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Betaine lipids: Biosynthesis, functional diversity and evolutionary perspectives

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ABSTRACT

Betaine lipids (BL) are relatively understudied non-phosphorus glycerolipids. They are predominantly found in algae but have also been detected in other unicellular eukaryotes, fungi, bacteria, and some bryophytes and pteridophytes. These extraplasmidic lipids are considered as substitutes for phospholipids in organisms, particularly under phosphate (Pi) deficiency. This review provides a broader perspective on the roles and functions of BL, revealing their functional diversity across species and environments. It also discusses the biosynthetic pathways of BL. Indeed, the pathway for DGTS (1(3),2-diacylglycerol-3(1)-O-4'-(*N,N,N*-trimethyl)-homoserine), the most widespread and studied BL form, is completely known, whereas the pathway for DGTA (1(3),2-diacylglycerol-3(1)-O-2'-(*N,N,N*-trimethyl)- β -alanine) is only partially understood. In this review, the role of the *BTA1* gene, responsible for the synthesis of DGTS, is discussed. It is revealed to be essential in DGTA synthesis as it enables the production of its intermediate, DGTS. A phylogenetic analysis, conducted on *BTA1* gene, does not seem to link the phylogenetic position of *BTA1* with the BL species produced but confirms the distribution trends, with a BL diversification in marine environments and the gradual disappearance of DGTS in the evolution of the green lineage. Further research is needed to elucidate the specific roles and biosynthetic pathways of BL across different species.

1. Introduction

Glycerolipids are key structural and functional components of cellular membranes. In photosynthetic organisms, membrane glycerolipids can be categorized into three main classes: phospholipids, glyco-glycerolipids and betaine lipids (BL). Phospholipids are predominantly found in the endomembrane system and mitochondria whereas glyco-glycerolipids are primarily located in the chloroplast membranes. While the former classes are ubiquitous among photosynthetic organisms, the same cannot be said for BL, which exhibit a more specific distribution. BL are a poorly known class of glycerolipids present in algae, some fungi, bacteria and some bryophytes and pteridophytes. To date, three BL species have been described: DGTS (diacylglycerol trimethylhomoserine), DGTA (diacylglycerol-hydroxymethyl-trimethylalanine) and DGCC (diacylglycerol carboxyhydroxymethylcholine). These compounds have been identified in a large number of organisms and characterized as both functional and structural lipids, although many uncertainties remain regarding their synthesis,

functions and distribution. Their discovery in the seventies was relatively recent compared to that of phospholipids, which have been the subject of more extensive research. As BL do not contain phosphorus, they are generally accepted as substitutes for phospholipids and few studies contributed to the understanding of their specific biological relevance.

The role of BL as substitutes for phospholipids has been specifically studied in the context of organisms' adaptation to phosphate (Pi) deficiency. These mechanisms have notably been reported within phytoplankton communities of certain oligotrophic oceanic regions characterized by low phosphorus concentration [1]. In these organisms, BL synthesis appeared to increase in response to Pi limitation to replace the degraded phospholipids [1–5]. However, the dynamics of BL synthesis in such conditions vary among species and seem to be implicated in other abiotic stresses, such as nitrogen starvation or cold temperatures [2,6–8]. Thus, the synthesis of BL seems indicative of a specific adaptation to environmental conditions. Although their distribution and proportions in species are highly variable and poorly understood, some

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research has shown that habitat could play a significant role in these aspects [9]. The progressive disappearance of BL during plant evolution also supports this hypothesis [10].

This review covers the current knowledge of the structural and metabolic functions of BL in organisms. Additionally, BL biosynthetic processes are reviewed, including new findings regarding DGTA specific pathway. Finally, the distribution of BL across species is discussed, along with a phylogenetic analysis of a gene involved in their synthesis.

2. Discovery and structure

Betaine lipids structure is characterized by a specific polar head composed by a derivative of a hydroxyamino acid linked through an ether bond to a diacylglycerol at the *sn*-3 position of the glycerol moiety. These compounds are zwitterionic at neutral pH due to the presence of a negatively-charged carboxyl group and a positively-charged trimethylated ammonium group in their polar head [11] (Fig. 1). DGTS and DGTA were discovered in 1969 in lipid extracts from the stramenopile *Ochromonas danica* [12]. These two compounds were first mentioned as unknown lipids before the elucidation of their chemical structure in 1974 for DGTS [13] and in 1990 for DGTA [14]. DGTS was described as 1(3),2-diacylglycerol-3(1)-*O*-4'-(*N,N,N*-trimethyl)-homoserine and DGTA as 1(3),2-diacylglycerol-3(1)-*O*-2'-(*N,N,N*-trimethyl)- β -alanine (Fig. 1). It is interesting to note that the position of the carboxyl group in DGTS and DGTA polar heads differs from one carbon to the next, making these two lipids structural isomers. Further studies isolated DGTS in several taxonomic groups like green algae [15–17], pteridophytes [18], bryophytes [19], bacteria [20] and fungi [21], whereas DGTA was mostly identified in phaeophyceae [22]. The third betaine lipid class, DGCC, was first described in 1994 as 1,2-diacylglycerol-3(1)-*O*-carboxy-(hydroxymethyl)-choline from the haptophyte *Dicranema lutheri* [23]. Its structure can be distinguished from DGTS and DGTA by the presence of an acetal carbon at the origin of the carboxyl group (Fig. 1). DGCC was detected in other haptophyte species and seemed to be restricted to this clade [24]. Since their discovery, BL have been identified in a broadening array of species through to the development of physicochemical detection methods based on mass spectrometry [11]. DGTS is the most widespread BL among organisms due to its detection in several distant phylogenetic groups. Further details on BL distribution are discussed in section 5.

3. BL functions

3.1. Membrane structural role

The initial studies presented BL as major lipid components of extraplastidial membranes. These lipids can represent a high portion of polar lipids composition. In *O. danica*, DGTS and DGTA account for almost 50 % of the total glycerolipids, with DGTS being the most abundant [26]. In the phaeophyceae *Fucus vesiculosus*, DGTA also represents the highest percentage among glycerolipids [27]. From a general point of view, BL are thought to be abundant in species with low levels of phospholipids, while being less prevalent in species producing high

amounts of phospholipids. A negative correlation between DGTA and phosphatidylcholine (PC) content was suggested in several studies on phaeophyceae [22,28,29]. This trend was also reported concerning the relative proportions of DGTS and PC in fungi, bryophytes and green algae [30–32], although no quantitative correlation was detected between the amounts of these two lipids [30]. The inverse relationship between PC and BL is particularly evident in organisms that produce BL to the total exclusion of PC, such as the haptophyte *D. lutheri* or the green alga *Chlamydomonas reinhardtii* [16,33]. Based on these findings, it has been generally accepted that BL replace phospholipids as major membrane constituents, especially regarding DGTS and PC. These two lipids have a similar structure and seem to have a connected evolution since PC progressively replaced DGTS during the evolution of the green lineage [10]. It has been suggested that these lipids fulfill similar functions [34–36] and can be interchangeable, as shown by [37] in an engineered strain of *Saccharomyces cerevisiae* producing DGTS instead of PC. This study demonstrated that DGTS production had no negative impact on the membrane biogenesis or the yeast growth, suggesting that DGTS could perfectly substitute to PC in this organism [37]. However, certain doubts have been raised regarding the interchangeable nature of DGTS and PC. In green lineage organisms, DGTS and PC do not share the same fatty acid composition and may therefore play different roles [32]. A recent study on artificial membranes also questioned DGTS and PC substitutability, showing that these two lipids have different physical properties [9]. DGTS bilayers were shown to be thicker and more sensitive to hydration than bilayers composed of PC, likely due to the differences in their polar head structure. It was proposed that coexistence of DGTS and PC in the same membrane can be achieved by changing the fatty acid composition between these two lipids, thereby restoring the membrane thickness. This mechanism is proposed to occur in marine organisms in which DGTS is enriched in very-long chain polyunsaturated fatty acids (VLC-PUFAs) in comparison to PC [9]. This fatty acid composition change may not apply to freshwater or terrestrial organisms that produce only medium-chain fatty acid and no VLC-PUFAs. In this situation, DGTS and PC might not be good substitutes for each other because membrane properties and thickness cannot be maintained. This could explain weak coexistence of DGTS and PC observed in the green lineage [9]. Indeed, freshwater organisms with significant betaine lipids content usually have little or no PC [32]. Even some algae contain only betaine lipids and no PC such as *Chlamydomonas reinhardtii* whereas some other do not contain betaine lipid and only PC such as *Chlorella sorokinia* in Pi repleted condition [38,39].

3.2. Role in the lipid metabolism

Apart from their structural role in membranes, BL have also been studied for their involvement in the lipid metabolism. Once again, they are thought to function similarly to PC as acyl carriers for fatty acid editing and subsequent lipid synthesis. This hypothesis is well developed in [40], with a comparison of what is known about acyl editing on PC in land plants and the evidence for acyl editing of betaine lipids. Prior ¹³C-labeling studies have demonstrated that DGTS contributes substantial fatty acid flux toward TAG synthesis in *Chlamydomonas reinhardtii* [41].

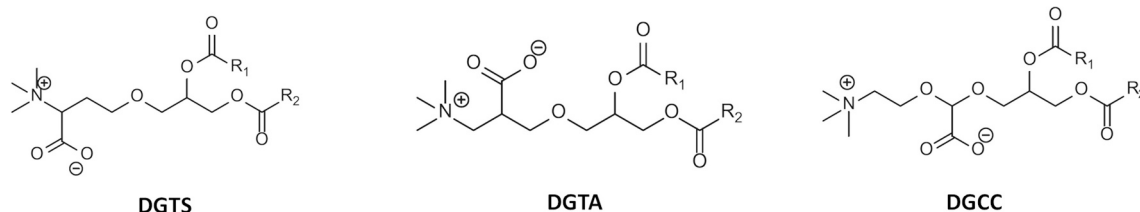


Fig. 1. Structure of the three known betaine lipids (DGTS:1(3),2-diacylglycerol-3(1)-*O*-4'-(*N,N,N*-trimethyl)-homoserine, DGTA: 1(3),2-diacylglycerol-3(1)-*O*-2'-(*N,N,N*-trimethyl)- β -alanine, DGCC: 1,2-diacylglycerol-3(1)-*O*-carboxy-(hydroxymethyl)-choline). Adapted from [25]. R1 and R2 correspond to the fatty acid esterified at the *sn*-1 and *sn*-2 positions, respectively.

However, the mechanism by which betaine lipids contributes to TAG accumulation likely differs from that of PC. Indeed, since phospholipase D and C act on the phosphate moiety absent in betaine lipids, they are not expected to be active on this lipid class. Furthermore, enzymes capable of breaking the ether bond linking the betaine polar head to the glycerol backbone have not yet been identified, but they are presumed to exist in order for cells to catabolize DGTS.

In plant, PC is known to participate in the synthesis of plastidic lipids via the “eukaryotic pathway”. PC acts initially as a substrate for the desaturation of oleic acid (18:1) into linoleic acid (18:2) before its degradation into diacylglycerol (DAG), which is converted into monogalactosyldiacylglycerol (MGDG) within the chloroplast [42]. As many algae contain little or no PC, BL were thought to play a similar role [40,43]. Labelling experiments in *C. reinhardtii* and *O. danica* showed that DGTS was a substrate for oleic acid desaturations, leading to the production of 18:2, 18:3 and 18:4 fatty acids [26,34,35]. Similar observations were noted for DGTA in *O. danica* and *F. vesiculosus* [26,27]. In accordance with these studies, BL were suggested to redistribute saturated acyl chains for the synthesis of other polar lipids, notably MGDG [44]. Pulse chase experiments indicated that 18:1 and 18:2 fatty acids were transferred from DGCC to MGDG in *D. lutheri* [43]. This hypothesis was also supported in *C. reinhardtii* and *Dictyopterus membranacea* concerning DGTS and DGTA, respectively [34,44]. In *M. gaditana*, 20:5 fatty acid (eicosapentaenoic acid or EPA) from DGTS was proposed to be imported to the plastid for MGDG formation through the “omega pathway” [45]. In *P. tricorutum*, mutants affected in plastidial desaturation exhibited higher amounts of EPA and extraplastidial glycerolipids including DGTA. These results suggest that DGTA might play a role in the omega pathway as a desaturation platform for EPA production and import into the chloroplast [46]. However, these recent studies provided only indirect evidence for BL contribution to MGDG synthesis. In marine algae, BL are primarily regarded as final acceptors for VLC-PUFAs, including the high valuable EPA and 22:6 fatty acids (docosahexaenoic acid or DHA) [47–51]. BL have also been reported to be actively involved in the lipid metabolism under certain stress conditions; this last point is discussed in the following sections 3.3 to 3.5.

