World J Microbiol Biotechnol (2017) 33:74
DOI 10.1007/s11274-017-2236-3

REVIEW

Lipid metabolism and potentials of biofuel and high added-value oil production in red algae

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Received: 29 December 2016 / Accepted: 1 March 2017 / Published online: 16 March 2017

Abstract Biomass production is currently explored in microalgae, macroalgae and land plants. Microalgal biofuel development has been performed mostly in green algae. In the Japanese tradition, macrophytic red algae such as *Pyropia yezoensis* and *Gelidium crinale* have been utilized as food and industrial materials. Researches on the utilization of unicellular red microalgae such as *Cyanidioschyzon merolae* and *Porphyridium purpureum* started only quite recently. Red algae have relatively large plastid genomes harboring more than 200 protein-coding genes that support the biosynthetic capacity of the plastid. Engineering the plastid genome is a unique potential of red microalgae. In addition, large-scale growth facilities of *P. purpureum* have been developed for industrial production of biofuels. *C. merolae* has been studied as a model alga for cell and molecular biological analyses with its completely determined genomes and transformation techniques. Its acidic and warm habitat makes it easy to grow this alga axenically in large scales. Its potential as a biofuel producer is recently documented under nitrogen-limited conditions. Metabolic pathways of the accumulation of starch and triacylglycerol and the enzymes involved therein are being elucidated. Engineering these regulatory mechanisms will open a possibility of exploiting the full capability of production of biofuel and high added-value oil. In the present review, we will describe the characteristics and potential of these algae as biotechnological seeds.

Keywords *Cyanidioschyzon merolae* · Lipid · Triacylglycerol · *Porphyridium purpureum*

Introduction

Biomass production has been important resources for human activity both as nutrition, materials and energy source. Traditionally, crop plants served as nutritional sources, whereas woody plants or trees were used as construction materials and fuels. Algae have rarely been regarded as probable materials for either nutrition or construction, while no one had ever imagined using algae as fuels before the break of current trends in biofuel production (Menetrez 2012; De Bhowmick et al. 2015). Currently, extensive efforts have been made on the biotechnology of microalgae, notably, green algae such as *Chlamydomonas reinhardtii* (Merchant et al. 2012; Li-Beisson et al. 2015), or marine algae such as *Nannochloropsis* (Pal et al. 2011; Iwai et al. 2015).

In the Japanese tradition, however, people have long been exploiting various macrophyte marine algae from the beginning of the ancient imperial era (more than 1300 years ago as documented in the oldest chronicle *Kojiki* published in 712): the red alga *Pyropia yezoensis* known as "(Asakusa) Nori" ("Asakusa" refers to the algae grown in Tokyo Bay in Edo Era), the brown algae in Laminariaceae, notably *Saccharina japonica* known as "Konbu", the brown algae *Sargassum fusiforme* (formerly *Hizikia*) known as

Electronic supplementary material The online version of this article (doi:10.1007/s11274-017-2236-3) contains supplementary material, which is available to authorized users.

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"Hiziki" and *Undaria pinnatifida* known as "Wakame" are still common food stuff in daily Japanese life (Fig. 1). The red algae in Gelidiaceae, typically *Gelidium crinale* known as "Tengusa", are used to produce agar or "Kanten", which is used as gelling additives in food production or industry (as well as in microbiology laboratories!). Cyanobacterial mat of *Aphanothece sacrum* known as "Suizenji nori" is at the crisis of extinction in the natural habitats, but has been used as delicacy in the local cuisine.

In spite of this tradition of seaweed or algal consumption, Japanese people did not try to use seaweeds or algae as fuels, not only because marine products are wet, but also due to their low content of oil. They were mainly used as food and mineral resources. This does not mean that the algae do not produce oil. In the present review, we will present recent understandings on the lipid biosynthesis and the production of triacylglycerol (TAG) in red algae, or specifically in red microalgae in the hope of using this knowledge in biotechnological developments. Oils can be used as either high added-value products or biofuel depending on its composition. Readers are referred to some reviews on algal lipids in general (see *e.g.*, Guschina and Harwood 2006; Qin et al. 2012; Zienkiewiz et al. 2016).

Two types of red algal oils

Red algae are taxonomically classified into two classes, Cyanidiales that include unicellular species that are blue-green in appearance and found in hot springs (such as *Cyanidioschyzon merolae*) and Rhodophytina that include marine species of both unicellular (such as *Porphyridium*) and macrophyte (such as *Pyropia*) forms. Apart from taxonomic classification, there are two types of red algae that produce different kinds of oil. Most marine red algae (and some Cyanidiales species) produce oil containing high levels of polyunsaturated fatty acids (PUFA), whereas the oil of *C. merolae* contains no PUFA and suited for biofuel. PUFA such as arachidonic (20:4 or ARA) and eicosapentaenoic (20:5 or EPA) acids are more suited for nutrients or healthy products rather than biofuel. We first explain general characteristics of lipid biosynthesis in red algae, and then present individual red algae and their potential biotechnological use.

