.03.007

Hölzl, G., & Dörmann, P. (2007). Structure and function of glycoglycerolipids in plants and bacteria. *Progress in Lipid Research*, *46*(5), 225–243. https://doi.org/10.1016/j.plipres.2007.05.001

Howard Hughes Medical Institute. (2018). *Phage Discovery Guide*. https://seaphagesphagediscoveryguide.helpdocsonline.com/copyright

Ivanov, A. G., Miskiewicz, E., Clarke, A. K., Greenberg, B. M., & Huner, N. P. (2007). Protection of photosystem II against UV-A and UV-B radiation in the cyanobacterium *Plectonema boryanum*: The role of growth temperature and growth irradiance. *Photochemistry and Photobiology*, *72*(6), 772–779. https://doi.org/10.1562/0031-8655(2000)0720772popiau2.0.co2

Konopka, A., & Brock, T. D. (1978). Effect of temperature on blue-green algae (cyanobacteria) in Lake Mendota. *Applied and Environmental Microbiology*, *36*(4), 572–576. https://doi.org/10.1128/aem.36.4.572-576.1978

Kutter, E., Bryan, D., Ra, G., Brewster, E., Blasdel, B., & Guttman, B. (2018). From host to phage metabolism: Hot tales of phage T4's takeover of *E. coli*. *Viruses*, *10*(7), 387. https://doi.org/ https://doi.org/10.3390/v10070387

Leblond, J. D., & Chapman, P. J. (2000). Lipid class distribution of highly unsaturated long chain fatty acids in marine dinoflagellates. *Journal of Phycology*, *36*(6), 1103–1108. https://doi.org/10.1046/j.1529-8817.2000.00018.x

Leblond, J. D., Timofte, H. I., Roche, S. A., & Porter, N. M. (2010). Mono- and digalactosyldiacylglycerol composition of glaucocystophytes (Glaucophyta): A modern interpretation using positive-ion electrospray ionization/mass spectrometry/mass spectrometry. *Phycological Research*, *58*(3), 222–229. https://doi.org/10.1111/j.1440-1835.2010.00582.x

Leblond, J. D., & Dahmen, J. L. (2012). Mono- and digalactosyldiacylglycerol composition of dinoflagellates. v. the galactolipid profile of *Alexandrium tamarense* (dinophyceae) during the course of infection by the parasitic syndinian dinoflagellate *Amoebophrya* sp. *European Journal of Phycology*, *47*(4), 490–497. https://doi.org/10.1080/09670262.2012.742140

Leblond, J. D., Dodson, J., & Dahmen, J. l. (2013). Mono- and digalactosyldiacylglycerol composition of dinoflagellates. vii. evidence against galactolipid production and plastid presence in the heterotrophic, basal dinoflagellate, *Oxyrrhis Marina*. *European Journal of Phycology*, *48*(3), 309–317. https://doi.org/10.1080/09670262.2013.833297

Lee, W. M., & Ahlquist, P. (2003). Membrane synthesis, specific lipid requirements, and localized lipid composition changes associated with a positive-strand RNA virus RNA replication protein. *Journal of Virology*, *77*(23), 12819–12828. https://doi.org/10.1128/jvi.77.23.12819-12828.2003

Lefler, F. W., Berthold, D. E., & Laughinghouse, H. D. (2020). The occurrence of *Affixifilum* gen. nov. and *Neolyngbya* (Oscillatoriaceae) in South Florida (USA), with the description of *A. floridanum* sp. nov. and *N. biscaynensis* sp. *Journal of Phycology*, *57*(1), 92–110. https://doi.org/10.1111/jpy.13065

Lefkowitz, E. J., Dempsey, D. M., Hendrickson, R. C., Orton, R. J., Siddell, S. G., & Smith, D. B. (2018). Virus taxonomy: The database of the International Committee on Taxonomy of Viruses (ICTV). *Nucleic acids research*, *46*(1), 708-717. https://doi.org/10.1093/nar/gkx932

Mackenzie, J. M., Khromykh, A. A., & Parton, R. G. (2007). Cholesterol manipulation by West Nile Virus perturbs the cellular immune response. *Cell Host & Microbe*, *2*(4), 205–277. https://doi.org/https://doi.org/10.1016/j.chom.2007.09.003

Mazzon, M., & Mercer, J. (2014). Lipid interactions during virus entry and infection. *Cellular Microbiology*, *16*(10), 1493–1502. https://doi.org/10.1111/cmi.12340

Murata, N., & Wada, H. (1995). Acyl-lipid desaturases and their importance in the tolerance and acclimatization to cold of cyanobacteria. *Biochemical Journal*, *308*(1), 1–8. https://doi.org/10.1042/bj3080001

National Center for Biotechnology Information (2022). PubChem compound summary for CID 91820596, 1-18:2-2-16:1-monogalactosyldiacylglycerol. Retrieved March 22, 2022, from https://pubchem.ncbi.nlm.nih.gov/compound/1-18_2-2-16_1-Monogalactosyldiacylglycerol.