3.3. Adaptation to phosphate deficiency

The involvement of BL in adaptation to phosphate deficiency has been well documented since the 1990s. The first discovery of betaine lipid accumulation during phosphate starvation was made in *Rhodobacter sphaeroides* by Benning et al., [20]. As mentioned before, BL are generally considered as substitutes for phospholipids, especially under phosphate scarcity. Since they do not contain phosphorus, they seem specifically adapted to replace phospholipids in extraplastidial membranes, allowing organisms to lower their phosphorus demand and maintain their cellular growth. This mechanism was first illustrated in eukaryotic phytoplankton living in oligotrophic regions of the ocean [1]. These organisms were able to substitute PC and phosphatidylethanolamine (PE) by BL in response to phosphate starvation [1]. Similar observations were recorded in other marine microalgae such as the diatom *Phaeodactylum tricorutum* [2,52] and several green algae including *Ostreococcus tauri* [4,5]. In these organisms, the level of DGTA significantly increased under phosphate-starved conditions, concomitant with a decrease in phospholipid levels. The same trend was observed concerning DGTS behaviour in the eustigmatophytes *Nannochloropsis oceanica* and *Microchloropsis gaditana* [4,53,54], as well as in the green alga *Picochlorum atomus* [4]. Apart from marine organisms, DGTS was also shown to be accumulated under phosphate limitation in bacteria [20,55], snow algae [56] and fungi [37]. In addition to its role as a structural substitute for phospholipids under phosphate starvation, DGTS was also suggested to replace phospholipids metabolic functions in this condition. In *N. oceanica*, DGTS was proposed to serve as an acyl carrier for C₁₈ fatty acids desaturation during phosphate starvation, as PC in normal conditions. DGTS could also provide EPA acyl chains to

produce chloroplastic lipids and triacylglycerol (TAG) [53]. In the eustigmatophyte *Monodus subterraneus*, DGTS was shown to replace PE as a substrate for C₂₀ fatty acids desaturation in response to phosphate starvation. It was suggested that DGTS acyl chains were subsequently incorporated into chloroplastic digalactosyldiacylglycerol (DGDG), as PE under standard conditions [57]. However, no change was observed in PC level in this organism [57].

The previously mentioned species were shown to increase their BL content during phosphate limitation, while producing basal level of BL under normal conditions. Some organisms seem to have evolved differently in this adaptation process by synthesizing BL only in nutrient deprivation. This is the case for the green alga *Chlorella kessleri*, in which the gene involved in DGTS synthesis is specifically induced under phosphate-deficient conditions [58]. In the haptophyte *Emiliania huxleyi*, DGCC synthesis also appears to occur solely under phosphate limitation [59]. A different evolutionary strategy can be observed in other organisms, in which BL are constitutively produced and reflect the low-phosphate environmental content. This phenomenon has been documented in some Icelandic bryophytes, where DGTS large distribution seems to be an intrinsic response to the low phosphorus levels in Iceland [31]. Conversely, the BL level may be independent of phosphate availability, as shown in several marine haptophytes in which betaine lipid amount did not increase during phosphate starvation [3,4,60]. In these species, phospholipids are intrinsically not abundant. In this case, BL can be considered major membrane components rather than substitute lipids under certain stress conditions. Thus, BL role in phosphate starvation cannot be assumed in every species [4].

3.4. Adaptation to nitrogen deficiency

BL involvement in the nitrogen limitation response has been discussed in a few studies on microalgae. Contrary to the phosphate deprivation response where phospholipids are specifically used as phosphorus storage, nitrogen stress does not imply the recycling of membrane lipids despite the presence of nitrogen in BL, PE and PC. The lipid remodeling due to nitrogen deprivation rather implies the use of acyl chains from glycerolipids for TAG accumulation, in addition to the de novo synthesized fatty acids. The role of glycerolipids in this mechanism seems to be mostly attributed to MGDG in nitrogen-deprived algae [2,6,61], even if some studies evoked the role of BL in this response. In the haptophyte *Tisochrysis lutea*, DHA from DGTS was suggested to contribute to TAG synthesis upon nitrogen depletion [3]. Similar observations were reported in *P. tricorutum* [62] but most of the studies have been conducted in *C. reinhardtii*. In this organism, DGTS seems to be a major source of fatty acids for TAG formation, in addition to MGDG and DGDG [36,63,64]. It seems that only specific molecular species of DGTS are involved in this process, notably DGTS 16:0/18:3, which contributes to 18:3 accumulation in TAG [65,66]. Contrary to MGDG, DGTS glycerol backbone does not appear to participate in TAG formation and instead undergoes deacylation to redistribute fatty acids [67]. This could explain why the overall DGTS content is weakly affected under nitrogen limitation [6,36].

3.5. Adaptation to low temperatures

Low temperatures are known to induce an increase in fatty acid desaturation within glycerolipids [68]. This mechanism allows the maintenance of membrane fluidity and plays a key role in the acclimation of plants and algae to cold environment [69]. A first study on *D. lutheri* reported BL involvement in this process [70]. At 15 °C, DGTS increased four-fold compared to cells grown at 25 °C, with an enrichment in VLC-PUFAs such as EPA and DHA [70]. In *N. oceanica*, DGTS was also shown to be accumulated at lower temperatures [7,8]. Contrary to PC, the EPA content in DGTS and MGDG strongly increased in cold conditions, at the expense of 16:0 and 16:1 fatty acids. These results suggest the specific role of DGTS as an important stock of EPA in

extraplastidial membranes, in coordination with MGDG in the chloroplast compartment [7]. The absence of DGTS synthesis in a mutant strain of *N. oceanica* led to a reduction in cellular growth and EPA content at low temperatures [8]. These observations further support the crucial role of DGTS in temperature tolerance in this organism. The potential role of BL in cold stress acclimation remains poorly investigated in other species.

4. BL biosynthesis

The biosynthesis of BL has been explored in several studies but remains partially understood. DGTS is the only BL for which the entire pathway is known. Some hypotheses have been proposed concerning DGTA synthesis and there is no existing knowledge regarding DGCC. In the following sections 4.1 and 4.2, we will discuss DGTS synthesis discovery and current explorations into DGTA pathway.

4.1. DGTS biosynthesis

In vivo labeling experiments in *C. reinhardtii* first demonstrated that methionine was a precursor for DGTS synthesis. Radiolabeled L-methionine was incorporated into both homo-Serine moiety and N-methyl units of the DGTS headgroup [71]. Similar experiments confirmed these observations in the bryophyte *Marchantia polymorpha* [72], the algae *O. danica* and *Cryptomonas* [26,73] and the bacterium *Rhodobacter sphaeroides* [74]. Methionine was then revealed to originate from S-adenosyl-L-Methionine (SAM), which contributed to both homo-Serine and methyl moieties of the DGTS headgroup [75]. The elucidation of DGTS pathway progressed further with the identification of the genes involved in DGTS formation in bacteria. Mutants of *R. sphaeroides* were used to identify an operon containing *btaA* and *btaB*, two essential genes for DGTS synthesis [76]. *btaA* was proposed to encode an S-adenosylmethionine:diacylglycerol 3-amino-3-carboxypropyl transferase, responsible for the attachment of the homo-Serine moiety to a DAG backbone. *btaB* was proposed to catalyse the next three methylation steps as an S-adenosylmethionine-dependent N-methyltransferase (Fig. 2) [76]. These results provided the first evidence of the two-step formation of DGTS from a presumed DAG substrate, with the use of SAM in both reactions. The BtaA and BtaB enzymes from *R. sphaeroides* (BtaA_{RS} and BtaB_{RS}) were then characterized by heterologous expression in *Escherichia coli*, which does not produce DGTS in natural conditions.

The co-expression led to DGTS formation and confirmed DGTS pathway in *R. sphaeroides* [77]. On the basis of protein similarity, eukaryotic orthologs of BtaA_{RS} and BtaB_{RS} were found in a subsequent study in *C. reinhardtii* [78]. The *C. reinhardtii* BTA1 gene (BTA1_{Cr}) was identified and suggested to encode a bifunctional protein sufficient for DGTS synthesis. BTA1_{Cr} protein was shown to carry the two catalytic domains BtaA-like and BtaB-like, presumed to be functional homologs of BtaA_{RS} and BtaB_{RS}, respectively (Fig. 2) [78]. Enzymatic characterization of each domain validated this hypothesis, suggesting that eukaryotic BTA1 protein carries out the entire pathway of DGTS formation. Binding sites to SAM were also identified in both domains and proved essential for enzyme activity [78]. Eukaryotic BTA1 homologs were then identified in several fungi [37,79,80] and algae [8,58]. It is interesting to note that the arrangement of the domains on the eukaryotic BTA1 protein can be of two types, namely type A characterized by a BtaB-BtaA domain, and type B for which the arrangement is opposite [8]. A phylogenetic analysis revealed that type A BTA1 was predominant in freshwater organisms while type B was more prevalent among marine organisms, suggesting a correlation between habitat and BTA1 configuration [9]. Moreover, it is interesting to note that the BTA1 gene sometimes exists in two copies within the genome. In *N. oceanica*, a short version of BTA1, which contains only the BtaB domain, is present in addition to the full-length BTA1 gene [8]. However, so far, no enzymatic data except the primary works from Riekhof [77,78] are available on the different configurations of BTA1. In our recent work, we show that type B Bta1 from *Microchloropsis gaditana* and *Phaeodactylum tricorutum* do not have the same efficiency in heterologous expression in *Nicotiana Benthamiana* although they have the same configurations whereas type A Bta1 from *Chlamydomonas reinhardtii* or *Kluyveromyces lactis* seem to be as efficient as *M. gaditana* enzyme [81]. It would be interesting to compare activities of different isoforms and to assess whether AlphaFold prediction together with evolutionary data can provide insights on this particular organization of the two domains.

Cell fractionation and immunomicroscopy studies conducted on the pteridophyte *Lycopodium annotinum* and the algae *C. reinhardtii* and *O. danica* provided initial evidence of DGTS subcellular localization [30]. DGTS occurred mainly in non-plastidial structures, suggesting its presence in the ER [30]. In accordance with these results, BTA1 synthesis pathway was presumed to occur in the endoplasmic reticulum (ER) in *C. reinhardtii* [78]. Fluorescence microscopy experiments further confirmed the ER localization of BTA1 enzyme in the fungus *Fusarium*

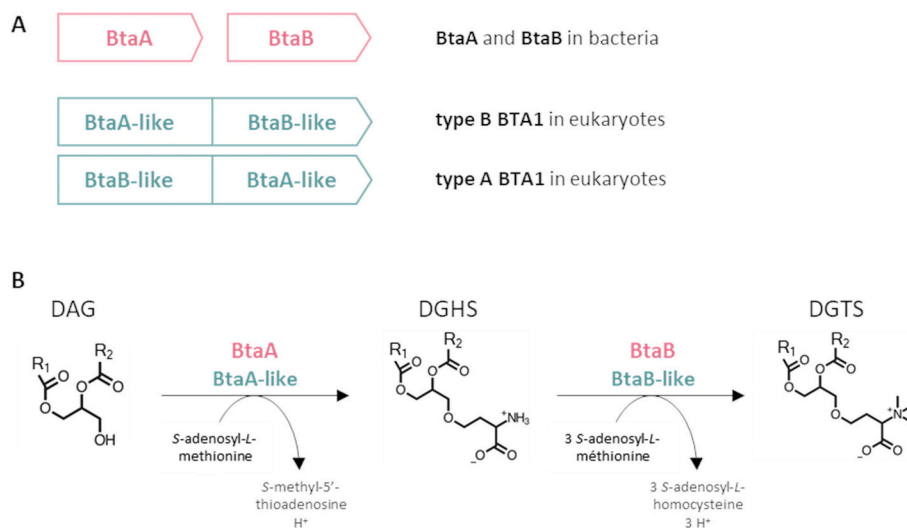


Fig. 2. DGTS biosynthesis in organisms. A. Protein domains organization. BtaA-like and BtaB-like domains of BTA1 proteins are respective homologs of bacterial BtaA and BtaB proteins. Two configurations are found in eukaryotes, type A and type B, corresponding to different organization of the BtaA-like and BtaB-like domains. B. DGTS biosynthetic pathway in bacteria (pink) and eukaryotes (green). DAG, diacylglycerol; DGHS, diacylglycerylhomoserine. Adapted from [37]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

graminearum [82]. Following overexpression of *M. gaditana* BTA1 in the ER of *Nicotiana Benthamiana* leaves, DGTS is excluded of chloroplastic membrane and its accumulation induces a proliferation of ER membrane [81]. Positional distribution of fatty acids has also been used to investigate the site of DGTS synthesis. A large number of viridiplantae species possess unsaturated C₁₈ fatty acids at the *sn*-2 position of DGTS glycerol backbone [28]. This composition being representative of lipids synthesized through the eukaryotic pathway, DGTS was assumed to be produced in the ER [28]. Similar conclusions were reported in *C. kessleri* and *C. reinhardtii*, in which *sn*-2 position of DGTS is occupied almost exclusively by C₁₈ unsaturated fatty acids [38,58,83]. Enzymatic test in *C. reinhardtii* organelles also confirmed this hypothesis, showing that DGTS synthesis activity was associated with the ER [75]. Moreover, the fatty acid composition of DGTS synthesized in response to Pi starvation was shown to be similar to that of PC and PE in *C. kessleri*, indicating that DGTS might be partially synthesized from degraded PC and PE in the endoplasmic reticulum [58]. However, some of the DGTS content in *C. reinhardtii* was reported to be located within the chloroplast [36],

raising the possibility of a potential DGTS transport in the chloroplastic compartment.