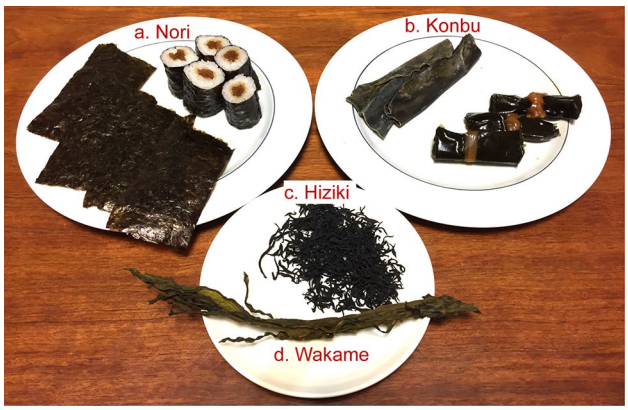

Fig. 1 Marine macro algae used in daily Japanese meal. **a** Nori (*Pyropia yezoensis*) as dried sheets (*left*) and Nori rolls (*right*) with rice and Kanpyo (a cucurbit *Lagenaria siceraria* var. *hispida*) inside, a kind of Sushi; **b** Konbu (*Saccharina japonica*) as dried thalli (*left*) and Kobumaki (*right*), cooked in soy sauce and sugar and served with Kanpyo (a dish for New Year); **c** dried Hiziki (*Sargassum fusiforme*), commonly cooked with soy beans in soy sauce and sugar; **d** dried Wakame (*Undaria pinnatifida*), commonly used in soy soup for Japanese breakfast

Genomics-based elucidation of lipid biosynthesis in red algae

Composition and biosynthesis of lipids have been intensively studied in model algae such as *Chlamydomonas reinhardtii* (Merchant et al. 2012; Sakurai et al. 2014; Li-Beisson et al. 2015; Zienkiewicz et al. 2016) or related species such as *C. debaryana* (Toyoshima and Sato 2015), but understanding of entire pathways of biosynthesis of lipids, including TAG, was quite limited in red algae until recently. Simple lists of genes involved in lipid synthesis have been repeatedly presented: such as Riekof et al. (2005) and Li-Beisson et al. (2015) for *C. reinhardtii*, Sato and Moriyama (2007) for *C. merolae*, and Misra et al. (2012) for several algal species including these algae and two species of *Ostreococcus*.

Genomic data are the key information for the estimation of metabolic pathways and hence metabolic engineering. All the 121 genes involved in lipid metabolism in *C. merolae* were estimated (Mori et al. 2016) by comparative genomics based on homolog clustering (Sato 2009). Intracellular localization of 113 enzymes involved in the metabolism of fatty acids and lipids in *C. merolae* (excepting those encoded by the plastid genome) was studied by Mori et al. (2016) using transient expression of GFP fusion proteins. The results confirmed the previous results of Sato and Moriyama (2007), and enabled construction of the complete metabolic map of lipid metabolism in *C. merolae*, which is essentially applicable to other red algae in Cyanidiales, as well as to red algae in general with minor modifications (Fig. 2). We believe that this model on red algae can even be extended, with minor modifications, to Chromophyta (brown algae, diatoms, Eustigmatophyceae etc) that originated by secondary endosymbiosis of red algal cell (Cavalier-Smith 2003). In this model, some interesting characteristics emerge: lack of stearate desaturation in the plastid and phycobilisomes as a source of TAG accumulation.

