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(54) **Title:** ALGAL OIL AND BIOFUEL AND METHODS OF PRODUCING SAME

(57) **Abstract:** Methods of producing algal oil and algal biofuel are provided. Accordingly there is provided a method of producing oil, the method comprising infecting *Emiliania huxleyi* microalgae with a Coccolithovirus Eh V201; and extracting the oil from the microalgae 24-72 hours following the infecting. Also provided is a method of producing biofuel, the method comprising extracting oil from virus infected *Emiliania huxleyi* microalgae; and processing the oil by a reaction that splits the fatty acids chains of a triacylglycerol (TAG) comprised in the oil from its glycerin backbone. Also provided are oil, biofuel and cake from an *Emiliania huxleyi* microalgae and methods of producing same.



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ALGAL OIL AND BIOFUEL AND METHODS OF PRODUCING SAME

FIELD AND BACKGROUND OF THE INVENTION

The present invention, in some embodiments thereof, relates to algal oil and
5 biofuel and method of producing same.

Petroleum, a natural resource composed primarily of hydrocarbons, is currently the most common source of fuels. Extracting petroleum oil from the earth, its transportation and processing into usable fuels are expensive, dangerous and often at the expense of the environment. Furthermore, worldwide reservoirs of petroleum
10 oil rapidly decrease. Biofuel, such as biodiesel, has been identified as a possible alternative to petroleum-based fuels. Biodiesel refers to monoalkyl esters of long chain fatty acids derived from lipids present in animal, plant or microorganism, primarily triacylglycerides (TAG), which can be used in diesel-engine vehicles.

Microalgae are groups of photosynthetic microorganisms found in freshwater
15 and marine biosystems that can utilize inorganic nutrients (e.g., carbon, nitrogen, phosphorus) from the environment to produce organic compounds, such as protein, pigment, and oil. Algal blooms are regulated by both abiotic factors, such as nutrient supply, temperature and light, and biotic interactions with grazers and viruses. Viral infection of microalgae is typically accompanied by coordinated modulation of flux
20 through host metabolic pathways to supply building blocks such as fatty acids, amino acids and nucleotides to facilitate replication and assembly of the virus. On the other hand viruses can expand the metabolic capabilities of their host by expressing metabolic genes encoded by their own genomes with unique biochemical features. Therefore, viral infection may redefine the chemical composition and the metabolic
25 profile of the infected microalgae and release the synthesized metabolites to the microenvironment during the lytic phase of infection [see e.g., Fulton et al., (2014) Environ Microbiol 16: 1137-1149; and Rosenwasser et al., (2014) The Plant cell 26, 2689-2707].

Microalgae are recognized as an excellent source for the production of
30 economically viable biofuel given their fast growth rate, the fact that they can be cultured in sea water, in salty or in waste water in areas not suitable for agriculture and can also serve as biofilters for flue gas emitted by power plants and other carbon dioxide emitting industries. In addition, byproducts of microalgae following oil

extraction can be utilized as a source of protein for animal and fish feed, as fertilizers in agriculture and as sources for high-value pigments and vitamins.

Lipid composition and content vary between different microalgal species and depends on the different stages of the life cycle and environmental and culture
5 conditions. However, many microalgae have the ability to produce substantial amounts of TAGs under adverse environmental conditions. Indeed, the most common strategies to induce TAGs accumulation include stress induction e.g., nitrogen, sulfate or phosphate limitation, silicon limitation, low temperature or high light intensities. Thus, high levels of TAGs are accumulated only under growth limiting conditions
10 which maximize lipid productivity. These conditions, however, inhibit cell division and biomass production, resulting in overall limited lipid productivity per liter culture (Hu Q. et al., 2008. Plant J 54:621-639). As a result, the overall lipid productivity of most algal species per liter culture decreases at growth limiting conditions, due to the large decrease in biomass productivity.

15 Additional background art includes International Patent Application Publication No. WO 2008083352; U.S. Patent Application Publication No. U.S. 20120165490; and U.S. Patent Nos. 8557514, 8636815 and 8450090.

SUMMARY OF THE INVENTION

20 According to an aspect of some embodiments of the present invention there is provided a method of producing oil, the method comprising:

- (a) infecting *Emiliana huxleyi* microalgae with a Coccolithovirus EhV201; and
- (b) extracting the oil from the microalgae 24-72 hours following the
25 infecting,
thereby producing oil.

According to an aspect of some embodiments of the present invention there is provided a method of producing an algal lysate, the method comprising:

- (a) treating an *Emiliana huxleyi* microalgae with an agent that increases
30 triacylglycerols (TAGs) in the microalgae;
- (b) producing a lysate from the treated microalgae, the lysate being capable of increasing TAGs in the algal culture.

According to some embodiments of the invention, the agent is a pathogen.

According to some embodiments of the invention, the pathogen is a virus.

According to some embodiments of the invention, the virus is a lytic virus.

According to some embodiments of the invention, the lytic virus is a Coccolithovirus.

5 According to some embodiments of the invention, the Coccolithovirus virus is EhV201 or EhV86.

According to some embodiments of the invention, the pathogen comprise bacteria.

10 According to some embodiments of the invention, the bacteria comprise Roseobacter bacteria.

According to some embodiments of the invention, the method further comprises fractionating the lysate so as to obtain a fraction which is able to increase TAGs in the algae.

15 According to some embodiments of the invention, the fraction is pathogen-free.

According to some embodiments of the invention, the lysate consists of 100 kDa molecules or lower.

According to an aspect there is provided a composition of matter comprising a pathogen-free algal lysate capable of increasing TAGs content in an algal culture.

20 According to some embodiments of the invention, the pathogen-free algal lysate consists of molecules of 100 kDa or lower.

According to some embodiments of the invention, the composition further comprises a preservative.

25 According to some embodiments of the invention, the preservative is selected from the group consisting of antioxidants or protease inhibitors.

According to some embodiments of the invention, the algal culture comprises *Emiliana huxleyi* microalgae.

According to some embodiments of the invention, the extracting is effected following virion release.

30 According to some embodiments of the invention, the extracting is effected prior to virion release.

According to some embodiments of the invention, the method comprising extracting oil from virions released from the microalgae following the infecting.

According to some embodiments of the invention, the infecting is effected at a multiplicity of infection (MOI) of about 1 viral particle per 1 cell.

According to some embodiments of the invention, the infecting is effected in a microalgae bioreactor.

5 According to some embodiments of the invention, there is provided an oil produced according to the method.

According to an aspect of some embodiments of the present invention there is provided an *Emiliana huxleyi* microalgae oil having an increased content of triacylglycerols (TAGs); as compared to an oil of *Emiliana huxleyi* microalgae not
10 infected with a Coccolithovirus EhV201 and/or an oil of *Emiliana huxleyi* microalgae following nitrogen deprivation.

According to some embodiments of the invention, the TAGs comprise saturated and/or mono-unsaturated TAGs.

According to an aspect of some embodiments of the present invention there is
15 provided a method of producing biofuel, the method comprising:

(a) extracting oil from virus infected *Emiliana huxleyi* microalgae; and
(b) processing the oil by a reaction that splits the fatty acids chains of a triacylglycerol (TAG) comprised in the oil from its glycerin backbone,
thereby producing the biofuel.

20 According to some embodiments of the invention, the reaction is selected from the group consisting of transesterification, hydrocracking and hydrogenation.

According to some embodiments of the invention, the extracting is effected 24-72 hours following infection with the virus.

25 According to some embodiments of the invention, the extracting is effected following virion release.

According to some embodiments of the invention, the extracting is effected prior virion release.

30 According to some embodiments of the invention, the method comprising extracting oil from virions released from the microalgae following infection with the virus.

According to some embodiments of the invention, the microalgae were infected with the virus at a multiplicity of infection (MOI) of about 1 viral particle per 1 cell.

According to some embodiments of the invention, the microalgae were infected with the virus in a microalgae bioreactor.

According to some embodiments of the invention, the method comprising purifying the biofuel following the processing.

5 According to some embodiments of the invention, the method comprising isolating a glycerol following the processing.

According to some embodiments of the invention, there is provided a biofuel produced according to the method.

10 According to an aspect of some embodiments of the present invention there is provided a biofuel from *Emiliana huxleyi* microalgae having an increased content of saturated and/or mono-unsaturated fatty acids; as compared to biofuel from *Emiliana huxleyi* microalgae not infected with a virus and/or biofuel from *Emiliana huxleyi* microalgae following nitrogen deprivation.

According to some embodiments of the invention, the biofuel is biodiesel.

15 According to an aspect of some embodiments of the present invention there is provided a method of producing an algal cake, the method comprising:

- (a) infecting *Emiliana huxleyi* microalgae with a virus; and
 - (b) removing the oil from the microalgae,
- thereby producing cake.

20 According to some embodiments of the invention, the removing is effected 24-72 hours following the infecting.

According to some embodiments of the invention, there is provided an algal cake produced according to the method.

25 According to an aspect of some embodiments of the present invention there is provided an algal cake comprising virus infected *Emiliana huxleyi* microalgae.

According to some embodiments of the invention, the virus is a lytic virus.

According to some embodiments of the invention, the virus is a Coccolithovirus.

30 According to some embodiments of the invention, the Coccolithovirus is EhV201.

Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although methods and materials similar or equivalent to those

described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

Some embodiments of the invention are herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of embodiments of the invention. In this regard, the description taken with the drawings makes apparent to those skilled in the art how embodiments of the invention may be practiced.

In the drawings:

FIGs. 1A-B show graphs demonstrating the effect of viral infection on *E. huxleyi* cell survival. Host cell numbers (Figure 1A) and extracellular viral numbers (Figure 1B) are presented at the indicated time points following infection (hours post infection, denoted hereinafter hpi) with lytic or non-lytic virus as compared to non-infected control cells.

FIG. 2 shows lipidome chromatograms illustrating total lipidome analysis derived from *E. huxleyi* cultures 48 hpi with lytic or non-lytic virus as compared to non-infected control cells. The retention time area of the major lipid classes (described in Table 1) is marked and the number of species identified for each class is specified in brackets.

FIG. 3 shows principal component analysis (PCA) graphs illustrating distinct segregation in the lipid composition derived from *E. huxleyi* cultures infected with lytic or non-lytic virus as compared to non-infected control cells at the indicated time points following infection (hpi).

FIG. 4 is a clustergram representation of the alterations in the contents of 200 lipids derived from *E. huxleyi* cultures infected with lytic or non-lytic virus as compared to non-infected control cells at the indicated time points following infection (hpi). The right panel represents the association of the detected lipids to the five major lipid classes: phospholipids (PL), sphingolipids (SPL), betaine lipids (BL),

glycolipids (GL) and triacylglycerols (TAG). The change in the abundance of viral-derived sphingolipid (vGSL) is marked with an arrow.

FIGs. 5A-B are pie charts demonstrating that TAGs are over produced during lytic viral infection and accumulated in the isolated virions. Figure 5A illustrates the relative abundance of lipids associated with the major lipid classes derived from *E. huxleyi* cultures infected with lytic or non-lytic virus as compared to non-infected control cells at the indicated time points (hpi). Figure 5B illustrates the relative abundance of lipids associated with the major lipid classes derived from the isolated virions of the lytic (EhV201) virus 72 hpi.

FIG. 5C illustrates a transmission electron micrographs (TEM) of the sample of EhV201 virions after purification by filtration and concentration (see Methods). Scale of inset 200nm.

FIGs. 6A-D demonstrate that neutral lipids are accumulated in lipid droplets during lytic viral infection. Figure 6A shows fluorescent microscopy photomicrographs illustrating the accumulation of lipids in *E. huxleyi* cultures 24 and 48 hpi with lytic virus, as compared to non-infected control. The images are composites of bright field (BF) and BODIPY fluorescence (green) alone or combined with chlorophyll auto-fluorescence (red). Figure 6B is a graph illustrating neutral lipids levels in *E. huxleyi* cultures infected with lytic virus, as compared to non-infected control in the indicated time points following infection, as determined by flow cytometry with BODIPY staining. Figure 6C is a graph illustrating the area of high intensity BODIPY staining in control cells (blue) and cells infected with the lytic virus (red) plotted against the bright detail intensity of the BODIPY staining, as assessed by imaging flow cytometry. The two treatments were found to be significantly different (MANOVA, $F_{1,4026}=571$, $p < 0.001$). Figure 6D shows fluorescent microscopy photomicrographs acquired during imaging flow cytometry analysis of control cells or cells infected with the lytic virus for 48 hpi. Images are composites of bright field (BF), BODIPY fluorescence (green), chlorophyll auto-fluorescence (red) and a merged image.

FIG. 7 illustrates the abundance of TAGs with various saturation levels in *E. huxleyi* cultures 72 hpi with lytic virus (left panel) and in the isolated virions (right panel).

FIG. 8 illustrates the expression profile of genes encoding for TAGs biosynthesis proteins, 1 and 24 hpi with lytic virus as compared to non-lytic virus; indicating *de-novo* TAG biosynthesis during lytic viral infection.

FIGs. 9A-F are graphs demonstrating that lytic viral infection resulted in higher TAGs amounts and saturation levels compared to nitrogen starvation. *E. huxleyi* cells were subjected to lytic viral infection or nitrogen starvation for 96 hours and total lipidome was analyzed using LC-MC. Total lipid content (Figures 9A-B), TAGs levels (Figures 9C-D) and saturation level (Figures 9E-F) at the indicated time points following viral infection (Figures 9A, 9C and 9E) and nitrogen starvation (Figures 9B, 9D and 9F) are presented.

FIG. 10 shows calibration curves of TAG standards generated to calculate the data presented in Table 7, presenting the area under peak of each TAG versus the concentration.

FIGs. 11A-B are bar graphs showing that virus-free lysate (VFL) can mimic viral infection and induce TAGs formation without induction of cell death. Figure 11A depicts neutral lipids levels in *E. huxleyi* cultures, 24 hours after supplemented with either VFL or control lysate, as compared to untreated control culture or *E. huxleyi* cultures that were infected with lytic virus for 24h (24hpi), as determined by BODIPY staining in the flow cytometry. Figure 11B depicts cell death levels in *E. huxleyi* cultures, 24 hours after supplemented with either VFL or control lysate, as compared to untreated control culture or *E. huxleyi* cultures that were infected with lytic virus for 24h (24hpi), as assessed by Sytox green staining in the flow cytometry.

FIG. 12 is a bar graph showing that VFL can mimic viral infection and induce specific TAGs formation in *E. huxleyi*. The graph illustrating the level of 9 TAGs in *E. huxleyi* cultures, 72 hours after supplemented with VFL, as compared to untreated control culture or *E. huxleyi* cultures that were infected with lytic virus for 72h (72hpi), as determined by LC-MS.

FIG. 13 is a bar graph showing that VFL can mimic viral infection and induce TAGs formation in *E. huxleyi*. Relative abundance of 9 different TAGs as determined by LC-MS in control *E. huxleyi* cultures, in infected *E. huxleyi* cultures for 72 h or after supplemented with VFL for 72 h (Percent gated (%)).

DESCRIPTION OF SPECIFIC EMBODIMENTS OF THE INVENTION

The present invention, in some embodiments thereof, relates to algal oil and biofuel and method of producing same.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not necessarily limited in its application to the details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways.

Microalgae are recognized as an excellent source for the production of economically viable biofuel, such as biodiesel, that can serve as a possible alternative to petroleum-based fuels. In addition, byproducts of microalgae following oil extraction can be utilized as a source of protein for animal and fish feed, as fertilizers in agriculture and as sources for high-value pigments and vitamins. However, in most algal species high levels of triacylglycerides (TAGs) are accumulated only under growth limiting conditions such as nitrogen, sulfate or phosphate limitation, low temperature or high light intensities which maximize lipid productivity but on the other hand inhibit cell division and biomass production, resulting in overall limited lipid productivity per liter culture.

Whilst reducing the present invention to practice, the present inventors have uncovered that infection of *Emiliania huxleyi* microalgae by the Coccolithovirus EhV201 induces accumulation of highly saturated TAGs (i.e. TAGs composed of saturated and/or mono-unsaturated fatty acyl chains) in the infected microalgae and the released virions. The TAG content and the high proportion of saturated and monounsaturated fatty acids, is considered optimal for e.g., high quality biofuel and more specifically for biodiesel production.

Taken together, the present teachings suggest the use of viral infection in obtaining microalgae oil that can further be used for production of biofuel or in the food, feed and agricultural industries.

As is illustrated herein under and in the examples section, which follows, the present inventors have shown that EhV201 viral infection of *E. huxleyi* cultures induces growth arrest 24 hours post infection, accumulation of viral particles in the medium and subsequent lysis 72 hours post infection (Example 1, Figures 1A-B). Following, the present inventors have uncovered a significant increase in lipid abundance accompanied by a shift in lipid composition between *E. huxleyi* cultures

infected with the lytic virus EhV201 as compared to non-infected cultures or cultures infected with non-lytic virus (EhV163), evident starting 24 hours post infection. Specifically, EhV201 viral infection induced accumulation of TAGs, phospholipids (PL) and viral specific GSLs (Example 1, Figures 2-4 and Tables 2-3). Thus, for example, 48 hours post infection TAGs were found to account for 20 % of the total detected lipids in the EhV201-infected cells, in contrary to only 2 % in the non-infected and the EhV163-infected cells (Example 1, Figure 5A). Lipidome analysis of the purified released virions demonstrated that the viral particles were enriched with TAGs as well (Example 1, Figures 5B-5C and Table 4). Furthermore, the present inventors have shown that TAGs accumulated in the EhV201-infected cells and in the purified virions were significantly enriched with saturated and mono-unsaturated TAGs (Example 1, Figure 7). These results were further supported by an increase in total non-polar lipids, the level of lipid droplets during infection and with the up-regulation of genes encoding for the *de-novo* TAG biosynthesis (Example 1, Figures 6A-D and 8 and Table 5).

In addition, the present inventors have shown that TAGs abundance and saturation level were higher during EhV201 viral infection as compared to nitrogen starvation which is commonly used to enhance TAGs production for biofuel (Example 2, Figures 9A-F, 10 and Tables 6 and 7). Hence, the method of the present invention is advantageous over commonly used method for inducing TAGs accumulation by nutrient starvation such as nitrogen starvation.

The present inventors further shown that the induction of TAGs formation can also be done by supplementing an algal culture with a virus-free lysate (VFL) prepared by isolating a 100 kDa fraction of algal culture infected with the virus (Figures 11-13). This is of significance value as the induction of TAGs without free virions substantially reduces cell death (Figure 11B).

Consequently, the present teachings provide *E. huxleyi* microalgae oil enriched with TAGs, its uses and methods of producing same.

Thus, according to a first aspect of the present invention, there is provided a method of producing oil, the method comprising:

(a) infecting *Emiliania huxleyi* microalgae with a Coccolithovirus EhV201; and

(b) extracting the oil from said microalgae 24-72 hours following said infecting,
thereby producing oil.

As used herein, the term “*Emiliana huxleyi* (*E. huxleyi*) microalgae” refers to a species within the Coccolithophores which are a class of unicellular eukaryotic microalgae belonging to the phylum *haptophytes*, and may possess calcium carbonate plates (or scales) called coccoliths. According to specific embodiments, the *E. huxleyi* is of a strain selected from the group consisting of CCMP2090, 374, 92F, 1516, CCMP 1516, or RCC 1216 [Allen et al., Environmental Microbiology, 9(4) 971-982 (2007)]. According to a specific embodiment, the *E. huxleyi* is CCMP2090. According to a specific embodiment, the *E. huxleyi* strain is infectable by EhV201.

As used herein the term “Coccolithovirus *E. huxleyi* virus 201”, “Coccolithovirus EhV201” or “EhV201” refers to an EhV201 strain of a dsDNA lytic virus within the monophyletic *Phycodnaviridae* capable of infecting *E. huxleyi*. Following infection the EhV201 can replicate, produce virions, induce lysis of the infected *E. huxleyi* cell membrane and release the progeny virions which can infect other *E. huxleyi* cells. According to specific embodiments the infection cycle ranges between 4-120 hours, 4-96 hours, 4-72 hours, 20-96 hours, 20-72 hours, 48-120 hours or 48-96 hours.

As used herein, the terms “infecting”, “infection” and “infected”, which are used interchangeably, refer to the step of incubating *E. huxleyi* microalgae with a virus under conditions which allow the virus to invade the microalgae. According to a specific embodiment, the conditions include ratios of the number of virus particles to the number of target algal cells [i.e. multiplicity of infection (MOI)] used for infection which include but are not limited to at least 1 viral particle per 100 cells, at least 1 viral particle per 10 cells, at least 1 viral particle per 1 cell, at least 5 viral particles per 1 cell, at least 10 viral particles per 1 cell or at least 100 viral particles for 1 cell.

According to specific embodiments, the infection is effected at a multiplicity of infection (MOI) of 0.5-10 viral particles per 1 cell.

According to specific embodiments, the infection is effected at a multiplicity of infection (MOI) of about 1 viral particle per 1 cell.

Methods of evaluating viral infection are well known in the art and include determining viral adsorption by e.g., counting free viral particles immediately after viral addition and at early time points (e.g., 30 minutes) following viral addition; determining viral replication activity by e.g., DNA sequencing and Southern blot analysis; determining viral lytic activity by e.g., optical density and dye indicators for viability.

According to specific embodiments, the infection is effected in a microalgae bioreactor.

As used herein, the term “bioreactor” refers to a structure that supports the growth of *E. huxleyi* microalgae. The bioreactor may be a sterile or a non-sterile bioreactor. According to specific embodiments, the bioreactor is a non-sterile bioreactor. Typically *E. huxleyi* microalgae cultivation requires water, carbon dioxide, light and minerals (also referred to herein as growth medium). According to specific embodiments the bioreactor contains at least 100 liters, at least 1 cube, at least 100 cubes or at least 1000 cubes of growth medium comprising *E. huxleyi* microalgae.

Any algal bioreactor known in the art can be used including open or closed bioreactor operated in a batch, semi-batch or a continuous mode (see e.g., U.S. Department of Energy, 2009 *National Algal Biofuels Technology Roadmap*. Washington, D.C.: U.S. Department of Energy; and Sheehan, J. et al., (1998). *A Look Back at the U.S. Department of Energy's Aquatic Species Program - Biodiesel from Algae*. Golden: National Renewable Energy Laboratory; and Benemann, J. R. (2008). *Open Ponds and Closed Photobioreactors – Comparative Economics*. 5th Annual World Congress on Industrial Biotechnology & Bioprocessing. Chicago).

According to specific embodiments, the bioreactor is an open bioreactor.

As used herein, the term “open bioreactor” refers to an uncovered pond, in which the surface of the growth media is directly exposed to the surrounding environment. In a special type of pond, called a raceway pond or high-rate pond, growth media is circulated to provide mixing.

According to specific embodiments, the bioreactor is a closed bioreactor. As used herein, the term “closed bioreactor” refers to a closed system which partially isolates the microalgae by circulating growth media through a system of tubes or other containers.

As used herein, the term “batch mode” refers to a process in which a reactor is filled with a growth medium, inoculated with microalgae, and then left to grow.

As used herein, the term “semi-batch mode” refers to a process in which a reactor is filled with a growth medium, inoculated with microalgae, and then a
5 fraction of the reactor volume is replaced during each reactor cycle.

As used herein, the term “continuous mode” refers to a process in which a reactor is inoculated with microalgae and then fresh media is added multiple times following predetermined time points while bioreactor fluid containing waste products is constantly removed following predetermined time points, while leaving at least an
10 inoculum.

Typically, the bioreactor contains growth medium which comprises nutrients required for the growth of the *E. huxleyi* microalgae. According to specific embodiments, the growth medium is a liquid medium. The growth medium used by the present invention can be a water-based medium which includes a combination of
15 substances such as salts, nutrients, minerals, vitamins, amino acids, nucleic acids, proteins and small molecules all of which are needed for microalgae proliferation and survival. According to specific embodiments the growth medium is based on seawater, such as disclosed for example in Keller et al., Journal of Phycology (1987) 23, 633-638. According to other specific embodiments, the growth medium is based
20 on municipal wastewater. For example, a culture medium can be a synthetic growth medium such as K/2 medium or F/2 medium, as described by Keller et al., Journal of Phycology (1987) 23, 633-638 and can be purchased from e.g., Florida-Aqua-Farms.

As mentioned, following infection, oil is extracted.

As used herein the term “extracting oil” refers to the process in which oil is
25 removed from the infected microalgae and/or the virions released from same. Exemplary time ranges for extracting the oil following infection include but are not limited to 12-72 hours post infection, 20-72 hours post infection, 24-72 hours post infection, 24-48 hours post infection, 48-72 hours post infection, 24-96 hours post infection, or 48-96 hours post infection.

30 According to specific embodiments the oil is extracted from the infected microalgae 24-72 hours following infection.

According to specific embodiments the oil is extracted from the infected microalgae following virion release.

According to specific embodiments the oil is extracted from the infected microalgae prior to virion release.

Oil or crude oil refers to the hydrophobic liquid containing TAGs which can be extracted from virus infected *E. huxleyi* microalgae (e.g., lytic Coccolithovirus such as EhV201) and/or from virions (e.g., Coccolithovirus virions such as EhV201 virions) released from *E. huxleyi* microalgae. According to a specific embodiment oil refers to crude oil (e.g., emulsions) obtained by extraction without further steps of enrichment for specific lipids or refinement or manipulation (e.g., by splitting).

As used herein the term “oil” includes derivatives thereof, including racemic mixtures, enantiomers, diastereomers, hydrates, salts, solvates, metabolites, analogs, and homologs.

As used herein the term “triacylglyceride (TAG)” refers to a neutral lipid composed of a glycerol molecule esterified with three fatty acids. A TAG may comprise saturated, mono-unsaturated or polyunsaturated fatty acids. Methods of determining TAGs amount and composition are known in the art and include chromatography such as high performance liquid chromatography (HPLC) and Mass spectrometry (MS) analysis.

As used herein, the term “fatty acid” refers to a carboxylic acid having an aliphatic tail which is either saturated or unsaturated. The fatty acid may be in a free stated (i.e. non-esterified) or in an esterified form such as part of a TAG.

As used herein, the term “saturated fatty acid” refers to a fatty acid with no double bonds along the carbon chain.

As used herein, the term “mono-unsaturated fatty acid” refers to a fatty acid with one carbon-carbon double bond along the carbon chain.

As used herein, the term “polyunsaturated fatty acid” refers to a fatty acid with more than one carbon-carbon double bond along the carbon chain. As used herein, the terms “glycerol” and “glycerin”, which are interchangeably used, refer to an organic trihedral alcohol with the formula $C_3H_5(OH)_3$.

According to specific embodiments the TAGs comprise saturated and/or mono-unsaturated fatty acids, i.e. saturated and/or mono-unsaturated TAGs.

According to specific embodiments the mono-unsaturated TAGs comprise TAG46:1, TAG48:1 and TAG50:1.

Methods of extracting oil from microalgae are known in the art such as disclosed in e.g., Nagle, N. and Lemke, P. (1989) *Microalgal Fuel Production Processes: Analysis of Lipid Extraction and Conversion Methods*, Aquatic Species Program Annual Report 1989, SERI/SP-231-3579. Golden, CO: Solar Energy
5 *Research Institute*; US Patent No. US 8,313,648; and International Application Publication No. WO 2013005209.

According to specific embodiments the microalgae biomass is concentrated prior to oil extraction. Concentration can be accomplished by e.g., filtration, screening, centrifugation, flotation or sedimentation which can be combined with
10 coagulation and flocculation (see e.g., Benemann, et al., *Algae Biomass* (1980) 457-495; and Metcalf and Eddy. (2003). *Wastewater Engineering: Treatment and Reuse*. New York: McGraw-Hill).

Exemplary oil extraction methods involve cell lysis by mechanical, thermal, enzymatic or chemical methods. These methods result in emulsions, requiring an
15 expensive cleanup process. The emulsion is a complex mixture, containing neutral lipids, polar lipids, proteins, and other algal products, necessitating refining processes to isolate the neutral lipids (e.g., TAGs).

Thus, for example mechanical cell disruption may be effected by bead milling, homogenizing or sonication (see e.g., Doucha, J., & Livansky, K. (2008).
20 *Biotechnological Products and Process Engineering* , 431-440; and Shuler, M. L. (2002). *Bioprocess Engineering: Basic Concepts*. Upper saddle River, NJ: Prentice-Hall, Inc.).