4.2. DGTA biosynthesis

Early studies tried to decipher DGTA biosynthetic pathway after the elucidation of its chemical structure [84]. DGTA being a structural isomer of DGTS, it has been suggested that the synthesis of both lipids was linked. Pulse-chase labeling experiments in *Cryptomonas* first demonstrated that the C-3 and C-4 carbons of DGTS were incorporated into DGTA polar head, suggesting the potential role of DGTS as a precursor for DGTA formation [47]. Similar experiments in *O. danica* confirmed this hypothesis and postulated that DGTS conversion to DGTA occurred through modifications in DGTS polar head. The carboxyl group of DGTS might be removed and replaced by a new one onto the adjacent carbon through a sequential process of decarboxylation and carboxylation (Fig. 3A) [26]. In contrast, the notable differences between the DGTS and DGTA fatty acid compositions indicate a potential remodeling of

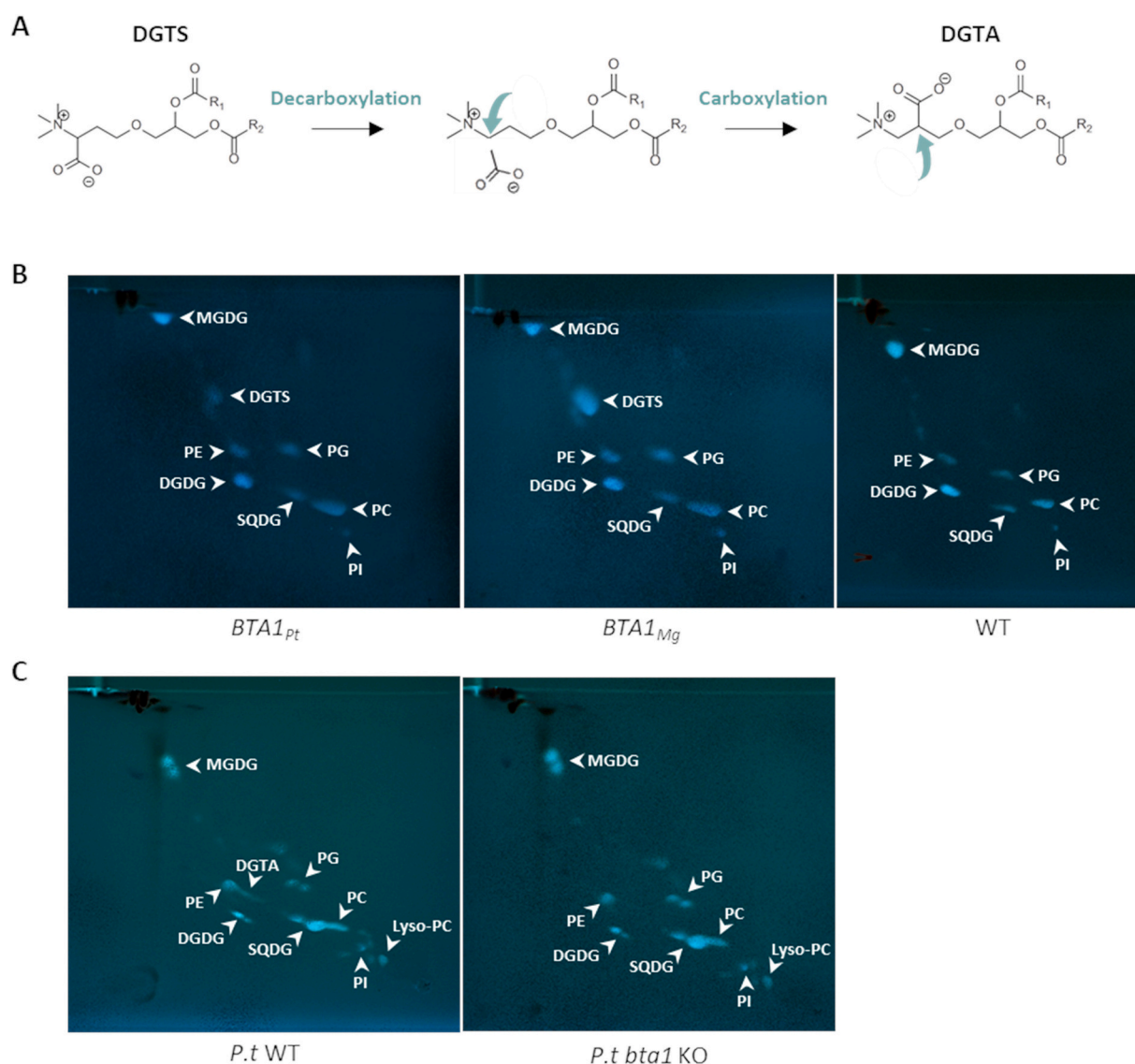


Fig. 3. DGTA biosynthesis. A. Hypothetical conversion of DGTS into DGTA. B. Separation by TLC of the glycerolipids from *N. benthamiana* leaves agroinfiltrated with BTA1 gene of *P. tricornutum* (BTA1_{Pt}) and *Microchloropsis gaditana* (BTA1_{Mg}) (Naga_100016g36). BTA1_{Mg} was used as a control of a DGTS producing organism. WT: wild type. Constructs are described in [81] C. Separation by TLC of the glycerolipids from *P. tricornutum* wild-type (WT) and KO mutant of BTA1 gene (Phatr3_J42872)(P.t bta1 KO). TLC analysis was performed as described in [2]. SQDG: sulfoquinovosyldiacylglycerol, DGDG: digalactosyldiacylglycerol, PG: phosphatidylglycerol, PI: phosphatidylinositol. TLC: Thin Layer Chromatography.

acyl chains. In *O. danica*, DGTA is mainly composed of C₂₀ and C₂₂ fatty acids, while DGTS predominantly contains C₁₄ and C₁₈ fatty acids. A complete exchange of acyl groups through deacylation and re-acylation was then predicted during DGTS conversion to DGTA [26]. These differences in the fatty acid molecular distributions are also found in other species, in which high VLC-PUFAs content is characteristic of DGTA rather than DGTS [33,85].

More recently, the hypothesis of DGTS conversion to DGTA has gained support with genetic and transcriptomic analyses. In the fungus *Cryptococcus neoformans*, the *BTA1* gene mutation resulted in a decrease in both DGTS and DGTA production [80]. In *P. tricornutum*, which only produces DGTA, a putative *BTA1* gene was identified and shown to be overexpressed during phosphate starvation, resulting in an increase in DGTA synthesis [86]. This last result suggests that the *BTA1* gene in DGTA producing organisms is present and actively involved in the metabolism, probably allowing the production of DGTS followed by its complete conversion into DGTA. In *Cryptomonas* and *O. danica*, the amount of DGTA was shown to be governed by the rate of DGTS transformation to DGTA, which seemed to be faster under a high DGTA/DGTS cellular ratio. It was then expected that DGTS conversion to DGTA occurred even more rapidly in organisms producing only DGTA, thus explaining the absence of DGTS detection [26]. The putative *BTA1* gene from the DGTA producing diatom *P. tricornutum* (Phatr3_J42872), initially identified by [86], was expressed in the plant *N. benthamiana* and resulted in DGTS production (Fig. 3B) [81]. This new result confirms the functionality of *BTA1* enzyme as a DGTS synthase and not as a DGTA synthase. Moreover, lipidomics analyses of *P. tricornutum* *BTA1* knock-out mutants revealed the suppression of DGTA production (Fig. 3C and Supplementary Methods section 7.1). This further demonstrates the involvement of *BTA1* in DGTA synthesis by the production of DGTS as an intermediate.

5. BL distribution and evolution

5.1. BL distribution among organisms

The literature has been reviewed in order to gain insight into the global distribution of BL in species. BL producing organisms were gathered and classified based on their phylogenetic affiliation, with the aim to provide an overview of the main groups in which each type of BL is found. The resulting list is presented in Table 1. Because DGTS and DGTA are structural isomers, both molecules can be confounded if mass spectrometry analysis is used and performed solely on the 236 fragment [87]. Furthermore, as described above, DGTA synthesis pathway is not fully known and relies on the presence of *BTA1*. Sometimes, by genomic analysis, organisms are described as DGTS producers when *BTA1* is detected. This can lead to uncertainties in the detection of DGTS and DGTA in organisms such as *P. tricornutum* [62]. Thus, the selection of organisms presented here took into account the method used for BL detection, which is either performed by thin layer chromatography (TLC) analysis [88] or by mass spectrometry with the identification of DGTS or DGTA specific fragments [89,90]. Finally, it is important to consider that, unlike other pathways with identified synthesis genes, research for BL detection cannot rely on genomic analyses like high-throughput transcriptomics. Since BL can only be revealed by metabolic analyses, their reported detection in the literature may not fully cover the actual extent of their distribution within organisms.

As previously mentioned, DGTS is the BL the most widely distributed among the organisms, with a representation in marine, freshwater and terrestrial ecosystems. It is detected in taxonomically diverse and distant groups, including plants, green algae, fungi, bacteria, Chromista and Amoebozoa (Table 1). Several publications listed a significant number of DGTS producing organisms around the 1990s, notably in plants such as Bryophytes and Pteridophytes [18,28,30,32,91] and in various algae and fungi [15,30,32,92]. Among algae, DGTS seems to be characteristic of the green lineage, with a high representation in Chlorophyta and

Rhodophyta. DGTS was also detected in numerous chromista marine organisms such as Cryptophytes and Haptophytes (Table 1), although it appears to be excluded from certain groups within chromista such as phaeophyceae [29]. Because of its widespread distribution and hypothetical involvement in DGTA synthesis, DGTS was speculated to be the first BL to appear during evolution [30]. Bolik et al. (2023) [9] also supported this hypothesis, suggesting that DGTA and DGCC could have been acquired later in the evolution in marine organisms. As discussed above, DGTS and PC are considered as good substitutes for each other in marine species, allowing the replacement of one lipid by the other depending on the phosphate availability. The selection pressure on DGTS in marine organisms was therefore different than the one exerted in freshwater organisms, where the coexistence of PC and DGTS is rare and results in the predominance of one lipid over the other. This could have led marine species to diversify their BL production through the emergence of DGTA and DGCC [9]. On the contrary, it is possible that DGTS remained the only BL produced in freshwater organisms and all species that emerged from the green lineage. DGTS has been progressively replaced by PC during the evolution of plants and is completely absent in vascular plants [10,18,30,48], suggesting its gradual loss during plant terrestrialization. The evolution of the tracheophytes is marked by the loss of vegetative desiccation tolerance [93] and it seems that DGTS disappearance occurred concomitantly with this event [9]. DGTS is detected in tracheophytes but several studies reported that this lipid was degraded during drought periods in these organisms [94–96], suggesting that DGTS might be deleterious in terrestrial environments where water is limiting. Thus, the losses of DGTS production and desiccation tolerance might be linked and could explain why seed plants do not synthesize DGTS [9].

DGTA was initially identified in a significant number of phaeophyceae species (Table 1) and described as a characteristic lipid of this group, although certain suborders lack DGTA [22,28–30]. DGTA was also detected in other Stramenopiles like Dinoflagellates, as well as in Cryptophytes and Haptophytes (Table 1). Most of these species live in marine environments, with the exception of several freshwater organisms such as *O. danica* and *Cryptomonas* CR-1 (Table 1). Despite the overrepresentation of DGTS in the green lineage, DGTA was reported in a few green algae species like *O. tauri* and *Micromonas pusilla*. Apart from the Chromista and green algae groups, DGTA was also detected in the fungus *Cryptococcus neoformans*, which also produces DGTS, and in the Euglenozoa *Diplonema papillatum*, a marine eukaryote (Table 1). Thus, DGTA appears to be primarily distributed among marine Chromists. Although it has been detected in some species more phylogenetically distant, the distribution of DGTA remains much less extensive than that of DGTS. These observations are consistent with the hypothesis that DGTS is the most primitive BL. DGTA synthesis could have been acquired later in the evolution in certain DGTS producing organisms [9], as DGTS was shown to be involved in DGTA synthesis. This could have led to the emergence of organisms producing both DGTS and DGTA or DGTA only (Table 1).

DGCC distribution seems to be even more restrictive than that of DGTA, as it is detected only in marine Chromist species (Table 1). This lipid was first detected in several Haptophytes before being identified in other representatives of the SAR (Stramenopiles, Alveolates, Rhizaria) supergroup, such as Chlorarachniophytes [87], Dinophyceae and diatoms [90] (Table 1). It is interesting to note that DGCC is often found in combination with DGTS or DGTA. It can also be produced with both DGTS and DGTA, although DGCC was usually detected in small amounts in this case [87]. Because the genes involved in DGCC synthesis are not known, no phylogenomic study could be achieved.

5.2. *BTA1* phylogeny

In the present review, we sought at identifying possible evolutionary pathways of betaine lipid-producing organisms by analyzing the amino acid sequences of the *BTA1* protein. We began with sequences recently

Table 1

Representative distribution list of the three BL species among organisms, based on data from the literature. This work relies on the detection (x) of BL produced by TLC methods or mass spectrometry with the identification of specific fragments of DGTS and DGTA. Absence of an "x" means that the betaine lipid was not detected because either it is absent or it is below the detection level of the used technique. The colors blue, green, pink, yellow, light pink, and orange represent the general phylogenetic classification of the listed species, corresponding respectively to Chromista, green lineage organisms, Euglenozoa, Amoebozoa, fungi, and bacteria. The unique identifier for each species is provided by NCBI taxonomy.

