Chapter 10
The Bioactivity and Chemotaxonomy of Microalgal Carotenoids

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Abstract Microalgae are a diverse group of photosynthetic microorganisms which inhabit a wide variety of freshwater and marine environments. They play a key role in the Earth's biogeochemical cycles and hold great potential for their application in the fields of environmental remediation, biotechnology and nanotechnology. The bioactivity of carotenoids warrants their applications as nutraceuticals, cosmaceuticals or biopharmaceuticals in the treatment and prevention of chronic and age-related diseases. Their photosynthetic pigment signatures are typically taxon-specific, facilitating their application as chemotaxonomic biomarkers and provide a complementary approach to the morphogenetic taxonomy in the characterisation of new strains. This chapter provides a brief overview of carotenoids structural diversity, their bioactivity and biosynthesis. In addition, it aims to introduce the endosymbiotic theory of protist evolution which gave rise to 11 algal divisions. At present, there are 27 reported classes of photosynthetic algae representing 44 pigment types based on the distribution of their chlorophyll and carotenoid pigments.

Keywords Microalgae · Phylogeny · Chemotaxonomy · Pigment types · Carotenoids · Antioxidants · Nutraceuticals · Biopharmaceuticals

10.1 Introduction

Algae are a broad term for organisms other than higher plants that obtain energy from autotrophic photosynthesis assimilating carbon through the ribulose bisphosphate pathway (Clavin cycle). Algae are classified into divisions (phylogenetic affix: *-phyta*) based on their cellular and biochemical features such as; pigment combinations, storage products, cell wall structure (silica frustule or theca), flagella

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structure and other specialised cellular structures (Tomas 1997; Jeffrey et al. 2011). As a result, they encompass an enormous diversity of organism ranging from unicellular prokaryotes (cyanobacteria and prochlorophytes) and eukaryotic protists to multicellular macroalgae (seaweeds) (Metting 1996). There are 80,000–100,000 known algae species of which currently over 200 microalgae species are culture worldwide for different sectors ranging from aquaculture, biotechnology, nanotechnology and bioremediation services (Enzing et al. 2014).

The diversity of unicellular microalgae provides an ideal source for bioprospecting novel metabolites. They have a wide distribution of habitats including; terrestrial, freshwater and marine, some are extremophiles, while others form symbiotic relationships with fungi, tropical corals and plants (Gupta et al. 2013). Their ability to adapt stressful and competitive environments is in part owed to their photosynthetic capabilities including the biosynthesis of antioxidant carotenoids with novel biopharmaceutical applications. *Chlorella* and *Arthrospira* species are commonly cultured for the production of vitamins and as health supplements. Pigments from algae have been shown to act as powerful antioxidants and have been routinely applied to food, cosmetic and pharmaceutical products. These include phycocyanin (*Spirulina*), phycoerythrin (Rhodophyta spp.), astaxanthin (*Haematococcus pluvialis*) and fucoxanthin (Phaeophyceae spp.) as well as β-carotene (*Dunaliella* spp.) (Cuellar-Bermudez et al. 2015; Chokshi et al. 2017). The following sections explore the microalgae chemotaxonomy and their bioactive carotenoid pigments.

10.2 Carotenoid Structure and Function

At present, there are over 750 carotenoids reported in nature, of which 30 have a functional role in the photosynthetic light-harvesting complexes (Takaichi 2011). Carotenoids are tetraterpenoid pigments derived from eight isoprene units, terminating in cyclic β-ionone rings (Britton et al. 2004). The central C_{40} conjugated double-bond system of the carotenoid polyene chain acts as a "chromophore" absorbing blue light (400–500 nm) for photosynthesis and giving them their characteristic yellow, orange or red colour. Carotenoids are either classified as carotenes comprising solely of hydrocarbons or oxygenate xanthophylls (Britton et al. 2004). Oxygenated functional groups present at terminal β-ionone rings of xanthophylls act to protect the cell from oxidative damage by quenching single oxygen species (Namitha and Negi 2010). Additional functional groups include allene ($C{=}C{=}C$), acetylene ($C{\equiv}C$) or acetylated ($–O–CO–CH_3$) (Fig. 10.1). The allene moiety is present in fucoxanthin associated with the Chromophyceae algae, peridinin associated with dinoflagellates and 9′-*cis*-neoxanthin predominately associated with the green algal lineage. Xanthophylls containing acetylene functional groups ($C{\equiv}C$) are unique to algae, which to date have been found in the Cryptophyta specific carotenoids alloxanthin, crocoxanthin and monadoxanthin in addition to the Chromophyceae xanthophyll cycle pigments diadinoxanthin and