Other exemplary oil extraction methods include, but not limited to, the use of solvents or chemicals to extract lipids from a growing algal culture. Thus, for example
25 the Bligh and Dyer method or a variation thereof, of lipid extraction uses a chloroform-methanol solvent system (Bligh & Dyer, 1959; and Van Mooy et al., (2006), Proc. Natl. Acad. Sci. USA 103, 8607). The Soxhlet extraction uses hexane as the standard extraction solvent. At industrial scale, various mixtures of short-chain alcohols and alkanes are more commonly used for extractions, for example,
30 methylene chloride. Following extraction the solvent can be removed by e.g., a reduced pressure distillation.

According to specific embodiments, the TAG fraction or a specific TAG population may be separated from the oil by e.g., preparative chromatography, such

as high performance liquid chromatography (HPLC) or with solid phase extraction methods (SPE).

According to specific embodiments, at least 70 %, at least 80 %, at least 90 %, at least 95 % or at least 99 % of the purified fraction is TAGs.

5 As the present inventors have uncovered that EhV201 virions released from the *E. huxleyi* microalgae are characterized by a modified oil composition (i.e. enriched with TAGs), the present teachings suggest extracting the oil from the released virions.

Thus, according to specific embodiments, the method comprising extracting oil
10 from virions released from the microalgae following viral infection.

Methods of extracting oil from viruses are known in the art such as disclosed e.g., in Kalvodova et al., J Virol. 2009 Aug; 83(16): 7996–8003; US Patent No: US 8,506,968; and International Application Publication No. WO 2005101966; and include the use of solvents effective in solubilizing lipids in the viral envelope such as
15 alcohols, hydrocarbons, amines, ethers, phenols, esters, halohydrocarbons, halocarbons and combinations thereof. Exemplary solvent systems are the Bligh and Dyer method or a variation thereof, as described hereinabove, or the Folch extraction as described by Folch et al., J Biol Chem 1957, 226, 497.

According to specific embodiments, the virions are purified from the algal cells
20 before oil extraction.

Methods of isolating virions are known in the art and include density gradient separation (e.g., OptiPrep gradient) such as disclosed e.g., in Lawrence JE, Steward GF. Purification of viruses by centrifugation. In: Wilhelm SW, Weinbauer MG, Suttle CA, editors. Manual of aquatic viral ecology. College Station, TX, USA: ASLO; 2010.
25 pp. 166–181.

According to specific embodiments, following purification the virions are present in a preparation which is devoid of intact algal cell, e.g., less than 30 %, less than 20 %, less than 15 %, less than 10 %, less than 5 % or less than 1 % algal cells.

According to other specific embodiments, following purification the algal cells
30 are present in a preparation which is devoid of intact virions, e.g., less than 30 %, less than 20 %, less than 15 %, less than 10 %, less than 5 % or less than 1 % virions.

The microalgae oil generated according to the above teachings is characterized by a modified lipid composition.

Thus, according to an aspect of some embodiments of the invention, there is provided oil produced by the method of some embodiments of the invention.

According to another aspect of the present invention, there is provided an *Emiliana huxleyi* microalgae oil having an increased content of TAGs; as compared
5 to an oil of *Emiliana huxleyi* microalgae not infected with a Coccolithovirus EhV201 and/or an oil of *Emiliana huxleyi* microalgae following nitrogen deprivation.

According to specific embodiments the increased content of TAGs in the oil is of at least 5 %, at least 10 %, at least 20 %, at least 30 %, at least 40 %, at least 50 %, at least 60 %, at least 70 %, at least 80 %, at least 90 % or more than 100 % higher
10 than that found in an oil of *Emiliana huxleyi* microalgae of the same strain not infected with a Coccolithovirus EhV201 and/or an oil of *Emiliana huxleyi* microalgae of the same strain following nitrogen deprivation, as determined by e.g., HPLC and/or MS.

As according to specific embodiments the TAGs comprise saturated and/or
15 mono-unsaturated TAGs, according to specific embodiments the increased content of saturated and/or mono-unsaturated TAGs in the oil is of at least 5 %, at least 10 %, at least 20 %, at least 30 %, at least 40 %, at least 50 %, at least 60 %, at least 70 %, at least 80 %, at least 90 % or more than 100 % higher than that found in an oil of *Emiliana huxleyi* microalgae of the same strain not infected with a Coccolithovirus
20 EhV201 and/or an oil of *Emiliana huxleyi* microalgae of the same strain following nitrogen deprivation, as determined by e.g., HPLC and/or MS.

According to specific embodiments, the increased content of TAGs and/or the increased content of saturated and/or mono-unsaturated TAGs is determined in the crude oil (e.g., emulsions) obtained by extraction without further steps of enrichment
25 for specific lipids or refinement or manipulation (e.g., by splitting).

As used herein, the term “nitrogen deprivation” refers to nitrogen conditions which are less than optimal, in the case of *E. huxleyi* below 300 μ M. Exemplary time ranges for nitrogen deprivation include, but are not limited to, 96 hours, 72 hours, 48 hours and 24 hours.

30 According to specific embodiments the nitrogen deprivation is effected in the absence of nitrogen for 72 hours.

As used herein, the term “nitrogen” refers to nitrogen that can be assimilated by *E. huxleyi* microalgae to produce proteins.

The remaining mass of algal cells following oil removal, which comprise proteins and carbohydrates, can be dried and pressed into *E. huxleyi* microalgae cake.

As mentioned, the present inventors have further uncovered that a lysate of an algal culture free of viruses is sufficient to increase TAGs formation in an algal culture
5 treated therewith.

Thus according to a further aspect of the invention there is provided a method of producing an algal lysate, the method comprising

- (a) treating a microalgal microalgae culture (e.g., *E. huxleyi*) with an agent capable of increasing triacylglycerols (TAGs) in the microalgae
- 10 (b) producing a lysate from the agent treated [e.g., virus-infected algal culture or bacterial-infected algal culture, or from algal culture growing under specific conditions known to induce TAGs], the lysate being capable of increasing TAGs in the algal culture.

Examples of agents that can be used to increase the TAGs content in the
15 microalgae include, but are not limited to, a pathogenic agent and or a growth condition.

Examples of pathogenic agents include a viral infection or a bacterial infection.

According to some embodiments, the agent is a pathogen.

According to some embodiments, the pathogen is a virus.

20 According to some embodiments, the virus is a lytic virus.

According to some embodiments, the lytic virus is a Coccolithovirus.

According to some embodiments, the Coccolithovirus virus is EhV201 or EhV86.

According to some embodiments, the pathogen is bacteria.

25 According to some embodiments, the bacteria comprise Roseobacter bacteria.

According to some embodiments the bacteria is Sulfitobacter sp. or Phaeobacter sp.

Examples of growth conditions include, but are not limited to nitrogen limitation, stationary phase and other stress conditions. Typically, Oleaginous algae
30 produce TAGs during Stationary phase (i.e aging culture) or when placed under stress conditions imposed by chemical or physical environmental stimuli like nutrient starvation (e.g. nitrogen limited medium). Additional factors that may affect TAG formation include salinity and growth-medium pH and also temperature and light

intensity (Hu *et al.* 2008 Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. *Plant J* 54, 621-639).

The treatment may cause the lysis and thus lysate formation.

5 Infection may be effected according to the teachings provided herein or as known to those of skills in the art.

As used herein “lysate” refers to a preparation that does not include intact organisms or viable or replicative organisms such as virions, bacteria and/or algae.

As used herein “virus/bacteria-free algal lysate” or “VFL”/ “BFL” refers to an algal free, virus free, bacterial free preparation. The lysate may be a result of a lytic
10 virus or pathogenic bacteria or a result of an artificial (man-made) induced lysis of algal cells.

As used herein “free” refers to below 1-10, 10-100, 100-1000 virions per mL, or below 1-10, 10-100, 100-1000 intact algal cells per mL or below 1-10, 10-100, 100-1000 intact bacteria per mL.

15 In order to produce an active preparation, the lysate may be further subjected to fractionation so as to obtain a fraction which is able to increase TAGs in said algae. These methods are typically based on fractionation of the original VFL according to size (by filtration or by size-exclusion chromatography), polarity/hydrophobicity, charge or other chemical properties.

20 Methods of qualifying such fractions are known in the art and are further described as an exemplary embodiment (though generally applied) in Example 3 of the Examples section.

According to a specific embodiment, the virus-free algal lysate consists of 100 kDa molecules or lower. It may be supplemented with other macromolecules (above
25 100 kDa) from a heterologous source (i.e., not the virus of the algae-infected therewith).

Thus, according to a further aspect of the invention, there is provided a composition of matter comprising a virus-free algal lysate (e.g., belonging to Coccolithophores, to Diatoms, to Nannochloropsis, or to other microalgae) capable of
30 increasing TAGs content in an algal culture.

As mentioned, according to a specific embodiment, the composition comprises a virus-free algal lysate (VFL) or bacteria-free algal lysate (BFL) consisting of

molecules of 100 kDa or lower. However, molecules of higher molecular weight may also be present in the composition.

According to a specific embodiment, the composition may further comprise an agent such as a preservative.

5 The composition may be used fresh or stored e.g., cryo-preserved or kept in the dark.

According to a specific embodiment, the preservative is selected from the group consisting of antioxidants or protease inhibitors.

10 The compositions described herein (e.g., comprising the VFL/BFL) can be used to increase TAGs content in an algal culture, negating the need for infection and reducing algal death (as a result of pathogen infection) significantly.

As used herein “reduced cell death” refers to less than 10%, 20%, 30%, dying cells out of the total cells, as determined by as assessed by methods well known in the art e.g., Sytox green staining in flow cytometry

15 Thus, according to a further aspect of the invention, there is provided a method of increasing content of triacylglycerols (TAGs) in algae, the method comprising treating an algal culture (belonging to Coccolithophores, Diatoms, Nannochloropsis, or to other microalgae) with the composition comprising the VFL, as described herein, thereby increasing content of triacylglycerols (TAGs) in algae.

20 According to a specific the VFL may be of an algal source identical to the algal culture treated therewith (i.e., same algal species).

According to a specific the VFL may be of an algal source different than the algal culture treated therewith (i.e., different algal species).

Any algal culture stage can be treated with the composition described herein.

25 According to a specific embodiment, the algal culture is at the exponential stage.

According to a specific embodiment, the algal culture is at the stationary stage.

Also provided herein is an oil or a cake produced using the VFL, as described herein.

30 Thus, also provided is a method of producing oil without viral infection, the method comprising:

(a) treating an algal culture with the composition (comprising the VFL, as described herein), thereby increasing content of triacylglycerols (TAGs) in algae; and

- (b) extracting the oil from said algal culture.

According to some embodiments of the invention description of the oil and methods of extraction provided herein apply in full to this aspect as well.

5 Thus, according to another aspect of the present invention there is provided a method of producing an algal cake, the method comprising:

- (a) infecting *Emiliania huxleyi* microalgae with a virus; and
(b) removing the oil from said microalgae,
thereby producing cake.

10 Alternatively or additionally, there is provided a method of producing an algal cake, the method comprising:

- (a) producing oil according to the method described herein (i.e., virus infection or treating with an agent); and
(b) removing the oil from said microalgae,
15 thereby producing cake.

As used herein, the term “algal cake” refers to a microalgae product obtained as the residue from oil extraction. The cake can be used for e.g., animal feed, fish feed, nutritional supplements (e.g., vitamins and antioxidants), fertilizer, a dry fuel (i.e. “green coal”), or in the generation of bioethanol via carbohydrate fermentation.

20 Oil removal is effected by extracting the oil from the microalgae as disclosed in details hereinabove.

According to specific embodiments, oil removal is effected 24-72 hours following infection.

25 According to specific embodiments the virus is a lytic virus. As used herein, the term “lytic virus” refers to a virus capable of infecting *E. huxleyi* and follows the lytic pathway. Following infection the lytic virus can replicate, produce virions, induce lysis of the infected *E. huxleyi* cell membrane and release the progeny virions which can infect other *E. huxleyi* cells.

According to specific embodiments, the virus is a Coccolithovirus.

30 As used herein the term “Coccolithovirus” refers to a dsDNA virus within the monophyletic *Phycodnaviridae* capable of infecting *E. huxleyi*. According to specific embodiments, the Coccolithovirus is a Coccolithovirus *E. huxleyi* virus (EhV). According to specific embodiments the Coccolithovirus is a lytic Coccolithovirus.

According to specific embodiments, the Coccolithovirus is selected from the group consisting of EhV1, EhV84, EhV86, EhV88, EhV163, EhV201, EhV202, EhV203, EhV204, EhV205, EhV206, EhV207, EhV208, EhV209, EhV-V2 and EhVice.

According to specific embodiments, the Coccolithovirus is EhV201.

5 The microalgae cake generated according to the above teachings is characterized by the presence of viral (e.g., lytic Coccolithovirus e.g., EhV201) DNA.

Thus, according to an aspect of some embodiments of the invention, there is provided an algal cake produced by the method of some embodiments of the invention.

10 According to another aspect of the present invention there is provided an algal cake comprising virus infected *Emiliana huxleyi* microalgae. Methods of determining the presence of viral DNA (e.g., EhV201) in the cake are well known in the art and include e.g., PCR, DNA sequencing and Southern blot.

Also contemplated are processed products of the microalgae oil of some
15 embodiments of the invention including but not limited to biofuel, oil for food, food additive, feed, nutritional supplement, cosmetics, soap, detergent, candle, paint, a personal care product, a medicinal product and a veterinary product.

Thus, according to an aspect of the present invention there is provided a method of producing biofuel, the method comprising:

- 20 (a) extracting oil from virus infected *Emiliana huxleyi* microalgae; and
 (b) processing said oil by a reaction that splits the fatty acids chains of a triacylglycerol (TAG) comprised in said oil from its glycerin backbone, thereby producing the biofuel.

Alternatively or additionally, there is provided a method of producing biofuel,
25 the method comprising:

- (a) producing oil as described herein (e.g., virus infection or treatment with an agent); and
 (b) processing said oil by a reaction that splits the fatty acids chains of a triacylglycerol (TAG) comprised in said oil from its glycerin backbone,
30 thereby producing the biofuel.

As used herein the term “biofuel” refers to an organic fuel derived from *E. huxleyi* microalgae. According to specific embodiments, the biofuel is biodiesel.

As used herein, the term “biodiesel” refers to monoalkyl (methyl, propyl or ethyl) esters of long chain fatty acids derived from lipids (e.g., TAGs) of *E. huxleyi* microalgae, which can be used in diesel-engine vehicles. The properties of biodiesel, such as ignition quality, cold-flow properties and oxidative stability, are largely
5 determined by the structure of its component fatty acid esters. For example, saturated fats produce a biodiesel with superior oxidative stability and a higher cetane number, but rather poor low-temperature properties. Biodiesels produced using these saturated fats are more likely to gel at ambient temperatures.

Typically, methods of producing biofuel (e.g., biodiesel) from *E. huxleyi*
10 microalgae comprise extracting oil from the microalgae and subjecting the oil to a reaction that splits the fatty acids chains of a triacylglycerol (TAG) comprised in the oil from its glycerin backbone. Such reactions are well known in the art and are disclosed e.g., in Gardner: Chapter 8: Oil Seed and Algal Oils as Biofuel Feedstocks 121-143; US Patent No. US 8313648; and US Application Publication No US
15 20100081835, the contents of which are incorporated herein by reference in their entirety.

According to specific embodiments the reaction is selected from the group consisting of transesterification, hydrocracking and hydrogenation.

As used herein, the term “transesterification” refers to the process of reacting a
20 TAG molecule with an excess of alcohol, typically methanol, in the presence of a catalyst to produce glycerin and alkyl esters (i.e. biodiesel). Transesterification can be accomplished by using traditional chemical processes such as acid or base catalyzed reactions [such as sulfuric acid (H_2SO_4) and hydrochloric acid (HCl)] or by using enzyme-catalyzed reactions (such as lipase). See e.g., Nagle, N. and Lemke, P.
25 (1989) Microalgal Fuel Production Processes: Analysis of Lipid Extraction and Conversion Methods, Aquatic Species Program Annual Report 1989, SERI/SP-231-3579. Golden, CO: Solar Energy Research Institute; and Park et al., Bioresour Technol. (2015)184:267-75, the contents of which are incorporated herein by reference in their entirety.

30 It has been proposed that direct transesterification can be used to produce biodiesel directly from wet microalgal biomass without the need for the oil extraction step (e.g., Park et al., Bioresour Technol. (2015)184:267-75). Thus, according to another aspect of the present invention there is provided a method of producing

biofuel, the method comprising transesterifying *Emiliania huxleyi* microalgae infected with a lytic Coccolithovirus, thereby producing the biofuel.

As used herein, the term “hydrocracking” refers to a catalytic cracking process assisted by the presence of an elevated partial pressure of hydrogen gas. The process
5 employs high pressure, high temperature, a catalyst, and hydrogen. Hydrocracking is normally facilitated by a bifunctional catalyst that is capable of rearranging and breaking hydrocarbon chains as well as adding hydrogen to aromatics and olefins to produce naphthenes and alkanes. The products of this process are saturated hydrocarbons that can be used as e.g., jet fuel. See e.g., US Patent No. US 8313648,
10 the contents of which are incorporated herein by reference in their entirety.

As used herein, the term “hydrogenation” refers to a process comprising hydrogenation of the C=C bonds and further reaction with hydrogen to split the fatty acids chains from the glycerin backbone. The saturated fatty acid chains can undergo dehydration, decarbonylation or decarboxylation reactions to produce normal alkanes
15 that can be used as renewable diesel or renewable jet fuel, sometimes branded “green diesel” or “green jet”. The glycerol backbone is hydrogenated to propane so there is substantially no glycerol produced as a byproduct. See e.g., Huber (2007) Applied Catalysis A: General. 329:120-129; and US Patent No. US 8636815, the contents of which are incorporated herein by reference in their entirety.

Following, the biofuel may be isolated and purified. Thus, according to
20 specific embodiments the method comprising purifying the biofuel following the processing. Methods of purifying the biofuel (e.g., biodiesel) are known in the art and disclosed e.g., in Gerpen, J. V. (2005). *Fuel Processing Technology*, 1097-1107. According to specific embodiments, the glycerol is isolated from the biofuel
25 following the processing. Glycerol is denser than biodiesel and can be drained out of a reactor. Other impurities can be removed by washing the product with water, using ion exchange resins or solid adsorbents. Residual methanol can be removed by distillation.

The glycerol obtained according to the methods of some embodiments of the
30 present invention can further be used in the production of e.g., food, food additive, feed, nutritional supplement, cosmetic, soap, detergent, toothpaste, explosives, candle, paint, tobacco, emulsifiers, a personal care product, a medicinal product and a veterinary product.

The biofuel generated according to the above teachings is characterized by a modified fatty acid composition.

Thus, according to an aspect of some embodiments of the invention, there is provided a biofuel produced by the method of some embodiments of the invention.

5 According to another aspect of the present invention there is provided a biofuel from *Emiliana huxleyi* microalgae having an increased content of saturated and/or mono-unsaturated fatty acids; as compared to biofuel from *Emiliana huxleyi* microalgae not infected with a virus and/or biofuel from *Emiliana huxleyi* microalgae following nitrogen deprivation.

10 According to specific embodiments the increased content of saturated and/or mono-unsaturated fatty acids in the biofuel is of at least 5 %, at least 10 %, at least 20 %, at least 30 %, at least 40 %, at least 50 %, at least 60 %, at least 70 %, at least 80 %, at least 90 % or more than 100 % higher than that found in biofuel from *Emiliana huxleyi* microalgae of the same strain not infected with a virus (e.g., lytic
15 Coccolithovirus e.g., EhV201) and/or biofuel from *Emiliana huxleyi* microalgae of the same strain following nitrogen deprivation, as determined by e.g., HPLC and/or MS.

As mentioned, the properties of biodiesel are largely determined by the structure of its component fatty acid esters. Thus, as the biofuel of the present
20 invention is characterized by increased content of saturated and/or mono-unsaturated fatty acids, according to specific embodiments, the biofuel (i.e. biodiesel) of the present invention present a better quality biofuel demonstrating e.g., higher oxidative stability and higher cetane number as compared to biofuel from *Emiliana huxleyi* microalgae not infected with a virus (e.g., lytic Coccolithovirus e.g., EhV201) and/or
25 biofuel from *Emiliana huxleyi* microalgae following nitrogen deprivation.

It is expected that during the life of a patent maturing from this application many relevant methods of producing biofuel from algal oil will be developed and the scope of the phrase "reaction that splits the fatty acids chains of a TAG comprised in the oil from its glycerin backbone" is intended to include all such new technologies *a*
30 *priori*.

As used herein the term "about" refers to ± 10 %.

The terms "comprises", "comprising", "includes", "including", "having" and their conjugates mean "including but not limited to".

The term "consisting of" means "including and limited to".

The term "consisting essentially of" means that the composition, method or structure may include additional ingredients, steps and/or parts, but only if the additional ingredients, steps and/or parts do not materially alter the basic and novel characteristics of the claimed composition, method or structure.

As used herein, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a compound" or "at least one compound" may include a plurality of compounds, including mixtures thereof.

Throughout this application, various embodiments of this invention may be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

Whenever a numerical range is indicated herein, it is meant to include any cited numeral (fractional or integral) within the indicated range. The phrases "ranging/ranges between" a first indicate number and a second indicate number and "ranging/ranges from" a first indicate number "to" a second indicate number are used herein interchangeably and are meant to include the first and second indicated numbers and all the fractional and integral numerals therebetween.

As used herein the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts.

When reference is made to particular sequence listings, such reference is to be understood to also encompass sequences that substantially correspond to its complementary sequence as including minor sequence variations, resulting from, e.g.,

sequencing errors, cloning errors, or other alterations resulting in base substitution, base deletion or base addition, provided that the frequency of such variations is less than 1 in 50 nucleotides, alternatively, less than 1 in 100 nucleotides, alternatively, less than 1 in 200 nucleotides, alternatively, less than 1 in 500 nucleotides, alternatively, less than 1 in 1000 nucleotides, alternatively, less than 1 in 5,000 nucleotides, alternatively, less than 1 in 10,000 nucleotides.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination or as suitable in any other described embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

Various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below find experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions illustrate some embodiments of the invention in a non limiting fashion.

Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific American Books, New York; Birren et al., (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202;

4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Culture of Animal Cells - A Manual of Basic Technique" by Freshney, Wiley-Liss, N. Y. (1994), Third Edition; "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al., (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994);
 5 Mishell and Shiigi (eds), "Selected Methods in Cellular Immunology", W. H. Freeman and Co., New York (1980); available immunoassays are extensively described in the patent and scientific literature, see, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262;
 10 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521; "Oligonucleotide Synthesis" Gait, M. J., ed. (1984); "Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and Translation" Hames, B. D., and Higgins S. J., eds. (1984); "Animal Cell Culture" Freshney, R. I., ed. (1986); "Immobilized Cells and Enzymes"
 15 IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR Protocols: A Guide To Methods And Applications", Academic Press, San Diego, CA (1990); Marshak et al., "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996); all of which are incorporated by reference as if fully set
 20 forth herein. Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader. All the information contained therein is incorporated herein by reference.

25 MATERIALS AND METHODS

Culture Growth and Viral Infection - Cells of the non-calcifying *Emiliania huxleyi* (*E. huxleyi*) strain CCMP2090 were cultured in K/2 medium (Keller et al., 1987) and incubated at 18 °C with a 16 : 8 hours light / dark illumination cycle. A light intensity of 100 μM photons / m^2s was provided by cool white fluorescent lights.
 30 For N-depletion conditions, cells were gently pelleted by centrifugation for 10 minutes at 3,000 g and re-suspended in K/2 medium (control) or modified K/2 not containing NaNO_3 and NH_4Cl (N-depletion). *E. huxleyi* virus EhV201 (lytic) and EhV163 (non-lytic) (Schroeder et al., 2002) were used to infect *E. huxleyi* cultures

using a 1 : 100 volumetric ratio of viral lysate to culture (multiplicity of infection of ~1 : 1 viral particles per cell). All experiments were performed with exponential phase cultures (5×10^5 to 10^6 cells / ml).

Enumeration of Cell and Virus Abundance - Cells were monitored and
5 quantified using an Eclipse (iCyt) flow cytometer, equipped with 405- and 488- nm solid state-air cooled lasers (both 25 mW on the flowCell) and standard filter set-up. Algae were identified by plotting chlorophyll fluorescence in the red channel (737-663 nm) versus green fluorescence (500-550 nm) or side scatter. For extracellular viral production, samples were fixed with glutaraldehyde at a final concentration of
10 0.5 % for 30 min at 4 °C, followed by freezing in liquid nitrogen and storage at -80 °C until analysis. Following thawing, a 2 : 75 ratio of fixed sample was stained with SYBER gold (Invitrogen) prepared in Tris-EDTA buffer (5 µl SYBER gold in 50 ml Tris-EDTA) following manufacturer's instructions, then incubated for 20 minutes at 80 °C and cooled down to room temperature. Flow cytometry analysis was performed
15 with excitation at 488 nm and emission at 525 nm.

Isolation and concentration of virions - Three liters of viral lysate (EhV201) were obtained 72 hours following infection by filtering the infected culture through a 0.45 µM PVDF filter (Millex-HV, Millipore). The lysate was concentrated and the viruses were isolated as described by (Schatz et al., 2014).

20 **Neutral Lipids Staining** - 200 ng / µL BODIPY® 493/503 dye (Life Technologies, D2191) were dissolved in DMSO and samples were dyed with 1 µl dye / 200 µl sample (Hallenbeck et al., 2015).

Microscopy and Flow cytometry - Fluorescence microscopy images were obtained using IX71S1F-3-5 motorized inverted Olympus microscope, equipped with
25 a 60x objective, with the following filter systems: BODIPY (ex:470/40nm, em:525/50nm), chlorophyll auto-fluorescence (ex:500/20nm, em:650nm LP). Images were captures using an EXi Blue™ (Q Imaging).

Flow cytometry quantitative data was obtained using Eclipse iCyt flow cytometer, equipped with 488nm solid state air cooled lasers with 25mW on the flow
30 cell and with standard filter set-up, whereby BODIPY was measured in the 525/50nm and chlorophyll auto-fluorescence was measured using 488nm excitation and emission was measured in 665/30nm. Leakage of chlorophyll auto-florescence into the green channel was subtracted by measurement of unstained cells.

For multispectral imaging flow-cytometry (ImageStreamX) analysis, cells were stained for BODIPY as described above and imaged using multispectral Imaging Flow Cytometry (ImageStreamX markII flow cytometer; Amnis Corp, part of EMD millipore, Seattle, WA) using a 60X lens. The dye was excited by the 488nm laser (100mW) and imaged on channel 2 (480-560nm), and the chlorophyll was excited by the 405nm laser and measured on channel 10 (595-640nm). Approximately 5×10^3 cells were collected from each sample and data were analyzed using image analysis software (IDEAS 6.1; Amnis Corp). Images were compensated for fluorescent dye overlap by using single-stain controls. Cells were gated for single cells using the area and aspect ratio features, and for focused cells using the Gradient RMS feature (George et al., 2006). BODIPY staining was quantified using 2 features: Area_Threshold 60 (Area in μm^2 of the 60 % highest intensity pixels) and Bright Details Intensity R3 (Intensity of localized bright spots that are 3 pixels in radius or less).

Statistical analysis of the data obtained by the imaging flow-cytometry was performed with Multivariate analysis of variance test (MANOVA, $F_{1,4026}=571$, $p<0.001$) for the log-transformed data.

Chemicals and internal standards - Liquid chromatography grade solvents were purchased from Merck (Germany) and Bio-Lab (Israel). Ammonium acetate was purchased from Sigma-Aldrich (St. Louis, MO, USA). Internal standards were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL, USA). A lipid internal standard (Avanti Polar lipids, Alabaster, Alabama) was added to the extract solution employed in the initial extraction step.

Lipid extraction for LC-MS analysis - Lipids were extracted from non-infected *E. huxleyi* cells and *E. huxleyi* cells infected with either EhV201 (lytic) or EhV163 (non-lytic) 1, 4, 24, 32, 48 and 72 hours post infection (hpi). 50 ml of cultures in three biological replicates were collected on filters (Vardi et al., 2009). Preparation of the lipid samples for LC-MS analysis was performed as previously described in (Hummel et al., 2011) with some modifications: filters containing algae were placed in 15 ml glass tube and extracted with 3 ml of a pre-cooled (-20°C) homogenous methanol: methyl-tert-butyl-ether (TMBE) 1 : 3 (v/v) mixture containing $0.1 \mu\text{g} / \text{ml}$ of Glucosyl Ceramide (d18:1/12:0) which was used as internal standard. The tubes were shaken for 20 minutes at 4°C and then sonicated for 30 minutes. 1.5

ml of water (UPLC grade) : methanol in a ratio of 3 : 1 v/v were added to the tubes followed by centrifugation. The upper organic phase (1.2 ml) was transferred into a 2 ml Eppendorf tube. The polar phase containing algae debris and filter pieces was re-extracted with 0.5 ml of TMBE. The organic phases were combined and dried under N₂ stream and then stored at -80 °C until analysis. The dried lipid extracts were re-suspended in 300 µl mobile phase buffer B (acetonitrile: isopropanol in a ratio of 7 : 3, with 1 % 1 M NH₄Ac and 0.1 % acetic acid) and centrifuged again at 10,000 g at 4 °C for 5 min. The supernatant was transferred to an autosampler vial and an aliquot of 3 µl was subjected to UPLC-MS (Ultra performance liquid chromatography - mass spectrometry) analysis.