Species name	Classification	Taxonomy ID	Reference	Habitat	DGTS	DGTA	DGCC
<i>Ascophyllum nodosum</i>	Chromista, Stramenopile, Ochrophyta	NCBI: txid52969	Jones and Harwood, 1992 [97]	marine water		x	
<i>Bigelowiella natans</i>	Chromista, Rhizaria	NCBI: txid227086	Roche and Leblond, 2010 [87]	marine water	x	x	x
<i>Calyptrosphaera sphaeroidea</i>	Chromista, Haptophyta	NCBI: txid35139	Kato et al., 1996 [24]	marine water			x
<i>Chattonella antiqua</i>	Chromista, Stramenopile, Ochrophyta	NCBI: txid859642	Sato et al., 1987 [98]	marine water	x		
<i>Chroomonas placoides</i>	Chromista, Cryptophyceae	NCBI: txid173977	Cañavate et al., 2016 [99]	marine water		x	
<i>Chroomonas salina</i>	Chromista, Cryptophyceae	NCBI: txid402395	Eichenberger et al., 1996 [100]	marine water		x	
<i>Conticribra weissflogii</i>	Chromista, Stramenopile, Ochrophyta	NCBI: txid1577725	Li et al., 2016 [101]	brackish water			x
<i>Cryptomonas strain CR-1</i>	Chromista, Cryptophyceae		Sato, 1991 [47]	marine, brackish and freshwater, terrestrial	x	x	
<i>Diacronema lutheri (or Pavlova lutheri)</i>	Chromista, Haptophyta	NCBI: txid2081491	Kato et al., 1995 [33]	marine water		x	x
<i>Diacronema vlkianum</i>	Chromista, Haptophyta	NCBI: txid70452	Armada et al., 2013 [90]	marine and freshwater	x	x	x
<i>Dictyota dichotoma</i>	Chromista, Stramenopile, Ochrophyta	NCBI:txid2876	Araki et al., 1991 [22]	marine water		x	
<i>Emiliania huxleyi</i>	Chromista, Haptophyta	NCBI:txid2903	Shemi et al., 2016 [59]	marine water	x		x
<i>Fucus vesiculosus</i>	Chromista, Stramenopile, Ochrophyta	NCBI: txid49266	Jones and Harwood, 1992 [97]	marine water		x	
<i>Gomphonema parvulum</i>	Chromista, Stramenopile, Ochrophyta	NCBI: txid97227	Künzler and Eichenberger, 1997 [102]	marine water		x	
<i>Gymnochlora stellata</i>	Chromista, Rhizaria	NCBI: txid67809	Roche and Leblond, 2010 [87]	marine water	x	x	x
<i>Hizikia fusiformis (or Sargassum fusiforme)</i>	Chromista, Stramenopile, Ochrophyta	NCBI: txid590727	Araki et al., 1991 [22]	marine water		x	
<i>Ishige okamurai</i>	Chromista, Stramenopile, Ochrophyta	NCBI: txid233772	Araki et al., 1991 [22]	marine water		x	
<i>Isochrysis galbana</i>	Chromista, Haptophyta	NCBI: txid37099	Cañavate et al., 2017 [60]	marine water	x		x
<i>Microchloropsis gaditana</i>	Chromista, Stramenopile, Ochrophyta	NCBI: txid72520	Cañavate et al., 2016 [99]	marine water	x		
<i>Monodus subterraneus (or Monodopsis subterranea)</i>	Chromista, Stramenopile, Ochrophyta	NCBI: txid44434	Khazin-Goldberg and Cohen, 2006 [57]	freshwater	x		
<i>Nannochloropsis granulata</i>	Chromista, Stramenopile, Ochrophyta	NCBI: txid43926	Banskota et al., 2013 [49]	marine water	x		
<i>Nannochloropsis oculata</i>	Chromista, Stramenopile, Ochrophyta	NCBI: txid1259847	Li et al., 2014 [50]	marine and brackish water	x		
<i>Ochromonas danica</i>	Chromista, Stramenopile, Ochrophyta	NCBI:txid2986	Vogel and Eichenberger, 1992 [26]	freshwater	x	x	
<i>Pachydictyon coriaceum (or Dictyota coriacea)</i>	Chromista, Stramenopile, Ochrophyta	NCBI: txid499617	Araki et al., 1991 [22]	marine water		x	
<i>Padina arborescens</i>	Chromista, Stramenopile, Ochrophyta	NCBI: txid200423	Araki et al., 1991 [22]	marine water		x	
<i>Phaeodacylum tricorutum</i>	Chromista, Stramenopile, Ochrophyta	NCBI:txid2850	Abida et al., 2015 [2]	marine water		x	
<i>Prorocentrum micans</i>	Chromista, Alveolata	NCBI:txid2945	Kato et al., 1996 [24]	marine water			x
<i>Pseudoisochrysis paradoxa</i>	Chromista, Haptophyta	NCBI: txid185006	Armada et al., 2013 [90]	marine water	x		x

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Table 1 (continued)

Species name	Classification	Taxonomy ID	Reference	Habitat	DGTS	DGTA	DGCC
<i>Rhodomonas baltica</i>	Chromista, Cryptophyceae	NCBI: txid295837	Cañavate et al., 2016 [99]	marine water		x	
<i>Sargassum horneri</i>	Chromista, Stramenopile, Ochrophyta	NCBI: txid74089	Araki et al., 1991 [22]	marine water		x	
<i>Sargassum ringgoldianum</i>	Chromista, Stramenopile, Ochrophyta	NCBI: txid127524	Araki et al., 1991 [22]	marine water		x	
<i>Sargassum thunbergii</i>	Chromista, Stramenopile, Ochrophyta	NCBI: txid127542	Araki et al., 1991 [22]	marine water		x	
<i>Thalassiosira pseudonana</i>	Chromista, Stramenopile, Ochrophyta	NCBI: txid35128	Martin et al., 2011 [103]/Cañavate et al., 2016 [99]/Hunter et al., 2018 [104]	marine water	(x)		x
<i>Tisochrysis lutea</i>	Chromista, Haptophyta	NCBI: txid1321669	Huang et al., 2019 [3]	marine water	x	x	x
<i>Acetabularia mediterranea</i>	Archaeplastida, Chlorophyta	NCBI: txid35845	Thompson, 1996 [105]	marine water	x		
<i>Asplenium viride</i>	Archaeplastida, Embryophyta	NCBI: txid147940	Künzler and Eichenberger, 1997 [102]	terrestrial	x		
<i>Bazzania yoshinagana</i>	Archaeplastida, Embryophyta	NCBI: txid989914	Sato and Furuya, 1985 [32]	terrestrial	x		
<i>Chlamydomonas reinhardtii</i>	Archaeplastida, Chlorophyta	NCBI:txid3055	Eichenberger, 1982 [15]	freshwater	x		
<i>Chlorella kessleri</i>	Archaeplastida, Chlorophyta	NCBI:txid3074	Oishi et al., 2022 [58]	freshwater	x		
<i>Chlorella minutissima</i>	Archaeplastida, Chlorophyta		Haigh et al., 1996 [106]	freshwater	x		
<i>Chlorococcum amblyostomatis</i>	Archaeplastida, Chlorophyta	NCBI: txid1008953	Conde et al., 2021 [107]	freshwater	x		
<i>Cladophora japonica</i>	Archaeplastida, Chlorophyta	NCBI: txid162069	Sato and Furuya, 1985 [32]	marine, brackish and freshwater, terrestrial	x		
<i>Codium fragile</i>	Archaeplastida, Chlorophyta	NCBI:txid3133	Sato and Furuya, 1985 [32]	marine water	x		
<i>Coleochaete scutata</i>	Archaeplastida, Streptophyta	NCBI:txid3125	Sato and Furuya, 1985 [32]	freshwater	x		
<i>Dunaliella salina</i>	Archaeplastida, Chlorophyta	NCBI:txid3046	Norman and Thompson, 1985 [108]	marine and freshwater	x		
<i>Fritschiella tuberosa</i>	Archaeplastida, Chlorophyta	NCBI: txid56004	Eichenberger, 1982 [15]	freshwater	x		
<i>Lycopodium clavatum</i>	Archaeplastida, Embryophyta	NCBI:txid3252	Sato and Furuya, 1984 [18]	terrestrial	x		
<i>Lygodium japonicum</i>	Archaeplastida, Embryophyta	NCBI: txid13824	Sato and Furuya, 1984 [18]	terrestrial	x		
<i>Marchantia polymorpha</i>	Archaeplastida, Embryophyta	NCBI:txid3197	Lu et al., 2023 [31]	terrestrial	x		
<i>Mastocarpus stellatus</i>	Archaeplastida, Rhodophyta	NCBI: txid29227	Künzler and Eichenberger, 1997 [102]	marine water	x		
<i>Micromonas pusilla</i>	Archaeplastida, Chlorophyta	NCBI: txid38833	Maat et al., 2015 [109]	marine water	x	x	
<i>Ostreococcus tauri</i>	Archaeplastida, Chlorophyta	NCBI: txid70448	Degraeve-Guilbault et al., 2017 [5]	marine water		x	
<i>Phyllophora pseudoceranooides</i>	Archaeplastida, Rhodophyta	NCBI: txid508189	Künzler and Eichenberger., 1997 [102]	marine water	x		
<i>Picochlorum atomus</i>	Archaeplastida, Chlorophyta	NCBI: txid133490	Cañavate et al., 2016 [99]	marine water	x		
<i>Polytoma uvella</i>	Archaeplastida, Chlorophyta	NCBI: txid40532	Eichenberger, 1982 [15]	freshwater	x		
<i>Racomitrium lanuginosum</i>	Archaeplastida, Embryophyta	NCBI: txid140628	Lu et al., 2023 [31]	terrestrial	x		
<i>Selaqinella kraussiana</i>	Archaeplastida, Embryophyta	NCBI: txid81964	Künzler and Eichenberger, 1997 [102]	terrestrial	x		
<i>Tetraselmis suecica</i>	Archaeplastida, Chlorophyta	NCBI: txid270643	Cañavate et al., 2016 [99]	marine water		x	
<i>Ulva lactuca</i>	Archaeplastida, Chlorophyta	NCBI: txid63410	Dembitsky and Rozentsvet, 1996 [92]	marine water	x		
<i>Ulva pertusa</i>	Archaeplastida, Chlorophyta	NCBI:txid3120	Sato and Furuya, 1985 [32]	marine water	x		
<i>Volvox carteri</i>	Archaeplastida, Chlorophyta	NCBI:txid3067	Moseley and Thompson, 1980 [110]	freshwater	x		
<i>Diplonema papillatum</i>	Euglenozoa	NCBI: txid91374	Škodová-Sveráková et al., 2021 [111]	marine water		x	
<i>Acanthamoeba castellanii</i>	Amoebozoa	NCBI:txid5755	Furlong et al., 1990 [112]	terrestrial	x		
<i>Boletus edulis</i>	Fungi, Basidiomycota	NCBI: txid36056	Vaskovsky et al., 1998 [113]	terrestrial	x		
<i>Cryptococcus neoformans</i>	Fungi, Basidiomycota	NCBI:txid5207	Lev et al., 2019 [80]	terrestrial	x	x	

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Table 1 (continued)

Species name	Classification	Taxonomy ID	Reference	Habitat	DGTS	DGTA	DGCC
<i>Fusarium graminearum</i>	Fungi, Ascomycota	NCBI:txid5518	Zhang et al., 2016 [82]	terrestrial	x		
<i>Xylaria hypoxylon</i>	Fungi, Ascomycota	NCBI:txid37992	Künzler and Eichenberger, 1997 [102]	terrestrial	x		
<i>Cereibacter sphaeroides</i> (or <i>Rhodobacter sphaeroides</i>)	Bacteria	NCBI:txid1063	Hofmann and Eichenberger, 1996 [74]/ Benning et al., 1995 [20]	marine and freshwater	x		

published by Bolik et al. (2023) [9]. We then compared this dataset with Table 1 to fill the gaps. Most organisms listed in Table 1 either lacked a sequenced genome or had an unidentifiable BTA1 gene. We utilized a bioinformatics approach to include the maximum number of species in our analysis (see Supplementary Methods section 7.2). This effort resulted in the addition of 25 sequences to the Bolik et al. (2023) dataset [9], often requiring us to work with non-annotated genomes or transcriptomes. Given the differing configurations of the BtaA- and BtaB-like domains, which correspond to the pfam DUF3419 and S-adenosyl-L-methionine-dependent methyltransferase domains, respectively, we aligned and analyzed each domain separately (Fig. 4), as detailed in Bolik et al. (2023) [9].

In the final alignment, the sequence of the fern *Diphasiastum complanatum* was included due to its phylogenetic proximity to the fern *Lygodium japonicum*, despite no indication of its ability to produce betaine lipids. A similar rationale was used for the inclusion of the dinoflagellate *Symbiodinium microadriaticum* sequence; although belonging to the order Suessiales, it can be hypothesized that it produces DGCC like *Prorocentrum micans*, which belongs to the order Proccentrales. Notably, the sequences from *Acanthamoeba castellanii*, *Chara brunii*, *Haematococcus lacustris*, *Lygodium japonicum*, and *Symbiodinium microadriaticum* contained only the DUF3419 domain, similar to bacteria. In the *Lycopodium clavatum* genome, two genes bore a DUF3419 domain: one containing both the SAM and DUF3419 domains, and another bearing only the DUF3419 domain. After alignment and preliminary phylogenetic analyses, the sequence harboring only the DUF3419 domain was removed because it was too short and possibly represented a truncation or a pseudogene or a case of independent horizontal gene transfer.

Three main exceptions to the hypothesis proposed by Bolik et al. (2023) [9]—which suggests that marine species show a DUF3419-SAM configuration (type B) while freshwater and terrestrial species show the opposite arrangement (type A)—should be noted: 1) the freshwater diatoms *Alphora coffeiformis*, *Nitzschia inconspicua*, *Fragilaria crotonensis*, and *Mayamaea pseudoterrestris*; 2) the freshwater Chrysophyte *Ochromonas danica*; and 3) the marine unicellular Chlorophyte *Chloropicon primus*. The first two exceptions, although living in freshwater, show a marine arrangement of the domains. Notably, both belong to the SAR supergroup, suggesting that SARs may have evolved in marine environments and subsequently moved back to freshwater. Conversely, *Chloropicon primus*, although a marine species, shows a freshwater configuration.