O'Neil, J. M., Davis, T. W., Burford, M. A., & Gobler, C. J. (2012). The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change. *Harmful Algae*, *14*, 313–334. https://doi.org/10.1016/j.hal.2011.10.027

Padan, E., Ginzburg, D., & Shilo, M. (1970). The reproductive cycle of cyanophage LPP1-G in *Plectonema boryanum* and its dependence on photosynthetic and respiratory systems. *Virology*, *40*(3), 514–521. https://doi.org/10.1016/0042-6822(70)90194-7

Pal, M., Yesankar, P. J., Dwivedi, A., & Qureshi, A. (2020). Biotic control of harmful algal blooms (HABs): A brief review. *Journal of Environmental Management*, *268*, 110687. https://doi.org/10.1016/j.jenvman.2020.110687

Proctor, L. M., & Fuhrman, J. A. (1990). Viral mortality of marine bacteria and cyanobacteria. *Nature*, *343*(6253), 60–62. https://doi.org/10.1038/343060a0

Rajaneesh, K. M., Naik, R. K., Roy, R., & D'Costa, P. M. (2020). Cyanobacteria in tropical and subtropical marine environments: Bloom formation and ecological role. *Advances in Cyanobacterial Biology*, 35–46. https://doi.org/10.1016/b978-0-12-819311-2.00003-6

Safferman, R. S., & Morris, M. E. (1963). Algal virus: Isolation. *Science*, *140*(3567), 679–680. https://doi.org/10.1126/science.140.3567.679

Safferman, R. S., & Morris, M. E. (1964). Growth characteristics of the blue-green algal virus LPP-1. *Journal of Bacteriology*, *88*(3), 771–775. https://doi.org/10.1128/jb.88.3.771-775.1964

Sánchez-Baracaldo, P., Bianchini, G., Wilson, J. D., & Knoll, A. H. (2022). Cyanobacteria and biogeochemical cycles through Earth history. *Trends in Microbiology*, *30*(2), 143–157. https://doi.org/10.1016/j.tim.2021.05.008

Schoch, C. L., Ciufo, S., Domrachev, M., Hotton, C. L., Kannan, S., Khovanskaya, R., Leipe, D., Mcveigh, R., O'Neill, K., Robbertse, B., Sharma, S., Soussov, V., Sullivan, J. P., Sun, L., Turner, S., & Karsch-Mizrachi, I. (2020). NCBI Taxonomy: A comprehensive update on curation, resources and tools. *Database: The Journal of Biological Databases and Curation*, *2020*. https://doi.org/10.1093/database/baaa062

Sündermann A., Eggers L.F., Schwudke D. (2016) Liquid Extraction: Bligh and Dyer. In M. Wenk (Eds.), *Encyclopedia of Lipidomics* (pp. 1-4). Springer. https://doi.org/10.1007/978-94-007-7864-1_88-1

Welti, R., Li, W., Li, M., Sang, Y., Biesiada, H., Zhou, H. E., Rajashekar, C. B., Williams, T. D., & Wang, X. (2002). Profiling membrane lipids in plant stress responses. *Journal of Biological Chemistry*, *277*(35), 31994–32002. https://doi.org/10.1074/jbc.m205375200

Wurtsbaugh, W. A., Paerl, H. W., & Dodds, W. K. (2019). Nutrients, eutrophication and harmful algal blooms along the freshwater to marine continuum. *WIREs Water*, *6*(5), 1-27. https://doi.org/10.1002/wat2.1373

Zepke, H.D., Heinz, E., Radunz, A., Linscheid, M.W., & Pesch, R. (2004). Combination and positional distribution of fatty acids in lipids from blue-green algae. *Archives of Microbiology, 119*, 157-162. https://doi.org/10.1007/BF00964267

Züllig, T., & Köfeler, H. C. (2020). High resolution mass spectrometry in lipidomics. *Mass Spectrometry Reviews*, *30*(3), 162–176. https://doi.org/https://doi.org/10.1002/mas.21627