Lipids extraction from virions was performed by Bligh and Dyer method (Bligh and Dyer 1959).

UPLC-q-TOF MS for lipidomics analysis - Lipid extracts were analyzed using a Waters ACQUITY UPLC system coupled to a SYNAPT G2 HDMS mass spectrometer (Waters Corp., MA, USA). Chromatographic conditions were as described in (Hummel et al., 2011). Briefly, the chromatographic separation was performed on an ACQUITY UPLC BEH C8 column (2.1×100 mm, i.d., 1.7 µm) (Waters Corp., MA, USA). The mobile phases consisted of water (UPLC grade) with 1 % 1 M NH₄Ac, 0.1 % acetic acid (mobile phase A), or acetonitrile: isopropanol (7 : 3) with 1 % 1 M NH₄Ac, 0.1 % acetic acid (mobile phase B). The column was maintained at 40 °C with mobile phase flow rate of 0.4 ml / min. MS parameters were as follows: the source and de-solvation temperatures were maintained at 120 °C and 450 °C, respectively. The capillary voltage and cone voltage were set to 1.0 kV and 40 V, respectively. Nitrogen was used as the de-solvation gas and cone gas at a flow rate of 800 l / h and 20 l / h, respectively. The mass spectrometer was operated in full scan MS^E positive resolution mode over a mass range of 50 Da-1500 Da.

For the high energy scan function, a collision energy ramp of 15-35 eV was applied and for the low energy scan function a collision energy ramp of 4 eV was applied. Leucine-enkephalin was used as lock-mass reference standard.

LC-MS Data Analysis - LC-MS data was analyzed and processed using QuanLynx (Version 4.1, Waters Corp., MA, USA). Areas of metabolites were normalized to the area of the internal standard GlcCer (d18:1/12:0) and to the amount of algae used for analysis. Quantification of triacylglycerols (TAGs) was performed

by calculating the ratio of the peak areas of the endogenous lipids and the corresponding internal TAGs standards: TAG 44:1, 48:1, 50:0, 58:7 and 58:10 by producing a standard curve for each of the 5 TAGs (plotting signal intensity vs. amount).

5 **RNA extraction for RNAseq transcriptome analysis** - RNA was extracted from host *E. huxleyi* 1 and 24 hours following infection with the lytic virus (EhV201) or with the non-lytic virus (EhV163) as described previously by (Schatz et al., 2014).

10 **Annotation and Expression Level of Genes Encoding Enzymes Involved in TAG biosynthesis** - Genes encoding for TAGs biosynthesis were annotated based on the *E. huxleyi* genome (Read et al., 2013) and *de novo* transcriptome assembly (Feldmesser et al., 2014). The list of the annotated *E. huxleyi* genes and their homologous is shown in Table 5 below. Expression data for each of the TAG biosynthesis gene was generated based on RNAseq transcriptome analysis of the *E. huxleyi* cells infected with the lytic virus or with the non-lytic virus, and
15 normalized to the expression levels detected in uninfected cells at the same time point (Rosenwasser et al., 2014).

EXAMPLE 1

VIRAL INFECTION OF ALGAE INDUCES ACCUMULATION OF TAGS IN 20 THE INFECTED ALGAE AND ISOLATED VIRIONS

Viral infection of algae triggers a global remodeling of the host lipidome

Remodeling of host primary metabolism toward lipid biosynthesis was found to be critical for successful infection of *E. huxleyi* cell by EhV (Rosenwasser et al., 2014). To determine the consequences of this metabolic remodeling, profiling the
25 lipidome of infected host and purified virions was effected by LC/MS-based global lipidomics approach.

To this end, a comparative host-virus system was utilized in which a single host *E. huxleyi* (strain CCMP2090) was exposed to either the lytic virus (EhV201) or the non-lytic virus (EhV163) and growth dynamics was followed over the infection
30 time course. Cultures of *E. huxleyi* not infected with virus served as control. As shown in Figure 1A, while cultures infected with the lytic virus exhibited growth arrest 24 hours post infection (denoted hereinafter as hpi) and subsequent lysis 72 hpi, the control cultures as well as the cultures infected with the non-lytic virus exhibited

exponential growth throughout the experiment. In addition, accumulation of viral particles in the medium was observed only during infection with the lytic virus, reaching a maximal extracellular level 48 hpi (see Figure 1B).

In order to characterize a specific pattern of lipids associated with distinct phases of the infection, cells from the different cultures were sampled for lipidome profiling at various time points along the experiment (i.e., 1, 4, 24, 32, 48 and 72 hpi). In addition, the viral particles fraction of the culture infected by the lytic virus was sampled and purified 72 hpi in order to characterize the lipids contents of the virions. The lipids extracted from the different samples were separated by liquid chromatography and analyzed by mass-spectroscopy using a targeted approach, by which the identification of pre-selected lipids was validated according to retention time, accurate mass and expected fragmentation. Using this approach, the relative level of ~200 different lipid species that belong to 5 main lipid classes (Figure 2, and Table 1 below) including phospholipids (PL), sphingolipids (SPL), betaine lipids (BL), glycolipids (GL) and the neutral lipids triacylglycerols (TAGs) was determined. General overview of the lipid chromatograms produced for the different cultures suggested a major shift of the lipid profile of the cultures infected with the lytic virus as compared to the un-infected (control) cells and cultures infected with the non-lytic virus (Figure 2). Principal component analysis (PCA) of the identified lipids (Figure 3) showed a clear separation between the samples of cells infected with the lytic virus as compared with cells of either the control or cells infected with the non-lytic virus. More specifically, while samples derived from earlier stages of infection (1-4 hpi) were not significantly different in their lipid composition, a clear separation between the samples was evident 24 hpi, and this separation was even more pronounced towards later stages of infection (i.e. 48 hpi). These results indicated a substantial modulation of the lipidome during the mid-late phase of lytic viral infection and towards maximal viral release (Tables 2 and 3 below provide detailed description of the identified lipids and their relative abundance during infection).

Table 1: Association of the identified metabolites with lipid classes

Lipid Type	Description	Class	# of Species Identified
BLL	Betaine like lipids	Betaine lipids (BL)	4

Cer	Ceramides	Sphingolipids (SPL)	11
DAG	Diacylglycerol	Glycolipids (GL)	3
DGCC	Diacylglyceryl carboxyhydroxymethylcholine	Betaine lipids (BL)	4
DGDG	Digalactosil-diacylglycerol	Glycolipids (GL)	21
DGTS	Diacylglycerol-trimethyl-homoserine	Betaine lipids (BL)	9
GSL	Glycosphingolipids	Sphingolipids (SPL)	5
LysoDGDG	Lyso-Digalactosil-diacylglycerol	Glycolipids (GL)	4
LysoMGDG	Lyso-Monogalactosil-diacylglycerol	Glycolipids (GL)	6
LysoPC	Lyso-Phosphatidylcholine	Phospholipid (PL)	4
LysoPE	Lyso-Phosphatidylethanolamine	Phospholipid (PL)	2
MGDG	Monogalactosil-diacylglycerol	Glycolipids (GL)	23
PC	Phosphatidylcholine	Phospholipid (PL)	12
PE	Phosphatidylethanolamine	Phospholipid (PL)	17
PDPT	Phosphatidyl dimethylpropanethiol	Phospholipid (PL)	4
SQDG	Sulphoquinovosyl-diacylglycerol	Glycolipids (GL)	14
TAG	Triacylglycerol	Neutral lipids (TAG)	56
TOTAL			199

E. huxleyi 2090										E. huxleyi 2090 infected with Non-Lytic Virus EhV163				E. huxleyi 2090 infected with Lytic Virus EhV201				EhV201 virions	
1		4		24		32		48		1		4		24		32		48	
Lipids:	0.0055	0.0055	0.0051	0.0056	0.0049	0.0057	0.0058	0.0054	0.0055	0.0049	0.0056	0.0052	0.0033	0.0033	0.0018	0	0.00172	0.00172	0
MGDG 28:0	0.0328	0.0351	0.0316	0.0321	0.031	0.0329	0.0364	0.0314	0.0327	0.0314	0.0332	0.036	0.0399	0.0423	0.0357	0.00172	0.00172	0	0
MGDG 32:1	0.0372	0.0394	0.0379	0.0394	0.0365	0.0376	0.0397	0.0372	0.0385	0.0366	0.0377	0.037	0.02	0.0146	0.0076	0	0	0	0
MGDG 32:4	0.0007	0.0007	0.0009	0.0009	0.0013	0.0007	0.0007	0.0009	0.0009	0.0013	0.0007	0.0006	0.0004	0.0007	0	0	0	0	0
MGDG 32:5	0.0132	0.0128	0.0179	0.0153	0.018	0.0141	0.0135	0.0189	0.0156	0.0176	0.014	0.0117	0.0102	0.0082	0.0072	0	0	0	0
MGDG 36:8	0.0594	0.0543	0.0592	0.0517	0.0552	0.0587	0.054	0.0589	0.051	0.0557	0.06	0.052	0.0393	0.036	0.0348	0	0	0	0
MGDG 36:9	0.0942	0.0899	0.0904	0.084	0.0887	0.091	0.0911	0.0888	0.0844	0.0874	0.0942	0.0905	0.079	0.0794	0.0704	0	0	0	0
MGDG 36:10	0.0027	0.0031	0.0031	0.0041	0.0039	0.0027	0.0032	0.003	0.0043	0.0039	0.0027	0.003	0.0034	0.0032	0.003	0	0	0	0
MGDG 40:11	0.0232	0.0231	0.0168	0.0193	0.0132	0.0237	0.0235	0.0166	0.0201	0.0126	0.0238	0.0233	0.0141	0.0116	0.0053	0	0	0	0
MGDG 44:12	0.0051	0.0062	0.0039	0.0049	0.0028	0.0053	0.006	0.0038	0.0046	0.0028	0.0053	0.0061	0.0039	0.0034	0.0021	0.00022	0.00022	0.00022	0.00022
DGDG 28:0	0.0228	0.0253	0.0196	0.0221	0.0162	0.023	0.0248	0.0195	0.0216	0.0162	0.0226	0.0239	0.0164	0.0143	0.0133	0.00019	0.00019	0.00019	0.00019
DGDG 32:1	0.0051	0.0083	0.0047	0.0091	0.0054	0.0056	0.0082	0.0047	0.0094	0.0053	0.0052	0.0079	0.0047	0.0026	0.0015	0	0	0	0
DGDG 32:4	0.0099	0.0126	0.0102	0.0144	0.0124	0.01	0.0114	0.0101	0.0144	0.0118	0.0097	0.0115	0.0086	0.006	0.0034	0	0	0	0
DGDG 32:5	0.0767	0.0751	0.0773	0.0722	0.0728	0.076	0.0758	0.0761	0.0724	0.0721	0.0772	0.0762	0.0716	0.0718	0.0577	0	0	0	0
DGDG 36:10	0.0104	0.0109	0.0113	0.0117	0.0124	0.0108	0.0107	0.0116	0.0114	0.0122	0.0108	0.0109	0.0107	0.0112	0.0111	0.00233	0.00233	0.00233	0.00233
SQDG 28:0	0.0032	0.0032	0.0037	0.0038	0.0043	0.0033	0.003	0.0037	0.0035	0.0041	0.0031	0.003	0.0029	0.0034	0.0039	0.00148	0.00148	0.00148	0.00148
SQDG 30:0	0.022	0.0218	0.0247	0.0242	0.0256	0.0212	0.0219	0.0242	0.024	0.0251	0.0209	0.0222	0.0195	0.0179	0.0148	0.00072	0.00072	0.00072	0.00072
SQDG 32:3	0.0021	0.002	0.0028	0.0027	0.0043	0.0022	0.002	0.003	0.0027	0.004	0.0021	0.0019	0.002	0.0019	0.0023	0	0	0	0
SQDG 32:4	0.0079	0.0082	0.009	0.01	0.0106	0.0079	0.0081	0.0093											

SQDG 36:6 (2)	0.002	0.0014	0.0025	0.0014	0.0023	0.0019	0.0015	0.0027	0.0014	0.0022	0.002	0.0014	0.0009	0.0007	0.0006	0
SQDG 36:7	0.0195	0.0197	0.022	0.0227	0.026	0.0195	0.0197	0.0222	0.0227	0.0254	0.0194	0.0192	0.0176	0.0164	0.0147	0.00012
PPDMS 36:6	0.0784	0.073	0.0705	0.0647	0.0658	0.0779	0.0742	0.0708	0.0653	0.0666	0.0781	0.0742	0.0629	0.0639	0.0558	0.03009
PPDMS 38:6	0.0249	0.0256	0.0265	0.0266	0.0271	0.0256	0.0256	0.0269	0.0258	0.0274	0.0248	0.0257	0.0293	0.0288	0.0287	0.01319
PPDMS 40:7	0.0105	0.0103	0.0148	0.0127	0.0172	0.011	0.0103	0.0153	0.0123	0.0172	0.0106	0.0106	0.0256	0.0275	0.0263	0.01926
PPDMS 44:12	0.0149	0.0151	0.0129	0.0134	0.0124	0.0158	0.0157	0.0132	0.0139	0.0123	0.0152	0.0146	0.0113	0.0098	0.0097	0.00678
PE 30:1	0	0	0	0	0	0	0	5E-06	1E-05	2E-05	0	0	6E-05	0.0001	0.0005	0.00291
PE 31:1	0	0	0	0	0	0	0	5E-05	7E-05	0.0001	0	0	8E-05	0.0002	0.001	0.0099
PE 32:1	0	0	0	0	0	0	0	0	0	0	2E-05	4E-05	0.0007	0.0022	0.005	0.00263
PE 33:1	0	0	0	0	0	0	0	0	0	0	0	0	6E-05	0.0001	0.0012	0.00051
PE 34:1	0	0	0	0	0	0	0	0	0	0	0	6E-06	0.0002	0.0006	0.0025	0.00035
DGCC 36:6	0.0656	0.0622	0.0617	0.0594	0.0601	0.0647	0.0632	0.0611	0.0601	0.0612	0.0658	0.0627	0.0531	0.0555	0.0504	0.01948
DGCC 38:6	0.0202	0.0222	0.0249	0.0282	0.0277	0.021	0.0223	0.0253	0.0285	0.0291	0.0207	0.0214	0.0205	0.0179	0.0219	0.00704
DGCC 40:7	0.005	0.0049	0.0079	0.0066	0.0095	0.0051	0.0048	0.0081	0.0067	0.0098	0.0051	0.0048	0.0102	0.0112	0.0145	0.00606
DGCC 44:12	0.0463	0.0433	0.0468	0.0432	0.0462	0.0457	0.0434	0.0459	0.0433	0.0464	0.0463	0.0437	0.0396	0.0377	0.0364	0.00727
BLL 36:6	0.0003	0.0004	0.0003	0.0005	0.0005	0.0004	0.0003	0.0004	0.0006	0.0009	0.0004	0.0023	0.0226	0.0242	0.0235	0.0012
BLL 38:6	0.0114	0.0137	0.0106	0.0135	0.0109	0.0135	0.0104	0.0113	0.0131	0.0102	0.0118	0.01	0.0009	0.0007	0.0007	0.00126
BLL 40:7	0.0054	0.0067	0.0059	0.0079	0.0081	0.0067	0.0052	0.0066	0.0076	0.0076	0.0059	0.0054	0.0017	0.0013	0.0012	0
BLL 44:12	0.0012	0.0019	0.0012	0.0021	0.0013	0.0013	0.0019	0.0012	0.0022	0.0014	0.0014	0.0018	0.0022	0.0026	0.0044	0.00701
DGTS 28:0	2E-05	2E-05	2E-05	2E-05	2E-05	2E-05	2E-05	1E-05	2E-05	2E-05	2E-05	3E-05	0.0003	0.0004	0.0005	0.00071
DGTS 30:1	0.0002	0.0003	0.0003	0.0003	0.0004	0.0002	0.0003	0.0003	0.0003	0.0004	0.0002	0.0003	0.0003	0.0003	0.0004	0
DGTS 32:1	0.0336	0.0313	0.0399	0.0358	0.0413	0.0323	0.0319	0.0384	0.0357	0.0418	0.0325	0.032	0.0387	0.0418	0.0474	0.03889
DGTS 32:2	0.0029	0.003	0.0043	0.0043	0.0056	0.0029	0.0031	0.0042	0.0043	0.0057	0.0029	0.003	0.0026	0.0022	0.002	0.00084
DGTS 32:3	0.0023	0.0021	0.0027	0.0025	0.0032	0.0022	0.0022	0.0026	0.0025	0.0032	0.0022	0.0021	0.0027	0.0031	0.0046	0.00035
DGTS 32:4	0.0039	0.0039	0.0049	0.0052	0.0078	0.0038	0.0039	0.0048	0.0052	0.0078	0.0037	0.0037	0.0026	0.0032	0.0052	7.9E-05

DGTS 33:4	0.0003	0.0002	0.0005	0.0004	0.0003	0.0004	0.0007	0.0003	0.0002	0.0003	0.0002	0.0003	0.0003	0.0006	0
DGTS 34:4	0.0001	0.0001	0.0002	0.0001	0.0001	0.0002	0.0002	0.0003	0.0001	0.0001	0.0001	1E-04	8E-05	0.0001	0
DGTS 36:6	0.0004	0.0004	0.0005	0.0004	0.0004	0.0005	0.0005	0.0004	0.0004	0.0004	0.0004	0.0006	0.0005	0.0005	0
hGSL22:2 dl9:3LCB	0.0175	0.0163	0.0206	0.0199	0.0245	0.0171	0.0202	0.0201	0.0243	0.0175	0.0162	0.0169	0.0169	0.0197	0.00139
vGSL	0	0	0	0	0	0.0001	0.0002	0.0003	0.0007	0.0002	0.0009	0.0091	0.0113	0.0144	0.01862
sGSL 22:0 dl8:2 LCB	0.0009	0.0013	0.0008	0.0012	0.0008	0.001	0.0009	0.0007	0.0009	0.0008	0.0007	0.0006	0.0007	0.0004	0.00675
sGSL 22:0 dl8:1 LCB	4E-05	9E-05	3E-05	8E-05	6E-05	6E-05	7E-05	4E-05	7E-05	4E-05	2E-05	0	7E-06	3E-06	0.00088
TAG 42:0	8E-05	5E-05	6E-05	3E-05	3E-05	6E-05	6E-05	4E-05	4E-05	6E-05	4E-05	0.0002	0.0002	0.0002	0.01775
TAG 42:1	6E-05	4E-05	3E-05	2E-05	1E-05	4E-05	2E-05	2E-05	1E-05	3E-05	3E-05	6E-05	7E-05	1E-04	0.01371
TAG 44:0	0.0001	0.0001	1E-04	6E-05	6E-05	0.0001	8E-05	8E-05	7E-05	1E-04	9E-05	0.0003	0.0005	0.0007	0.02948
TAG 44:1	0.0002	0.0001	8E-05	5E-05	5E-05	0.0001	9E-05	6E-05	5E-05	9E-05	0.0001	0.0003	0.0003	0.0004	0.02902
TAG 46:0	0.0002	0.0002	0.0002	1E-04	9E-05	0.0002	0.0001	0.0001	1E-04	0.0002	0.0001	0.0003	0.0004	0.0004	0
TAG 46:1	0.001	0.0008	0.0007	0.0006	0.0006	0.0008	0.0007	0.0007	0.0007	0.0008	0.0014	0.0089	0.0103	0.0096	0.06068
TAG 46:2	0.0003	0.0002	0.0001	1E-04	8E-05	0.0002	0.0001	0.0001	9E-05	0.0002	0.0002	0.0005	0.0005	0.0005	0.0289
TAG 46:3	7E-05	6E-05	5E-05	3E-05	3E-05	6E-05	4E-05	4E-05	4E-05	4E-05	8E-05	0.0005	0.0006	0.0009	0
TAG 48:0	0.0002	0.0002	0.0001	8E-05	9E-05	0.0002	0.0001	0.0001	9E-05	0.0001	0.0001	0.0002	0.0002	0.0003	0.04463
TAG 48:1	0.001	0.0008	0.0006	0.0005	0.0004	0.0009	0.0007	0.0006	0.0006	0.0008	0.0016	0.0081	0.0096	0.0094	0.07353
TAG 48:2	0.0006	0.0005	0.0003	0.0002	0.0002	0.0005	0.0004	0.0003	0.0002	0.0004	0.0005	0.0014	0.0016	0.0016	0.05143
TAG 48:3	0.0003	0.0002	0.0002	0.0001	9E-05	0.0002	0.0002	0.0002	0.0001	0.0002	0.0003	0.0015	0.0019	0.0026	0.01409
TAG 48:4	0.0002	0.0001	0.0001	8E-05	7E-05	0.0002	0.0001	0.0001	9E-05	0.0001	0.0002	0.0013	0.0016	0.0029	0
TAG 50:0	0.0001	8E-05	8E-05	4E-05	4E-05	9E-05	6E-05	6E-05	5E-05	7E-05	7E-05	0.0001	0.0002	0.0003	0.01945
TAG 50:1	0.0008	0.0006	0.0005	0.0004	0.0003	0.0007	0.0005	0.0005	0.0004	0.0004	0.0009	0.004	0.0052	0.0054	0.06668

TAG 50:2	0.0013	0.001	0.0007	0.0005	0.0005	0.0005	0.0007	0.0003	0.0005	0.0005	0.0005	0.0011	0.0009	0.0007	0.0006	0.0006	0.0005	0.0011	0.0013	0.0042	0.0046	0.0042	0.05409
TAG 50:3	0.0005	0.0004	0.0003	0.0003	0.0003	0.0003	0.0003	0.0003	0.0002	0.0002	0.0002	0.0004	0.0004	0.0003	0.0003	0.0003	0.0002	0.0004	0.0006	0.0017	0.002	0.0028	0.02133
TAG 50:4	0.0008	0.0007	0.0006	0.0005	0.0005	0.0005	0.0006	0.0006	0.0005	0.0005	0.0006	0.0007	0.0007	0.0006	0.0006	0.0006	0.0006	0.0007	0.0011	0.0051	0.0056	0.0062	0
TAG 50:5	0.0004	0.0003	0.0004	0.0003	0.0003	0.0003	0.0004	0.0004	0.0003	0.0003	0.0003	0.0003	0.0003	0.0004	0.0003	0.0003	0.0004	0.0003	0.0005	0.0043	0.0047	0.0061	0
TAG 50:6	0.0005	0.0004	0.0006	0.0004	0.0004	0.0004	0.0006	0.0006	0.0005	0.0005	0.0004	0.0004	0.0004	0.0005	0.0004	0.0005	0.0005	0.0004	0.0007	0.0057	0.0066	0.0094	0
TAG 50:7 (1)	7E-05	6E-05	7E-05	5E-05	6E-05	6E-05	7E-05	7E-05	6E-05	6E-05	6E-05	6E-05	6E-05	7E-05	6E-05	6E-05	8E-05	7E-05	9E-05	0.0015	0.0018	0.003	0
TAG 50:7 (2)	4E-05	3E-05	2E-05	2E-05	2E-05	2E-05	2E-05	2E-05	2E-05	2E-05	2E-05	3E-05	3E-05	3E-05	3E-05	2E-05	2E-05	3E-05	4E-05	0.0001	0.0001	0.0002	0
TAG 50:8 (1)	4E-05	3E-05	5E-05	4E-05	4E-05	4E-05	5E-05	5E-05	4E-05	4E-05	4E-05	4E-05	3E-05	4E-05	3E-05	4E-05	5E-05	3E-05	4E-05	0.0006	0.0008	0.0016	0
TAG 50:8 (2)	2E-05	3E-05	3E-05	1E-05	2E-05	2E-05	3E-05	3E-05	2E-05	2E-05	2E-05	3E-05	3E-05	2E-05	2E-05	2E-05	4E-05	3E-05	5E-05	0.0009	0.0011	0.0023	0
TAG 50:9	6E-05	5E-05	8E-05	4E-05	4E-05	4E-05	8E-05	8E-05	6E-05	6E-05	6E-05	6E-05	4E-05	7E-05	6E-05	5E-05	9E-05	6E-05	8E-05	0.0013	0.0015	0.0027	0
TAG 52:0	7E-05	6E-05	6E-05	3E-05	3E-05	3E-05	6E-05	6E-05	4E-05	4E-05	4E-05	7E-05	5E-05	5E-05	6E-05	4E-05	4E-05	6E-05	5E-05	0.0001	0.0002	0.0004	0.01844
TAG 52:1	0.0003	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0001	0.0001	0.0001	0.0003	0.0002	0.0002	0.0002	0.0002	0.0001	0.0002	0.0003	0.0014	0.002	0.0023	0.02167
TAG 52:2	0.0004	0.0003	0.0004	0.0002	0.0002	0.0002	0.0004	0.0004	0.0002	0.0002	0.0002	0.0004	0.0003	0.0003	0.0003	0.0002	0.0002	0.0004	0.0004	0.0014	0.0015	0.0014	0.05089
TAG 52:3	0.0012	0.0009	0.0006	0.0005	0.0005	0.0005	0.0006	0.0006	0.0003	0.0003	0.0003	0.0011	0.001	0.0006	0.0006	0.0006	0.0004	0.0011	0.0013	0.003	0.003	0.0028	0.03006
TAG 52:4	0.0013	0.001	0.0007	0.0006	0.0006	0.0006	0.0007	0.0007	0.0004	0.0004	0.0004	0.0012	0.0011	0.0007	0.0007	0.0007	0.0005	0.0012	0.0017	0.0061	0.0067	0.0065	0.01478
TAG 52:5	0.001	0.0008	0.0006	0.0006	0.0006	0.0006	0.0006	0.0006	0.0004	0.0004	0.0004	0.0009	0.0008	0.0006	0.0006	0.0006	0.0005	0.001	0.0013	0.0055	0.0057	0.0057	0
TAG 52:6	0.0005	0.0004	0.0003	0.0003	0.0003	0.0003	0.0003	0.0003	0.0002	0.0002	0.0002	0.0004	0.0004	0.0003	0.0003	0.0003	0.0003	0.0005	0.0009	0.0045	0.0049	0.0061	0
TAG 52:7 (1)	0.0002	0.0002	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0002	0.0002	0.0002	0.0002	0.0001	0.0001	0.0002	0.0002	0.0015	0.0019	0.0027	0
TAG 52:7 (2)	0.0002	0.0002	0.0002	0.0001	0.0001	0.0001	0.0002	0.0002	1E-04	1E-04	1E-04	0.0002	0.0002	0.0001	0.0001	0.0001	0.0001	0.0002	0.0003	0.001	0.001	0.0014	0
TAG 52:8	0.0002	0.0001	0.0001	9E-05	9E-05	9E-05	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0002	0.002	0.0025	0.004	0
TAG 52:9	0.0001	0.0001	0.0001	8E-05	8E-05	8E-05	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0002	0.0001	0.0002	0.0019	0.0023	0.0036	0
TAG 54:1	0.0006	0.0006	0.0003	0.0003	0.0003	0.0003	0.0003	0.0003	0.0002	0.0002	0.0002	0.0006	0.0005	0.0003	0.0003	0.0004	0.0004	0.0006	0.0007	0.0052	0.0083	0.0111	0.0125
TAG 54:2	0.0008	0.0008	0.0006	0.0005	0.0005	0.0005	0.0006	0.0006	0.0003	0.0003	0.0003	0.0008	0.0008	0.0006	0.0006	0.0006	0.0003	0.0008	0.0009	0.0014	0.0016	0.0018	0.0117
TAG 54:3	0.0007	0.0005	0.0006	0.0003	0.0003	0.0003	0.0006	0.0006	0.0002	0.0002	0.0002	0.0007	0.0005	0.0004	0.0004	0.0004	0.0002	0.0006	0.0006	0.0016	0.0016	0.0021	0.03043
TAG 54:4	0.0015	0.0011	0.0009	0.0007	0.0007	0.0007	0.0009	0.0009	0.0004	0.0004	0.0004	0.0014	0.0012	0.0009	0.0009	0.0008	0.0006	0.0014	0.0015	0.0033	0.0035	0.0037	0.01891