The Bayesian phylogenetic inference (Fig. 4) showed no correlation of the betaine lipid synthesized with the phylogenetic position of the BTA1 protein, indeed DGTA and DGTS producers can sit in the same clade, like the haptophytes *Diacronema lutheri* and *Emiliania huxleyi*. The present follow-up to Bolik et al. (2023) [9] work gives insights about the inconsistency between the molecular evolution of genes and proteins and the corresponding metabolites. Nonetheless, our analyses highlight the wider metabolic diversity showed by unicellular algae compared to the green lineage with the latter producing mainly exclusively DGTS.

As previously stated, the hypothesis that DGTA derives from DGTS has recently gained strength. It is believed that decarboxylation/carboxylation reactions are fundamental to the conversion of DGTS into DGTA. However, this decarboxylation/carboxylation must occur extremely quickly, at least in *P. tricorutum* and other species that contain only DGTA, as no traces of DGTS have ever been identified in the

lipidomes. This suggests that the enzyme/s involved in the conversion of DGTS to DGTA may be closely associated with BTA1. An alternative approach could involve a comprehensive in silico structural comparative analysis of the full BTA1 amino acid sequences from species that synthesize DGTS and phylogenetically related ones that synthesize DGTA. This could help identify potential domains responsible for interactions with other enzymes.

If the full-length sequence of the BTA1 enzyme could provide insights into the mechanism of DGTA production from DGTS, another phylogenetic analysis was conducted on the 33 type B BTA1 sequences, as illustrated in Fig. 5. In this analysis, the full-length sequences were aligned and curated then followed by a Bayesian phylogenetic analysis as detailed in the Supplementary Methods. Compared to the previous analysis, which was based on a reduced number of residues, this approach offered better statistical support for certain nodes. The latter analysis reveals representatives of the SAR ‘supergroup’ dispersed throughout the entire Bayesian phylogenetic tree. For instance, diatoms form a robust, self-standing clade, closely related to Bigyra (*Cafeteria roenbergensis*) and the euglenophyte *Diplonema papillatum*. Eustigmatophytes, such as *Nannochloropsis* and *Microchloropsis*, cluster with the cryptophyte *Guillardia theta* in a sister clade to choanoflagellates *Monosiga brevicollis* and *Salpingoeca rosetta*. The distant phylogenetic position of eustigmatophytes from other stramenopiles, particularly from diatoms, was previously noted for the phospholipid:diacylglycerol acyltransferase (PDAT) [114] and diacylglycerol acyltransferase (DGAT) [115] enzymes. It has been hypothesized that such discrepancies between taxonomic assignments and the phylogenetic positions of certain proteins may indicate different evolutionary histories in closely related taxa. In eustigmatophytes, the secondary host-originated gene may have been retained, whereas in other stramenopiles, the red algal gene was retained.

Regardless of the evolutionary pathway taken by the gene encoding the type B BTA1 protein, the organism’s ability to synthesize either DGTS or DGTA did not follow the same route. Therefore, the enigma of the enzymes capable of converting DGTS into DGTA remains unresolved, requiring further experimental and computational investigations to explore this intriguing issue.

6. Concluding remarks

BL are non-phosphorus glycerolipids predominantly found in extraplastidial membranes of unicellular algae. In this review, a thorough literature survey was conducted to summarize the available knowledge on these poorly understood lipids. Discovered in the 1970s, betaine lipids have been identified in an increasing number of organisms since the development of mass spectrometry techniques. They have been detected in diverse unicellular eukaryotes – mainly microalgae – as well as certain bacteria, fungi, macroalgae and some bryophyte and pteridophyte. Among the three identified species, DGTS is the most widespread and extensively studied form. It is considered an ancestral glycerolipid to PC, progressively replaced by the latter during the evolution of the green lineage.

According to the literature, BL are regarded as substitute lipids for phospholipids, particularly under conditions of phosphorus deficiency. This mechanism has been extensively studied in numerous species, where the synthesis of betaine lipids increases concomitantly with the degradation of phospholipids in response to Pi deficiency. However, the

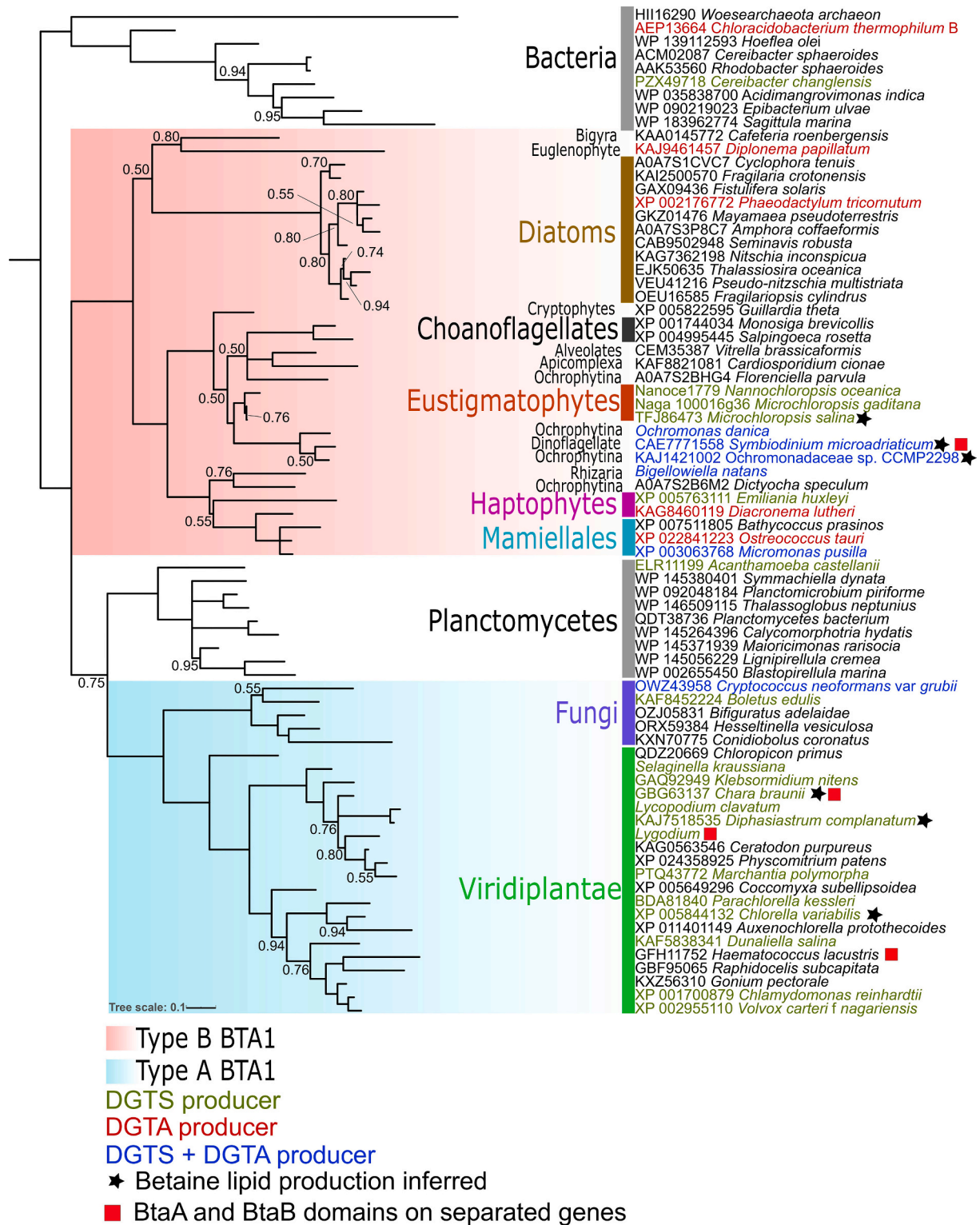


Fig. 4. BTA1 protein Bayesian phylogenetic inference of the BtaA domain. Posterior Probability values below 0.95 are reported at each node. Type B BTA1 proteins are shaded in red, while type A BTA1 proteins are shaded in blue. Sequence accession numbers precede species names. Species indicated in green identify DGTS producers, in red DGTA producers, in blue DGTS and DGTA producers. For species indicated in black, information about the betaine lipid synthesized is absent. A star indicates closely related species that synthesize the corresponding betaine lipids. A red rectangle denotes species where BtaA and BtaB domains are encoded on separate genes. The BtaA domain phylogeny was computed as described by Bolik et al., 2023 [9] using a partitioned model, one including the amino acid sequence and the others for the betaine lipid produced and the BTA1 type A or B. 4 million generations were imposed. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

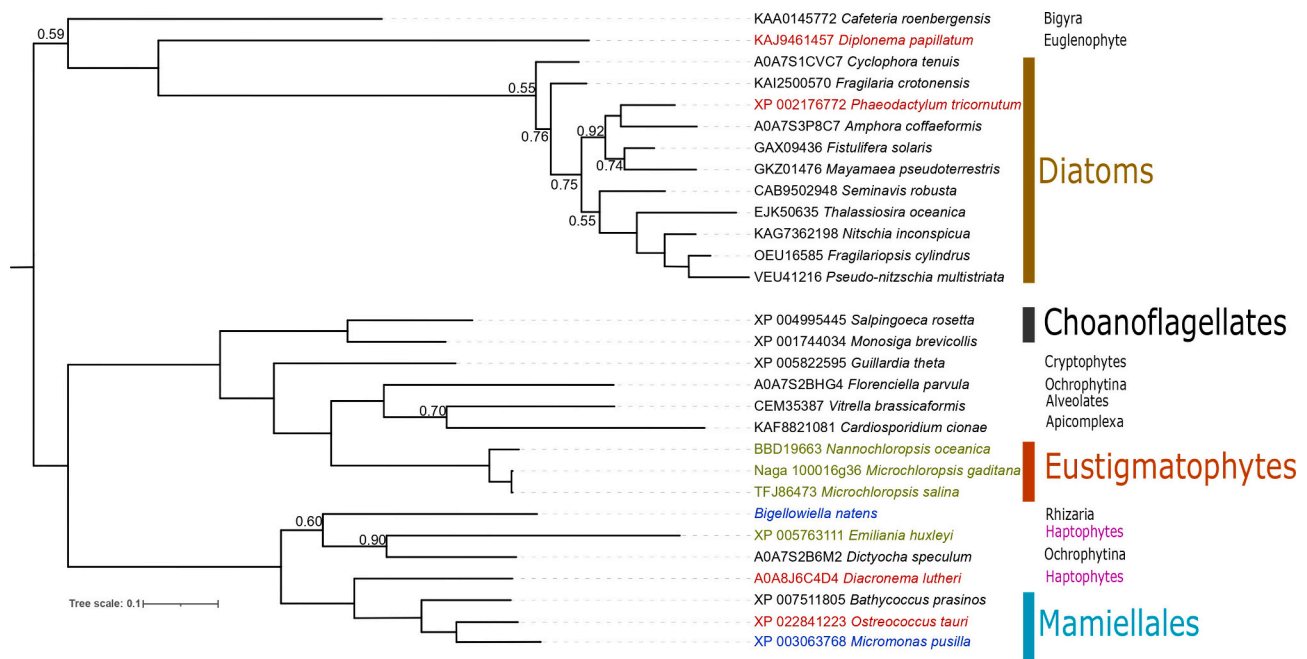


Fig. 5. Bayesian phylogenetic inference of the full-length protein of the type B BTA1 only (shaded in red in Fig. 4). Posterior Probability values below 0.95 are reported at each node. The phylogenetic analysis employed an alignment comprising 452 positions. The inference was performed using 8 Markoff chains (one cold and 7 heated) and 2 million generations. Colour code follows Fig. 4. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

relationship between BL and phospholipids appears to be more complex than a simple substitution. In some species, the synthesis of BL remains constant and independent of Pi levels in the environment, with phospholipids being intrinsically low in abundance. Several studies have highlighted the specific role of BL in the lipid metabolism. They are believed to participate in the redistribution of EPA among plastidial lipids via the omega pathway, although these hypotheses stem from indirect evidence. In marine algae, BL are primarily considered as final acceptors of VLC-PUFAs. They also seem to play a role in adaptation to cold temperatures and nitrogen deficiency, although these roles have been investigated in a very limited number of species. Indeed, most studies have been conducted in the green alga *C. reinhardtii* or certain stramenopile model species, primarily focused on DGTS or DGTA. Further investigations would be needed to better understand the specific roles of the three BL in organisms.

Regarding the biosynthesis of BL, all studies seem to indicate the essential role of the *BTA1* gene in the synthesis of DGTS as well as DGTA. While the biosynthetic pathway of DGTS is fully resolved, some uncertainties remain regarding DGTA synthesis. Nevertheless, recent findings in *P. tricornutum* represent a significant advance in elucidating the DGTA pathway; the *BTA1* gene is indeed involved in its synthesis by enabling the production of DGTS as an intermediate. Further analyses need to be conducted to identify genes involved in the conversion of DGTS to DGTA. The phylogenetic studies presented in this review do not seem to indicate a potential link between the evolution of the BTA1 protein and the type of BL produced. However, they have confirmed previous findings [9] regarding the distribution of BL among organisms: while the green lineage predominantly contains DGTS, marine organisms exhibit greater diversity in the BL they produce, thereby supporting the hypothesis of BL diversification in the marine environment and the gradual disappearance of DGTS in the evolution of the green lineage.