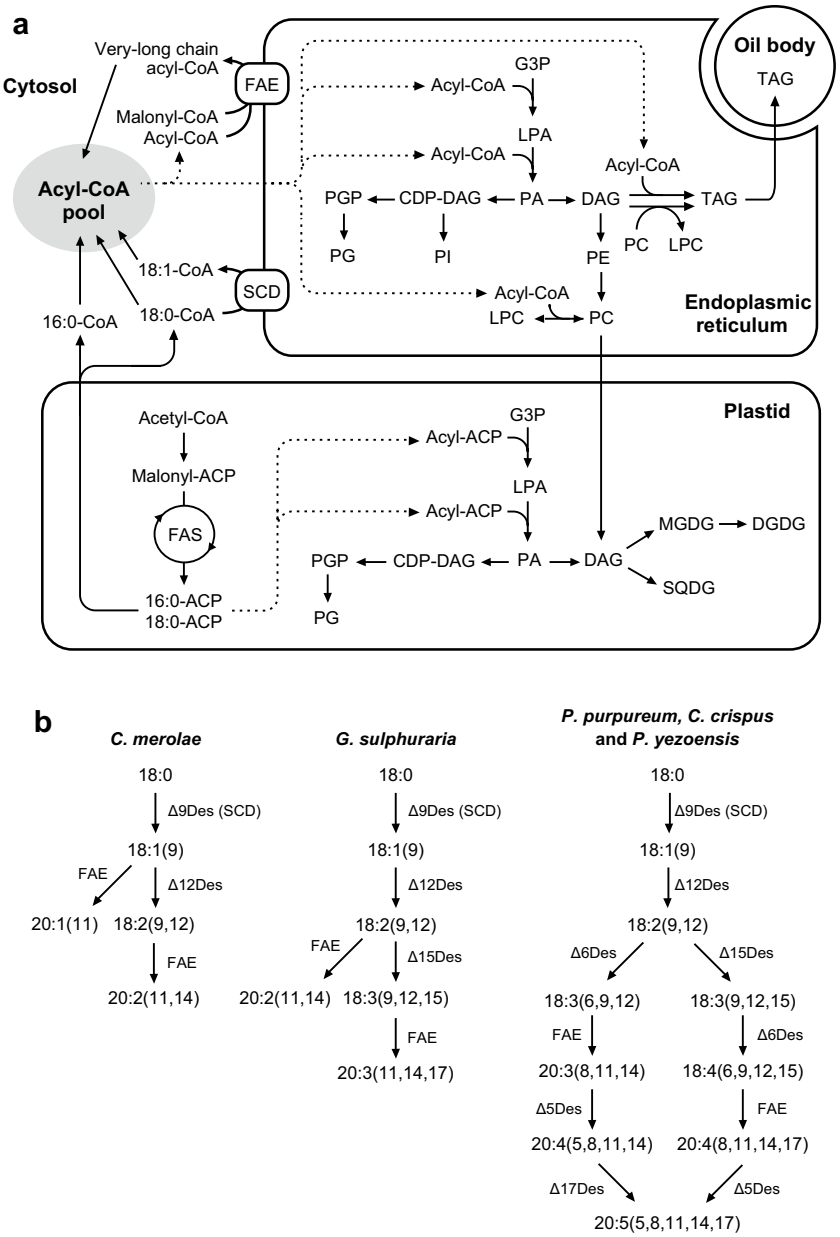

Fig. 2 Generalized pathway of lipid biosynthesis in red algae. **a** Generalized lipid metabolism in red algae. The model was based on Mori et al. (2016), but modified and simplified according to the comparative genomic data (Table S1). **b** Comparison of fatty acid elongation (FAE) and desaturation (Des) pathways in five red algae. Note that the pathway starts from 18:0, but not 18:1, because stearoyl-ACP desaturase is not present in the plastid in red algae. Δ + number indicates the position of desaturation. Abbreviations that are not defined in the text (see also legends for Tables 2 and 3): *G3P* glycerol 3-phosphate, *CDP* cytidine diphosphate, *DAG* diacylglycerol, *PGP* phosphatidylglycerol phosphate, *LPA* lysophosphatidic acid, *LPC* lysophosphatidylcholine, *SCD* stearoyl-CoA Δ9-desaturase

Lack of stearate desaturation in the red algal plastid

The most important trait of red algae in lipid biosynthesis is the lack of stearoyl acyl-carrier-protein (ACP) desaturase (Fig. 2a, Table S1), which is ubiquitous in green algae and land plants, as well as some actinobacteria (see Cluster 1324 of Dataset Gclust2012_42 in the Gclust comparative genomic database at http://gclust.c.u-tokyo.ac.jp/, Sato 2009). Since this soluble enzyme is localized in the plastid stroma, the primary products of plastid fatty acid synthesis (FAS) are palmitic acid (16:0) and oleic acid (18:1) in green algae and plants (See the legends for Tables 2, 3 for the names of lipids and fatty acids). The plastid FAS in red algae produces saturated acids, such as 16:0 and stearic acid (18:0), which are then transported to cytosol, and activated to become acyl CoAs, which are further used in acyl lipid synthesis, desaturation or elongation in ER. The products of elongation and desaturation are dependent on algal species (Fig. 2b): In *C. merolae*, the end product of elongation and desaturation is 20:2, whereas trienoic acids are also produced in *G. sulphuraria*. In marine red algae, the products are predominantly C20 PUFA, such as ARA and EPA (Guschina and Harwood 2006), which are then transported back to plastids for the synthesis of galactolipids, which retain most of ARA and EPA within the cell.

Many previous labeling studies used labeled 18:1 or other unsaturated fatty acids as precursors (Nichols and Appleby 1969; Shiran et al. 1996; Khozin et al. 1997) to

show the desaturation and elongation pathways, which are now turned out to be localized in the ER, or outside plastids. That is why a flow of fatty acids from TAG to monogalactosyl diacylglycerol (MGDG) was detected in *P. cruentum* (Khozin-Goldberg et al. 2000). In contrast, labeling with acetate resulted in rapid labeling of 16:0 in MGDG in *C. merolae* (Sato and Moriyama 2007). Linoleic acid (18:2) is, however, provided for MGDG synthesis by the rapid turnover of phosphatidylcholine (PC) in the ER (Sato and Moriyama 2007; Sato et al. 2016; Toyoshima et al. 2016). The collaboration of plastid and ER for the synthesis of plastid MGDG is a unique characteristic of this alga.