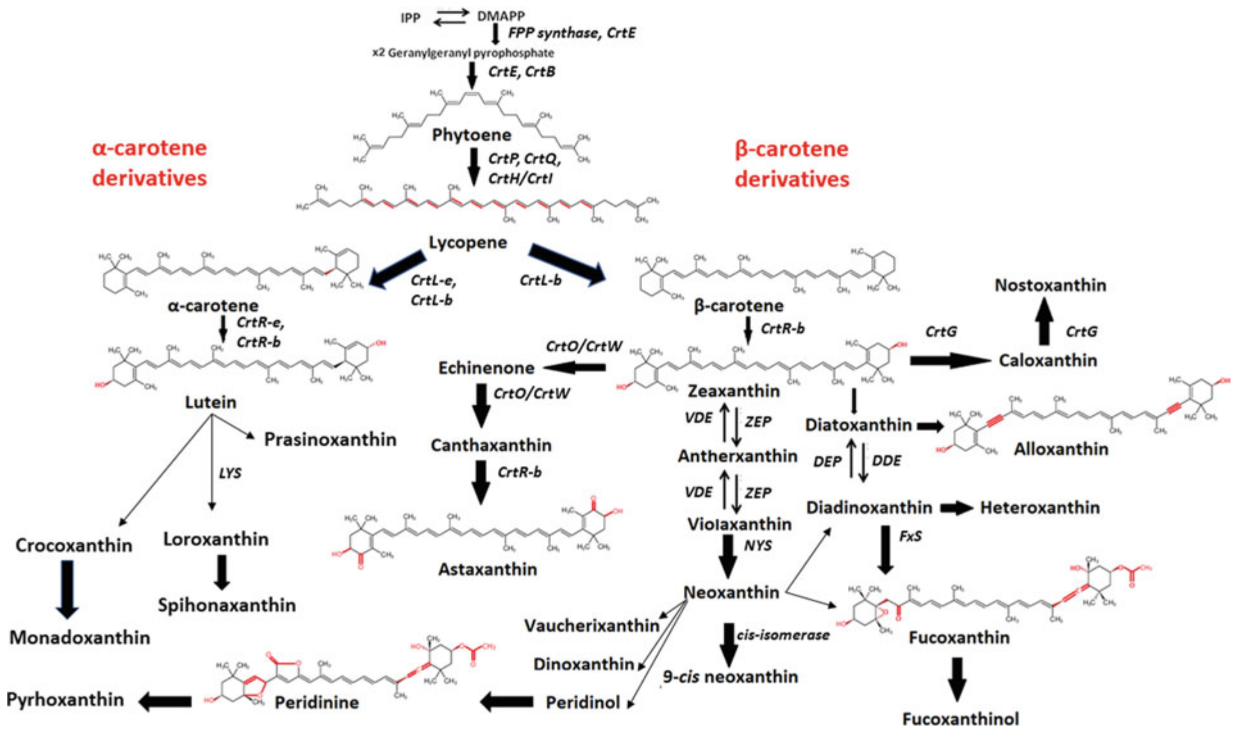

Fig. 10.1 Biosynthetic pathway of chemotaxonomically relevant and high-value carotenoids in microalgae. IPP (isopentenyl diphosphate), FPP synthase (farnesyl pyrophosphate), DMAPP (dimethylallyl diphosphate), CrtE (geranylgeranyl diphosphate synthase), CrtB (phytoene synthase), CrtP/CrtI (phytoene desaturase), CrtQ (ζ-carotene desaturase), CrtL (lycopene β-cyclase), CrtH (*cis*-to-*trans* carotene isomerase), CrtR (β-carotene hydroxylase), CrtG (2,2′-β hydroxylase), CrtO (β-carotene monoketolase), CrtW (β-carotene diketolase), VDE (violaxanthin de-epoxidase), ZEP (zeaxanthin epoxidase), DEP (diatoxanthin epoxidase), DDE (diadinoxanthin de-epoxidase), LYS (loroxanthin synthase) and FxS (fucoxanthin synthase)

diatoxanthin. Fucoxanthin, peridinin and dinoxanthin contain a novel acetylated functional group (–O–CO–CH_3) which may contribute to the bioactivity and light-harvesting capabilities of these carotenoids.