7. Supplementary methods

7.1. *P. t bta1* KO mutants generation by CRISPR-Cas9 method

Single guide RNA (sgRNA) design was achieved using crispor.tefor

website (<http://crispor.tefor.net/>) with the reference genome of *P. tricornutum*, choosing NGG as Protospacer Adjacent Motif (PAM) sequence, and allowing CRISPR targets to start with any nucleotide. The selected sgRNA 5'→3' sequence was GCAAGCCAAGGGTTACGACG, located between nucleotide 1407 and 1429 of the coding sequence of the gene. The sgRNA insert was obtained by annealing forward primer TCGAGCAAGCCAAGGGTTACGACG and reverse primer AAACCGTCG-TAACCCCTGGCTTGC, generating cohesive ends for subsequent ligation with the episome PtPuc3_diaCas9_sgRNA acquired from addgene (Plasmid #109219). To generate the sgRNA insert, 2.5 μL of each primer at 100 μM were mixed with 45 μL of annealing buffer (1 mM EDTA, 50 mM NaCl, 10 mM Tris pH 7.6) for a final concentration of 5 μM, then heated at 95 °C for 4 min, and cooled down at room temperature for 45 min for hybridization. The episome PtPuc3_diaCas9_sgRNA was digested by *BsaI* enzyme, generating linearized vector with cohesive ends complementary to the prepared insert. 1 μL of prepared sgRNA insert and 100 ng of linearized vector were ligated using T4 DNA ligase (New England BioLabs). The ligation product was transformed into *E. coli* NEB5α (NEB 5-alpha Competent *E. coli*, New England BioLabs) for vector multiplication. Transformed bacteria were screened for the insertion of the sgRNA into the vector via PCR.

For bacterial conjugation, *P. tricornutum* culture at 5.10^8 cellules/mL was spread onto conjugation-based solid agar medium (45 % ESAW, 1 % agar, 5 % LB) within 6-well cell culture plates. The plates were then incubated overnight at 20 °C under constant light. Transformations were conducted via bacterial conjugation, involving the transfer of the episome PtPuc3_diaCas9_sgRNA from conjugation-competent bacteria to *P. tricornutum*. The conjugation-competent bacteria used were *E. coli* EPI300, which are designed for conjugal transfer of plasmids [116]. *E. coli* EPI300 were transformed with the generated PtPuc3_diaCas9_sgRNA episome carrying the sgRNA and selected on agar plates. The day prior to transformation, several random colonies from the selection plates were used to inoculate 3 mL of LB containing suitable antibiotics. Following overnight incubation, the preculture was used to initiate a culture with an optical density OD-600 nm of 0.1 in 12.5 mL of LB and appropriate antibiotics. After 3–4 h of growth until reaching an OD-600 nm of 0.8–1, the culture was centrifuged at 3000 xg for 15 min

to pellet the cells. The pellet was then gently resuspended in 100 μ L of SOC media using a vortex. The *E. coli* suspension was then pipetted into the wells containing *P. tricorutum*. Wells were placed in darkness at 30 °C for 90 min and subsequently returned to 20 °C and exposed to continuous light for 48 h. Following incubation, each well was scraped with ESAW medium to collect the cells in a 2 mL Eppendorf tube, and then re-plated onto selection plates containing suitable selective media. The plates were then allowed to grow for 10–14 days or until colonies were visible.

Colony PCR (utilizing Phire Plant Direct PCR Master Mix, F160, ThermoFisher Scientific) was conducted to analyze the mutation profile through sequencing (Macrogen). Forward primer GATACGGATTTT-CACACGC and reverse primer TGCGAGACATTAGTATGCGC were designed to amplify a region approximately 1000 nucleotides surrounding the Cas9 protein's cleavage site.

For the assessment of mutant colonies, ICE (Inference of CRISPR Edits v2) online software was utilized to analyze the composition of the mutation profile. Mosaic colonies were spread onto plates supplemented with Carbenicillin and Zeocin. Following one to two weeks, the same steps of PCR and sequencing were conducted. The pure mutant colony displayed a 1-nucleotide deletion in the sgRNA sequence (GCAAGC-CAAGGGTTACACG), generating a stop codon at 40 nucleotides after the deletion and a truncated protein of 487 amino acids instead of 715 amino acids.

7.2. Phylogeny

For species with annotated genomes or transcriptomes, we performed a BLAST search using the sequence of the *Ostreococcus tauri* BTA1 protein, selecting and downloading proteins with over 70 % identity and a *p*-value below 0.05 (Supplementary File 1). Using these high-identity proteins, we created a local BLAST database and performed another BLAST search. We verified the presence of the BTA1 domain in each BLAST match using InterProScan [117]. For species without annotated genomes or transcriptomes, we created a FASTA file with all identified BTA1 protein sequences and aligned them against the non-annotated genomes using Exonerate (<https://www.ebi.ac.uk/a bout/vertebrate-genomics/software/exonerate>) with a protein-to-genome model. For each Exonerate match, we created a FASTA file containing the region's sequence similar to the BTA1 proteins. We then used MAKER [118] to annotate the extracted regions, using the retrieved protein and transcript sequences as local databases for MAKER. We confirmed the presence of the BTA1 domain in each protein identified by MAKER using InterProScan. Finally, we repeated the last steps, including all retrieved BTA1 proteins in the initial FASTA file. Once all the protein sequences were collected, InterProScan was used to identify the boundaries of the DUF3149 and the S-adenosyl-L-methionine-dependent methyltransferase (SAM) domains, corresponding to the BtaA and BtaB domains, respectively.

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CRedit authorship contribution statement

Sarah Salomon: Writing – review & editing, Writing – original draft, Formal analysis. **Océane Oliva:** Formal analysis. **Alberto Amato:** Writing – review & editing, Investigation, Formal analysis. **Olivier Bastien:** Writing – review & editing, Investigation, Formal analysis. **Morgane Michaud:** Writing – review & editing, Supervision, Methodology. **Juliette Jouhet:** Writing – review & editing, Supervision, Project

administration, Funding acquisition.

Declaration of competing interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.plipres.2025.101320>.

References

- [1] Van Mooy BAS, Fredricks HF, Pedler BE, Dyhrman ST, Karl DM, Koblížek M, et al. Phytoplankton in the ocean use non-phosphorus lipids in response to phosphorus scarcity. *Nature* 2009;458:69–72. <https://doi.org/10.1038/nature07659>.
- [2] Abida H, Dolch LJ, Mei C, Villanova V, Conte M, Block MA, et al. Membrane glycerolipid remodeling triggered by nitrogen and phosphorus starvation in *Phaeodactylum tricorutum*. *Plant Physiol* 2015;167:118–36. <https://doi.org/10.1104/pp.114.252395>.
- [3] Huang B, Marchand J, Thiriet-Rupert S, Carrier G, Saint-Jean B, Lukomska E, et al. Betaine lipid and neutral lipid production under nitrogen or phosphorus limitation in the marine microalga *Tisochrysis lutea* (Haptophyta). *Algal Res* 2019;40. <https://doi.org/10.1016/j.algal.2019.101506>.
- [4] Cañavate JP, Armada I, Hachero-Cruzado I. Interspecific variability in phosphorus-induced lipid remodelling among marine eukaryotic phytoplankton. *New Phytol* 2017;213:700–13. <https://doi.org/10.1111/nph.14179>.
- [5] Degraeve-Guilbault C, Bréhélin C, Haslam R, Sayanova O, Marie-Luce G, Jouhet J, et al. Glycerolipid characterization and nutrient deprivation-associated changes in the green picoalga *Ostreococcus tauri*. *Plant Physiol* 2017;173:2060–80. <https://doi.org/10.1104/pp.16.01467>.
- [6] Fan J, Andre C, Xu C. A chloroplast pathway for the de novo biosynthesis of triacylglycerol in *Chlamydomonas reinhardtii*. *FEBS Lett* 2011;585:1985–91. <https://doi.org/10.1016/j.febslet.2011.05.018>.
- [7] Ferrer-Ledo N, Stegemüller L, Janssen M, Wijffels RH, Barbosa MJ. Growth and fatty acid distribution over lipid classes in *Nannochloropsis oceanica* acclimated to different temperatures. *Front Plant Sci* 2023;14. <https://doi.org/10.3389/fpls.2023.1078998>.
- [8] Murakami H, Nobusawa T, Hori K, Shimojima M, Ohta H. Betaine lipid is crucial for adapting to low temperature and phosphate deficiency in *nannochloropsis*. *Plant Physiol* 2018;177:181–93. <https://doi.org/10.1104/pp.17.01573>.
- [9] Bolik S, Schlaich A, Mukhina T, Amato A, Bastien O, Schnecke E, et al. Lipid bilayer properties potentially contributed to the evolutionary disappearance of betaine lipids in seed plants. *BMC Biol* 2023;21:1–16. <https://doi.org/10.1186/s12915-023-01775-z>.
- [10] Cannell N, Emms DM, Hetherington AJ, MacKay J, Kelly S, Dolan L, et al. Multiple metabolic innovations and losses are associated with major transitions in land plant evolution. *Curr Biol* 2020;30. <https://doi.org/10.1016/j.cub.2020.02.086>. 1783–1800.e11.
- [11] Dembitsky VM. Betaine ether-linked glycerolipids: chemistry and biology. *Prog Lipid Res* 1996;35:1–51. [https://doi.org/10.1016/0163-7827\(95\)00009-7](https://doi.org/10.1016/0163-7827(95)00009-7).
- [12] Nichols BW, Appleby RS. The distribution and biosynthesis of arachidonic acid in algae. *Phytochemistry* 1969;8:1907–15.
- [13] Brown A, Elovson J. Isolation and characterization of a novel lipid, 1(3),2-diacylglyceryl-(3)-O-4'-(N,N,N-trimethyl)homoserine, from *Ochromonas danica*. *Biochemistry* 1974;34:76–82.
- [14] Vogel G, Woznica M, Gfeller H, Müller C, Stämpfli AA, Jenny TA, et al. 1(3),2-Diacylglyceryl-(3(1)-O-2'-(hydroxymethyl)(N,N,N-trimethyl)- β -alanine (DGTA): a novel betaine lipid from *Ochromonas danica* (Chrysophyceae). *Chem Phys Lipids* 1990;52:99–109. [https://doi.org/10.1016/0009-3084\(90\)90154-J](https://doi.org/10.1016/0009-3084(90)90154-J).
- [15] Eichenberger W. Distribution of (diacylglyceryl-O-4'-N,N,N-trimethyl)-homoserine in different algae. *Plant Sci Lett* 1982;24:91–5.
- [16] Eichenberger W, Boschetti A. Occurrence of 1(3),2-diacylglyceryl-(3)-O-4'-(N,N,N-trimethyl)-homoserine in *Chlamydomonas reinhardtii*. *FEBS Lett* 1978;88:201–4. [https://doi.org/10.1016/0014-5793\(78\)80173-2](https://doi.org/10.1016/0014-5793(78)80173-2).
- [17] Evans RW, Kates M, Wood GW. Identification of diacylglycerol-O-(N,N,N-trimethyl)-homoserine in the halotolerant alga, *Dunaliella parva*. *Chem Phys Lipids* 1982;31:331–8. [https://doi.org/10.1016/0009-3084\(82\)90069-X](https://doi.org/10.1016/0009-3084(82)90069-X).
- [18] Sato N, Furuya M. Distribution of diacylglyceryltrimethylhomoserine in selected species of vascular plants. *Phytochemistry* 1984;23:1625–7. [https://doi.org/10.1016/S0031-9422\(00\)83454-8](https://doi.org/10.1016/S0031-9422(00)83454-8).
- [19] Dembitsky V, Rezanka T. Distribution of diacylglycerylhomoserines, phospholipids and fatty acids in thirteen moss species from southwestern Siberia. *Biochem Syst Ecol* 1995;23:71–8.
- [20] Benning C, Huang Z-H, A. Cage D. Accumulation of a novel glycolipid and a betaine lipid in cells of *Rhodobacter sphaeroides* grown under phosphate limitation. *Arch Biochem Biophys* 1995;317:103–11.
- [21] Yamada T, Nozawa Y. An unusual lipid in the human pathogenic fungus *Epidermophyton floccosum*. *Biochim Biophys Acta* 1979;574:433–9.
- [22] Araki S, Eichenberger W, Sakurai T, Sato N. Distribution of Diacylglycerylhydroxymethyltrimethyl- β -alanine (DGTA) and