The lack of stearoyl ACP desaturase is universal in red algae and Chromophyta. The abundant ARA and EPA, or docosahexaenoic acid (22:6 or DHA) present in marine red algae or chromophyte algae are all produced by the elongation and desaturation pathway starting from 18:0 (but not from 18:1, as usually mentioned) in the ER. The only desaturase present in the red algal plastids is FAD4 (Cluster 7570 in Gclust database; Gao et al. 2009), which introduces a *trans* double bond in 16:0 to produce Δ3-*trans*-hexadecenoic acid (16:1). This acid is specifically present at the *sn*-2 position of plastid phosphatidylglycerol (PG) in all photosynthetic eukaryotes, and the desaturation is believed to occur within PG molecule.

Phycobilisomes as a source of TAG accumulation

TAG is accumulated under nitrogen deprivation in *C. merolae*, as in many other algae (Fig. 3f, g; Toyoshima et al. 2016; Takusagawa et al. 2016). Both oil bodies and starch granules are accumulated in the cytosol, because starch (or glycogen) synthesis takes place in the cytosol in red algae (Ball et al. 2011). Comparison of fatty acid composition suggested that the acyl groups for the synthesis of TAG comes from PC, or acyl CoA pool which is in rapid equilibrium with PC. Labeling studies on *C. merolae* using [^{32}P]

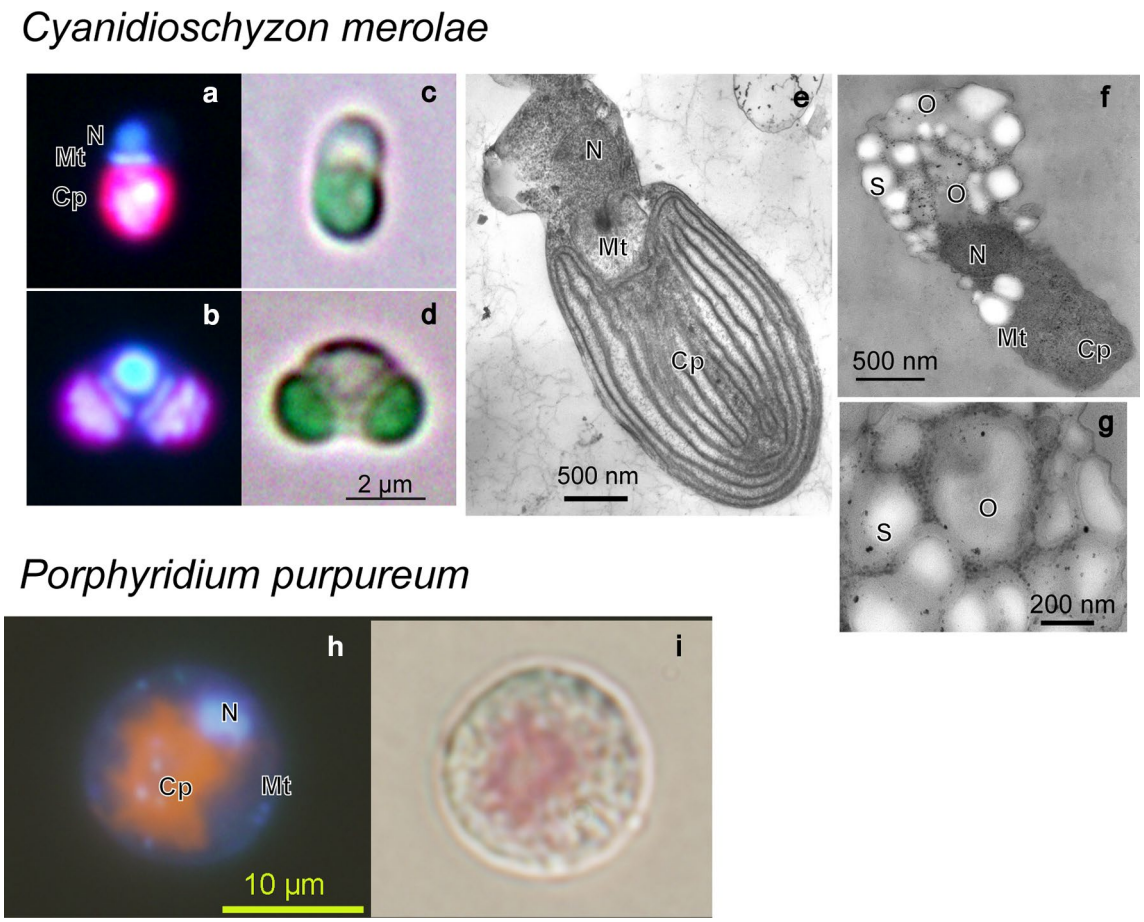

Fig. 3 Unicellular model red algae. *Upper panels* (**a**–**g**), *Cyanidioschyzon merolae; lower panels* (**h, i**), *Porphyridium purpureum* **a, b, h** are fluorescence micrographs of 4′,6-diamidino-2-phenylindole (DAPI)-stained cells; **c, d, i** are corresponding differential interference images. *Panels* **b, d** show a dividing cell. *Panels* **e**–**g** are electron micrographs of *C. merolae* cells in stationary culture (**e**), and after nitrogen deprivation for 2 days (**f, g**). *Cp* chloroplast (plastid), *Mt* mitochondrion, *N* nucleus, *O* oil body, *S* starch granule