10.3 Bioactivity of Carotenoids

The bioactive potential of microalgal pigments has led to a strong market demand for their application as natural colouring agents and functional foods (Ambati et al. 2018). The global carotenoid market in 2016 had an estimated value of US$124 billion, which is projected to reach US$1.53 billion by 2021 (Markets and Markets 2016). Carotenoids commonly used as colouring agents include astaxanthin, canthaxanthin, violaxanthin, lutein, zeaxanthin and β-carotene. In vitro, in vivo and epidemiological studies have shown that consumption of the carotenoids lycopene, β-carotene, lutein, zeaxanthin, violaxanthin, astaxanthin, canthaxanthin and fucoxanthin is directly associated with the prevention of tumour progression in a range of cancers including prostate, colon, breast, lung and mammary (Chew et al. 1996; Park et al. 1998; Kotake-Nara et al. 2001; Nishino et al. 2002; Granado et al. 2003; Guerin et al. 2003; Pasquet et al. 2011; Baudelet et al. 2013; Bertram 2018). However, evidence suggests that synthetic carotenoids and the consumers lifestyle can have conflicting and detrimental health effects including raising the risk of developing cancer (Heinonen et al. 1994).

Beta-carotene is a naturally occurring primary photosynthetic carotenoid present in the reaction centre of photosystem II, where it functions to shuttle excitation energy to chlorophyll and scavenging reactive oxygen species (Telfer 2002). It is commercially marketed as the natural orange colourant E160a and is viewed as a nutraceutical due to its provitamin A and antioxidant activity (Murthy et al. 2005). Commercial production of β-carotene is predominately synthesised chemically in its *trans*-isomeric configuration. The 9′-*cis*-β-carotene isomer biosynthesised from the halotolerant microalgae *Dunaliella salina* is more favourable due to its enhanced bioavailability, antioxidant and anti-tumour activity than its *trans*-isomer (Demmig-Adams and Adams 2002; Gomez and Gonzalez 2005; García-González et al. 2005; Prieto et al. 2011). As a result, there is a strong market demand from natural 9′-*cis*-β-carotene, which is expected to reach US$334 million by 2018 (BCC Research 2015).

Similarly, the lutein supplement market is experiencing an annual growth rate of 3.6%, which is anticipated to reach US$309 million by 2018 (BCC Research 2015). Lutein is favoured over its geometric isomer zeaxanthin due to its higher percentage distribution in human serum (Alves-Rodrigues and Shao 2004). Epidemiological evidence suggests that a diet rich in lutein and zeaxanthin reduces the risk of developing cataracts and age-related macular degeneration (Moeller et al. 2008). This is a consequence of their accumulation within the macula and lens of the eye, where they act to filter out short-wavelength light and quench reactive oxygen species (Seddon et al. 1994; Krinsky et al. 2003). In addition to their function as macular pigments, lutein and zeaxanthin are also believed to play a critical role in the prevention of lung cancer and in protecting the skin from damaging UV-B radiation (Chew et al. 1996; Park et al. 1998; Nishino et al. 2002; Granado et al. 2003).

Traditionally, lutein and zeaxanthin have been extracted from the marigold flower *Tagetes erecta* as an oleoresin marketed under E161b (Delgado-Vargas et al. 2000; Krinsky et al. 2003). Microalgae such as *Murellopsis* and *Scenedesmus* spp. offer a more sustainable source of lutein due to their fast growth rates and production capacity, which is tenfold higher than marigold plants (Sanchez et al. 2008a, b).

Astaxanthin is a secondary keto-carotenoid which accumulates as lipid vesicles in some *Chlorophyceae* microalgae as a survival strategy in response to unfavourable conditions (Table 10.1). The antioxidant capacity of astaxanthin has been reported to be 10-fold higher than that of β-carotene (Guerin et al. 2003). The esterification of astaxanthin in fatty acids protects lipids from reactive oxygen species (ROS)-induced peroxidation and enhances its bioavailability (Saw et al. 2013; Zuluaga et al. 2018). It is widely applied as a colouring agent for the characteristic pink/orange colour in farmed salmon and rainbow trout (Choubert et al. 2006; Chitchumroonchokchai and Failla 2017). Commercial astaxanthin production is predominated by synthetic racemic mixture of *3S, 3′S*; *3R, 3′S* and *3R, 3′ R* stereoisomers and it is marketed at a value of over US$240 million per annum (Han et al. 2013).