Appendix

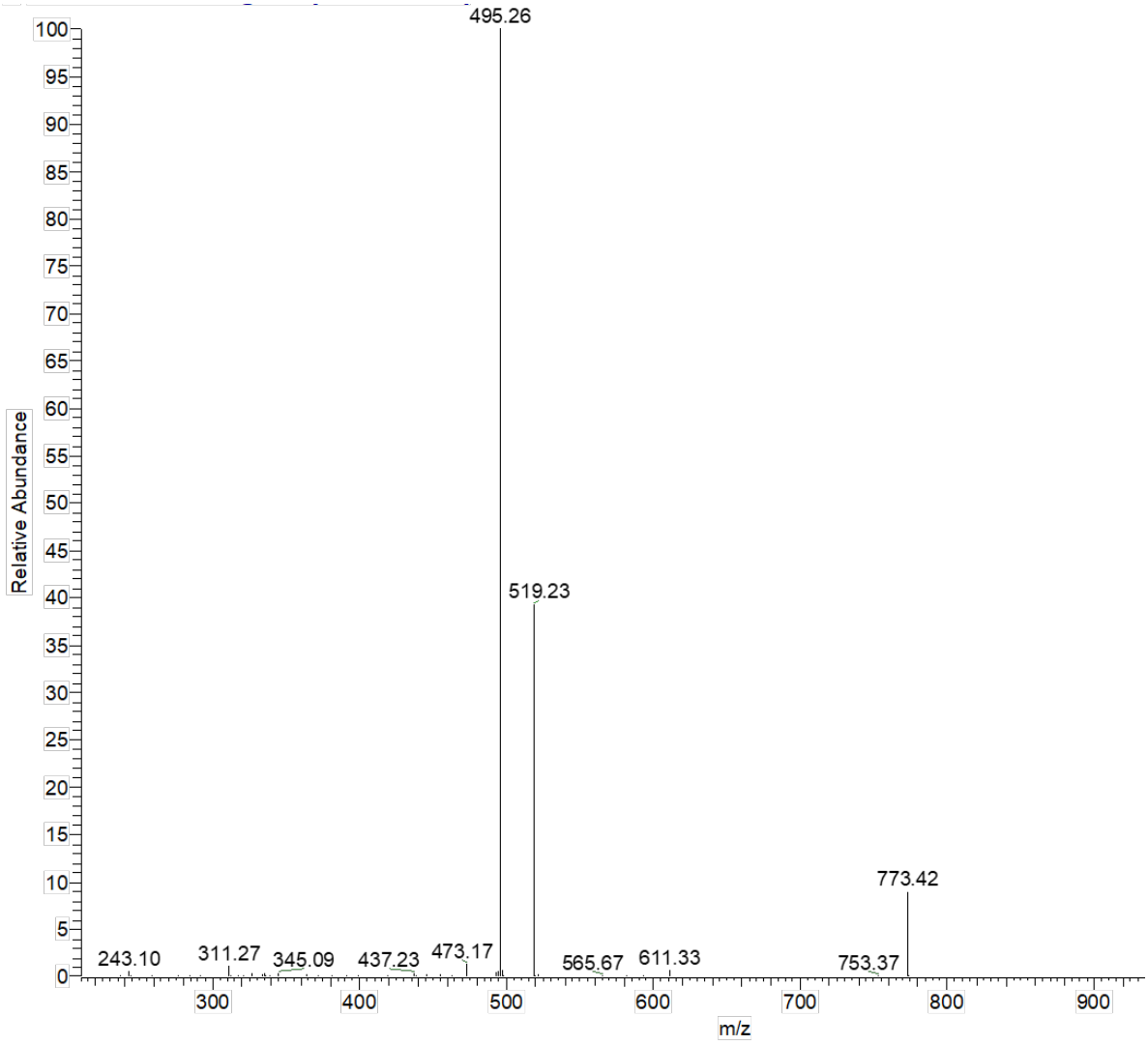

Figure 2. ESI/MS/MS spectrum showing 18:3/16:1 MGDG (*m/z* 773) and its associated fatty acid fragments. This spectrum was used to identify the regiochemistry, carbon length, and number of double bonds in the acyl chains of 18:3/16:1 MGDG. The *m/z* 495 represents the 18:3 *sn*-1 acyl chain and *m/z* 519 represents the 16:1 *sn*-2 acyl chain.