phosphate indicated slow labeling of PC (Sato et al. 2016), whereas the acyl groups are rapidly turned over (Sato and Moriyama 2007). This indicates important metabolic roles of PC in desaturation and lipid remodeling to provide fatty acids for TAG synthesis (Sato et al. 2016; Toyoshima et al. 2016). Similar active turnover of PC is known in plants (Bates 2016). In contrast with *C. reinhardtii*, nitrogen deprivation did not result in large-scale degradation of plastid lipids or global degeneration of plastids in *C. merolae* at least in the initial phase (Toyoshima et al. 2016). Phycobilisomes (light-harvesting pigment-protein complexes on the thylakoid membranes) are, however, degraded to provide nitrogen source for the cellular metabolism and carbon source for the synthesis of lipids and starch. The conversion of phycobilisomes to storage materials could be a good strategy of efficient oil production in red algae.

Comparative genomics of lipid biosynthesis

Enzymes involved in major lipid biosynthesis in *C. merolae* were estimated from the genome sequences (Sato and Moriyama 2007; Mori et al. 2016). As a result, enzymes for the fatty acid and lipid biosynthesis were identified. The list of all genes involved in lipid metabolism was extended to include five red algae, and the results are provided as Table S1. In the current map, we do not include betaine lipid biosynthesis, because this pathway was not detected in the complete genome sequences (Table S1). The comparative genomic database for red algae is available as Dataset Gclust2016R in the Gclust server.

Various red algae and their biotechnological potentials

Table 1 presents comparison of model red algae with model green algae, *Chlamydomonas reinhardtii* and their relatives. Roughly speaking, the two types of algae are comparable in the capacity of production of oil and carbohydrate. But the actual growth conditions are quite different, and this difference can be exploited for better cultivation. We briefly explain characteristics of representative red algae.

Cyanidioschyzon merolae

Cyanidioschyzon merolae is a small unicellular red alga, having a very simple cell structure, comprising one each of mitochondrion, plastid, and microbody per cell (Fig. 3a–e). It lives in acidic hot springs containing sulfuric acid (at about 40–50 °C, at pH 1.5–2.5). This growth condition allows culturing under open air without special cares such as autoclaving of the medium. Tolerance to high concentration (up to 100%) of CO_2 as well as nitrate and sulfate/sulfite allows use of exhaust gas of industry for the culture. Temperatures higher than 40 °C favorable for its growth can be obtained also by exhaust heat from the industry, but this

Table 1 Comparison of red microalgae and green microalgae as biotechnological resources

	Red algae		Green algae
	Cyanidioschyzon merolae	*Porphyridium purpureum*	*Chlamydomonas reinhardtii* or other relatives
Lipid content (% dry weight biomass)	10–20	9–14	10–20
Productivity (mg L^{-1} day^{-1})			
Lipid	50–80	35	15–40
Carbohydrate	5–20	150–180	15–150
Growth			
Sea water	Up to 1/3 SW	Yes	Up to 1/3 SW
Fresh water	Yes	Not tested	Yes
pH range	1.5–4.0	Neutral pH	Neutral pH but acidophiles exist
Temperature range (°C)	25–50	4–35	10–30
Doubling rate (d^{-1})	1–2.5	About 1	1–2
Open air (no sterilization)	Yes	No	No
Genetic manipulation	Routinely	Chloroplast	Routinely
Homologous recombination of nuclear genome	Yes	Unknown	No
Major products	TAG for biofuel Starch Phycocyanin	TAG (ARA and EPA) Phycoerythrin Mucoid	TAG for biofuel Starch

References on productivity: *C. merolae* Takusagawa et al. (2016), Toyoshima et al. (2016), Sumiya et al. (2015), *P. purpureum* Rodolfi et al. (2009), Satyanarayana et al. (2011), *Chlamydomonas* Karpagam et al. (2015), Toyoshima and Sato (2015), Satyanarayana et al. (2011), Siaut et al. (2011)

alga can also grow at ambient temperatures. All these properties are suited for its use in biotechnology. The nuclear (16,546,747 bp), mitochondrial (32,211 bp) and plastid (149,987 bp) genomes have been completely sequenced (Matsuzaki et al. 2004; Nozaki et al. 2007). Molecular genetic tools for genetic manipulation are now available for transient expression of fluorescent proteins (Ohnuma et al. 2014; Moriyama et al. 2014a, b) as well as targeted gene disruption (Fujiwara et al. 2013).