Naturally, derived astaxanthin is preferred due to increasing concerns over the safety and consumer demand for natural products. Currently, natural astaxanthin is obtained from *Haematococcus pluvalis* under controlled stress conditions, where it undergoes transition from green vegetative cells to red palmelloid cysts, accumulating up to 2–5% dry weight astaxanthin as a single chiral 3S, 3′S stereoisomer.

Table 10.1 Astaxanthin accumulating microalgae strains

Strain	Astaxanthin content[a]	References
Haematococcus pluvalis	4–5%	Han et al. (2013)
Botryococcus braunii	3–8%	Rao et al. (2010)
Chlamydocapsa spp.	0.04%	Leya et al. (2009)
Chlorococcum spp.	0.7%	Ma and Chen (2001)
Chlorella zofingiensis	0.7%	Orosa et al. (2000)
Chlamydomonas nivalis	0.004%	Leya et al. (2009)
Neochloris wimmeri	1.9%	Orosa et al. (2000)
Protosiphon botryoides	1.4%	Orosa et al. (2000)
Scenedesmus spp.	0.3%	Qin et al. (2008)
Scotiellopsis oocystiformis	1.1%	Orosa et al. (2000)
Tovellia sanguinea	7 pg/cell	Frassanito et al. (2006)
Euglena sanguinea	0.53%	Grung and Liaaen-Jensen (1993)

[a] % DW astaxanthin

However, production of astaxanthin from *Haematococcus pluvalis* is costly due to its fastidious growth rates and difficulties with the extraction of the carotenoid from encysted cells (Hata et al. 2001). In order to remain competitive with synthetic market, alternative strains, cultivation conditions and biorefinery technologies are sought for economical and sustainable astaxanthin production (Hanagata and Dubinsky 1999; Orosa et al. 2001; Ahmed et al. 2014; Mao et al. 2018).

Fucoxanthin is an abundant pigment in nature responsible for the golden-brown colour in *Heterokontophyta* and *Haptophyta* (Jeffrey et al. 2011). Among the carotenoids, fucoxanthin exhibits pronounced antioxidant and anti-inflammatory activity when compare to lycopene, β-carotene, lutein, zeaxanthin, β-cryptoxanthin and astaxanthin (Nomura et al. 1997; Ishikawa et al. 2008). Its potent bioactivity is attributed to the presence of an unusual allelic bond and numerous functional moieties (hydroxyl, epoxy, carbonyl and carboxyl) (Nomura et al. 1997; Hosokawa et al. 1999; Das et al. 2008; Nakazawa et al. 2009; Woo et al. 2010; Kim et al. 2010). It holds considerable promise as a nutraceutical and medical adjuvant for the treatment of diabetes, obesity, malaria and cancer (Afolayan et al. 2008; Woo et al. 2009, 2010; Hosokawa et al. 2010; Park et al. 2011; Wang et al. 2012; Rengarajan et al. 2013).

Abidov et al. (2010) carried out a 16-week randomised double-blind study on the effect of Xanthigen™ (brown marine algae fucoxanthin and pomegranate seed oil) on body weight, body fat, liver lipids and blood biochemistry on 151 non-diabetic, obese premenopausal women with non-alcoholic fatty liver disease. The study found that Xanthigen™ reduced body and liver fat content and improved liver function tests.

Fucoxanthin induces anti-proliferative and apoptotic effects on tumours through a range of molecular mechanisms including the stimulation of gap-junction cellular communication, immunomodulation of the JAK/STAT pathway, activation of capases-3, ROS generation, cell cycle arrest and anti-angiogenic effects (Zhang et al. 1991; Kim et al. 2010; Wang et al. 2012; Rengarajan et al. 2013). Its non-toxic pharmacokinetics warrants its application to alleviate the side effects of chemotherapy (Iio et al. 2011a, b). However, full-scale clinical trials are required to prove its safety and efficiency against a range of cancer therapies.