- phosphatidylcholine in Brown algae. *Plant Cell Physiol* 1991;32:623–8. <https://doi.org/10.1093/oxfordjournals.pcp.a078124>.
- [23] Kato M, Adachi K. A betaine lipid from *Pavlova lutheri*. *Phytochemistry* 1994;37:279–80.
- [24] Kato M, Sakai M, Adachi K, Ikemoto H, Sano H. Distribution of betaine lipids in marine algae. *Phytochemistry* 1996;42:1341–5. [https://doi.org/10.1016/0031-9422\(96\)00115-X](https://doi.org/10.1016/0031-9422(96)00115-X).
- [25] Kharbush JJ. Intact polar diacylglycerol biomarker lipids isolated from suspended particulate organic matter accumulating in an ultraoligotrophic water column. *Org Geochem* 2016;13.
- [26] Vogel G, Eichenberger W. Betaine Lipids in Lower Plants. Biosynthesis of DGTS and DGTA in *Ochromonas danica* (Chrysophyceae) and the Possible Role of DGTS in Lipid Metabolismvol. 33; 1992.
- [27] Jones AL, Harwood JL. Lipid composition of the brown algae *Fucus vesiculosus* and *Ascophyllum nodosum*. *Phytochemistry* 1992;31:3397–403.
- [28] Sato N. Betaine lipids. *Bot Mag Tokyo* 1992;105:185–97.
- [29] Eichenberger W, Araki S, Müller DG. Betaine lipids and phospholipids in brown algae. *Phytochemistry* 1993;34:1323–33. [https://doi.org/10.1016/0031-9422\(91\)80024-U](https://doi.org/10.1016/0031-9422(91)80024-U).
- [30] Künzler K, Eichenberger W, Adunzb AR. Intracellular Localization of Two Betaine Lipids by Cell Fractionation and Immunomicroscopyvol. 52; 1997.
- [31] Lu Y, Eiriksson FF, Thorsteinsdóttir M, Cronberg N, Simonsen HT. Lipidomes of Icelandic bryophytes and screening of high contents of polyunsaturated fatty acids by using lipidomics approach. *Phytochemistry* 2023;206. <https://doi.org/10.1016/j.phytochem.2022.113560>.
- [32] Sato N, Furuya M. Distribution of diacylglyceryltrimethylhomoserine and phosphatidylcholine in non-vascular green plants. *Plant Sci* 1985;38:81–5.
- [33] Kato M, Hajiro-Nakanishi K, Sano H, Miyachi S. Polyunsaturated Fatty Acids and Betaine Lipids from *Pavlova lutheri*vol. 36; 1995.
- [34] Schlapfer P, Eichenberger W. Evidence for the involvement of diacylglyceryl (N, N,N-trimethyl) homoserine in the desaturation of oleic and linoleic acids in *Chlamydomonas reinhardtii* (Chlorophyceae). *Plant Sci Lett* 1983;32:243–52.
- [35] Giroud C, Eichenberger W. Lipids of *Chlamydomonas reinhardtii*. Incorporation of [14C]acetate, [14C]palmitate and [14C]oleate into different lipids and evidence for lipid-linked desaturation of fatty acids. *Plant Cell Physiol* 1989;30:121–8. <https://doi.org/10.1093/oxfordjournals.pcp.a077705>.
- [36] Yang M, Meng Y, Chu Y, Fan Y, Cao X, Xue S, et al. Triacylglycerol accumulates exclusively outside the chloroplast in short-term nitrogen-deprived *Chlamydomonas reinhardtii*. *Biochim Biophys Acta Mol Cell Biol Lipids* 2018;1863:1478–87. <https://doi.org/10.1016/j.bbalip.2018.09.009>.
- [37] Riekhof WR, Naik S, Bertrand H, Benning C, Voelker DR. Phosphate starvation in fungi induces the replacement of phosphatidylcholine with the phosphorus-free betaine lipid diacylglyceryl-N,N,N-trimethylhomoserine. *Eukaryot Cell* 2014;13:749–57. <https://doi.org/10.1128/EC.00004-14>.
- [38] Giroud C, Gerber A, Eichenberger W. Lipids of *Chlamydomonas reinhardtii*. Analysis of molecular species and intracellular site(s) of biosynthesis. *Plant Cell Physiol* 1988;29:587–95. <https://doi.org/10.1093/oxfordjournals.pcp.a077533>.
- [39] Widzowski J, Vogel A, Altrogge L, Pfaff J, Schoof H, Usadel B, et al. High light induces species specific changes in the membrane lipid composition of *Chlorella*. *Biochem J* 2020;477:2543–59. <https://doi.org/10.1042/BCJ20200160>.
- [40] Hoffmann DY, Shachar-Hill Y. Do betaine lipids replace phosphatidylcholine as fatty acid editing hubs in microalgae? *Front Plant Sci* 2023;14. <https://doi.org/10.3389/fpls.2023.1077347>.
- [41] Young DY, Shachar-Hill Y. Large fluxes of fatty acids from membranes to triacylglycerol and back during N-deprivation and recovery in *Chlamydomonas*. *Plant Physiol* 2021;185:796–814. <https://doi.org/10.1093/plphys/kiab071>.
- [42] Ohlrogge J, Browse J. Lipid biosynthesis. *Plant Cell* 1995;7:27–65. <https://doi.org/10.1002/9781119991311.ch2>.
- [43] Eichenberger W, Gribi C. Lipids of *Pavlova lutheri*: cellular site and metabolic role of DGCC. *Phytochemistry* 1997;45:1561–7. [https://doi.org/10.1016/S0031-9422\(97\)00201-X](https://doi.org/10.1016/S0031-9422(97)00201-X).
- [44] Hofmann M, Eichenberger W. Radiolabelling Studies on the Lipid Metabolism in the Marine Brown Alga *Dictyosphaeridia membranacea*vol. 39; 1998.
- [45] Billey E, Magneschi L, Leterme S, Bedhomme M, Andres-Robin A, Poulet L, et al. Characterization of the bubblegum acyl-CoA synthetase of *Microchloropsis gaditana*. *Plant Physiol* 2021;185:815–35. <https://doi.org/10.1093/PLPHYS/KIAA110>.
- [46] Smith R, Jouhet J, Gandini C, Nekrasov V, Marechal E, Napier JA, et al. Plastidial acyl carrier protein Δ9-desaturase modulates eicosapentaenoic acid biosynthesis and triacylglycerol accumulation in *Phaeodactylum tricornutum*. *Plant J* 2021;106:1247–59. <https://doi.org/10.1111/tpj.15231>.
- [47] Sato N. Lipids in Cryptomonas CR-1. II. Biosynthesis of Betaine Lipids and Galactolipidsvol. 32; 1991.
- [48] Makewicz A, Gribi C, Eichenberger W. Lipids of *Ectocarpus fasciculatus* (Phaeophyceae). Incorporation of [1-14 C]Oleate and the role of TAG and MGDG in lipid. *Metabolism* 1997;38.
- [49] Banskota AH, Stefanova R, Sperker S, McGinn PJ. New diacylglyceryltrimethylhomoserines from the marine microalga *Nannochloropsis granulata* and their nitric oxide inhibitory activity. *J Appl Phycol* 2013;25:1513–21. <https://doi.org/10.1007/s10811-012-9967-1>.
- [50] Li S, Xu J, Chen J, Zhou C, Yan X. The major lipid changes of some important diet microalgae during the entire growth phase. *Aquaculture* 2014;328–429:104–10. <https://doi.org/10.1016/j.aquaculture.2014.02.032>.
- [51] Pal D, Khozin-Goldberg I, Didi-Cohen S, Solovchenko A, Batushansky A, Kaye Y, et al. Growth, lipid production and metabolic adjustments in the euryhaline eustigmatophyte *Nannochloropsis oceanica* CCALA 804 in response to osmotic downshift. *Appl Microbiol Biotechnol* 2013;97:8291–306. <https://doi.org/10.1007/s00253-013-5092-6>.
- [52] Huang.. Nitrogen and phosphorus limitations induce carbon partitioning and membrane lipid remodelling in the marine diatom *Phaeodactylum tricornutum*. *Eur J Phycol* 2019;54:342–58. <https://doi.org/10.1080/09670262.2019.1567823>.
- [53] Meng Y, Cao X, Yang M, Liu J, Yao C, Xue S. Glycerolipid remodeling triggered by phosphorus starvation and recovery in *Nannochloropsis oceanica*. *Algal Res* 2019;39. <https://doi.org/10.1016/j.algal.2019.101451>.
- [54] Mühlroth A, Winge P, El Assimi A, Jouhet J, Maréchal E, Hohmann-Marriott MF, et al. Mechanisms of phosphorus acquisition and lipid class remodeling under P limitation in a marine microalga. *Plant Physiol* 2017;175:1543–59. <https://doi.org/10.1104/pp.17.00621>.
- [55] López-Lara IM, Gao JL, Soto MJ, Solares-Pérez A, Weissenmayer B, Sohlenkamp C, et al. Phosphorus-free membrane lipids of *Sinorhizobium meliloti* are not required for the symbiosis with alfalfa but contribute to increased cell yields under phosphorus-limiting conditions of growth. *Mol Plant-Microbe Interact* 2005;18:973–82. <https://doi.org/10.1094/MPMI-18-0973>.
- [56] Lu N, Wei D, Chen F, Yang ST. Lipidomic profiling reveals lipid regulation in the snow alga *Chlamydomonas nivalis* in response to nitrate or phosphate deprivation. *Process Biochem* 2013;48:605–13. <https://doi.org/10.1016/j.procbio.2013.02.028>.
- [57] Khozin-Goldberg I, Cohen Z. The effect of phosphate starvation on the lipid and fatty acid composition of the fresh water eustigmatophyte *Monodus subterraneanus*. *Phytochemistry* 2006;67:696–701. <https://doi.org/10.1016/j.phytochem.2006.01.010>.
- [58] Oishi Y, Otaki R, Iijima Y, Kumagai E, Aoki M, Tsuzuki M, et al. Diacylglyceryl-N, N,N-trimethylhomoserine-dependent lipid remodeling in a green alga, *Chlorella kesslerii*. *Commun Biol* 2022;5. <https://doi.org/10.1038/s42003-021-02927-z>.
- [59] Shemi A, Schatz D, Fredricks HF, Van Mooy BAS, Porat Z, Vardi A. Phosphorus starvation induces membrane remodeling and recycling in *Emiliania huxleyi*. *New Phytol* 2016;211:886–98. <https://doi.org/10.1111/nph.13940>.
- [60] Cañavate JP, Armada I, Hachero-Cruzado I. Aspects of phosphorus physiology associated with phosphate-induced polar lipid remodeling in marine microalgae. *J Plant Physiol* 2017;214:28–38. <https://doi.org/10.1016/j.jplph.2017.03.019>.
- [61] Simionato D, Block MA, La Rocca N, Jouhet J, Maréchal E, Finazzi G, et al. The response of *Nannochloropsis gaditana* to nitrogen starvation includes de novo biosynthesis of triacylglycerols, a decrease of chloroplast galactolipids, and reorganization of the photosynthetic apparatus. *Eukaryot Cell* 2013;12:665–76. <https://doi.org/10.1128/EC.00363-12>.
- [62] Popko J, Herrfurth C, Feussner K, Ischebeck T, Iven T, Haslam R, et al. Metabolome analysis reveals betaine lipids as major source for triglyceride formation, and the accumulation of sedoheptulose during nitrogen-starvation of *Phaeodactylum tricornutum*. *PLoS One* 2016;11. <https://doi.org/10.1371/journal.pone.0164673>.
- [63] Lee JW, Shin SY, Kim HS, Jin ES, Lee HG, Oh HM. Lipid turnover between membrane lipids and neutral lipids via inhibition of diacylglyceryl N,N,N-trimethylhomoserine synthesis in *Chlamydomonas reinhardtii*. *Algal Res* 2017;27:162–9. <https://doi.org/10.1016/j.algal.2017.09.001>.
- [64] Xu C, Andre C, Fan J, Shanklin J. Cellular organization of triacylglycerol biosynthesis in microalgae. *Lipids Plant Algae Dev* 2016;86:207–21.
- [65] Yang D, Song D, Kind T, Ma Y, Hoefkens J, Fiehn O. Lipidomic analysis of *Chlamydomonas reinhardtii* under nitrogen and sulfur deprivation. *PLoS One* 2015;10. <https://doi.org/10.1371/journal.pone.0137948>.
- [66] Yang M, Kong F, Xie X, Wu P, Chu Y, Cao X, et al. Galactolipid dgdg and betaine lipid dgts direct de novo synthesized linolenate into triacylglycerol in a stress-induced starchless mutant of *Chlamydomonas reinhardtii*. *Plant Cell Physiol* 2020;61:851–62. <https://doi.org/10.1093/pcp/pcaa012>.
- [67] Young DY, Pang N, Shachar-Hill Y. 13C-labeling reveals how membrane lipid components contribute to triacylglycerol accumulation in *Chlamydomonas*. *Plant Physiol* 2022;189:1326–44. <https://doi.org/10.1093/plphys/kiac154>.
- [68] Lynch DV, Thompson GA. Low temperature-induced alterations in the chloroplast and microsomal membranes of *Dunaliella salina*. *Plant Physiol* 1982;69:1369–75. <https://doi.org/10.1104/pp.69.6.1369>.
- [69] Murata N, Los DA. Membrane fluidity and temperature perception. *Plant Physiol* 1997;115:875–9. <https://doi.org/10.1104/pp.115.3.875>.
- [70] Tatsuzawa H, Takizawa E. Changes in lipid and fatty acid composition of *Pavlova lutheri*. *Phytochemistry* 1995;40:397–400. [https://doi.org/10.1016/0031-9422\(95\)00327-4](https://doi.org/10.1016/0031-9422(95)00327-4).
- [71] Sato N. Dual role of methionine in the biosynthesis of Diacylglyceryltrimethylhomoserine in *Chlamydomonas reinhardtii*. *Plant Physiol* 1988;86:931–4. <https://doi.org/10.1104/pp.86.3.931>.
- [72] Sato N, Kato K. Analysis and biosynthesis of diacylglyceryl-N,N,N-trimethylhomoserine in the cells of *Marchantia* in suspension culture. *Plant Sci* 1988;55:21–5. [https://doi.org/10.1016/0168-9452\(88\)90037-4](https://doi.org/10.1016/0168-9452(88)90037-4).
- [73] Sato N, Murata N. Transition of lipid phase in aqueous dispersions of diacylglyceryltrimethylhomoserine. *Biochim Biophys Acta* 1991;108–11.
- [74] Hofmann M, Eichenberger W. Biosynthesis of Diacylglyceryl-N,N,N-Trimethylhomoserine in *Rhodobacter sphaeroides* and Evidence for Lipid-Linked N Methylationvol. 178; 1996.
- [75] Moore TS, Du Z, Chen Z. Membrane lipid biosynthesis in *Chlamydomonas reinhardtii*. In vitro biosynthesis of Diacylglyceryltrimethylhomoserine. *Plant Physiol* 2001;125:423–9.
- [76] Klug RM, Benning C. Two enzymes of diacylglyceryl-O-4'-(N,N,N-trimethyl)-homoserine biosynthesis are encoded by btaA and btaB in the purple bacterium *Rhodobacter sphaeroides*. *Proc Natl Acad Sci USA* 2001;98:5910–5. <https://doi.org/10.1073/pnas.101037998>.