Lipid profile of *C. merolae* is quite simple (Table 2). Unlike many other eukaryotes, phosphatidylserine (PS) and cardiolipin (CL) are not detected (Sato and Moriyama 2007). TAG accumulates under nitrogen depletion (Table 2; Toyoshima et al. 2016; Takusagawa et al. 2016). Major fatty acids are 16:0, 18:1 and 18:2. No PUFAs are formed even at low temperatures (Sato and Moriyama 2007; Toyoshima et al. 2016: Table 3). The TAG accumulated under nitrogen deprivation is also rich in the three fatty acids, namely, 16:0, 18:1 and 18:2, and is suitable for biodiesel after conversion to fatty acid methyl esters. Currently, this is the only red alga that can produce TAG for biofuel. Because of its simple pathway of lipid synthesis, this alga could be a good seed for further manipulation in biotechnology.

Porphyridium purpureum

Porphyridium purpureum (previously *cruentum*) is a marine unicellular red alga, which has also been studied for a long time in basic studies (Fig. 3h, i). The nuclear genome (19.7 Mbp; Bhattacharya et al. 2013) and the plastid genome (217,694 bp; Tajima et al. 2014) have been sequenced. Genetic manipulation in *P. purpureum* was reported (Lapidot et al. 1999, 2002), but no further attempts have been made. The plastid genome of the red algae in general has a large capacity of protein-coding genes (more than 200, see Tajima et al. 2014). Red algal plastids retain many prokaryotic enzymes involved in the gene expression, and in this respect, they are distinct from green algae and plants (Sato 2001). This could be a target for engineering

Table 2 Lipid composition of red algae

Lipid class	*Cyanidioschyzon merolae* (normal)	*C. merolae* (*N*-depleted)	*Galdieria sulphuraria* (pH 2)	*Porphyridium purpureum*	*Chondrus crispus*	*Pyropia yezoensis*
MGDG	25	14	1	37	17	27
DGDG	27	20	2	22	15	25
SQDG	15	8	5	13	16	11
PG	10	5	3	9	8	19
PE	8	10	14	2	2	3
PC	10	15	26	9	30	12
PI	2	2	3	2	–	–
PA	1	1	2	5	2	–
TAG	3	26	Present	21	3	3
DGTS	–	–	16	–	Present	–
DGTA	–	–	25	–	–	–
PSC	–	–	2	–	Present	–
PS	–	–	1	–	–	–
Reference	[1]	[1]	[2]	[3]	[4]	[5]

Molar percentage values are presented as integers. – not detected or not listed

Names of lipids: *MGDG* monogalactosyl diacylglycerol, *DGDG* digalactosyl diacylglycerol, *SQDG* sulfoquinovosyl diacylglycerol, *PG* phosphatidylglycerol, *PE* phosphatidylethanolamine, *PC* phosphatidylcholine, *PI* phosphatidylinositol, *PA* phosphatidic acid, *TAG* triacylglycerol, *DGTS* diacylglyceryl-*N,N,N*-trimethylhomoserine, *DGTA* diacylglyceryl-*O*-2′-(hydroxymethyl)-(*N,N,N*-trimethyl)-β-alanine, *PSC* phosphatidylsulfocholine, *PS* phosphatidylserine

References:

[1] Toyoshima et al. (2016). Other data are available in Sato and Moriyama (2007)

[2] Vitova et al. (2016)

[3] Khozin-Goldbert et al. (2000). SQDG was analyzed in detail in Naumann et al. (2007)

[4] Pettitt et al. (1989). Values are in weight percentage. Value of PC + PSC is presented as PC. PI was not listed. Value of PA + DAG is presented as PA. Others include free fatty acids and sterol esters as well as unidentified lipids. Other data are available in Melo et al. (2015) that reported the presence of DGTS, galactosyl ceramide and inositol phosphorylceramide

[5] Araki et al. (1986). Other data are available in Araki et al. (1987), Araki et al. (1989)

Table 3 Fatty acid composition of red algal lipids

Fatty acid	*Cyanidioschyzon merolae* (plus nitrogen)		*Galdieria sulphuraria* (pH 2)		*Porphyridium purpureum*		*Chondrus crispus*		*Pyropia yezoensis*	
	MGDG	TAG	Total	TAG	MGDG	TAG	MGDG	TAG	MGDG	TAG
14:0	–	–	2	3	–	–	t	1	1	t
16:0	34	33	26	25	26	21	4	15	13	13
16:1	0	1	4	5	1	2	t	13	–	0
17:0	t	2	–	–	–	–	–	–	–	–
18:0	t	15	4	6	t	2	1	3	–	–
18:1 (9)	2	12	17	21	t	1	3	13	4	10
18:2 (9,12)	62	22	35	30	4	21	1	3	1	7
18:3 (9.12.15)	–	–	10	5	–	–	t	1	t	t
18:3 (6,9,12)	–	–	–	–	t	2	t	1	1	1
18:4	–	–	–	–	–	–	1	1	t	1
20:1 (11)	t	t	–	–	–	–	–	–	1	4
20:2 (11,14)	1	t	Present	–	–	–	t	1	1	2
20:3	–	–	Present	–	t	1	–	1	3	8
20:4	–	–	–	–	6	33	23	27	2	11
20:5	–	–	–	–	63	17	68	18	74	41
Others	–	–	4	4	–	–	t	3	t	1
Reference	[1]		[2]		[3]		[4]		[5]	