The highest reported fucoxanthin containing microalgae includes *Mallomonas* sp. SBV13, *Odentella aurita*, *Isochrysis galbana* and *Phaeodactylum tricornutum* which are capable of biosynthesising up to 26.6, 18.5, 18.2 and 8.6 mg g^{-1} DW fucoxanthin, respectively (Kim et al. 2012; Xia et al. 2013; Gómez-Loredo et al. 2016; Petrushkina et al. 2017). Current commercial sources of fucoxanthin are derived from macroalgae. However, the fucoxanthin content in microalgae is significantly higher than that reported for macroalgae (Xiao et al. 2012; Jaswir et al. 2013; Schmid et al. 2017). Cultivation of *Phaeodactylum tricornutum* under low light conditions with enriched nitrate medium led to significant increase in fucoxanthin content from 9.9 ± 4.2 mg g^{-1} to 59.2 ± 22.8 mg g^{-1} (McClure et al. 2018).

Other high-value carotenoids associated with brown algae (Chromophyceae) include violaxanthin and canthaxanthin which are commonly used as food colouring additive (E161g). Violaxanthin is a primary light-harvesting carotenoid and is the major xanthophyll pigment associated with Eustigmatophyceae (Owens et al. 2004). Violaxanthin isolated by bioassay-guided fractionation possessed strong anti-proliferative activity against human mammary cancer cells (Pasquet et al. 2011). *Nannochloropsis gaditana* is also capable of biosynthesising the high-value keto-carotenoids canthaxanthin and astaxanthin representing 0.7% of dry weight biomass (Lubián et al. 2000).

Another source of microalgae derived natural colouring agents are phycobiliproteins. These water-soluble fluorescent pigments function as accessory or antenna pigments to harvest light for photosynthesis in *Cyanobacteria*, *Cryptophyta* and *Rhodophyta* spp. (Sekar and Chandramohan 2008). They are linear tetrapyrrole chromophores (bilins) covalently bound to apoproteins via thioether links to the cysteine residues forming phycobiliproteins (Stadnichuk et al. 2015).

C-phycocyanin is a blue fluorescent pigment protein with a range of applications ranging from natural colouring in food and drink, fluorescent marker in molecular diagnostic or as a nutraceutical due to its antioxidant, anti-inflammatory, hepatoprotective and cholesterol-lowering effects (Eriksen 2008). Similarly, phycoerythrin is a fluorescent phycobiliprotein which possesses anti-inflammatory activity and has applications in anticancer photodynamic therapy as well as in the field of immunodiagnostics (Oi et al. 1982; Cian et al. 2012; Cai et al. 2014). Due to its novel properties, patent applications have been filled for its application in immunoassays and flow cytometry (Chiueh 2003; Chiueh et al. 2003).

10.4 Carotenoid Biosynthesis

Carotenogenesis in microalgae is undertaken via either the mevalonate (MVA) pathway or the 1-deoxy-D-xylulose-5-phosphate (DOXP) pathway initiating from the precursor isopentenyl pyrophosphate (IPP). IPP is enzymatically condensed and elongated to geranylgeranyl diphosphate (GGDP) farnesyl pyrophosphate via geranylgeranyl diphosphate synthase. The head–head condensation of two GGDP by phytoene synthase yields the C_{40} molecule, phytoene. Subsequent desaturation of phytoene forms the red coloured carotenoid, lycopene. This, in turn, acts as the template for synthesis of the dicyclic carotenoids, α-carotene and β-carotene, and their oxygenated xanthophylls derivatives (Takaichi 2011; Huang et al. 2017). Alpha- and beta-carotene undergo hydroxylation via β-carotene hydroxylase forming the xanthophylls lutein and zeaxanthin, respectively (Fig. 10.1). While the epoxidation of zeaxanthin at position 5,6 or 5′,6′ yields antheraxanthin and violaxanthin, respectively. Violaxanthin is converted to neoxanthin by the rearrangement of an ionone ring in produces two allenic double bonds. Neoxanthin subsequently acts as a precursor for the synthesis of acetylenic xanthophylls vaucheriaxanthin, peridinin, fucoxanthin and its 19′-acyloxy derivatives, diadinoxanthin, heteroxanthin, diatoxanthin and alloxanthin (Fig. 10.1). Lutein acts as a precursor for the acetylenic xanthophylls crocoxanthin, monadoxanthin and loroxanthin (Bhosale and Bernstein 2005; Takaichi 2011).