- [77] Riekhof WR, Andre C, Benning C. Two enzymes, BtaA and BtaB, are sufficient for betaine lipid biosynthesis in bacteria. *Arch Biochem Biophys* 2005;441:96–105. <https://doi.org/10.1016/j.abb.2005.07.001>.
- [78] Riekhof WR, Sears BB, Benning C. Annotation of genes involved in glycerolipid biosynthesis in *Chlamydomonas reinhardtii*: discovery of the betaine lipid synthase BTA1Cr. *Eukaryot Cell* 2005;4:242–52. <https://doi.org/10.1128/EC.4.2.242-252.2005>.
- [79] Senik SV, Maloshenko LG, Kotlova ER, Shavarda AL, Moiseenko KV, Bruskin SA, et al. Diacylglyceryltrimethylhomoserine content and gene expression changes triggered by phosphate deprivation in the mycelium of the basidiomycete *Flammulina velutipes*. *Phytochemistry* 2015;117:34–42. <https://doi.org/10.1016/j.phytochem.2015.05.021>.
- [80] Lev S, Rupasinghe T, Desmarini D, Kaufman-Francis K, Sorrell TC, Roessner U, et al. The PHO signaling pathway directs lipid remodeling in *Cryptococcus neoformans* via DGTS synthase to recycle phosphate during phosphate deficiency. *PLoS One* 2019;14. <https://doi.org/10.1371/journal.pone.0212651>.
- [81] Salomon S, Schilling M, Albrieux C, Si Larbi G, Jouneau P-H, Roy S, et al. Betaine lipids overproduced in seed plants are excluded from plastid membranes and promote endomembrane expansion. *J Exp Bot* 2024;erae458. <https://doi.org/10.1093/jxb/erae458>.
- [82] Zhang Y, He J, Jia LJ, Yuan TL, Zhang D, Guo Y, et al. Cellular tracking and gene profiling of fusarium graminearum during maize stalk rot disease development elucidates its strategies in confronting phosphorus limitation in the host Apoplast. *PLoS Pathog* 2016;12:1–33. <https://doi.org/10.1371/journal.ppat.1005485>.
- [83] Li-Beisson Y, Beisson F, Riekhof W. Metabolism of acyl-lipids in *Chlamydomonas reinhardtii*. *Plant J* 2015;82:504–22. <https://doi.org/10.1111/tpj.12787>.
- [84] Vogel G, Woznica M, Gfeller H, Müller C, Stimpfli AA, Jenny TA, et al. (3), 2-Diacylglyceryl-3 (1)-O-2-(hydroxymethyl)(N,N,N-tri-methyl)-β-3-alanine (DGTA): A novel betaine lipid from *Ochromonas danica* (Chrysophyceae)*Vol. 52; 1990.
- [85] Meireles LA, Guedes AC, Malcata FX. Lipid class composition of the microalga *Pavlova lutheri*: eicosapentaenoic and docosahexaenoic acids. *J Agric Food Chem* 2003;51:2237–41. <https://doi.org/10.1021/jf025952y>.
- [86] Cruz de Carvalho MH, Sun HX, Bowler C, Chua NH. Noncoding and coding transcriptome responses of a marine diatom to phosphate fluctuations. *New Phytol* 2016;210:497–510. <https://doi.org/10.1111/nph.13787>.
- [87] Roche SA, Leblond JD. Betaine lipids in chlorarachniophytes. *Phycol Res* 2010; 58:298–305. <https://doi.org/10.1111/j.1440-1835.2010.00590.x>.
- [88] Cañavate JP, Armada I, Ríos JL, Hachero-Cruzado I. Exploring occurrence and molecular diversity of betaine lipids across taxonomy of marine microalgae. *Phytochemistry* 2016;124:68–78. <https://doi.org/10.1016/j.phytochem.2016.02.007>.
- [89] Li Y, Lou Y, Mu T, Xu J, Zhou C, Yan X. Simultaneous structural identification of diacylglyceryl-N-trimethylhomoserine (DGTS) and diacylglycerylhydroxymethyl-N,N,N-trimethyl-β-alanine (DGTA) in microalgae using dual Li⁺/H⁺ adduct ion mode by ultra-performance liquid chromatography/quadrupole. *Rapid Commun Mass Spectrom* 2017;31:457–68. <https://doi.org/10.1002/rcm.7818>.
- [90] Armada I, Hachero-Cruzado I, Mazuelos N, Ríos JL, Machado M, Cañavate JP. Differences in betaine lipids and fatty acids between *Pseudoisochrysis paradoxa* VLP and *Diacronema vlkianum* VLP isolates (Haptophyta). *Phytochemistry* 2013; 95:224–33. <https://doi.org/10.1016/j.phytochem.2013.07.024>.
- [91] Dembitsky VM. Lipids of bryophytes. *Prog Lipid Res* 1993;32:281–356. [https://doi.org/10.1016/0163-7827\(93\)90010-T](https://doi.org/10.1016/0163-7827(93)90010-T).
- [92] Dembitsky VM, Rozentvet OA. Distribution of polar lipids in some marine, brackish and freshwater green macrophytes. *Phytochemistry* 1996;41:483–8. [https://doi.org/10.1016/0031-9422\(95\)00570-6](https://doi.org/10.1016/0031-9422(95)00570-6).
- [93] Oliver MJ, Tuba Z, Mishler BD. *The Evolution of Vegetative Desiccation Tolerance in Land Plants*vol. 151; 2000.
- [94] Guschina IA, Harwood JL, Smith M, Beckett RP. Abscisic acid modifies the changes in lipids brought about by water stress in the moss *Atrichum androgynum*. *New Phytol* 2002;156:255–64. <https://doi.org/10.1046/j.1469-8137.2002.00517.x>.
- [95] Rozentvet OA. Comparative examination of distribution of phospholipids and a betaine lipid DGTS in tropical fern species. *Biochem Syst Ecol* 2004;32:303–11. <https://doi.org/10.1016/j.bse.2003.07.003>.
- [96] Rozentvet OA, Saksonov SV, Filin VR, Dembitsky VM. Seasonal changes of lipid content in the leaves of some ferns. *Physiol Plant* 2001;113:59–63. <https://doi.org/10.1034/j.1399-3054.2001.1130108.x>.
- [97] Jones AL, Harwood JL. Lipid composition of the brown algae *Fucus vesiculosus* and *Ascophyllum nodosum*. *Phytochemistry* 1992;31:3397–403. [https://doi.org/10.1016/0031-9422\(92\)83693-S](https://doi.org/10.1016/0031-9422(92)83693-S).
- [98] Sato N, Nemoto Y, Furuya M. Lipids of *Chattonella Antiqua* (Raphidophyceae). In: Stumpf PK, Mudd JB, Nes WD, editors. *Metab. Struct. Funct. Plant Lipids*. Boston, MA: Springer New York; 1987. p. 661–3. https://doi.org/10.1007/978-1-4684-5263-1_118.
- [99] Cañavate JP, Armada I, Ríos JL, Hachero-Cruzado I. Exploring occurrence and molecular diversity of betaine lipids across taxonomy of marine microalgae. *Phytochemistry* 2016;124:68–78. <https://doi.org/10.1016/j.phytochem.2016.02.007>.
- [100] Eichenberger W, Gfeller H, Grey P, Gribo C, James Henderson R. Gas chromatographic-mass spectrometric identification of betaine lipids in *Chroomonas salina*. *Phytochemistry* 1996;42:967–72. [https://doi.org/10.1016/0031-9422\(96\)00055-6](https://doi.org/10.1016/0031-9422(96)00055-6).
- [101] Li Y, Ye M, Zhang R, Xu J, Zhou C, Yan X. Lipid compositions in diatom *Conticribra weissflogii* under static and aerated culture conditions. *Phycol Res* 2016;64:281–90. <https://doi.org/10.1111/pre.12144>.
- [102] Künzler K, Eichenberger W. Betaine lipids and zwitterionic phospholipids in plants and fungi. *Phytochemistry* 1997;46:883–92. [https://doi.org/10.1016/s0031-9422\(97\)81274-5](https://doi.org/10.1016/s0031-9422(97)81274-5).
- [103] Martin P, Van Mooy BA, Heithoff A, Dyhrman ST. Phosphorus supply drives rapid turnover of membrane phospholipids in the diatom *Thalassiosira pseudonana*. *ISME J* 2011;5:1057–60. <https://doi.org/10.1038/ismej.2010.192>.
- [104] Hunter JE, Brandsma J, Dymond MK, Koster G, Moore CM, Postle AD, et al. Lipidomics of *Thalassiosira pseudonana* under phosphorus stress reveal underlying phospholipid substitution dynamics and novel diglycosylceramide substitutes. *Appl Environ Microbiol* 2018;84. <https://doi.org/10.1128/AEM.02034-17.e02034-17>.
- [105] Thompson GA. Lipids and membrane function in green algae. *Biochim Biophys Acta BBA - Lipids Lipid Metab* 1996;1302:17–45. [https://doi.org/10.1016/0005-2760\(96\)00045-8](https://doi.org/10.1016/0005-2760(96)00045-8).
- [106] Haigh WG, Yoder TF, Ericson L, Pratum T, Winget RR. The characterisation and cyclic production of a highly unsaturated homoserine lipid in *Chlorella minutissima*. *Biochim Biophys Acta BBA - Lipids Lipid Metab* 1996;1299:183–90. [https://doi.org/10.1016/0005-2760\(95\)00205-7](https://doi.org/10.1016/0005-2760(95)00205-7).
- [107] Conde TA, Neves BF, Couto D, Melo T, Neves B, Costa M, et al. Microalgae as sustainable bio-factories of healthy lipids: evaluating fatty acid content and antioxidant activity. *Mar Drugs* 2021;19:357. <https://doi.org/10.3390/md19070357>.
- [108] Norman HA, Thompson GA. Quantitative analysis of *Dunaliella salina* diacylglyceryltrimethylhomoserine and its individual molecular species by high performance liquid chromatography. *Plant Sci* 1985;42:83–7. [https://doi.org/10.1016/0168-9452\(85\)90146-3](https://doi.org/10.1016/0168-9452(85)90146-3).
- [109] Maat DS, Bale NJ, Hopmans EC, Sinnighe Damsté JS, Schouten S, Brussaard CPD. Increasing P limitation and viral infection impact lipid remodeling of the picophytoplankton *Micromonas pusilla*. *Biogeosciences* 2016;13: 1667–76. <https://doi.org/10.5194/bg-13-1667-2016>.
- [110] Moseley KR, Thompson Jr GA. Lipid composition and metabolism of *Volvox carterii* 1. *Plant Physiol* 1980;65:260–5. <https://doi.org/10.1104/pp.65.2.260>.
- [111] Kodová-Sveráková I, Záhonová K, Juricová V, Danchenko M, Moos M, Baráth P, et al. Highly flexible metabolism of the marine euglenozoan protist *Diplonema papillatum*. *BMC Biol* 2021;19:1–21. <https://doi.org/10.1186/s12915-021-01186-y>.
- [112] Furlong ST, Leary JA, Costello CE, Dawidowicz EA. Isolation and identification of 1(3),2-diacylglyceryl-(3)-O-4'-(N,N,N-trimethyl)homoserine from the soil amoeba, *Acanthamoeba castellanii*. *J Lipid Res* 1990;27:1182–9. [https://doi.org/10.1016/S0022-2275\(20\)38754-X](https://doi.org/10.1016/S0022-2275(20)38754-X).
- [113] Vaskovsky VE, Khotimchenko SV, Boolugh EM. Distribution of diacylglyceroltrimethylhomoserine and phosphatidylcholine in mushrooms. *Phytochemistry* 1998;47:755–60. [https://doi.org/10.1016/S0031-9422\(97\)00624-9](https://doi.org/10.1016/S0031-9422(97)00624-9).
- [114] Yang J, Liu J, Pan Y, Maréchal E, Amato A, Liu M, et al. PDAT regulates PE as transient carbon sink alternative to triacylglycerol in nanochloropsis. *Plant Physiol* 2022;189:1345–62. <https://doi.org/10.1093/plphys/kiac160>.
- [115] Wang D, Ning K, Li J, Hu J, Han D, Wang H, et al. Nannochloropsis genomes reveal evolution of microalgal oleaginous traits. *PLoS Genet* 2014;10. <https://doi.org/10.1371/journal.pgen.1004094>.
- [116] Karas BJ, Diner RE, Lefebvre SC, McQuaid J, Phillips APR, Noddings CM, et al. Designer diatom episodes delivered by bacterial conjugation. *Nat Commun* 2015; 6:1–10. <https://doi.org/10.1038/ncomms7925>.
- [117] Jones P, Binns D, Chang HY, Fraser M, Li W, McAnulla C, et al. InterProScan 5: genome-scale protein function classification. *Bioinformatics* 2014;30:1236–40. <https://doi.org/10.1093/bioinformatics/btu031>.
- [118] Holt C, Yandell M. MAKER2: an annotation pipeline and genome-database management tool for second-generation genome projects. *BMC Bioinform* 2011; 12. <https://doi.org/10.1186/1471-2105-12-491>.