TAG 54:5	0.0027	0.0021	0.0014	0.0011	0.0008	0.0024	0.0022	0.0014	0.0014	0.0009	0.0025	0.0028	0.006	0.0064	0.0066	0.01424
TAG 54:6	0.0015	0.0015	0.0007	0.0009	0.0005	0.0014	0.0016	0.0008	0.001	0.0005	0.0014	0.0021	0.0036	0.0032	0.0025	0.00778
TAG 54:7	0.0047	0.0045	0.0036	0.0039	0.003	0.0041	0.0046	0.0033	0.004	0.0032	0.0043	0.0083	0.0087	0.0067	0.0074	0
TAG 54:8	0.0006	0.0005	0.0005	0.0004	0.0004	0.0006	0.0005	0.0005	0.0005	0.0004	0.0006	0.0008	0.0029	0.0033	0.0048	0
TAG 54:9	0.0005	0.0004	0.0005	0.0003	0.0003	0.0004	0.0004	0.0004	0.0004	0.0004	0.0005	0.0006	0.0036	0.004	0.0053	0
TAG 54:10	0.0002	0.0002	0.0004	0.0002	0.0002	0.0002	0.0002	0.0003	0.0002	0.0003	0.0003	0.0003	0.002	0.0017	0.0022	0
TAG 56:1	0	0	0	0	0	0	0	0	0	0	0	0	0.0017	0.0022	0.0028	0.00777
TAG 56:2	0.0003	0.0003	0.0002	0.0002	0.0001	0.0003	0.0003	0.0002	0.0002	0.0001	0.0003	0.0004	0.0006	0.0007	0.0009	0.00583
TAG 56:3	0.0001	9E-05	7E-05	6E-05	3E-05	0.0001	1E-04	7E-05	7E-05	5E-05	0.0001	1E-04	0.0004	0.0007	0.0015	0
TAG 56:4	0.0003	0.0003	0.0002	0.0001	9E-05	0.0003	0.0002	0.0002	0.0002	0.0001	0.0003	0.0003	0.001	0.0014	0.0027	0
TAG 56:5	0.0003	0.0003	0.0002	0.0001	0.0001	0.0003	0.0003	0.0002	0.0002	0.0001	0.0003	0.0004	0.001	0.0012	0.0015	0
TAG 56:6	0.0002	0.0002	0.0002	0.0001	0.0001	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0003	0.0011	0.0014	0.0021	0
TAG 56:7	0.0009	0.0009	0.0006	0.0006	0.0004	0.0008	0.0008	0.0005	0.0007	0.0004	0.0008	0.0017	0.0023	0.0019	0.0019	0
TAG 56:8	0.0006	0.0004	0.0004	0.0003	0.0002	0.0005	0.0004	0.0004	0.0003	0.0003	0.0005	0.0006	0.0016	0.0017	0.0006	0
TAG 58:2	0.0002	0.0002	0.0002	0.0001	0.0001	0.0002	0.0002	0.0002	0.0001	0.0001	0.0002	0.0002	0.0006	0.0008	0.0011	0.00316
TAG 58:3	0.0003	0.0002	0.0003	0.0001	0.0001	0.0003	0.0002	0.0002	0.0002	0.0001	0.0003	0.0002	0.0004	0.0005	0.0008	0
TAG 58:4	0.0007	0.0006	0.0003	0.0004	0.0002	0.0006	0.0006	0.0004	0.0004	0.0003	0.0007	0.0007	0.0017	0.0023	0.0036	0
TAG 58:5 (1)	0.0007	0.0007	0.0005	0.0005	0.0003	0.0007	0.0007	0.0005	0.0005	0.0003	0.0007	0.0006	0.0008	0.001	0.0018	0
TAG 58:5 (2)	0.0003	0.0003	0.0002	0.0002	0.0001	0.0003	0.0002	0.0002	0.0002	0.0002	0.0003	0.0003	0.0025	0.0038	0.0073	0
TAG 58:6 (1)	0.0006	0.0005	0.0004	0.0003	0.0003	0.0005	0.0005	0.0004	0.0004	0.0003	0.0006	0.0005	0.0011	0.0012	0.0017	0
TAG 58:6 (2)	0.0002	0.0002	0.0002	0.0001	0.0001	0.0002	0.0002	0.0002	0.0002	0.0003	0.0002	0.0002	0.0027	0.0043	0.0094	0
DGDG 32:1	0.0225	0.0249	0.0193	0.0218	0.0159	0.0227	0.0245	0.0192	0.0213	0.0159	0.0223	0.0236	0.0162	0.0141	0.013	0.00019
DGDG 32:2	0.003	0.0041	0.0027	0.004	0.0027	0.0032	0.0037	0.0028	0.0037	0.0028	0.0029	0.0035	0.0014	0.0009	0.0005	0
DGDG 32:3	0.0085	0.01	0.0079	0.0107	0.0083	0.0084	0.0095	0.008	0.0103	0.0082	0.008	0.0094	0.0065	0.0043	0.002	0
DGDG 32:4	0.0051	0.0083	0.0047	0.0091	0.0054	0.0056	0.0082	0.0047	0.0093	0.0053	0.0052	0.0079	0.0047	0.0026	0.0015	0

DGDG 34:1	0.0038	0.0048	0.0039	0.0051	0.0037	0.0039	0.0048	0.004	0.0052	0.0035	0.004	0.0046	0.0045	0.004	0.0032	0
DGDG 34:2	0.001	0.0012	0.001	0.0014	0.001	0.001	0.0012	0.0011	0.0013	0.001	0.001	0.0012	0.0008	0.0006	0.0006	0
DGDG 34:3	0.0014	0.0018	0.0015	0.0022	0.0015	0.0014	0.0017	0.0015	0.0018	0.0014	0.0014	0.0017	0.0013	0.0011	0.0007	0
DGDG 34:4	0.0031	0.004	0.0032	0.0036	0.0024	0.0031	0.0037	0.0033	0.0037	0.0023	0.003	0.0036	0.0024	0.0017	0.001	0
DGDG 34:5 (1)	0.0005	0.0007	0.0006	0.0009	0.0008	0.0005	0.0007	0.0006	0.001	0.0007	0.0004	0.0006	0.0003	0.0002	0.0001	0
DGDG 34:5 (2)	9E-05	0.0001	8E-05	0.0001	8E-05	0.0001	0.0001	8E-05	0.0001	9E-05	0.0001	0.0001	9E-05	9E-05	1E-04	0
DGDG 36:2	0.0011	0.0015	0.001	0.0016	0.001	0.0012	0.0012	0.0012	0.0013	0.001	0.0012	0.0011	0.0007	0.0006	0.0006	0
DGDG 36:3	0.0008	0.0009	0.0008	0.001	0.0008	0.0008	0.0009	0.0008	0.0011	0.0008	0.0008	0.0008	0.0004	0.0003	0.0003	0
DGDG 36:4	0.006	0.006	0.0054	0.0059	0.0042	0.0057	0.0055	0.0055	0.0059	0.0042	0.0056	0.0054	0.0022	0.0016	0.0014	0
DGDG 36:5	0.0009	0.001	0.0013	0.0013	0.0012	0.0009	0.0009	0.0012	0.0011	0.0013	0.0009	0.0008	0.0004	0.0004	0.0005	0
DGDG 36:6	0.0021	0.0021	0.0023	0.0037	0.0031	0.0021	0.002	0.0023	0.0027	0.0031	0.0021	0.002	0.0011	0.0008	0.0006	0
DGDG 36:7	0.0011	0.0012	0.001	0.0012	0.0013	0.0011	0.0011	0.001	0.0012	0.0012	0.0011	0.0011	0.0005	0.0004	0.0003	0
DGDG 38:3	7E-05	8E-05	7E-05	0.0001	9E-05	8E-05	8E-05	9E-05	9E-05	9E-05	7E-05	7E-05	4E-05	3E-05	3E-05	0
Lyso-DGDG 16:0	0	0	0	2E-06	0	0	0	0	0	0	0	0	1E-05	1E-05	0.0001	0
Lyso-DGDG 18:3	0	0	0	0	0	0	0	0	2E-06	0	0	0	0	1E-06	4E-05	0
Lyso-DGDG 18:2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1E-05	0
Lyso-DGDG 18:1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3E-05	0
MGDG 32:0	7E-05	1E-04	5E-05	7E-05	2E-05	9E-05	8E-05	5E-05	7E-05	3E-05	9E-05	9E-05	9E-05	0.0001	0.0001	0
MGDG 32:2	0.0015	0.0015	0.0012	0.0013	0.001	0.0016	0.0017	0.0013	0.0013	0.001	0.0017	0.0016	0.0006	0.0005	0.0003	0
MGDG 32:3	0.0045	0.0045	0.0055	0.0044	0.0052	0.0047	0.0044	0.0052	0.004	0.0052	0.0043	0.0039	0.0037	0.0032	0.0018	0
MGDG 34:1	0.0032	0.0038	0.0022	0.0025	0.0016	0.0031	0.0037	0.0023	0.0027	0.0016	0.0029	0.0038	0.0048	0.0046	0.0034	0
MGDG 34:2	0.0012	0.0013	0.0013	0.0013	0.0013	0.0013	0.0013	0.0013	0.0012	0.0012	0.0013	0.0013	0.001	0.0008	0.0008	0

[illegible]

Lyso-MGDG 18:3 (1)	2E-06	3E-06	3E-06	0	6E-06	0	1E-05	1E-05	1E-05	2E-05	5E-06	1E-05	2E-05	2E-05	9E-06	0.0004	0
Lyso-MGDG 18:3 (2)	3E-05	4E-05	4E-05	5E-05	4E-05	5E-05	2E-05	4E-05	3E-05	3E-05	2E-05	2E-05	2E-05	2E-05	2E-05	6E-05	0
SQDG 34:1	0.0001	0.0001	0.0002	0.0001	0.0002	0.0001	0.0001	0.0002	0.0001	0.0002	0.0002	0.0002	0.0001	0.0003	0.0003	0.0004	0
SQDG 34:3	0.0031	0.0032	0.0039	0.0042	0.0046	0.003	0.0031	0.0039	0.0041	0.0044	0.003	0.0031	0.003	0.003	0.0028	0.0025	0.0001
SQDG 36:5	0.0004	0.0003	0.0005	0.0003	0.0004	0.0004	0.0003	0.0005	0.0003	0.0004	0.0004	0.0005	0.0003	0	0	0	0
SQDG 36:6	0.0077	0.0081	0.0089	0.0099	0.0105	0.0078	0.0079	0.0091	0.0101	0.0104	0.0079	0.008	0.0055	0.0045	0.0033	0	0
SQDG 32:0	0.0002	0.0002	0.0002	0.0003	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0
SQDG 32:1	0.002	0.0018	0.0022	0.0016	0.0018	0.0021	0.002	0.0019	0.0017	0.0017	0.0021	0.0015	0.0028	0.0033	0.0032	0.0004	0
SQDG 32:2 (1)	0.0015	0.0014	0.0017	0.0013	0.0015	0.0015	0.0016	0.0018	0.0014	0.0015	0.0016	0.0015	0.0007	0.0005	0.0004	0	0
SQDG 32:2 (2)	0.0001	0.0001	0.0002	0.0002	0.0002	0.0001	0.0001	0.0002	0.0002	0.0002	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0
SQDG 32:3	0.022	0.0219	0.0247	0.0243	0.0257	0.0212	0.022	0.0243	0.024	0.0251	0.021	0.0222	0.0196	0.018	0.0148	0.00072	0
PC 32:1	0.0006	0.0004	0.0012	0.0005	0.001	0.0005	0.0003	0.0011	0.0004	0.0012	0.0004	0.0004	0.0003	0.0016	0.003	0.0057	0.00215
PC 32:2	0.0001	0.0001	0.0002	0.0001	0.0002	0.0001	0.0001	0.0002	0.0001	0.0002	0.0001	0.0001	1E-04	0.0001	0.0001	0.0002	0
PC 32:3	0.0002	0.0002	0.0004	0.0003	0.0005	0.0002	0.0002	0.0004	0.0003	0.0006	0.0002	0.0002	0.0003	0.0004	0.0006	0.0015	0
PC 34:1	0.0002	0.0001	0.0004	0.0002	0.0003	0.0002	0.0002	0.0004	0.0002	0.0004	0.0002	0.0002	0.0002	0.0006	0.0009	0.0017	0.00058
PC 34:2	9E-05	7E-05	0.0002	0.0003	0.0001	9E-05	8E-05	0.0001	6E-05	0.0001	5E-05	6E-05	6E-05	0.0002	0.0002	0.0004	0
PC 34:3	7E-05	5E-05	0.0001	0.0005	0.0001	5E-05	6E-05	0.0001	5E-05	0.0001	6E-05	5E-05	5E-05	0.0004	0.0006	0.0015	0
PC 34:4 (1)	3E-05	1E-05	3E-05	2E-05	3E-05	2E-05	2E-05	4E-05	2E-05	4E-05	2E-05	2E-05	2E-05	0.0001	0.0002	0.0005	0
PC 34:4 (2)	0.0003	0.0002	0.0005	0.0002	0.0004	0.0003	0.0002	0.0004	0.0002	0.0005	0.0003	0.0003	0.0002	0.0002	0.0002	0.0005	0
PC 36:2	0.0001	9E-05	0.0002	0.0001	0.0002	0.0001	9E-05	0.0002	9E-05	0.0002	0.0001	0.0001	0.0001	0.0004	0.001	0.0027	0.00116
PC 36:5	0.0006	0.0007	0.0008	0.0007	0.0008	0.0006	0.0007	0.0008	0.0006	0.0012	0.0005	0.0007	0.0016	0.0031	0.0056	0	0
PC 38:3	0	0	2E-05	0	5E-06	0	0	3E-05	4E-06	2E-05	0	0	7E-06	9E-05	0.0002	0.0006	0
PC 38:4	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0001	0.0004	0.0016	0

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Table 3: Lipids distribution in *E. huxleyi* following infection and in the virus virions.

Data is presented as arbitrary units per cell (AU)

AU/Million cells AVG (STDEV)	<i>E. huxleyi</i> 2090					<i>E. huxleyi</i> 2090 infected with Non-Lytic Virus <i>EhV163</i>					<i>E. huxleyi</i> 2090 infected with Lytic Virus <i>EhV201</i>				
	1	4	24	32	48	1	4	24	32	48	1	4	24	32	48
Lipids:															
MGDG 28:0	0.06 ± 0.01	0.08 ± 0.01	0.04 ± 0.01	0.06 ± 0.00	0.03 ± 0.00	0.07 ± 0.01	0.08 ± 0.01	0.05 ± 0.00	0.05 ± 0.00	0.03 ± 0.00	0.07 ± 0.00	0.07 ± 0.01	0.05 ± 0.00	0.05 ± 0.00	0.02 ± 0.00
MGDG 32:1	0.39 ± 0.03	0.50 ± 0.02	0.26 ± 0.01	0.32 ± 0.02	0.19 ± 0.00	0.42 ± 0.02	0.50 ± 0.01	0.28 ± 0.01	0.32 ± 0.02	0.19 ± 0.01	0.42 ± 0.02	0.50 ± 0.02	0.58 ± 0.06	0.58 ± 0.04	0.35 ± 0.04
MGDG 32:4	0.44 ± 0.03	0.56 ± 0.02	0.32 ± 0.02	0.39 ± 0.02	0.23 ± 0.00	0.48 ± 0.03	0.54 ± 0.02	0.33 ± 0.02	0.38 ± 0.01	0.22 ± 0.01	0.48 ± 0.02	0.51 ± 0.02	0.29 ± 0.00	0.20 ± 0.01	0.07 ± 0.01
MGDG 32:5	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
MGDG 36:8	0.15 ± 0.02	0.18 ± 0.01	0.15 ± 0.01	0.15 ± 0.01	0.11 ± 0.01	0.18 ± 0.01	0.18 ± 0.01	0.17 ± 0.00	0.15 ± 0.01	0.11 ± 0.01	0.18 ± 0.00	0.16 ± 0.01	0.15 ± 0.00	0.11 ± 0.00	0.07 ± 0.01
MGDG 36:9	0.70 ± 0.07	0.77 ± 0.01	0.50 ± 0.06	0.51 ± 0.03	0.35 ± 0.01	0.74 ± 0.07	0.74 ± 0.02	0.52 ± 0.04	0.50 ± 0.02	0.34 ± 0.03	0.76 ± 0.04	0.72 ± 0.01	0.56 ± 0.04	0.49 ± 0.01	0.34 ± 0.02
MGDG 36:10	1.11 ± 0.14	1.27 ± 0.06	0.76 ± 0.09	0.84 ± 0.08	0.56 ± 0.03	1.15 ± 0.13	1.24 ± 0.05	0.78 ± 0.08	0.83 ± 0.05	0.54 ± 0.05	1.20 ± 0.11	1.25 ± 0.02	1.14 ± 0.13	1.09 ± 0.11	0.69 ± 0.08
MGDG 40:11	0.03 ± 0.00	0.04 ± 0.01	0.03 ± 0.00	0.04 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.04 ± 0.01	0.03 ± 0.00	0.04 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.04 ± 0.01	0.05 ± 0.00	0.04 ± 0.00	0.03 ± 0.00
MGDG 44:12	0.27 ± 0.03	0.33 ± 0.02	0.14 ± 0.00	0.19 ± 0.01	0.08 ± 0.01	0.30 ± 0.01	0.32 ± 0.02	0.15 ± 0.00	0.20 ± 0.01	0.08 ± 0.00	0.30 ± 0.01	0.32 ± 0.02	0.20 ± 0.00	0.16 ± 0.01	0.05 ± 0.00
DGDG 28:0	0.06 ± 0.01	0.09 ± 0.01	0.03 ± 0.00	0.05 ± 0.00	0.02 ± 0.00	0.07 ± 0.01	0.08 ± 0.01	0.03 ± 0.00	0.05 ± 0.00	0.02 ± 0.00	0.07 ± 0.01	0.08 ± 0.00	0.06 ± 0.01	0.05 ± 0.00	0.02 ± 0.00
DGDG 32:1	0.27 ± 0.02	0.36 ± 0.01	0.16 ± 0.01	0.22 ± 0.02	0.10 ± 0.00	0.29 ± 0.01	0.34 ± 0.01	0.17 ± 0.01	0.21 ± 0.01	0.10 ± 0.00	0.29 ± 0.01	0.33 ± 0.02	0.24 ± 0.02	0.20 ± 0.01	0.13 ± 0.01
DGDG 32:4	0.06 ± 0.00	0.12 ± 0.01	0.04 ± 0.00	0.09 ± 0.00	0.03 ± 0.00	0.07 ± 0.00	0.11 ± 0.01	0.04 ± 0.00	0.09 ± 0.00	0.03 ± 0.00	0.07 ± 0.00	0.11 ± 0.01	0.07 ± 0.00	0.04 ± 0.00	0.01 ± 0.00
DGDG 32:5	0.12 ± 0.01	0.18 ± 0.02	0.09 ± 0.00	0.14 ± 0.01	0.08 ± 0.00	0.13 ± 0.00	0.16 ± 0.01	0.09 ± 0.00	0.14 ± 0.00	0.07 ± 0.00	0.12 ± 0.01	0.16 ± 0.01	0.12 ± 0.00	0.08 ± 0.00	0.03 ± 0.00
DGDG 36:10	0.90 ± 0.10	1.06 ± 0.04	0.65 ± 0.06	0.72 ± 0.07	0.46 ± 0.02	0.96 ± 0.11	1.03 ± 0.03	0.67 ± 0.06	0.71 ± 0.04	0.44 ± 0.04	0.98 ± 0.09	1.05 ± 0.03	1.03 ± 0.12	0.98 ± 0.08	0.57 ± 0.05
SQDG 28:0	0.12 ± 0.01	0.15 ± 0.01	0.09 ± 0.00	0.12 ± 0.01	0.08 ± 0.00	0.14 ± 0.00	0.15 ± 0.02	0.10 ± 0.00	0.11 ± 0.01	0.07 ± 0.00	0.14 ± 0.01	0.15 ± 0.01	0.15 ± 0.01	0.15 ± 0.01	0.11 ± 0.01
SQDG 30:0	0.04 ± 0.00	0.05 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	0.04 ± 0.01	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.05 ± 0.00	0.04 ± 0.00
SQDG 32:3	0.26 ± 0.02	0.31 ± 0.02	0.21 ± 0.01	0.24 ± 0.01	0.16 ± 0.00	0.27 ± 0.01	0.30 ± 0.02	0.21 ± 0.01	0.24 ± 0.01	0.15 ± 0.00	0.27 ± 0.01	0.31 ± 0.02	0.28 ± 0.01	0.24 ± 0.01	0.14 ± 0.01
SQDG 32:4	0.02 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.02 ± 0.00

SQDG 36:6 (1)	0.09 ± 0.01	0.12 ± 0.01	0.08 ± 0.01	0.10 ± 0.00	0.07 ± 0.00	0.10 ± 0.00	0.11 ± 0.01	0.08 ± 0.00	0.10 ± 0.00	0.06 ± 0.00	0.10 ± 0.00	0.11 ± 0.01	0.08 ± 0.00	0.06 ± 0.00	0.03 ± 0.00
SQDG 36:6 (2)	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
SQDG 36:7	0.23 ± 0.03	0.28 ± 0.03	0.18 ± 0.01	0.22 ± 0.01	0.16 ± 0.00	0.25 ± 0.02	0.27 ± 0.02	0.20 ± 0.00	0.22 ± 0.00	0.16 ± 0.00	0.25 ± 0.02	0.27 ± 0.03	0.25 ± 0.01	0.22 ± 0.01	0.14 ± 0.01
PPDMS 36:6	0.92 ± 0.09	1.03 ± 0.03	0.59 ± 0.05	0.64 ± 0.06	0.41 ± 0.02	0.99 ± 0.10	1.01 ± 0.03	0.63 ± 0.05	0.64 ± 0.05	0.41 ± 0.03	0.99 ± 0.07	1.03 ± 0.02	0.91 ± 0.11	0.87 ± 0.08	0.55 ± 0.06
PPDMS 38:6	0.29 ± 0.03	0.36 ± 0.02	0.22 ± 0.01	0.26 ± 0.01	0.17 ± 0.00	0.32 ± 0.02	0.35 ± 0.05	0.24 ± 0.00	0.25 ± 0.01	0.17 ± 0.00	0.32 ± 0.02	0.36 ± 0.02	0.42 ± 0.02	0.39 ± 0.02	0.28 ± 0.03
PPDMS 40:7	0.12 ± 0.03	0.15 ± 0.02	0.12 ± 0.00	0.13 ± 0.00	0.11 ± 0.01	0.14 ± 0.02	0.14 ± 0.03	0.13 ± 0.01	0.12 ± 0.00	0.11 ± 0.01	0.13 ± 0.02	0.15 ± 0.02	0.37 ± 0.01	0.38 ± 0.02	0.26 ± 0.02
PPDMS 44:12	0.17 ± 0.02	0.21 ± 0.03	0.11 ± 0.01	0.13 ± 0.00	0.08 ± 0.01	0.20 ± 0.01	0.21 ± 0.02	0.12 ± 0.00	0.14 ± 0.01	0.08 ± 0.00	0.19 ± 0.01	0.20 ± 0.02	0.16 ± 0.00	0.13 ± 0.00	0.10 ± 0.01
PE 30:1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
PE 31:1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00
PE 32:1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.03 ± 0.00	0.05 ± 0.01
PE 33:1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00
PE 34:1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.02 ± 0.01
DGCC 36:6	0.77 ± 0.09	0.88 ± 0.04	0.52 ± 0.05	0.59 ± 0.05	0.38 ± 0.02	0.82 ± 0.09	0.86 ± 0.02	0.54 ± 0.05	0.59 ± 0.04	0.38 ± 0.03	0.84 ± 0.07	0.87 ± 0.02	0.76 ± 0.08	0.76 ± 0.05	0.49 ± 0.04
DGCC 38:6	0.24 ± 0.03	0.31 ± 0.04	0.21 ± 0.01	0.28 ± 0.01	0.17 ± 0.00	0.27 ± 0.02	0.30 ± 0.04	0.22 ± 0.01	0.28 ± 0.01	0.18 ± 0.00	0.26 ± 0.01	0.30 ± 0.02	0.29 ± 0.01	0.24 ± 0.01	0.21 ± 0.01
DGCC 40:7	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.00	0.07 ± 0.00	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.00	0.07 ± 0.00	0.06 ± 0.00	0.06 ± 0.01	0.07 ± 0.01	0.15 ± 0.01	0.15 ± 0.01	0.14 ± 0.01
DGCC 44:12	0.54 ± 0.05	0.61 ± 0.02	0.39 ± 0.04	0.43 ± 0.03	0.29 ± 0.01	0.58 ± 0.05	0.59 ± 0.02	0.41 ± 0.03	0.43 ± 0.02	0.29 ± 0.02	0.59 ± 0.03	0.60 ± 0.00	0.57 ± 0.05	0.51 ± 0.03	0.36 ± 0.03
BLL 36:6	0.00 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.03 ± 0.00	0.32 ± 0.03	0.33 ± 0.02	0.23 ± 0.01
BLL 38:6	0.13 ± 0.02	0.19 ± 0.08	0.09 ± 0.01	0.13 ± 0.05	0.07 ± 0.01	0.17 ± 0.04	0.14 ± 0.02	0.10 ± 0.01	0.13 ± 0.03	0.06 ± 0.01	0.15 ± 0.00	0.14 ± 0.02	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
BLL 40:7	0.06 ± 0.01	0.10 ± 0.04	0.05 ± 0.01	0.08 ± 0.03	0.05 ± 0.00	0.08 ± 0.02	0.07 ± 0.01	0.06 ± 0.01	0.07 ± 0.02	0.05 ± 0.00	0.07 ± 0.00	0.07 ± 0.01	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00
BLL 44:12	0.01 ± 0.00	0.03 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	0.04 ± 0.00
DGTS 28:0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
DGTS 30:1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
DGTS 32:1	0.39 ± 0.03	0.44 ± 0.01	0.33 ± 0.03	0.36 ± 0.04	0.26 ± 0.01	0.41 ± 0.02	0.43 ± 0.01	0.34 ± 0.02	0.35 ± 0.02	0.26 ± 0.01	0.41 ± 0.02	0.44 ± 0.01	0.56 ± 0.06	0.57 ± 0.04	0.47 ± 0.05

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MGDG 32:0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
MGDG 32:2	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
MGDG 32:3	0.05 ± 0.01	0.06 ± 0.01	0.05 ± 0.00	0.04 ± 0.00	0.06 ± 0.00	0.06 ± 0.01	0.05 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.05 ± 0.00	0.05 ± 0.01	0.03 ± 0.00	0.03 ± 0.00	0.05 ± 0.00	0.04 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
MGDG 34:1	0.04 ± 0.00	0.05 ± 0.01	0.02 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	0.05 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	0.05 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.07 ± 0.00	0.06 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
MGDG 34:2	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
MGDG 34:3	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
MGDG 34:4	0.02 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
MGDG 34:6 (1)	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
MGDG 34:6 (2)	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
MGDG 36:1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
MGDG 36:2	0.06 ± 0.01	0.06 ± 0.01	0.04 ± 0.00	0.04 ± 0.00	0.07 ± 0.01	0.06 ± 0.01	0.05 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.07 ± 0.00	0.06 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
MGDG 36:3	0.09 ± 0.02	0.10 ± 0.02	0.06 ± 0.00	0.07 ± 0.00	0.10 ± 0.01	0.10 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.00	0.10 ± 0.01	0.10 ± 0.01	0.04 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
MGDG 36:4	0.32 ± 0.04	0.38 ± 0.04	0.20 ± 0.00	0.26 ± 0.01	0.36 ± 0.02	0.39 ± 0.03	0.22 ± 0.01	0.27 ± 0.01	0.27 ± 0.01	0.37 ± 0.01	0.38 ± 0.03	0.27 ± 0.00	0.27 ± 0.00	0.22 ± 0.02	0.22 ± 0.02	0.10 ± 0.01	0.10 ± 0.01
MGDG 36:5	0.04 ± 0.01	0.05 ± 0.01	0.03 ± 0.00	0.04 ± 0.00	0.05 ± 0.00	0.05 ± 0.01	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.05 ± 0.00	0.05 ± 0.01	0.03 ± 0.00	0.03 ± 0.00	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.00	0.02 ± 0.00
MGDG 36:7	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.00	0.03 ± 0.00	0.05 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.05 ± 0.00	0.04 ± 0.01	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
Lyo-MGDG 16:0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lyo-MGDG 16:1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lyo-MGDG 16:2	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lyo-MGDG 18:1 (1)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lyo-MGDG	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

[illegible]

[illegible]

[illegible]

[illegible]

Relative lipid abundance and composition following viral infection

In the next step, the main changes in lipid abundance and composition were evaluated by hierarchal clustering analysis performed on cultures infected with the lytic virus as compared to the un-infected (control) cells and cultures infected with the non-lytic virus at the 5 different time points along the experiment (Figure 4). This analysis indicated that the relative abundance of glycolipids (GL) [e.g., monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG) and sulfoquinovosyldiacylglycerol (SQDG)], the main membrane lipids of the thylakoids, was reduced over time to a similar extent in all tested cultures. On the other hand, the triacylglycerol (TAG) and phospholipids (PL) were present at very low levels in the control and the non-lytic infected cells, however their presence was greatly induced upon lytic viral infection. Interestingly, while TAGs level was induced significantly already 24 hpi, the levels of PLs (i.e. phosphatidylethanolamine, PE and phosphatidylcholine, PC) gradually increased during the infection cycle, reaching maximum levels at later stages of infection (48 hpi). In contrast to the prominent induction of TAGs and PLs levels during lytic infection, betaine lipids (BL) as well as sphingolipids (SPL) showed a mixed trend, where the levels of some of the lipid species were reduced upon lytic viral infection, while the levels of others were induced. In agreement with previous studies (Vardi et al., 2009, Fulton et al., 2014, Rosenwasser et al., 2014), the glycosphingolipid vGSL, which is a unique viral derived lipid, was exclusively detected in viral-infected cells during the onset of lytic viral infection. Importantly, vGSL (marked by an arrow in Figure 2B) was found to cluster along with the majority of the identified TAGs, further supporting the observed induction of TAG biosynthesis during lytic viral infection.