Peridinin and fucoxanthin are unique in that they contain an additional conjugated carbonyl functional group (Liaaen-Jensen 1979). In the case of lutein, the addition of carbonyl functional groups yields prasinoxanthin and siphonaxanthin. The addition of endo-cyclic keto functional groups to β-carotene yields the secondary carotenoids echinenone, canthaxanthin and astaxanthin (Bhosale and Bernstein 2005; Takaichi 2011).

10.5 Analysis of Microalgae Carotenoids

At present, high-performance liquid chromatography (HPLC) represents the gold standard analytical technique for the separation and quantification of photosynthetic pigments in microalgal cultures and phytoplankton community samples. Since the 1980s, various intercalibration initiatives carried out by NASA and the SORC/UNSECO have paved the way towards developing standardised protocols for the analysis of phytoplankton pigments (Wright et al. 1991; Claustre et al. 2004). Advances in HPLC methodologies for the analysis of different chlorophyll and carotenoid derivatives have been extensively reviewed by Garrido et al. (2011). Developments in the field include the application of mobile phase modifier (Zapata et al. 2000), a C_{16}-amide column (Jayaraman et al. 2011) or a pentafluorophenyloctadecyl silica column (Sanz et al. 2015) for the enhanced resolution of chemotaxonomically important monovinyl and divinyl chlorophyll pigment pairs in *Prochlorococcus* spp. High-throughput analysis has been achieved through the application of a monolithic column (Mc Gee et al. 2017) or using UHPLC systems (Suzuki et al. 2015).

In order to gain reliable pigment data, the SCOR working group set out guidelines for the “Minimum identification criteria for phytoplankton pigments”. These include the use of standards, biological reference material and the application of LC–MS to accurately identify chlorophylls and carotenoids present in extracts (Airs and Garrido 2011; Egeland 2011). The application of HPLC to the analyses of phytoplankton pigments has rapidly expanded our knowledge of pigment chemodiversity providing insights into species-specific chemotaxonomic biomarkers or class-specific pigments types (Zapata et al. 2004; Laza-Martinez et al. 2007; Paliwal et al. 2016; Serive et al. 2017). Various multivariant statistical analyses can be applied to the HPLC derived pigment matrices in order to determine phytoplankton biodiversity, estimate abundances and to characterise new strains (Mackey et al. 1996; Laza-Martinez et al. 2007; Paliwal et al. 2016; Serive et al. 2017; Mc Gee et al. 2017).

10.6 Chemotaxonomy

Current phylogenetic reconstructions propose that eukaryotic microalgae lineages have evolved through a series of endosymbiotic events (Fig. 10.2). A primary endosymbiotic event involving the phagocytosis of a primitive cyanobacterium by a heterotrophic eukaryote host gave rise to the Archaeplastida lineage, comprising the divisions Glaucophyta, Rhodophyta and Chlorophyta (Keeling 2013). Subsequent multiple secondary and tertiary eukaryotic–eukaryotic endosymbiotic events led to the diversification of microalgae plastids and the evolution of the hacrobians, SAR supergroup, Excavates lineages (Keeling 2013).

The unique distribution of photosynthetic plastids reflects the evolutionary history of the different photosynthetic microalgal lineages and is largely supported