Molar percentage values are presented as integers. *t* trace (<0.5%), – not detected or not listed

Chemical names of fatty acids: 14:0, myristic; 16:0, palmitic; 16:1, palmitoleic; 17:0, margaric; 18:0, stearic; 18:1(9), oleic; 18:2(9,12), linoleic; 18:3(9,12,15), α-linolenic; 18:3(6,9,12), γ-linolenic; 18:4, octadecatetraenoic; 20:1(11), eicosenoic; 20:2(11,14), eicosadienoic; 20:3, eicosatrienoic; 20:4, arachidonic; 20:5, eicosapentaenoic

References:

[1] Toyoshima et al. (2016). Other data available in Sato and Moriyama (2007)

[2] Vitova et al. (2016); 20:2, 20:3, 22:0, 24:0 were also reported in Sakurai et al. (2016)

[4] Pettitt et al. (1989). Values for MGDG are those for MGlyDG2 (major one). The nature of sugar was not examined by the authors. Other data are available in Melo et al. (2015)

[3] Khozin-Goldbert et al. (2000). Other data available in Nichols and Appleby (1969), Oh et al. (2009)

[5] Araki et al. (1986). Other data are available in Araki et al. (1987), Araki et al. (1989)

not only in *P. purpureum* (Lapidot et al. 2002) but also in *C. merolae*.

As this alga excretes highly viscous materials, characterization and synthesis of polysaccharides have been studied (Merchuk et al. 1998; Geresh et al. 2009). *Porphyridium* was found to contain semi-amylopectin rather than glycogen that was found in *Cyanidium* and *Galdieria* (Shimonaga et al. 2008). Lipid profile of *P. purpureum* (Khozin-Goldbert et al. 2000) is qualitatively similar to that of *C. merolae* (Table 2). In contrast, fatty acid profile is quite different: *P. purpureum* contains high levels of ARA and EPA (Table 3; Nichols and Appleby 1969; Ohta et al. 1992; Oh et al. 2009). Putative genes for the desaturases producing ARA and EPA were identified (Table S1). Detailed structural analysis of SQDG by mass spectrometry was reported (Naumann et al. 2007).

Process engineering of *P. purpureum* was studied in Posten laboratory in Germany (Fleck-Schneider et al. 2007; Sastre 2010). They developed a large plant for mass cultivation of this alga using sunlight, with least possible energy input for stirring and aeration. A problem of *P. purpureum* could be the production of viscous mucilage, which prevents harvesting and processing. We found that the cells can be grown at a reasonable rate at a temperature as high as 35 °C (Tajima et al. 2014), while the production of mucilage was reduced, although the color of the cells turns orange, in contrast with dark red at lower temperatures.

Other red algae

Galdieria sulphuraria is another thermophilic red alga belonging to Cyanidiales. Massive horizontal gene transfer (HGT) was found to contribute to the extremophilic properties of this alga (Schönknecht et al. 2013). HGT is not common in other thermophilic algae in Cyanidiales, such as *C. merolae* and *Cyanidium caldarium* (Ciniglia et al. 2004).

This could be the reason for the facultative heterotrophy of *G. sulphuraria* (Sakurai et al. 2016). Detailed lipidomic analysis (Vítová et al. 2016) indicated that this alga also contains uncommon lipids, such as diacylglyceryl-*N,N,N*-trimethylhomoserine (DGTS), diacylglyceryl-*O*-2′-(hydroxymethyl)-(*N,N,N*-trimethyl)-β-alanine (DGTA), and phosphosulfocholine (PSC) (Table 2). DGTS and DGTA are known as betaine lipids, which were initially not detected in red algae (Sato 1992), but later reported to be present in several species of red algae (Dembitsky 1996). Curiously, however, the gene *BTA1* encoding the enzyme catalyzing the biosynthesis of DGTS (Riekhof et al. 2005), or its bacterial homologs, *btaA* and *btaB* (the corresponding two domains are fused in BTA1), was not detected in the genome sequences of *G. sulphuraria* or *Chondrus crispus* (Table S1). Another point on the lipid composition of *G. sulphuraria* as reported by Vítová et al. (2016) is the unusual paucity of common plastid lipids, MGDG and DGDG, in autotrophic culture, in which large plastids must be present (Fig. 1 in Schönknecht et al. 2013). Further studies will be needed to elucidate these paradoxes. Differences in lipid and fatty acid composition in various growth conditions (Sakurai et al. 2016) were reported. Notably, TAG level markedly increased (up to 11 μg mL^{-1}) by addition of glucose in the heterotrophic culture.