Further inspection of the relative abundance of each lipid sub-classes (Figure 5A) revealed that 48 hpi, TAGs were found to account for 20 % of the total detected lipids in the lytic-infected cells, in contrary to only 2 % in the control cells. This accumulation of TAGs was coincided with a decrease in the relative abundance of MGDG, DGDG and SQDG in the lytic-infected cells: The MGDG fraction was only 20 % in the lytic-infected cells 48 hpi, compared to 31 % in the control cells; the DGDG accounted for 16 % in the control while only 11 % in the lytic-infected cells; and SQDG was accounted for 8 % in the lytic-infected cells as compared with 13 % in the control cells.

Importantly though, the amount of the glycolipids per cell was not different in the lytic-infected cells in comparison to the control cells (Table 2 above).

A small increase in the relative abundance of phospholipids (PE and PC) as well as sphingolipids was also evident in the cells infected with the lytic virus. No difference was observed in the relative abundance of the different lipid classes in the non-lytic infected cells relative to the control (Figure 5A).

Formation of lipid droplets

A common feature during accumulation of neutral lipids as TAGs is the formation of lipid droplets (LD). LD are specific organelles in the cell cytoplasm that store energy mainly in the form of TAGs and also serve as detoxification mechanism to prevent lipotoxicity upon overload of free fatty acids (FFA) (Kohlwein 2010). In order to test changes in LD formation during lytic infection, cells were stained with BODIPY 493/505, a specific stain for non-polar / neutral lipids (Traller and Hildebrand 2013, Hallenbeck et al., 2015). As demonstrated in Figure 6A, fluorescence microscopy imaging showed high number of LD with strong fluorescence signals in cells infected by lytic viruses compare to uninfected cells. Further quantification of the BODIPY signals by flow cytometry confirmed that high fraction of cells contained neutral lipid during lytic infection (Figure 6B): a significant increase in BODIPY staining started 24 hpi which was consistent also at 48 hpi. These results were also confirmed by using imaging flow cytometry analysis which revealed high localized BODIPY signal with a spot-like distribution within infected *E. huxleyi* cells. Low diffused BODIPY signal was detected in control cells (Figures 6C-D). These results are in agreement with the LC-MS data showing an increase in TAGs levels at these time point (Tables 2 and 3 above), suggesting that the lytic-infected cells stored TAGs in LD.

Accumulation of TAGs in the isolated virions

Since TAGs biosynthesis and accumulation was found to be the most significant remodeling in lipid composition following lytic viral infection, it was hypothesized that the newly produced viruses were enriched with TAGs. To this end, the lipidome of the isolated EhV201 virions was analyzed (Figure 5B and Table 4 below). The virions were purified by optiprep gradient which keeps the virions intact and infectious (Figure 5C). Interestingly, the relative abundance of the detected lipids varied markedly between the infected cells 48 hpi and the virions (Figures 5A-B): The lipidome of the viral particles

was composed of sphingolipids (3 %), betaine lipids (8 %), phospholipid (3 %) but mostly of TAGs (77 %), which were found to be highly enriched in the virion relative to the lytic-infected cells. These results clearly indicated a selective mode of membrane recruitment during viral assembly which differ from host membrane lipid composition, and may imply the budding of the virus from a specialized subcellular compartment (Liu et al., 2011).

Table 4: Relative quantification of the identified lipids in isolated EhV201 virions

Lipids:	<i>EhV201</i> virions	Lipids:	<i>EhV201</i> virions	Lipids:	<i>EhV201</i> virions	Lipids:	<i>EhV201</i> virions
MGDG 28:0		TAG 44:1	1.78E+6	TAG 58:5 (1)		SQDG 32:1	2.45E+4
MGDG 32:1	1.05E+5	TAG 46:0		TAG 58:5 (2)		SQDG 32:2 (1)	
MGDG 32:4		TAG 46:1	3.73E+6	TAG 58:6 (1)		SQDG 32:2 (2)	
MGDG 32:5		TAG 46:2	1.78E+6	TAG 58:6 (2)		SQDG 32:3	4.41E+4
MGDG 36:8		TAG 46:3		DGDG 32:1	1.14E+4	PC 32:1	1.32E+5
MGDG 36:9		TAG 48:0	2.74E+6	DGDG 32:2		PC 32:2	
MGDG 36:10		TAG 48:1	4.52E+6	DGDG 32:3		PC 32:3	
MGDG 40:11		TAG 48:2	3.16E+6	DGDG 32:4		PC 34:1	3.58E+4
MGDG 44:12		TAG 48:3	8.66E+5	DGDG 34:1		PC 34:2	
DGDG 28:0	1.32E+4	TAG 48:4		DGDG 34:2		PC 34:3	
DGDG 32:1	1.14E+4	TAG 50:0	1.20E+6	DGDG 34:3		PC 34:4 (1)	
DGDG 32:4		TAG 50:1	4.10E+6	DGDG 34:4		PC 34:4 (2)	
DGDG 32:5		TAG 50:2	3.33E+6	DGDG 34:5 (1)		PC 36:2	7.10E+4
DGDG 36:10		TAG 50:3	1.31E+6	DGDG 34:5 (2)		PC 36:5	
SQDG 28:0	1.43E+5	TAG 50:4		DGDG 36:2		PC 38:3	
SQDG 30:0	9.07E+4	TAG 50:5		DGDG 36:3		PC 38:4	
SQDG 32:3	4.41E+4	TAG 50:6		DGDG 36:4		PC 38:6	2.76E+5
SQDG 32:4		TAG 50:7 (1)		DGDG 36:5		lysoPC 16:0	1.27E+3
SQDG 36:6 (1)		TAG 50:7 (2)		DGDG 36:6		LysoPC 18:0	
SQDG 36:6 (2)		TAG 50:8 (1)		DGDG 36:7		LysoPC 18:1 (1)	
SQDG 36:7	7.38E+3	TAG 50:8 (2)		DGDG 38:3		LysoPC 18:1 (2)	
PPDMS 36:6	1.85E+6	TAG 50:9		Lyso-DGDG 16:0		lysoPC 18:3 (1)	
PPDMS 38:6	8.11E+5	TAG 52:0	1.13E+6	Lyso-DGDG 18:3		lysoPC 18:3 (2)	
PPDMS 40:7	1.18E+6	TAG 52:1	1.33E+6	Lyso-DGDG 18:2		PE 32:1 (1)	
PPDMS 44:12	4.17E+5	TAG 52:2	3.13E+6	Lyso-DGDG 18:1		PE 32:1 (2)	
PE 30:1	1.79E+5	TAG 52:3	1.85E+6	MGDG 32:0		PE 32:2	2.99E+5
PE 31:1	6.08E+5	TAG 52:4	9.09E+5	MGDG 32:2		PE 32:3	
PE 32:1	1.62E+5	TAG 52:5		MGDG 32:3		PE 34:1	2.12E+4
PE 33:1	3.12E+4	TAG 52:6		MGDG 34:1		PE 34:2	3.13E+4
PE 34:1	2.12E+4	TAG 52:7 (1)		MGDG 34:2		PE 34:3	

DGCC 36:6	1.20E+6	TAG 52:7 (2)		MGDG 34:3		PE 34:4 (1)	
DGCC 38:6	4.32E+5	TAG 52:8		MGDG 34:4		PE 34:4 (2)	
DGCC 40:7	3.72E+5	TAG 52:9		MGDG 34:6 (1)		PE 34:5	
DGCC 44:12	4.47E+5	TAG 54:1	7.68E+5	MGDG 34:6 (2)		PE 36:2	
BLL 36:6	7.35E+4	TAG 54:2	7.19E+5	MGDG 36:1		PE 36:3	
BLL 38:6	7.77E+4	TAG 54:3	1.87E+6	MGDG 36:2		PE 36:4	
BLL 40:7		TAG 54:4	1.16E+6	MGDG 36:3		PE 38:4	
BLL 44:12	4.31E+5	TAG 54:5	8.75E+5	MGDG 36:4	1.09E+4	LysoPE 16:0	
DGTS 28:0	4.35E+4	TAG 54:6	4.78E+5	MGDG 36:5		LysoPE 18:1 (1)	
DGTS 30:1		TAG 54:7		MGDG 36:7		LysoPE 18:1 (2)	
DGTS 32:1	2.39E+6	TAG 54:8		Lyso-MGDG 16:0		Cer t18:1/h20:0	
DGTS 32:2	5.14E+4	TAG 54:9		Lyso-MGDG 16:1		Cer t18:1/c22:0	
DGTS 32:3	2.17E+4	TAG 54:10		Lyso-MGDG 16:2		Cer d18:0/h22:0	
DGTS 32:4	4.83E+3	TAG 56:1	4.78E+5	Lyso-MGDG 18:1 (1)		Cer t18:0/c22:0	
DGTS 33:4		TAG 56:2	3.58E+5	Lyso-MGDG 18:1 (2)		Cer d18:0/c24:0	
DGTS 34:4		TAG 56:3		Lyso-MGDG 18:2 (1)		Cer t18:1/h22:0	
DGTS 36:6		TAG 56:4		Lyso-MGDG 18:2 (2)		Cer t18:0/h22:0	
hGSL22:2 d19:3LCB	8.57E+4	TAG 56:5		Lyso-MGDG 18:3 (1)		Cer t18:0/h24:0	
vGSL	1.14E+6	TAG 56:6		Lyso-MGDG 18:3 (2)		Cer d18:1/h26:1 Cer t18:1/c26:1 Cer t18:0/c26:2 Cer d18:2/h26:0	
sGSL 22:0 d18:2 LCB	4.15E+5	TAG 56:7		SQDG 34:1		Cer t18:0/c26:0	
sGSL 22:0 d18:1 LCB	5.42E+4	TAG 56:8		SQDG 34:3	6.29E+3	Cer t18:0/h26:0	
TAG 42:0	1.09E+6	TAG 58:2	1.94E+5	SQDG 36:5		GlcCer t18:1/h22:0	7.18E+4
TAG 42:1	8.43E+5	TAG 58:3		SQDG 36:6		DAG 36:3	
TAG 44:0	1.81E+6	TAG 58:4		SQDG 32:0		DAG 36:4	
						DAG 36:5	

Following viral infection, the TAGS composition is enriched with saturated and monounsaturated TAGs

- In order to better resolve the composition and abundance of the accumulated TAGs during infection, their saturation level was further analyzed. TAGs accumulated in the lytic-infected cells 72 hpi were significantly enriched with saturated and especially monounsaturated TAGs, which accounted for 57 % of the total TAGs detected (Figure 7, left panel). The most abundant TAGs in the lytic-infected cells were TAG46:1, TAG48:1 and TAG50:1 that reached their maximum level at 72 hpi.

Interestingly, the profile of TAGs identified in the purified virion (Figure 7, right panel) showed a similar, though more pronounced, preference towards the more saturated fatty acids in TAGs, as 80 % of the TAGs in the virions were either saturated or with 1-2 double bonds (relative to 70 % in the lytic-infected algal cell). This may imply a physiological role for saturated TAGs in determining the infectivity and decay rates of EhV.

***De novo* biosynthesis of TAGs is upregulated during viral infection**

In order to decipher the biosynthetic pathways involved in the lipidome remodeling during viral infection, the expression profile of genes predicted to be involved in the *de-novo* production of TAGs (Tanaka et al., 2015) was analyzed. Four enzymes catalyze the sequential acylation of the glycerol backbone to produce TAGs *de-novo* (Figure 8):

1. The acylation of glycerol-3-phosphate (G3P) is catalyzed by *sn*-glycerol-3-phosphate acyltransferase (GPAT, EC 2.3.1.15) to produce lysophosphatidic acid (LP), which is an important intermediate for the formation of storage and membrane lipids.
2. The second acylation is catalyzed by acyl-CoA:lyso-phosphatidic acid acyltransferase (LPAT, EC 2.3.1.51).
3. The generation of *sn*-1,2-diacylglycerol (DAG) is mediated by phosphatidic acid phosphatase (PAP, EC 3.1.3.4) which catalyzes the removal of the phosphate group from the phosphatidic acid (PA) (Deng et al., 2013).
4. The last, key rate-limiting, acyl-CoA-dependent acylation is catalyzed by acyl-CoA:diacylglycerol acyltransferase (DGAT, EC 2.3.1.20).

The analysis was based on RNAseq transcriptome of *E. huxleyi* 2090 at 1 and 24 hpi with the lytic and non-lytic virus and normalized to control cells (Rosenwasser et al., 2014) (Figure 8). Genes encoding for TAG biosynthesis were annotated based on the *E. huxleyi* genome (Read et al., 2013) and *de novo* transcriptome assembly (Feldmesser et al., 2014). The list of the annotated *E. huxleyi* genes and their homologous is shown in Table 5 below. Up regulation at 24 hpi was observed for acyl-CoA:lyso-phosphatidic acid acyltransferase (LPAT, 4 fold increase), phosphatidic acid phosphatase (PAP2, 14.5 fold increase) and some of the acyl-CoA:diacylglycerol acyltransferase orthologues genes (DGAT2, 3.5-4 fold increase), supporting the

induction of TAG biosynthesis during lytic infection. Down-regulation of phospholipid:diacylglycerol acyltransferase (PDAT, EC 2.3.1.158) which catalyzes TAG biosynthesis from phospholipids was also observed, resulting in a 4-fold decrease in its expression at 24 hpi of lytic infection. These data point for the involvement of the

5 *de-novo* biosynthesis pathway in the production of TAGs during lytic infection.

Table 5: Expression profiles of genes encoding for TAG biosynthesis enzymes. Data is presented as fold change normalized to control.

Gene Name	<i>E. huxleyi</i> gene sequence ID (From JGI*)	Lytic infection 1hr	Non-Lytic infection 1hr	Lytic infection 24hr	Non-Lytic infection 24hr	Best Hit (From NCBI**)
GPAT	441121	1.30	-1.26	-1.94	2.00	gi 46446952 ref YP_008317.1 putative glycerol-3-phosphate acyltransferase [Candidatus Protochlamydia amoebophila UWE25] > gi 46400593 emb CAF24042.1 putative glycerol-3-phosphate acyltransferase [Parachlamydia sp. UWE25] (model%: 74, hit%: 42, score: 444, %id: 55) [Candidatus Protochlamydia amoebophila UWE25]
GPAT	463748	1.20	-1.28	1.28	-1.30	gi 6319459 ref NP_009542.1 Glycerol 3-phosphate/dihydroxyacetone phosphate dual substrate-specific sn-1 acyltransferase of the glycerolipid biosynthesis pathway, prefers 16-carbon fatty acids, similar to Gpt2p, gene is constitutively transcribed; Sct1p [Saccharomyces cerevisiae] > gi 51704271 sp P32784 GPT1_YEAST Glycerol-3-phosphate O-acyltransferase 1 (G-3-P acyltransferase 1) (Dihydroxyacetone phosphate acyltransferase 1) (DHAP-AT 1) (Glycerol-3-phosphate / dihydroxyacetone phosphate acyltransferase 1) > gi 535999 emb CAA84831.1 SCT1 [Saccharomyces cerevisiae] (model%: 82, hit%: 71, score: 820, %id: 18) [Saccharomyces cerevisiae]
GPAT	450766	1.20	-1.19	-1.29	1.16	gi 115482728 ref NP_001064957.1 Os10g0497100 [Oryza sativa (japonica cultivar-group)]

						>gi 78708851 gb ABB47826.1 1-acyl-sn-glycerol-3-phosphate acyltransferase 1, chloroplast precursor, putative, expressed [<i>Oryza sativa</i> (japonica cultivar-group)] >gi 113639566 dbj BAF26871.1 Os10g0497100 [<i>Oryza sativa</i> (japonica cultivar-group)] (model%: 72, hit%: 66, score: 453, %id: 33) [no tax name]
LPAT	438276	1.07	1.08	4.17	-6.44	gi 118744049 ref ZP_01592047.1 1-acyl-sn-glycerol-3-phosphate acyltransferases [<i>Geobacter lovleyi</i> SZ] >gi 118683040 gb EAV89441.1 1-acyl-sn-glycerol-3-phosphate acyltransferases [<i>Geobacter lovleyi</i> SZ] (model%: 33, hit%: 54, score: 279, %id: 35) [no tax name]
PAP	210687	1.40	-1.38	1.87	-2.09	dre:431758 ppapdc1b : phosphatidic acid phosphatase type 2 domain containing 1B (model%: 82, hit%: 71, score: 324, %id: 39) [<i>Danio rerio</i>]
PAP2	193908	-1.20	1.16	-1.98	1.18	gi 73852549 ref YP_293833.1 putative lipid phosphate phosphatase [<i>Emiliana huxleyi</i> virus 86] >gi 72415265 emb CAI65502.2 putative lipid phosphate phosphatase [<i>Emiliana huxleyi</i> virus 86] (model%: 59, hit%: 74, score: 213, %id: 14) [<i>Emiliana huxleyi</i> virus 86]
PAP	233403	1.93	-2.13	14.47	-28.57	gi 78499696 gb ABB45850.1 hypothetical protein [<i>Theilungiella halophila</i>] (model%: 77, hit%: 46, score: 236, %id: 25) [<i>Theilungiella halophila</i>]
PAP	445286	-1.19	-1.08	-5.52	4.79	gi 116056575 emb CAL52864.1 putative phosphatidic acid phosphatase (ISS) [<i>Ostreococcus tauri</i>] (model%: 46, hit%: 45, score: 187, %id: 15) [no tax name]
DGAT1	64505	-1.14	-1.05	1.84	-1.73	i 50299542 gb AAT73629.1 acyl CoA:diacylglycerol acyltransferase [<i>Glycine max</i>] (model%: 93, hit%: 36, score: 500, %id: 49) [<i>Glycine max</i>]
DGAT2	115645	-1.15	1.10	-5.28	3.81	gi 39580537 emb CAE74669.1 Hypothetical protein CBG22470 [<i>Caenorhabditis briggsae</i>] (model%: 82, hit%: 83, score: 339, %id: 16) [<i>Caenorhabditis briggsae</i>]
DGAT2	194543	1.28	-1.44	1.38	-1.47	gi 114615126 ref XP_527842.2 PREDICTED: monoacylglycerol O-acyltransferase 3 isoform 3 [Pan

						troglodytes] (model%: 65, hit%: 74, score: 285, %id: 20) [no tax name]
DGAT2	462450	1.86	-1.24	-1.03	-1.09	gi 126342781 ref XP_001368584.1 PREDICTED: hypothetical protein [<i>Monodelphis domestica</i>] (model%: 61, hit%: 59, score: 369, %id: 23) [no tax name]
DGAT2	213149	1.09	-1.71	3.96	-6.62	gi 148222468 ref NP_001080699.1 diacylglycerol O-acyltransferase 2 like 1 [<i>Xenopus laevis</i>] > gi 27924233 gb AAH45058.1 Dgat2l1-a protein [<i>Xenopus laevis</i>] (model%: 78, hit%: 92, score: 563, %id: 26) [no tax name]
DGAT2	67639	1.03	-1.19	1.01	1.01	gi 86279636 gb ABC94473.1 type 2 diacylglycerol acyltransferase [<i>Vernicia fordii</i>] > gi 86279638 gb ABC94474.1 type 2 diacylglycerol acyltransferase [<i>Vernicia fordii</i>] (model%: 78, hit%: 50, score: 308, %id: 33) [<i>Vernicia fordii</i>]
DGAT2	440315	1.16	-1.31	3.52	-3.32	gi 115467862 ref NP_001057530.1 Os06g0326700 [<i>Oryza sativa</i> (japonica cultivar-group)] > gi 50725740 dbj BAD33251.1 putative mono- or diacylglycerol acyltransferase [<i>Oryza sativa</i> (japonica cultivar-group)] > gi 50725979 dbj BAD33505.1 putative mono- or diacylglycerol acyltransferase [<i>Oryza sativa</i> (japonica cultivar-group)] > gi 113595570 dbj BAF19444.1 Os06g0326700 [<i>Oryza sativa</i> (japonica cultivar-group)] (model%: 91, hit%: 39, score: 241, %id: 27) [no tax name]
DGAT2	112304	-1.08	-1.27	-2.34	2.03	ath:At3g51520 F26O13.160 : diacylglycerol acyltransferase family (model%: 55, hit%: 63, score: 297, %id: 34) [<i>Arabidopsis thaliana</i>]
PDAT	222113	1.02	-1.04	-4.31	3.98	gi 47208625 emb CAF91461.1 unnamed protein product [<i>Tetraodon nigroviridis</i>] (model%: 62, hit%: 75, score: 321, %id: 13) [<i>Tetraodon nigroviridis</i>]

* JGI database can be accessed through

[www.genome\(dot\)jgi-psf9\(dot\)org/Emihu1/Emihu1\(dot\)home\(dot\)html](http://www.genome(dot)jgi-psf9(dot)org/Emihu1/Emihu1(dot)home(dot)html) or

[www.genome9\(dot\)jgi-psf\(dot\)org/EmihuEXTC/EmihuEXTC\(dot\)home\(dot\)html](http://www.genome9(dot)jgi-psf(dot)org/EmihuEXTC/EmihuEXTC(dot)home(dot)html)

** NCBI database can be accessed through [www\(dot\)ncbi\(dot\)nlin\(dot\)nih\(dot\)gov](http://www(dot)ncbi(dot)nlin(dot)nih(dot)gov)

EXAMPLE 2

TAGS LEVELS AND COMPOSITION FOLLOWING VIRAL INFECTION AS COMPARED TO NITROGEN STARVATION

5 Nitrogen (N) starvation is commonly used to induce TAGs accumulation in algae (Breuer et al., 2012, Razeghifard 2013, Yang et al., 2013) for e.g., biofuel production. Thus, in order to examine the unique lipid composition of TAGs induced by lytic viral infection and to evaluate its suitability for biofuel production, TAGs production following viral infection was compared to the N-depletion in *E. huxleyi*
10 (Table 6 below).

As shown in Figures 9A-B, both induced stresses resulted in a significant increase in the total lipids detected per cell, reaching its maximum value at 72 following stress induction. However, the effect of viral infection was more pronounced as compared to N-starvation and a significant increase in the level of lipids detected per
15 cell was evident at late stages of viral infection. Specifically determining the amount of TAGs per cell, a similar pattern of induction was observed, wherein both treatments increased the levels of TAGs reaching its maximum value at 72 hours following stress induction (Figures 9C-D), a significantly higher amounts of TAGs were accumulated upon viral infection. These results were supported by the increase in BODIPY staining
20 of neutral lipids during the 5 days of infection and N-starvation (data not shown). Absolute quantification of five TAG markers (44:1, 48:1, 50:0, 58:7 and 58:10) normalized per cell was performed according to calibration curves of their corresponding standards (Figure 10). None of these TAGs were detected in the control samples. Of these five TAGs, the mono-unsaturated 48:1 TAG was found to be the most
25 abundant TAG in the viral-infected cells accumulating to 72.2 ± 4.2 fg/cell, while the unsaturated 58:10 TAG was the most abundant TAG in the N-deprived cells accumulating to 53 ± 5.8 fg/ cell (Table 7). Taken together, the results indicated accumulation of TAGs following both N-starvation and viral infection with the latter having a greater effect.

30 Interestingly, while the two conditions tested induced the accumulation of lipids to different extents, the induction of TAGs can account for only up to ca 50 % of the total lipids detected per cell regardless of the inducing stress. However, even though

the relative fraction of TAGs was similar between the N-limited cells and the viral infected cells, a pronounced difference between the TAG compositions was apparent. Absolute quantification of several TAGs according to their corresponding standards is presented in Table 6 below. As mentioned earlier, TAGs induced by the lytic viral infection showed a clear preference towards the saturated and mono-unsaturated TAGs (Figure 9E). In contrast, TAGs induced by N-depletion were more evenly distributed with regard to the number of double bonds (Figure 9F). The differences in the saturation characteristics of TAGs between the two conditions are not a general trait of the two lipidomes since the saturation of MGDG is similar between the two treatments, and no significant difference is apparent (Figures 9E-F). These results may reflect differences in the biosynthetic pathways that are involved in TAGs formation in N-depleted cells vs. lytic viral infected cells.

Table 6: Relative quantification (AU, normalized peak intensity per cell) of the identified TAGs in control compared to following lytic viral infection or N-starvations for 72 hours.