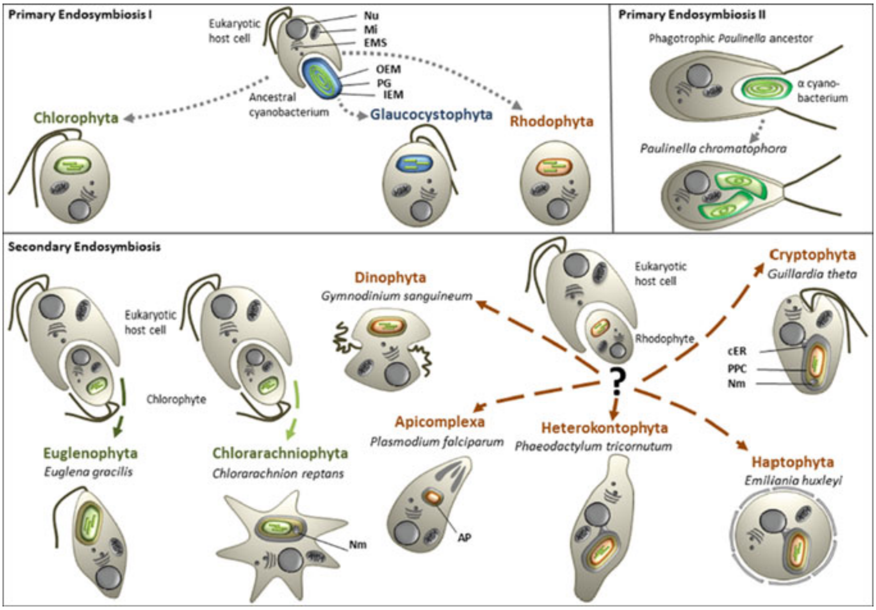

Fig. 10.2 Schematic of microalgae plastic evolution through endosymbiosis. Primary plastids originated from the phagocytotic engulfing of a cyanobacterium, resulting in two-membrane plastids in the Archaeplastida lineage (glaucophytes, red algae and green algae). The glaucocystophytes retained the peptidoglycan, which was lost in the other groups. An additional independent primary endosymbiotic event occurred between a α-cyanobacterial and *Paulinella chromatophora.* At least three independent eukaryote-eukaryote secondary endosymbiotic events occurred in which the plastid originated from either green or red algae. These subsequent events yielded plastids surrounded by three (euglenophytes, dinoflagellates) or four membranes (chlorarachniophytes, apicomplexans, heterokontophytes, haptophytes and cryptophytes). Nucleus (Nu), nucleomorph (Nm), mitochondrion (Mi), endomembrane system (EMS), outer envelope membrane (OEM), inner envelope membrane (IEM), peptidoglycan (PG), chloroplast ER (cER), apicoplast (AP), periplastidal compartment (PPC). Reprint from Gentil et al. (2017) with permission from Springer Publishing Group

Table 10.2 Chloroplast light-harvesting complexes in microalgae

	Light-harvesting complex (LHC)	Microalgal class
1.	*Chlorophyll a/ phycobiliprotein*	Cyanobacteria, Glaucophyceae, Rhodophyceae and Cryptophyceae
2.	*Chlorophyll a/c*	Haptophyceae, Heterokontophyceae, Cryptophyceae and Alevolates
3.	*Chlorophyll a/b*	Chlorophyceae and Euglenophyceae

by molecular phylogenetics (Keeling 2013). Microalgae can be classified into three hierarchical groups based on their light-harvesting complex (LHC) pigment signatures (Table 10.2).

These photosynthetic light-harvesting complexes (LHCs) harness solar light energy within the photosynthetically active radiation (PAR) spectrum ranging from 400 to 700 nm (Schulze et al. 2014). The carotenoids bound within these LHCs function in light harvesting, stabilising the photosystems and provide photoprotection from reactive oxygen species (Campbell 1996; Lohr and Wilhelm 1999; MacIntyre et al. 2002; Jahns and Holzwarth 2012; Depauw et al. 2012; Polimene et al. 2012; Musser et al. 2015).

The distribution of different carotenoids within these three hierarchical lineages is typically taxon-specific, facilitating their application as chemotaxonomic biomarkers. Certain pigments are class-specific diagnostic markers such as α-carotene and the acetylene xanthophylls alloxanthin, crocoxanthin and monadoxanthin in *Cryptophyceae* or the acetylated carotenoid, peridinin, in *Dinoflagellates.* Some pigments can also act as species-specific chemotaxonomic tracers; for example, the divinyl chlorophyll *a* for the marine cyanobacteria, *Prochlorococcus marinus* (Jeffrey et al. 2011). However, this distinction is not always so clearly defined, limiting the application of the single pigment biomarker approach (Zapata 2005; Laza-Martinez et al. 2007). This is evident in the distribution of fucoxanthin in the classes; *Heterokontophyta, Haptophyta and Dinoflagellate* pigment type DINO 2 and 3 (Jeffrey et al. 2011). To overcome this limitation, the total plastid pigment signature can be used to define class-specific pigment types (Zapata 2005; Jeffrey et al. 2011). At present, there are 44 pigment types representing 27 microalgal classes from 11 divisions (Jeffrey et al. 2011).