We have to mention two marine macrophytic red algae that are well characterized. Lipid analysis of *C. crispus* was reported (Pettitt et al. 1989), but detailed structural studies were recently published (Melo et al. 2015). Based on the genomic data now available (Collén et al. 2013), we can identify enzymes involved in lipid metabolism (Table S1). Another well-characterized red alga is *Pyropia* (formerly *Porphyra*) *yezoensis*, which is an important food in Japan (Fig. 1a). Commercially available sheets of this alga (laver of "Nori") are dry, but all lipid components are preserved without notable oxidation for a long time (Kayama et al. 1983). Lipid composition of *Pyropia yezoensis* was studied in detail (Araki et al. 1986, 1987, 1989). Lipid and fatty acid compositions in various species of *Gracilaria* were also reported from the same group (Araki et al. 1990). These macrophytic red algae contain high levels of ARA and EPA, especially in MGDG (Table 3). The contents in TAG are not, however, very high.

Potentials of red algae as biofuel feedstocks and high-added value products

Biofuel production in microalgae is now highlighted (De Bhowmick et al. 2015; Zienkiewicz et al. 2016), but conventional algal biotechnology focused on green algae, such as those in the genus *Chlamydomonas*. Table 1 compares red algae and *Chlamydomonas* for their potentials in biotechnology. Obviously, red algae have potentials as both biofuel feedstocks and producers of high-added value compounds. Representative values of lipid content and productivity of lipids and carbohydrates are comparable in red and green algae. Growth properties are markedly different: Cyanidiales such as *C. merolae* grows in acidic hot springs, and this property makes these groups of red algae unique organisms suitable for biotechnology. They are easy to grow in an acidic medium without sterilization, and exhaust gas and heat of industry can be used for their growth (Table 1). Sea water can be tolerated to some extent as stressful conditions to increase the content of oil. Marine red algae such as *P. purpureum* also provide good resource for biotechnology, because sea water is available everywhere in a country such as Japan. This microalga is usually grown in sterilized artificial sea water, but growth at high temperature (up to 35 °C) could be favorable for its maintenance in axenic state. In contrast, standard green algae such as *Chlamydomonas reinhardtii* grows in fresh water at neutral pH at ambient temperature. The growth medium must be sterilized for their growth. However, acid-tolerant strains of *Chlamydomonas* exist. These can be used more easily in large-scale cultivation. Growth rate of both red algae and green algae depends on light intensity. The microalgae can grow faster under the light with higher intensity, if enough CO_2 is provided. In this respect, very rapid growth is achieved in *C. merolae* under high light with high concentration of CO_2. This is realizable in open air with industrial exhaust gas and heat. In contrast, rapid growth and high yield of oil are difficult to achieve in *Chlamydomonas* even at high light and higher concentration of CO_2.

Genetic manipulation is now possible in both red and green algae. But homologous recombination is only possible in *C. merolae*. A promising example of genetic modification of *C. merolae* is the introduction of cyanobacterial acyl-ACP reductase, which increased the accumulation of TAG (Sumiya et al. 2015). Genetic engineering in completely sequenced, unicellular microalgae has a great advantage in efficient development. The genome sizes of red algae are, in general, small (10–30 Mbp), whereas the genomes of *Chlamydomonas* are fairly larger, namely, about 70–140 Mbp (Hirashima et al. 2016). Although there are other microalgae having small genomes, red algae are definitely the organisms of choice for genetic modification, because paucity of introns also characterizes red algae. *C. merolae* has only 27 introns in the entire 4775 protein-coding nuclear genes! We do not need to isolate cDNA, and we can manipulate the genome just like prokaryotic genomes.

Red algae provide two types of organisms, one suitable for biofuels and the other suitable for high added-value products. *C. merolae* does not contain PUFA, and this property is good as biofuel production. The simple metabolic pathway as well as ease in genetic manipulation makes this

alga a versatile photosynthetic organism to use in bioengineering. Metabolic maps of lipid metabolism (Mori et al. 2016) and carbon metabolism (Moriyama et al. 2014a) are ready to use in *C. merolae*. ARA and EPA are representative high added-value compounds produced by many red algae. If the flow of these acids into plastid MGDG could be switched to TAG synthesis, we would expect production of TAG enriched in ARA and EPA. The use of the desaturase genes in different organisms might also be promising.

Moreover, *C. merolae* serves as a model for engineering other red algae, both microalgae and macrophytes, in planning the strategy of genetic modification. Lipid biotechnology in microalgae has been mostly studied in green algae, but it now jumped up into a new era of red algal technology.

Acknowledgements The studies in the authors' laboratory have been supported in part by the CREST project on algal biotechnology from Japan Science and Technology Agency.

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