AU/10 ⁶ cells	Average		STD		AU/10 ⁶ cells	Average		STD	
Name	Control	Lytic infected cells	Control	Lytic infected cells	Name	Control	N-Depleted cells	Control	N-Depleted cells
TAG 42:0	0.00	0.58	0.00	0.06	TAG 42:0	0.00	0.02	0.00	0.01
TAG 42:1	0.00	0.15	0.00	0.04	TAG 42:1	0.00	0.02	0.00	0.01
TAG 44:0	0.00	1.38	0.00	0.05	TAG 44:0	0.00	0.03	0.00	0.01
TAG 44:1	0.00	0.43	0.00	0.08	TAG 44:1	0.00	0.09	0.00	0.04
TAG 46:0	0.00	1.79	0.00	0.15	TAG 46:0	0.01	0.03	0.00	0.01
TAG 46:1	0.02	4.02	0.00	0.21	TAG 46:1	0.03	0.99	0.00	0.51
TAG 46:2	0.00	0.30	0.00	0.03	TAG 46:2	0.00	0.08	0.00	0.03

TAG 46:3	0.00	0.05	0.00	0.02	TAG 46:3	0.00	0.02	0.00	0.01
TAG 46:4	0.00	0.04	0.00	0.02	TAG 46:4	0.00	0.01	0.00	0.00
TAG 46:5	0.00	0.04	0.00	0.01	TAG 46:5	0.00	0.00	0.00	0.00
TAG 48:0	0.00	1.50	0.00	0.16	TAG 48:0	0.01	0.02	0.00	0.00
TAG 48:1	0.02	3.88	0.00	0.35	TAG 48:1	0.02	0.56	0.00	0.23
TAG 48:2	0.01	0.78	0.00	0.01	TAG 48:2	0.01	0.18	0.00	0.06
TAG 48:3	0.00	0.17	0.00	0.02	TAG 48:3	0.00	0.03	0.00	0.01
TAG 48:4	0.00	0.06	0.00	0.01	TAG 48:4	0.00	0.04	0.00	0.02
TAG 48:5	0.00	0.07	0.00	0.02	TAG 48:5	0.00	0.00	0.00	0.00
TAG 48:6	0.00	0.00	0.00	0.00	TAG 48:6	0.00	0.01	0.00	0.00
TAG 50:0	0.00	0.90	0.00	0.03	TAG 50:0	0.00	0.01	0.00	0.00
TAG 50:1	0.01	4.65	0.00	0.37	TAG 50:1	0.02	0.42	0.00	0.18
TAG 50:2	0.01	1.48	0.00	0.12	TAG 50:2	0.03	0.82	0.00	0.33
TAG 50:3	0.00	0.35	0.00	0.03	TAG 50:3	0.01	0.23	0.00	0.12
TAG 50:4 (1)	0.00	0.30	0.00	0.01	TAG 50:4	0.01	0.30	0.00	0.16
TAG 50:4 (2)	0.00	0.11	0.00	0.03	TAG 50:4	ND	ND	ND	ND
TAG 50:5	0.01	0.28	0.00	0.06	TAG 50:5	0.00	0.17	0.00	0.07
TAG 50:6 (1)	0.00	0.03	0.00	0.01	TAG 50:6 (1)	0.00	0.01	0.00	0.01
TAG	0.00	0.00	0.00	0.00	TAG	0.00	0.02	0.00	0.01

50:6 (2)					50:6 (2)				
TAG 50:6 (3)	0.01	0.36	0.00	0.10	TAG 50:6 (3)	0.00	0.23	0.00	0.09
TAG 50:7	0.00	0.05	0.00	0.02	TAG 50:7	0.00	0.01	0.00	0.00
TAG 50:8 (1)	0.00	0.02	0.00	0.01	TAG 50:8 (1)	0.00	0.00	0.00	0.00
TAG 50:8 (2)	0.00	0.00	0.00	0.00	TAG 50:8 (2)	0.00	0.01	0.00	0.00
TAG 50:9	0.00	0.03	0.00	0.02	TAG 50:9	0.00	0.01	0.00	0.00
TAG 50:10	0.01	0.20	0.00	0.07	TAG 50:10	0.00	0.03	0.00	0.01
TAG 52:0	0.00	0.64	0.00	0.02	TAG 52:0	0.00	0.00	0.00	0.00
TAG 52:1	0.00	0.47	0.00	0.06	TAG 52:1	0.00	0.12	0.00	0.03
TAG 52:2	0.00	1.19	0.00	0.05	TAG 52:2	0.01	0.22	0.00	0.04
TAG 52:3	0.00	0.75	0.00	0.05	TAG 52:3	0.02	0.31	0.00	0.12
TAG 52:4	0.00	0.56	0.00	0.05	TAG 52:4	0.01	0.29	0.00	0.12
TAG 52:5	0.00	0.22	0.00	0.03	TAG 52:5	0.01	0.20	0.00	0.07
TAG 52:6	0.01	0.28	0.00	0.06	TAG 52:6	0.00	0.12	0.00	0.04
TAG 52:7 (1)	0.00	0.00	0.00	0.00	TAG 52:7 (1)	0.00	0.02	0.00	0.01
TAG 52:7 (2)	0.00	0.00	0.00	0.00	TAG 52:7 (2)	0.00	0.03	0.00	0.01
TAG 52:8 (1)	0.00	0.00	0.00	0.00	TAG 52:8 (1)	0.00	0.00	0.00	0.00
TAG 52:8 (2)	0.00	0.00	0.00	0.00	TAG 52:8 (2)	0.00	0.01	0.00	0.00
TAG 52:9 (1)	0.00	0.00	0.00	0.00	TAG 52:9 (1)	0.00	0.01	0.00	0.00

TAG 52:9 (2)	0.00	0.00	0.00	0.00	TAG 52:9 (2)	0.00	0.01	0.00	0.00
TAG 52:10 (1)	0.00	0.00	0.00	0.00	TAG 52:10 (1)	0.00	0.00	0.00	0.00
TAG 52:10 (2)	0.00	0.09	0.00	0.07	TAG 52:10 (2)	0.00	0.01	0.00	0.00
TAG 52:11	0.00	0.00	0.00	0.00	TAG 52:11	0.00	0.01	0.00	0.00
TAG 54:1	0.00	2.65	0.00	0.11	TAG 54:1	0.02	0.39	0.00	0.07
TAG 54:2	0.00	0.55	0.00	0.04	TAG 54:2	0.02	0.13	0.00	0.03
TAG 54:3	0.01	2.25	0.00	0.24	TAG 54:3	0.02	0.28	0.00	0.08
TAG 54:4 (1)	0.00	0.78	0.00	0.02	TAG 54:4	0.02	0.36	0.00	0.14
TAG 54:4 (2)	0.00	0.12	0.00	0.03	TAG 54:4	ND	ND	ND	ND
TAG 54:5 (1)	0.00	0.36	0.00	0.06	TAG 54:5 (1)	0.02	0.46	0.00	0.19
TAG 54:5 (2)	0.00	0.44	0.00	0.08	TAG 54:5 (2)	0.02	0.22	0.00	0.10
TAG 54:6 (1)	0.00	0.17	0.00	0.04	TAG 54:6 (1)	0.01	0.39	0.00	0.14
TAG 54:6 (2)	0.00	0.46	0.00	0.12	TAG 54:6 (2)	0.00	0.04	0.00	0.02
TAG 54:7 (1)	0.00	0.08	0.00	0.04	TAG 54:7 (1)	0.00	0.05	0.00	0.02
TAG 54:7 (2)	0.01	0.61	0.00	0.18	TAG 54:7 (2)	0.02	0.49	0.00	0.13
TAG 54:8 (1)	0.00	0.00	0.00	0.00	TAG 54:8 (1)	0.00	0.09	0.00	0.02
TAG 54:8 (2)	0.00	0.00	0.00	0.00	TAG 54:8 (2)	0.00	0.06	0.00	0.03
TAG	0.00	0.11	0.00	0.05	TAG	0.00	0.14	0.00	0.05

54:9 (1)					54:9 (1)				
TAG 54:9 (2)	0.00	0.00	0.00	0.00	TAG 54:9 (2)	0.00	0.01	0.00	0.00
TAG 54:10 (1)	0.00	0.00	0.00	0.00	TAG 54:10 (1)	0.00	0.08	0.00	0.01
TAG 54:10 (2)	0.00	0.15	0.00	0.09	TAG 54:10 (2)	0.00	0.02	0.00	0.01
TAG 54:11	0.00	0.08	0.00	0.05	TAG 54:11	0.00	0.09	0.00	0.02
TAG 54:12	0.00	0.00	0.00	0.00	TAG 54:12	0.00	0.01	0.00	0.00
TAG 54:13 (1)	0.00	0.00	0.00	0.00	TAG 54:13 (1)	0.00	0.00	0.00	0.00
TAG 54:13 (2)	0.00	0.00	0.00	0.00	TAG 54:13 (2)	0.00	0.01	0.00	0.00
TAG 54:14	0.00	0.00	0.00	0.00	TAG 54:14	0.00	0.01	0.00	0.00
TAG 54:15	0.00	0.00	0.00	0.00	TAG 54:15	0.00	0.01	0.00	0.00
TAG 56:1	0.00	0.82	0.00	0.07	TAG 56:1	0.00	0.10	0.00	0.00
TAG 56:2	0.00	0.14	0.00	0.01	TAG 56:2	0.00	0.04	0.00	0.00
TAG 56:3	0.00	0.22	0.00	0.04	TAG 56:3	0.00	0.02	0.00	0.00
TAG 56:4 (1)	0.00	0.09	0.00	0.02	TAG 56:4	0.00	0.07	0.00	0.02
TAG 56:4 (2)	0.00	0.05	0.00	0.00	TAG 56:4	ND	ND	ND	ND
TAG 56:5 (1)	0.00	0.06	0.00	0.02	TAG 56:5 (1)	0.00	0.07	0.00	0.02
TAG 56:5 (2)	0.00	0.05	0.00	0.02	TAG 56:5 (2)	0.00	0.06	0.00	0.01

TAG 56:5 (3)	ND	ND	ND	ND	TAG 56:5 (3)	0.00	0.00	0.00	0.00
TAG 56:6 (1)	0.00	0.01	0.00	0.02	TAG 56:6 (1)	0.00	0.05	0.00	0.01
TAG 56:6 (2)	0.00	0.22	0.00	0.10	TAG 56:6 (2)	0.00	0.03	0.00	0.01
TAG 56:7 (1)	0.00	0.00	0.00	0.00	TAG 56:7 (1)	0.01	0.14	0.00	0.06
TAG 56:7 (2)	0.00	0.16	0.00	0.08	TAG 56:7 (2)	0.01	0.17	0.00	0.03
TAG 56:8 (1)	0.00	0.00	0.00	0.00	TAG 56:8 (1)	0.00	0.05	0.00	0.02
TAG 56:8 (2)	ND	ND	ND	ND	TAG 56:8 (2)	0.00	0.03	0.00	0.01
TAG 56:9	0.00	0.00	0.00	0.00	TAG 56:9	0.00	0.05	0.00	0.02
TAG 56:10	0.00	0.00	0.00	0.00	TAG 56:10	0.00	0.02	0.00	0.01
TAG 56:11	0.00	0.00	0.00	0.00	TAG 56:11	0.00	0.01	0.00	0.00
TAG 56:12	0.00	0.00	0.00	0.00	TAG 56:12	0.00	0.02	0.00	0.00
TAG 56:13	0.00	0.00	0.00	0.00	TAG 56:13	0.00	0.01	0.00	0.00
TAG 56:14	0.00	0.00	0.00	0.00	TAG 56:14	0.00	0.00	0.00	0.00
TAG 56:15	0.00	0.00	0.00	0.00	TAG 56:15	0.00	0.00	0.00	0.00
TAG 58:1	0.00	0.00	0.00	0.00	TAG 58:1	0.00	0.00	0.00	0.00
TAG 58:2	0.00	0.25	0.00	0.04	TAG 58:2	0.01	0.09	0.00	0.01
TAG 58:3	0.00	0.08	0.00	0.02	TAG 58:3	0.01	0.09	0.00	0.01
TAG 58:4	0.00	0.24	0.00	0.10	TAG 58:4	0.01	0.23	0.00	0.04
TAG	0.00	0.30	0.00	0.08	TAG	0.01	0.13	0.00	0.02

58:5					58:5				
TAG 58:6 (1)	0.00	0.00	0.00	0.00	TAG 58:6 (1)	0.01	0.07	0.00	0.01
TAG 58:6 (2)	0.00	0.43	0.00	0.12	TAG 58:6 (2)	0.00	0.05	0.00	0.01
TAG 58:7 (1)	0.00	0.00	0.00	0.00	TAG 58:7 (1)	0.00	0.07	0.00	0.02
TAG 58:7 (2)	0.00	0.04	0.00	0.01	TAG 58:7 (2)	0.00	0.03	0.00	0.01
TAG 58:8 (1)	0.00	0.13	0.00	0.07	TAG 58:8 (1)	0.01	0.17	0.00	0.04
TAG 58:8 (2)	ND	ND	ND	ND	TAG 58:8 (2)	0.01	0.10	0.00	0.04
TAG 58:9	0.00	0.05	0.00	0.03	TAG 58:9	0.01	0.13	0.00	0.04
TAG 58:10 (1)	0.01	0.15	0.00	0.09	TAG 58:10 (1)	0.02	0.44	0.00	0.15
TAG 58:10 (2)	0.00	0.03	0.00	0.02	TAG 58:10 (2)	0.00	0.04	0.00	0.01
TAG 58:10 (3)	0.00	0.17	0.00	0.06	TAG 58:10 (2)	ND	ND	ND	ND
TAG 58:11	0.01	0.12	0.00	0.05	TAG 58:11	0.01	0.19	0.00	0.04
TAG 58:12	0.02	0.13	0.00	0.08	TAG 58:12	0.01	0.12	0.00	0.04
TAG 58:13	0.00	0.00	0.00	0.00	TAG 58:13	0.00	0.02	0.00	0.00
TAG 58:14 (1)	0.00	0.00	0.00	0.00	TAG 58:14 (1)	0.00	0.01	0.00	0.00
TAG 58:14 (2)	0.00	0.00	0.00	0.00	TAG 58:14 (2)	0.00	0.01	0.00	0.00
TAG	0.00	0.00	0.00	0.00	TAG	0.00	0.03	0.00	0.00

58:15					58:15				
TAG 58:16	0.01	0.07	0.00	0.04	TAG 58:16	0.00	0.06	0.00	0.01
TAG 60:2	0.00	0.06	0.00	0.03	TAG 60:2	0.00	0.00	0.00	0.00
TAG 60:4	0.00	0.00	0.00	0.00	TAG 60:4	0.00	0.01	0.00	0.00
TAG 60:5	0.00	0.00	0.00	0.00	TAG 60:5	0.00	0.01	0.00	0.00
TAG 60:6 (1)	0.00	0.00	0.00	0.00	TAG 60:6 (1)	0.00	0.01	0.00	0.00
TAG 60:6 (2)	0.00	0.05	0.00	0.02	TAG 60:6 (2)	0.00	0.01	0.00	0.00
TAG 60:7 (1)	0.00	0.00	0.00	0.00	TAG 60:7 (1)	0.00	0.00	0.00	0.00
TAG 60:7 (2)	0.00	0.00	0.00	0.00	TAG 60:7 (2)	0.00	0.01	0.00	0.00
TAG 60:8	0.00	0.00	0.00	0.00	TAG 60:8	0.00	0.01	0.00	0.00
TAG 60:9	0.00	0.00	0.00	0.00	TAG 60:9	0.00	0.02	0.00	0.01
TAG 60:10	0.00	0.00	0.00	0.00	TAG 60:10	0.00	0.01	0.00	0.00
TAG 60:11	0.00	0.00	0.00	0.00	TAG 60:11	0.00	0.01	0.00	0.00
Total TAGs	0.27	41.13				0.57	12.23		

Table 7: Absolute quantification of several TAGs following lytic viral infection or N-starvation. Data is presents as fg/cell.

TAG	Lytic virus 48 hpi		N depletion 48 h	
	Average	SD	Average	SD
TAG 44:1	2.90	0.08	5.00	0.66
TAG 48:1	72.19	4.20	31.35	6.15
TAG 50:0	2.98	0.07	0.55	0.04
TAG 58:7	6.76	0.48	23.85	5.84
TAG 58:10	4.20	0.28	52.96	5.85

EXAMPLE 3

VIRUS-FREE LYSATE CAN INCREASE TAGS IN NON-INFECTED ALGAE

Virus-Free Lysate (VFL) was produced by filtering *E. huxleyi* 2090 culture infected with EhV201 for 72 hours. The culture of 72 (hours post infection, hpi) was first filtered through a sterile 0.45µm PVDF filter (Stericaup 500ml Durapore, Milipore) and then through a 100kD filter device with Ultracel® low binding regenerated cellulose with 100,000NMWL cutoff (Amicon). The flow through of the two sequential filtration steps resulted in VFL. Control lysate was produced in a similar way from a non-infected *E. huxleyi* 2090 culture.

The VFL or the control lysate were added to an exponential growing culture ($1-2 \times 10^6$ cells/ ml) of *E. huxleyi* 2090 in a 1:1 v/v ratio. The *E. huxleyi* 2090 cultures that were supplemented with VFL or control lysate were monitored for TAGs formation using BODIPY staining quantified on the flow cytometry (Figure 11A) and for induction of death using Sytox Green staining quantified on the flow cytometry (Figure 11B). The *E. huxleyi* 2090 culture that was supplemented with VFL was also analyzed using LC-MC for the production of 9 most abundant TAGs (Malitsky et al., 2016) (Figure 12). The TAGs determined were: TAG 44:0, TAG 46:0, TAG 46:1, TAG 48:0, TAG 48:1, TAG 50:1, TAG 50:2, TAG 54:1, TAG 54:3. The integration area under the peaks of the selected TAGs was normalized against DGCC 38:6 or DGTS 32:4, lipids shown to be consistently abundant during exponential growth and during viral infection (Malitsky et al., 2016). As both normalizations showed similar results, only the DGTS 32:4 normalized data is presented in Figures 12-13.

The results obtained indicated the induction of TAGs formation by supplementing VFL to *E. huxleyi* culture, and especially TAG 46:1 (Figure 13).

Taken together, the data presented indicates that lytic viral infection results in remodeling of the algae host lipidome towards over production of highly saturated TAGs which are incorporated into the isolated virions; implying activation of *de-novo* TAGs biosynthesis during lytic infection.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all

such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

5 All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention. To the extent that section headings are used, they should not be construed as necessarily limiting.

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WHAT IS CLAIMED IS:

1. A method of producing oil, the method comprising:
 - (a) infecting *Emiliana huxleyi* microalgae with a Coccolithovirus EhV201;
and
 - (b) extracting the oil from said microalgae 24-72 hours following said infecting,
thereby producing oil.
2. A method of producing an algal lysate, the method comprising
 - (a) treating an *Emiliana huxleyi* microalgae with an agent that increases triacylglycerols (TAGs) in said microalgae;
 - (b) producing a lysate from said treated microalgae, said lysate being capable of increasing TAGs in said algal culture.
3. The method of claim 2, wherein said agent is a pathogen.
4. The method of claim 3, wherein said pathogen is a virus.
5. The method of claim 4, wherein said virus is a lytic virus.
6. The method of claim 5, wherein said lytic virus is a Coccolithovirus.
7. The method of claim 6, wherein said Coccolithovirus virus is EhV201 or EhV86.
8. The method of claim 3, wherein said pathogen comprises bacteria.
9. The method of claim 8, wherein said bacteria comprises *Roseobacter* bacteria.

10. The method of any one of claims 2-9, further comprising fractionating said lysate so as to obtain a fraction which is able to increase TAGs in said algae.
11. The method of claim 10, wherein said fraction is pathogen-free.
12. The method of any one of claims 2-9 wherein said lysate consists of 100 kDa molecules or lower.
13. A composition of matter comprising a pathogen-free algal lysate capable of increasing TAGs content in an algal culture.
14. The composition of claim 13, wherein said pathogen-free algal lysate consists of molecules of 100 kDa or lower.
15. The composition of any one of claims 13-14, further comprising a preservative.
16. The composition of claim 15, wherein said preservative is selected from the group consisting of antioxidants or protease inhibitors.
17. The method of any one of claims 2-12 or the composition of any one of claims 13-16, wherein said algal culture comprises *Emiliana huxleyi* microalgae.
18. A method of increasing content of triacylglycerols (TAGs) in algae, the method comprising treating an algal culture with the composition of any one of claims 13-17, thereby increasing content of triacylglycerols (TAGs) in algae.
19. The method of claim 18, wherein said algal culture is at the exponential stage.
20. The method of claim 18, wherein said algal culture is at the stationary stage.

21. A method of producing oil, the method comprising:
 - (a) treating an algal culture with the composition of any one of claims 13-16, thereby increasing content of triacylglycerols (TAGs) in algae; and
 - (b) extracting the oil from said algal culture.
22. The method of claim 1, wherein said extracting is effected following virion release.
23. The method of claim 1, wherein said extracting is effected prior to virion release.
24. The method of any one of claims 1-12 and 21-22, comprising extracting oil from virions released from said microalgae following said infecting.
25. The method of any one of claims 1-12 and 21-24, wherein said infecting is effected at a multiplicity of infection (MOI) of about 1 viral particle per 1 cell.
26. The method of any one of claims 1-12 and 21-25, wherein said infecting is effected in a microalgae bioreactor.
27. An oil produced according to the method of any one of claims 1 and 17-26.
28. An *Emiliania huxleyi* microalgae oil having an increased content of triacylglycerols (TAGs); as compared to an oil of *Emiliania huxleyi* microalgae not infected with a Coccolithovirus EhV201 and/or an oil of *Emiliania huxleyi* microalgae following nitrogen deprivation.
29. The oil of claim 28, wherein said TAGs comprise saturated and/or mono-unsaturated TAGs.

30. A method of producing biofuel, the method comprising:
- (a) producing oil according to the method of any one of claims 1 and 17-26;
- and
- (b) processing said oil by a reaction that splits the fatty acids chains of a triacylglycerol (TAG) comprised in said oil from its glycerin backbone, thereby producing the biofuel.
31. The method of claim 30, wherein said reaction is selected from the group consisting of transesterification, hydrocracking and hydrogenation.
32. The method of any one of claims 30-31, wherein said extracting is effected 24-72 hours following infection with said virus.
33. The method of any one of claims 30-32, wherein said extracting is effected following virion release.
34. The method of any one of claims 30-32, wherein said extracting is effected prior virion release.
35. The method of any one of claims 30-33, comprising extracting oil from virions released from said microalgae following infection with said virus.
36. The method of any one of claims 30-35, wherein said microalgae were infected with said virus at a multiplicity of infection (MOI) of about 1 viral particle per 1 cell.
37. The method of any one of claims 30-36, wherein said microalgae were infected with said virus in a microalgae bioreactor.
38. The method of any one of claims 30-37, comprising purifying said biofuel following said processing.

39. The method of any one of claim 30-38, comprising isolating a glycerol following said processing.

40. A biofuel produced according to the method of any one of claims 30-39.

41. A biofuel from *Emiliana huxleyi* microalgae having an increased content of saturated and/or mono-unsaturated fatty acids; as compared to biofuel from *Emiliana huxleyi* microalgae not infected with a virus and/or biofuel from *Emiliana huxleyi* microalgae following nitrogen deprivation.

42. The method of any one of claims 30-39, or the biofuel of any one of claims 40-41, wherein said biofuel is biodiesel.

43. A method of producing an algal cake, the method comprising:

- (a) producing oil according to the method of any one of claims 1, 17-26; and
- (b) removing the oil from said microalgae, thereby producing cake.

44. The method of claim 43, wherein said removing is effected 24-72 hours following said infecting.

45. An algal cake produced according to the method of any one of claims 43-44.

46. An algal cake comprising virus infected *Emiliana huxleyi* microalgae.

47. The method, composition, the biofuel or the algal cake of any one of claims 2-17 and 30-46, wherein said virus is a lytic virus.

48. The method, the biofuel or the algal cake of any one of claims 30-47, wherein said virus is a Coccolithovirus.

49. The method, the biofuel or the algal cake of claim 48, wherein said Coccolithovirus is EhV201.

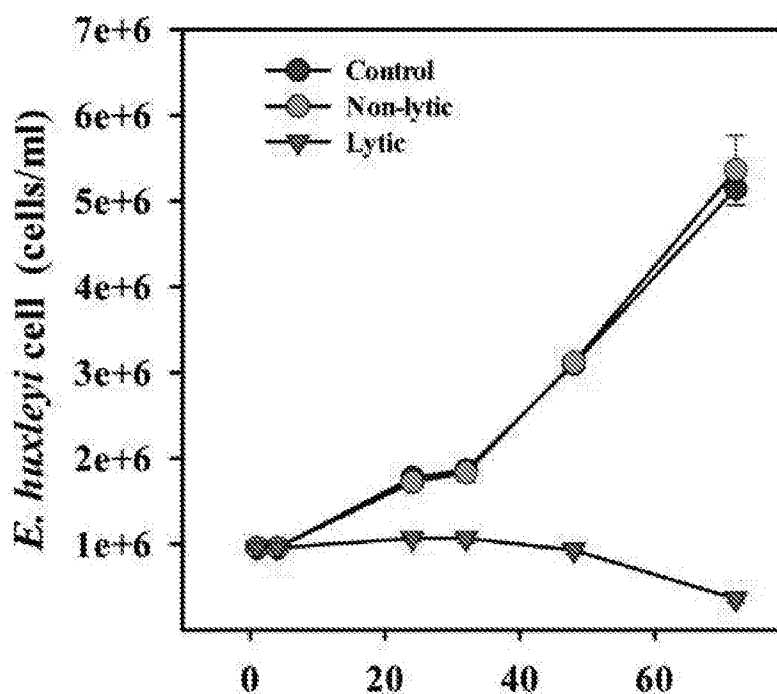


FIG. 1A

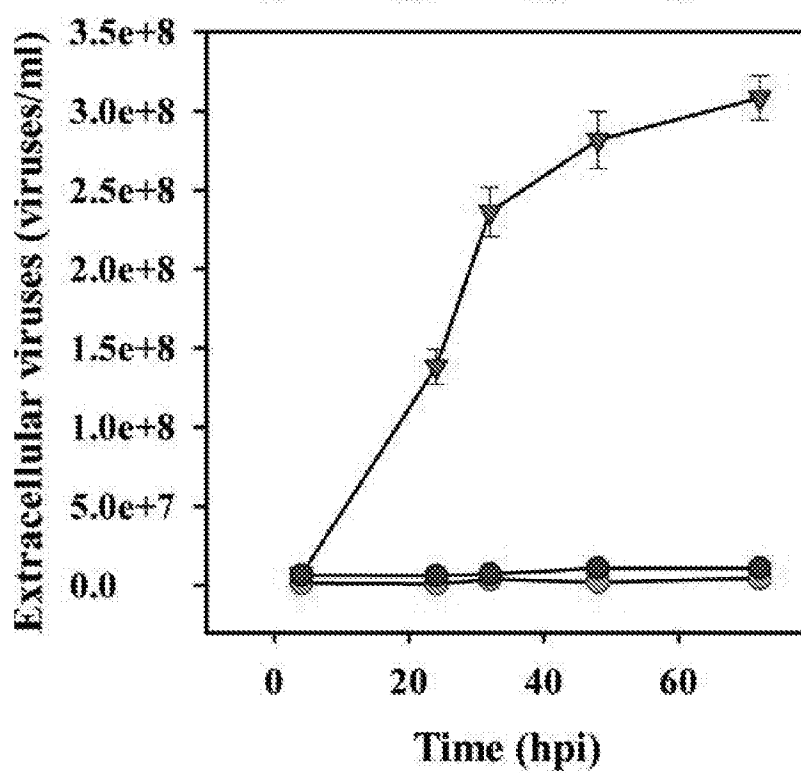
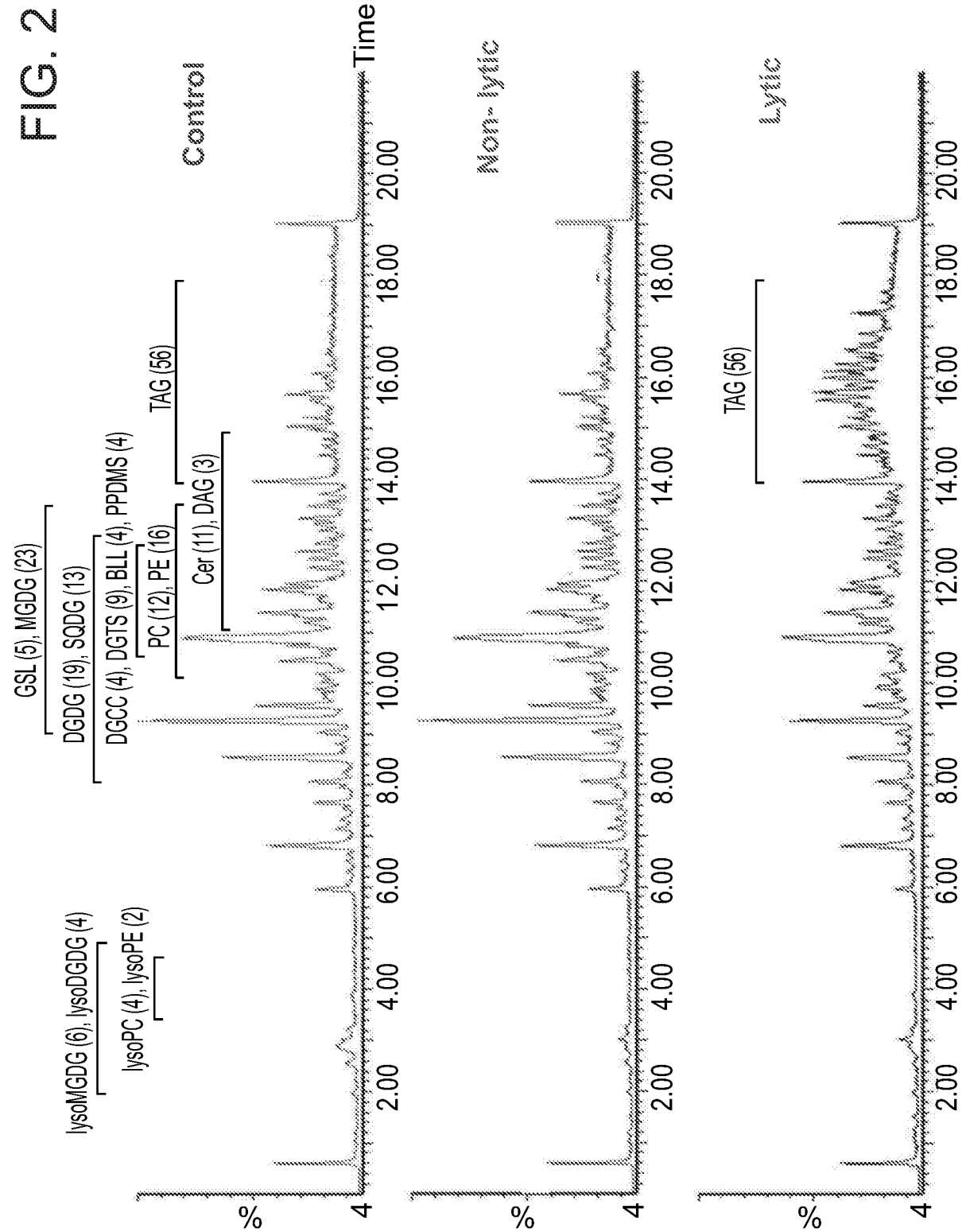


FIG. 1B

FIG. 2



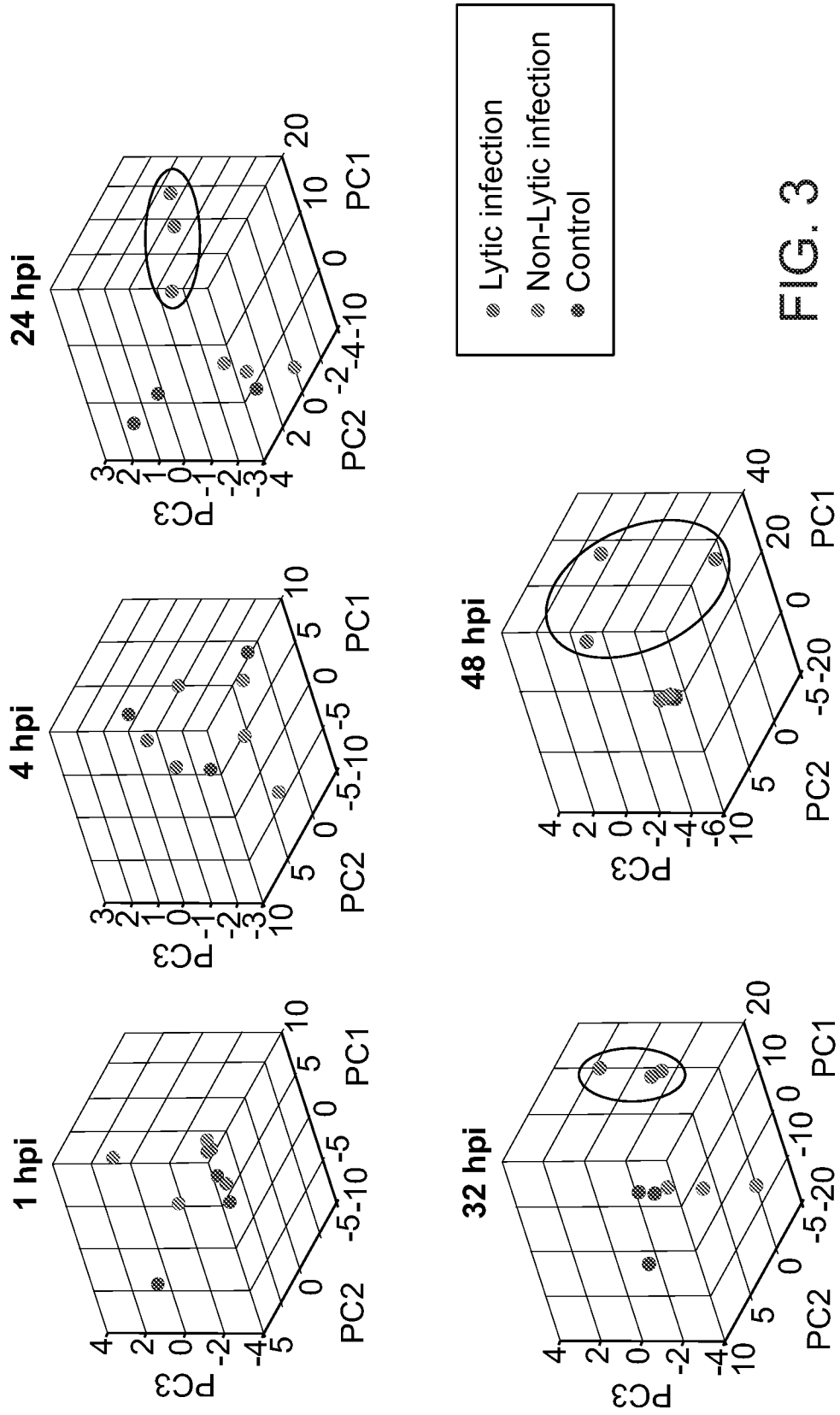


FIG. 3

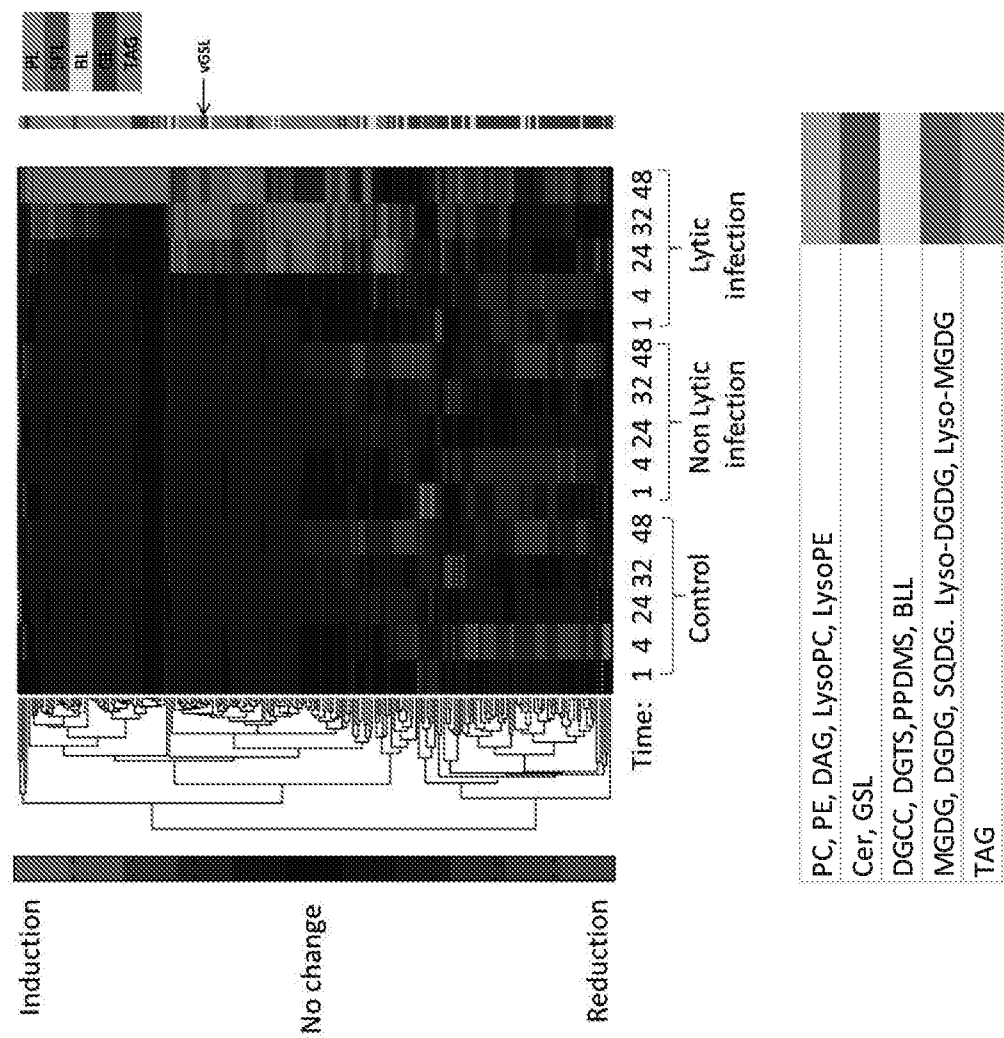


FIG. 4

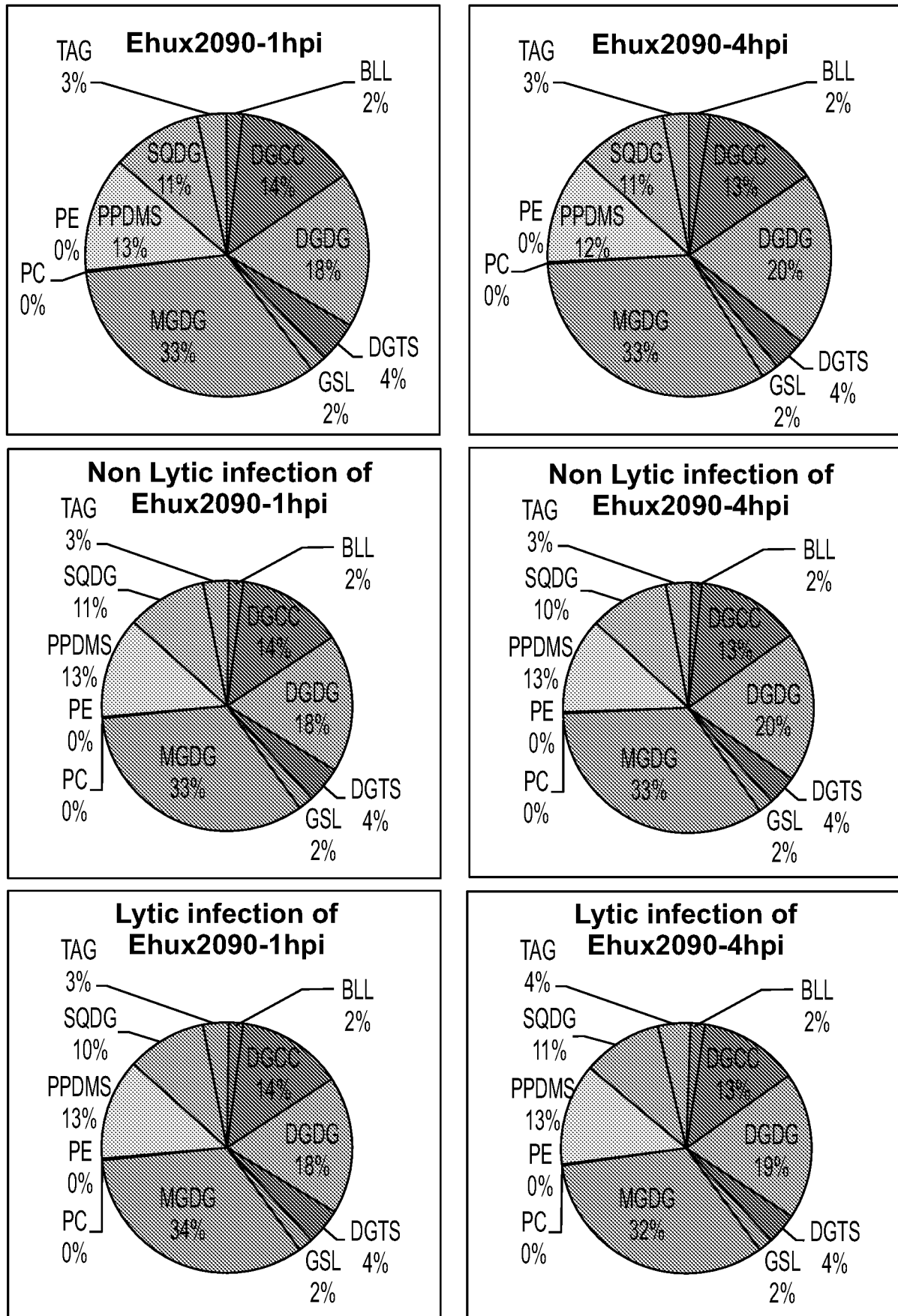


FIG. 5A

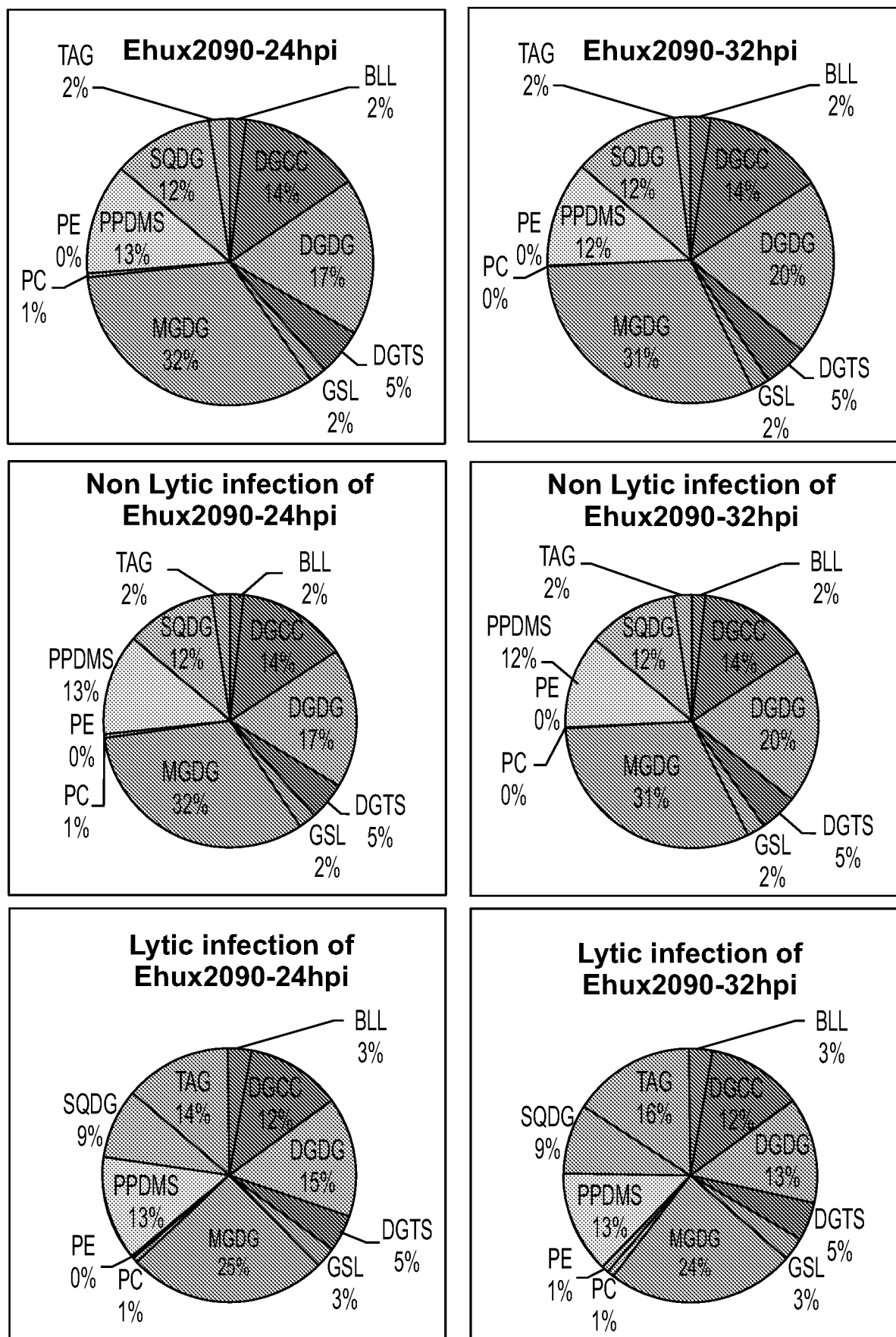


FIG. 5A (continued)

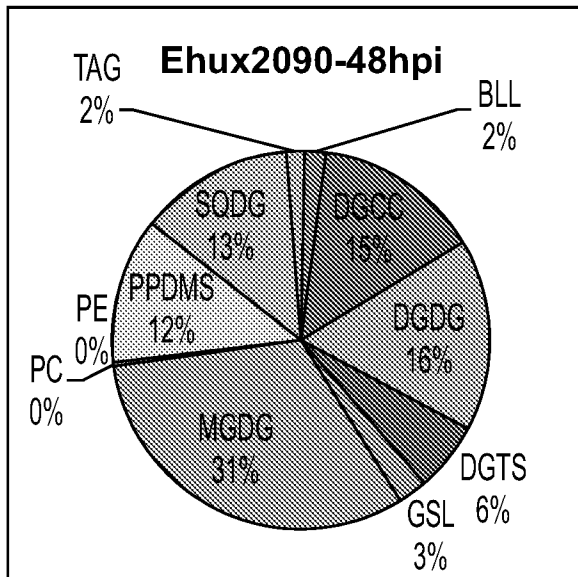
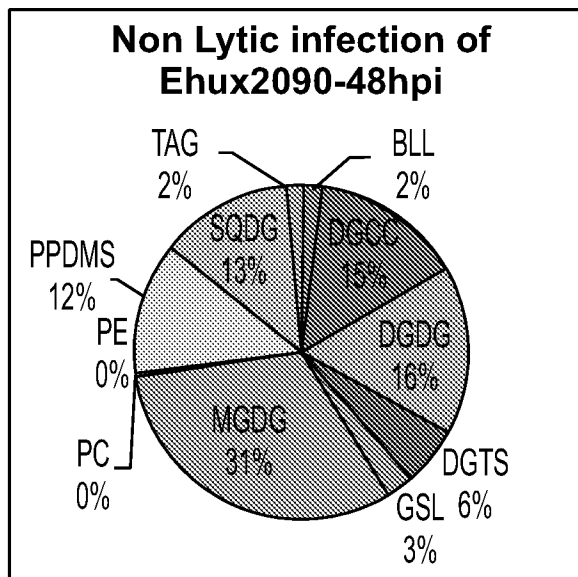
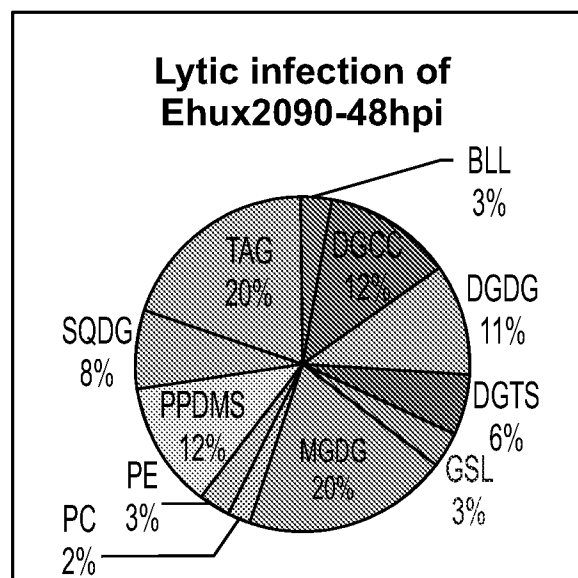


FIG. 5A (continued)

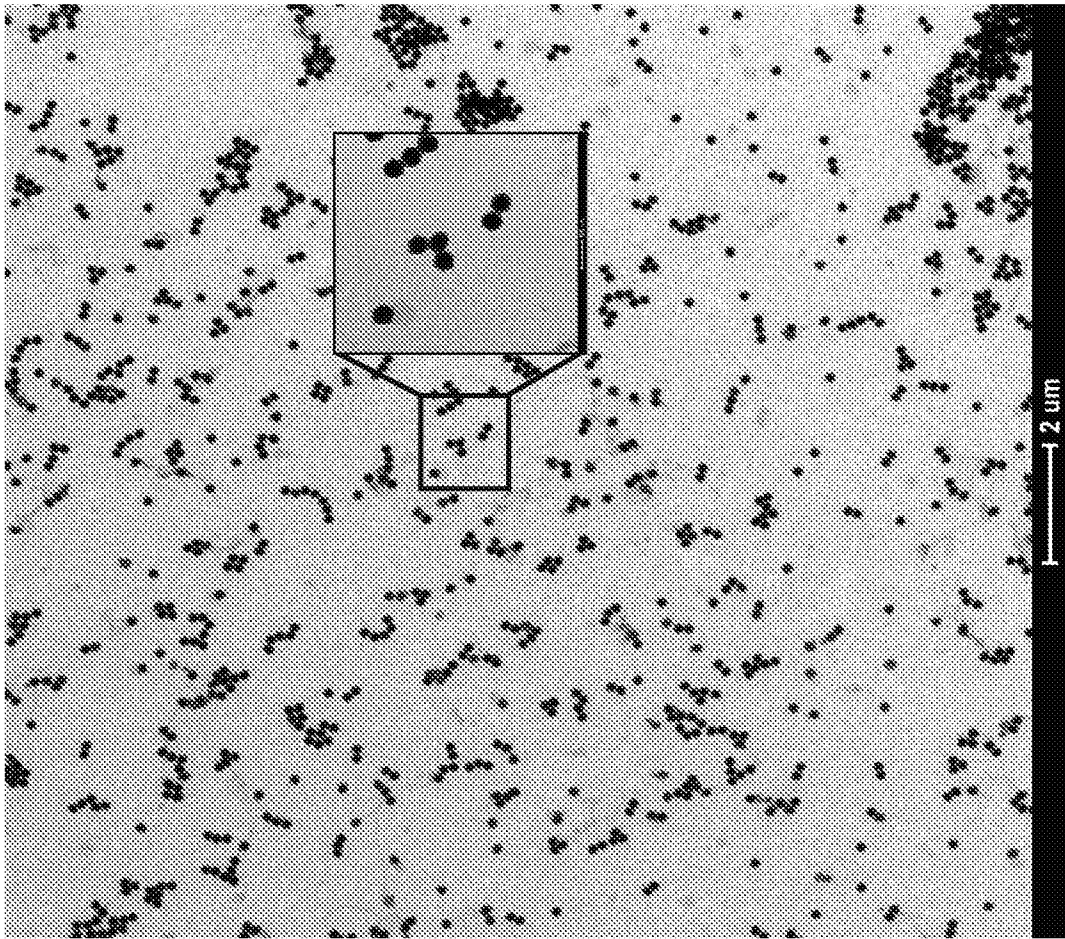


FIG. 5C

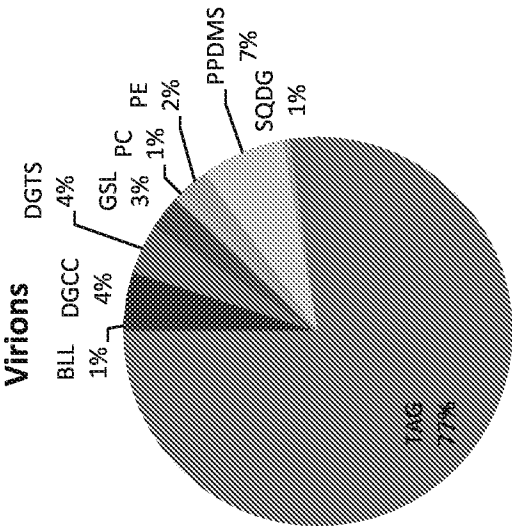


FIG. 5B

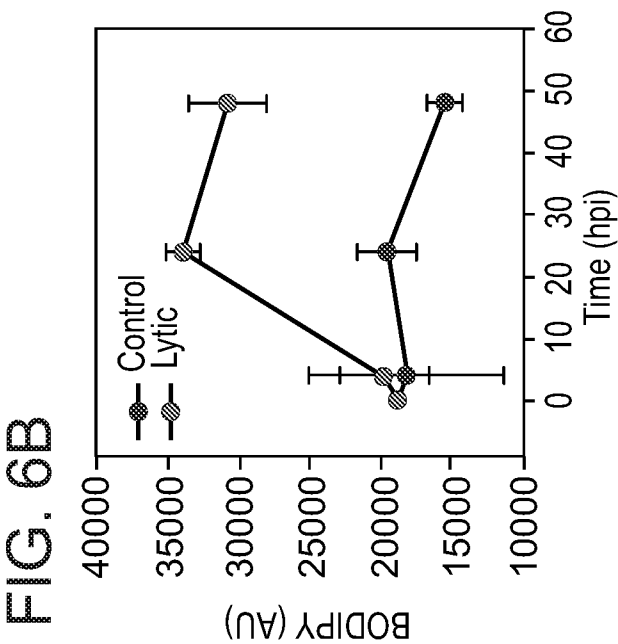
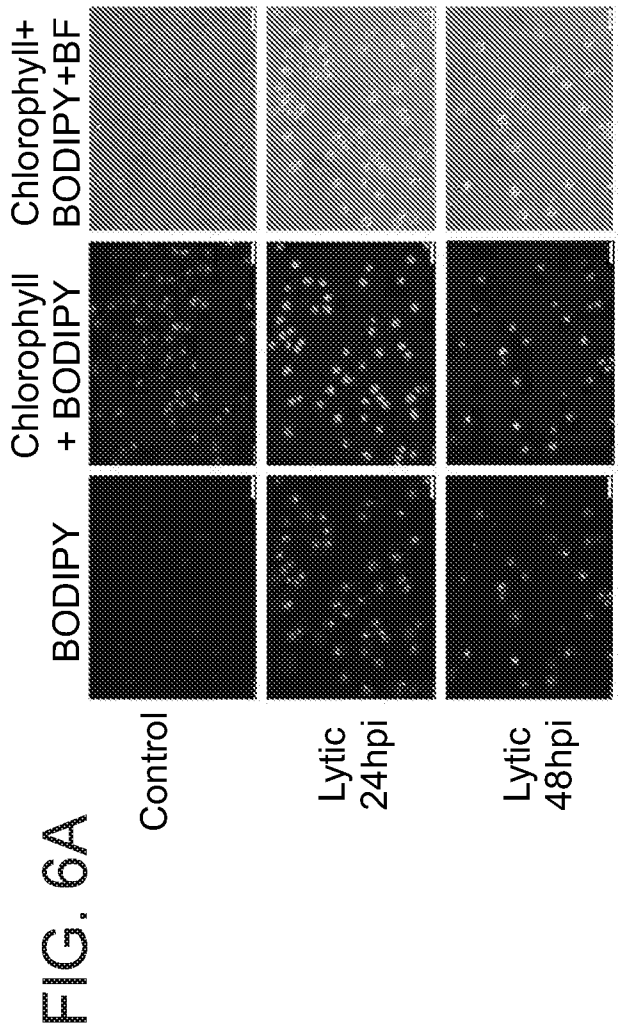
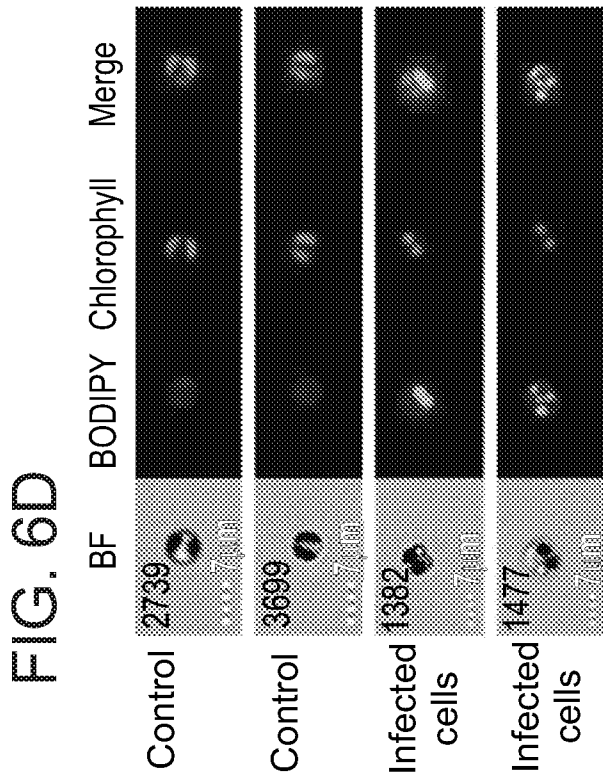
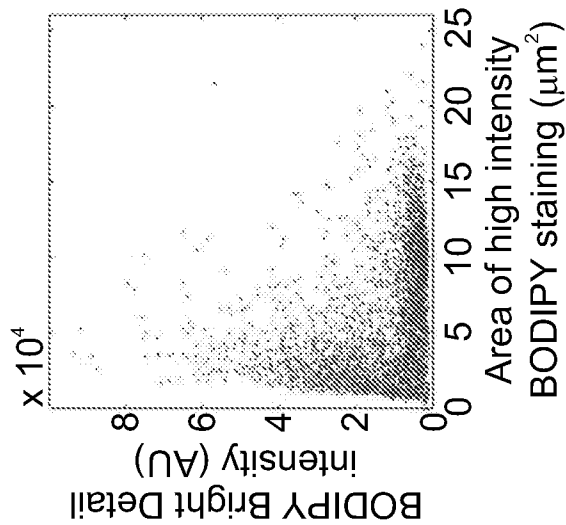
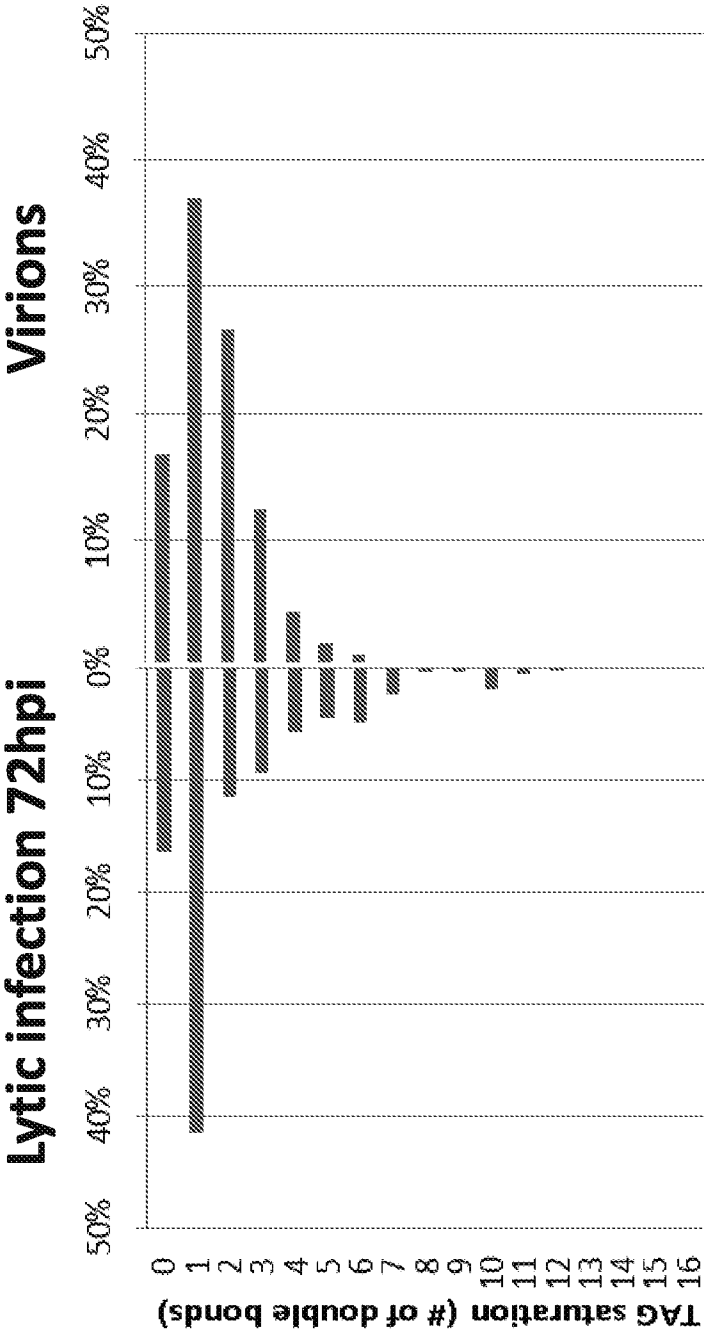


FIG. 7



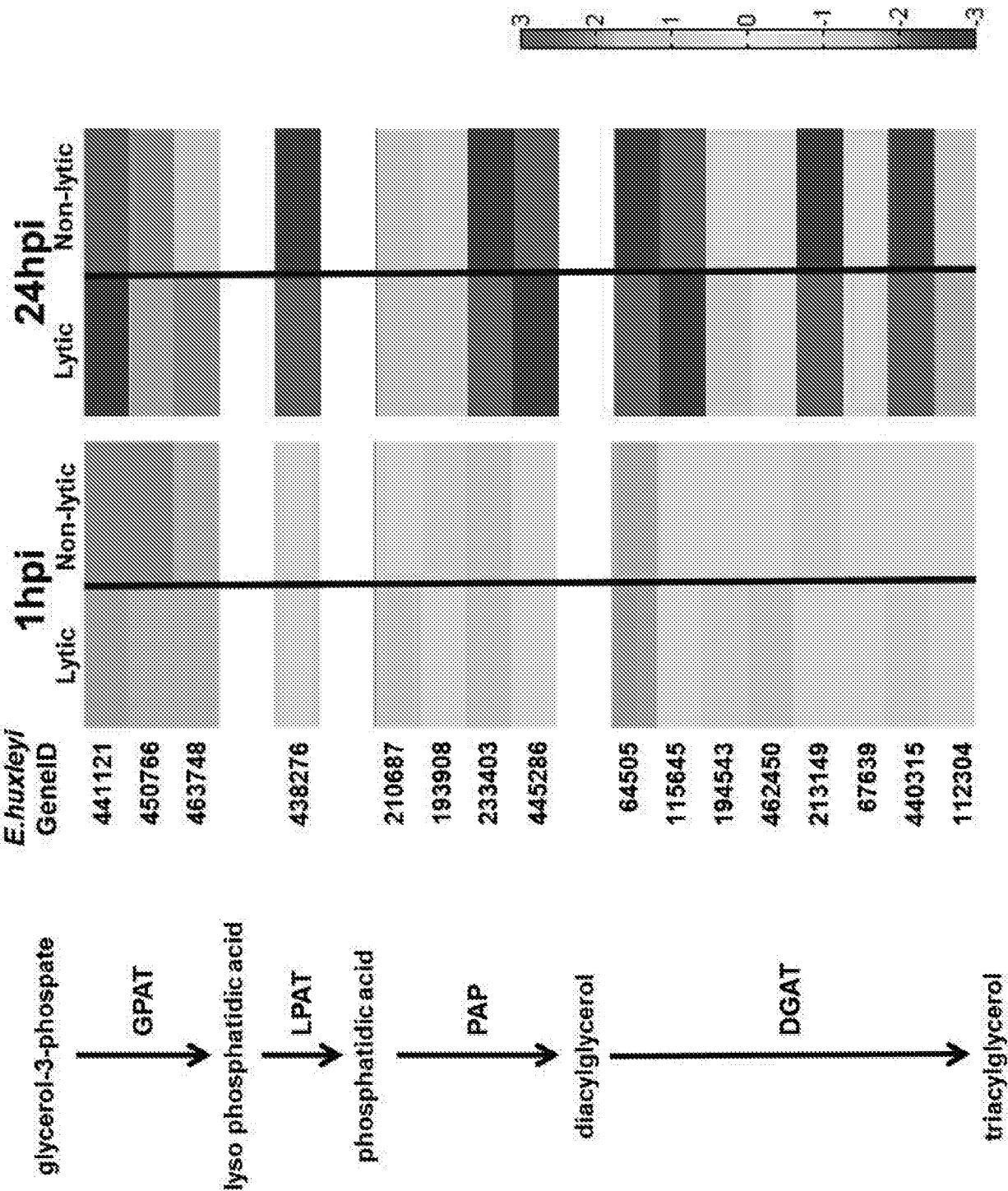
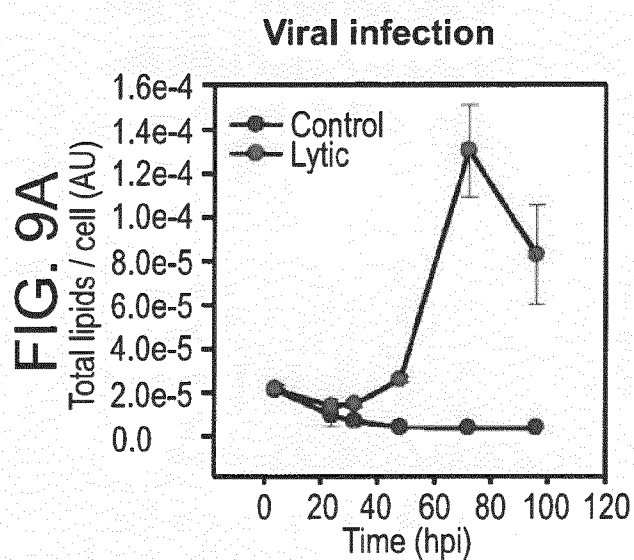
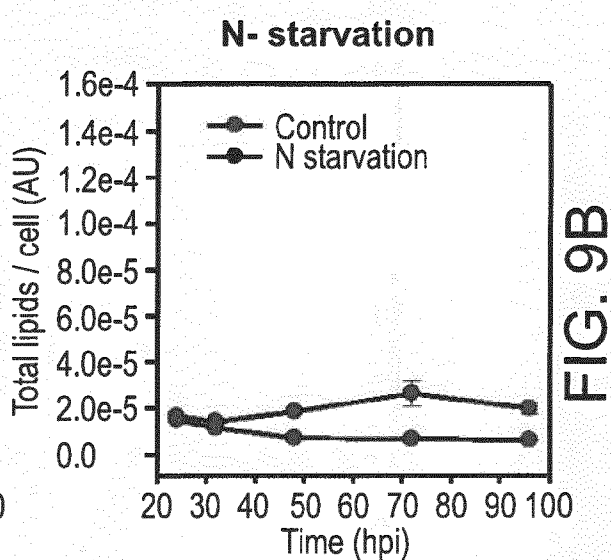
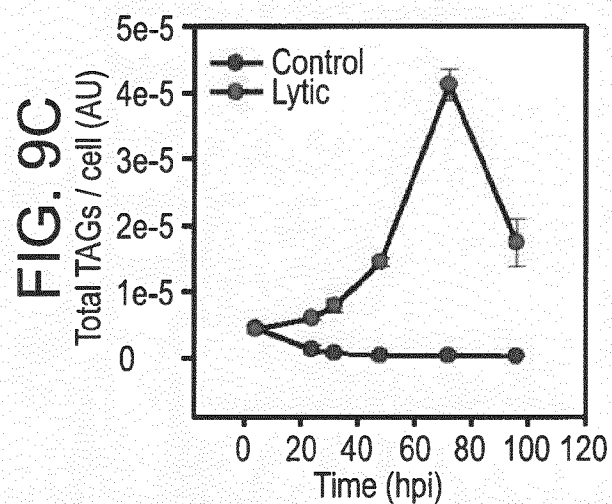
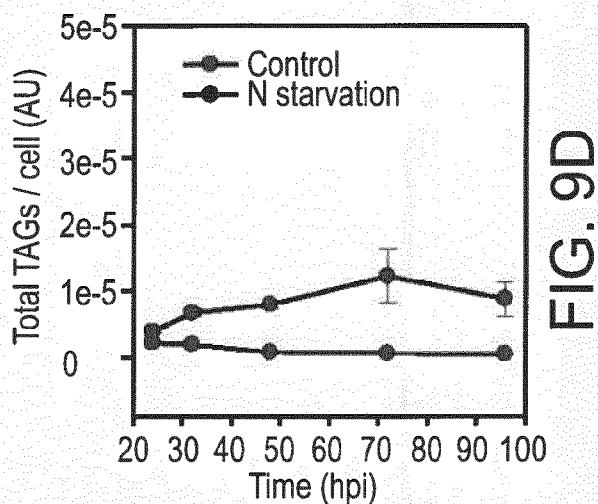
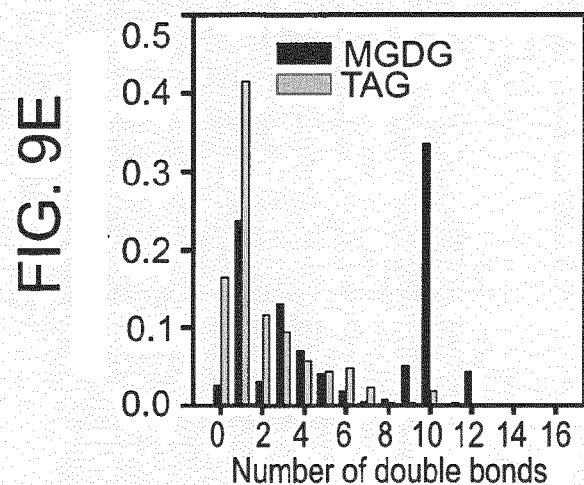
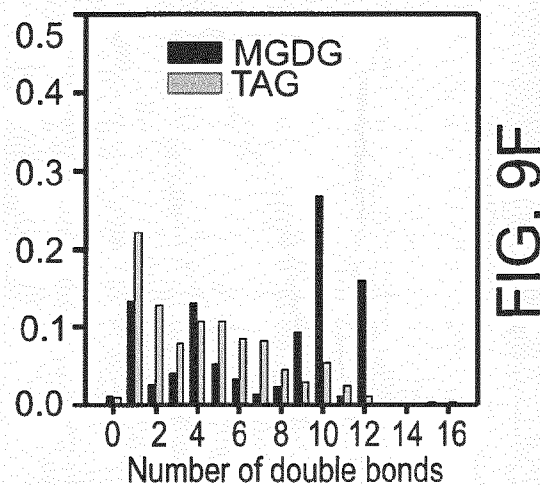


FIG. 8

12/15



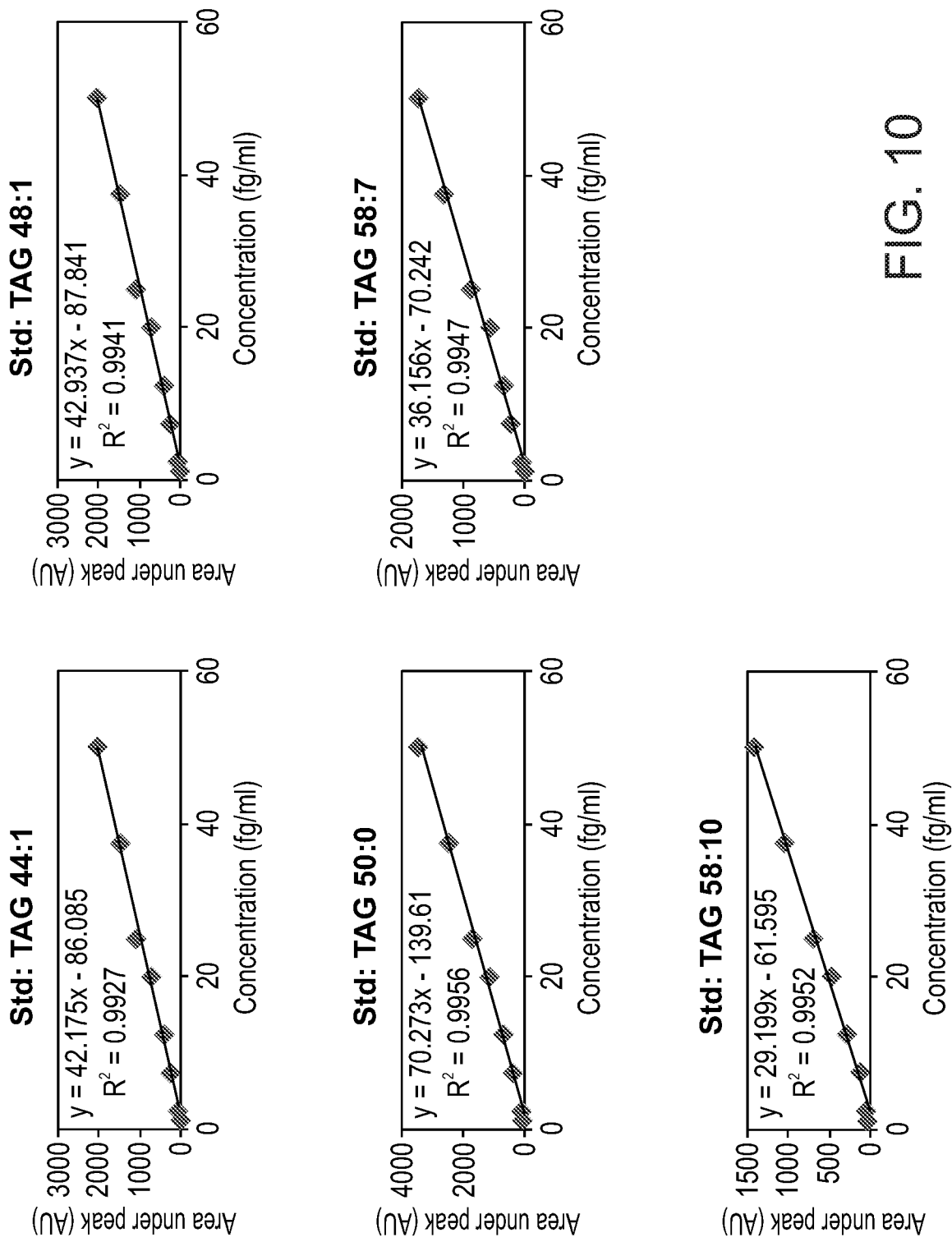


FIG. 10

FIG. 11A

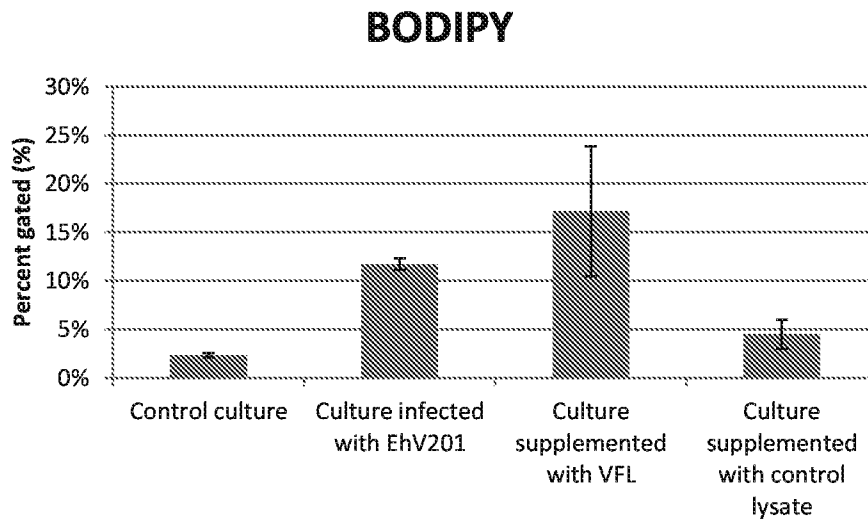
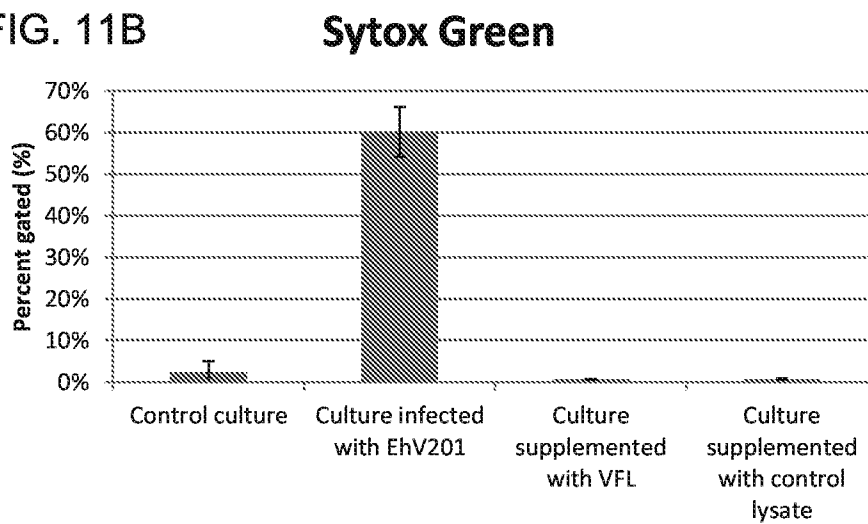


FIG. 11B



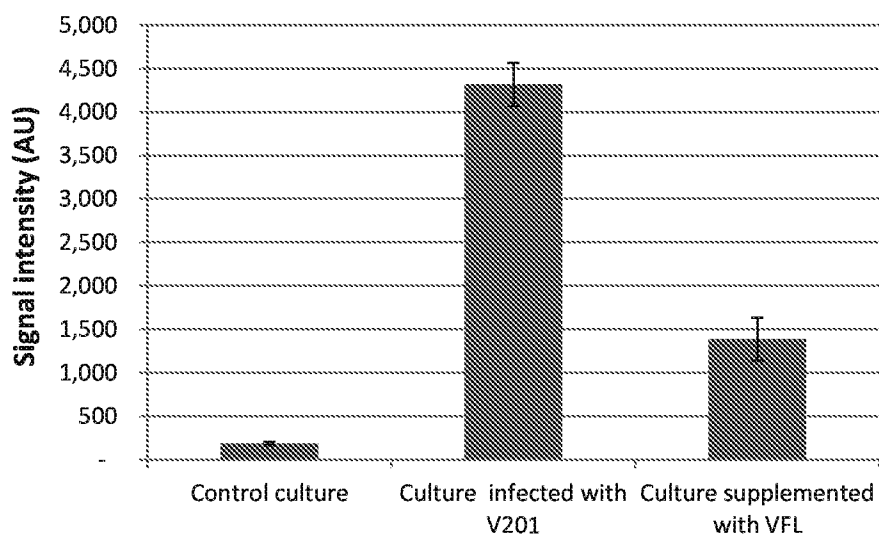


FIG. 12

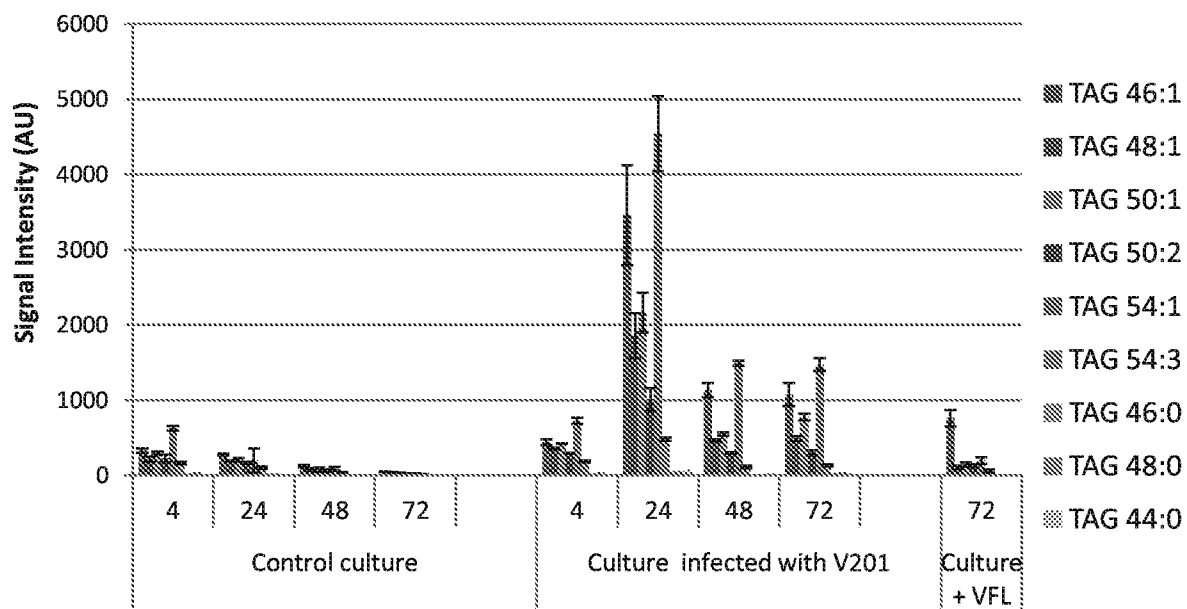


FIG. 13

INTERNATIONAL SEARCH REPORT

International application No

PCT/IL2016/050921

A. CLASSIFICATION OF SUBJECT MATTER
 INV. C12P7/64 C12N1/06
 ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 C12P C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, Sequence Search, FSTA, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ROSENWASSER S. ET AL: "Rewiring Host Lipid Metabolism by Large Viruses Determines the Fate of <i>Emiliana huxleyi</i> , a Bloom-Forming Alga in the Ocean", THE PLANT CELL, vol. 26, no. 6, 1 June 2014 (2014-06-01), pages 2689-2707, XP055317471, US ISSN: 1040-4651, DOI: 10.1105/tpc.114.125641 cited in the application the whole document	1-7,17, 22,23, 25, 27-29, 41,43-49
X	WO 2011/057132 A1 (WOODS HOLE OCEANOGRAPHIC INST [US]; UNIV RUTGERS [US]; BIDDLE KAY DANIE) 12 May 2011 (2011-05-12) the whole document ----- -/-	2-7,17, 27-29, 41,45-48



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

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Date of the actual completion of the international search

14 November 2016

Date of mailing of the international search report

24/11/2016

Name and mailing address of the ISA/

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Authorized officer

van de Kamp, Mart

INTERNATIONAL SEARCH REPORT

International application No

PCT/IL2016/050921

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2010/005560 A2 (WOODS HOLE OCEANOGRAPHIC INST [US]; UNIV RUTGERS [US]; BIDDLE KAY DANIE) 14 January 2010 (2010-01-14) the whole document	2-7,17, 27-29, 41,45-48
X	----- FULTON J.M. ET AL: "Novel molecular determinants of viral susceptibility and resistance in the lipidome of E miliania huxleyi", ENVIRONMENTAL MICROBIOLOGY, vol. 16, no. 4, 23 January 2014 (2014-01-23), pages 1137-1149, XP055317501, GB ISSN: 1462-2912, DOI: 10.1111/1462-2920.12358 cited in the application the whole document	2-7,17, 27-29, 41,45-48
X	----- EVANS C. ET AL: "Changes in Emiliania huxleyi fatty acid profiles during infection with E. huxleyi virus 86: physiological and ecological implications", AQUATIC MICROBIAL ECOLOGY, vol. 55, no. 3, 8 May 2009 (2009-05-08), pages 219-228, XP055319119, DE ISSN: 0948-3055, DOI: 10.3354/ame01295 the whole document	2-7,17, 27-29, 41,45-48
X	----- BREUER G. ET AL: "The impact of nitrogen starvation on the dynamics of triacylglycerol accumulation in nine microalgae strains", BIORESOURCE TECHNOLOGY, vol. 124, 10 August 2012 (2012-08-10), pages 217-226, XP028952675, ISSN: 0960-8524, DOI: 10.1016/J.BIORTECH.2012.08.003 cited in the application the whole document	27-29, 40-42,45
A	----- -/--	1,18,21, 30,43

INTERNATIONAL SEARCH REPORT

International application No

PCT/IL2016/050921

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HU Q. ET AL: "Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances", THE PLANT JOURNAL, vol. 54, no. 4, 1 May 2008 (2008-05-01), pages 621-639, XP009130325, ISSN: 0960-7412, DOI: 10.1111/J.1365-313X.2008.03492.X cited in the application	27-29, 40-42,45
A	the whole document	1,18,21, 30,43
X	----- YU W.-L. ET AL: "Modifications of the metabolic pathways of lipid and triacylglycerol production in microalgae", MICROBIAL CELL FACTORIES, vol. 10, no. 1, 2 November 2011 (2011-11-02), page 91, XP021112003, ISSN: 1475-2859, DOI: 10.1186/1475-2859-10-91	27-29, 40-42,45
A	the whole document	1,18,21, 30,43
X	----- SHARMA K. K. ET AL: "High lipid induction in microalgae for biodiesel production", ENERGIES, vol. 5, 18 May 2012 (2012-05-18), pages 1532-1553, XP002689116, ISSN: 1996-1073, DOI: 10.3390/EN5051532	27-29, 40-42,45
A	the whole document	1,18,21, 30,43
X	----- RAZEGHIFARD R.: "Algal biofuels", PHOTOSYNTHESIS RESEARCH, vol. 117, no. 1, 21 April 2013 (2013-04-21), pages 207-219, XP035355169, ISSN: 0166-8595, DOI: 10.1007/S11120-013-9828-Z [retrieved on 2013-04-21] cited in the application	27-29, 40-42
X	----- EP 2 899 281 A1 (COMMISSARIAT ENERGIE ATOMIQUE [FR]) 29 July 2015 (2015-07-29)	27-29, 40-42,45
A	the whole document	13
X	----- WO 2013/005209 A2 (YEDA RES & DEV [IL]; PICK URI [IL]; RACHUTIN ZALOGIN TATYANA [IL]) 10 January 2013 (2013-01-10)	27-29, 40-42,45
A	cited in the application the whole document	13
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INTERNATIONAL SEARCH REPORT

International application No

PCT/IL2016/050921

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2015/140619 A1 (REDDY CHRISTOPHER M [US] ET AL) 21 May 2015 (2015-05-21) the whole document	27,40,45
X	----- WO 2008/036654 A2 (UNIV ARIZONA [US]; HU QIANG [US]; SUMMERFELD MILTON [US]) 27 March 2008 (2008-03-27) the whole document	27,40,45
X	----- BELL M. V. ET AL: "Lipid composition during growth of motile and coccolith forms of <i>Emiliana huxleyi</i> ", PHYTOCHEMISTRY, vol. 41, no. 2, 1996, pages 465-471, XP002535800, ISSN: 0031-9422, DOI: 10.1016/0031-9422(95)00663-X	27,40,45
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