10.6.1 Cyanobacteria Pigment Signatures

Cyanobacteria are prokaryotic organisms which represent the oldest phototrophic life forms on earth, originating between 2600 and 3500 million years ago (Hedges et al. 2001). They comprise a diverse group of unicellular, filamentous and colonial microorganisms. Cyanobacteria LHC are comprised of chlorophyll and phycobiliproteins (phycocyanin, allophycocyanin and phycoerythrin). Cyanobacteria regulate the composition of these pigments by complementary chromatic adaptation and the distribution of light energy through the photosystems via state transitions to maximise their photosynthetic capacity in fluctuating light environments (Campbell 1996; McConnell 2002). There are five pigment types defining cyanobacteria and Prochlorophytes. The CYANO-2 pigment type comprises the basic cyanobacterial pigment signature (chlorophyll *a,* phycobillin's, zeaxanthin and β-carotene) and is predominately associated with picocyanobacterium species. Filamentous cyanobacteria are classified as CYANO-1 which contains the additional myxoxanthophyll, oscillaxanthin, nostoxanthin, aphanizophyll and 4-keto-myxoxanthophyll. The Prochlorophyceae species are classified into CYANO-3-5 pigment types defined by the presence of cryptoxanthin (α and β), divinyl chlorophylls and chlorophyll *d*, respectively (Jeffrey et al. 2011).

10.6.2 Primary Endosymbiont Pigment Signatures

Plastids of the lineage Archaeplastida are composed of the light-harvesting pigments chlorophyll *a* and phycobiliproteins (phycocyanin, phycoerythrin and allophycocyanin) in addition to accessory carotenoids. The presence of phycobiliproteins enables them to utilise light within the red, yellow and green electromagnetic spectrum (Schulze et al. 2014).

10.6.2.1 Glaucophyta

Glaucophyceae represent primitive primary endosymbionts which have been suggested to be an evolutionary connection between cyanobacteria, Rhodophyceae and Chlorophyceae (John et al. 2011). The phyla comprising of three main groups; *Cyanophora*, *Glaucocystis* and *Gloeochaete* based on cellular morphology, pigment composition and nuclear and plastid-encoded protein phylogeny (Reyes-Prieto and Bhattacharya 2007; John et al. 2011). They share features with cyanobacteria including peptidoglycan layer, carboxysomes, an ancestral form of fructose-1,6-bisphosphate aldolase and the phycobiliproteins. The GLAUCO-1 pigment type is similar to CYANO-2 comprising of chlorophyll *a*, phycobillin's, β-cryptoxanthin and zeaxanthin (Jeffrey et al. 2011).

10.6.2.2 Rhodophyta

Red algae lineage is comprised of 32 orders and 90 families and contains high species diversity with up to 6000 species of macroalgae reported to date, of which 97% are found in marine and benthic environments (Norton et al. 1996; Schneider and Wynne 2007). Microalgae within the red microalgae contain less biodiversity falling within three classes; Cyanidiales, Rhodellophyceae and Porphyridiophyceae.

Zeaxanthin is the predominant carotenoid in unicellular Rhodophyceae with trace levels of violaxanthin, antheraxanthin and β-cryptoxanthin (Schubert et al. 2006). In contrast to the cyanobacterium pigment type, phycoerythrin is the dominant accessory phycobiliprotein pigment in the light-harvesting antenna complex of red algae, bestowing upon them their characteristic dark red colouration. Rhodophyceae are commonly found in highly exposed open ocean sites and it has been proposed that phycoerythrin provides red algae with an advantage by enabling them to utilise the blue–green light spectrum more efficiently (Boney and Corner 1960).

10.6.2.3 Chlorophyta

The green algae comprise a diverse group of filamentous, colonial, motile and solitary cells within the classes Chlorophyceae, Charophyceae, Trebouxiophyceae, Ulvophyceae, Prasinophyceae, Mesostigmatophyceae (John et al. 2011). Microalgae within this lineage commonly used in research and industry include; *Chlamydomonas* spp., *Tetraselmis* spp., *Dunaliella* spp., *Lobosphaera* spp., *Botryococcus* spp., *Haematococcus* spp., *Coccomonas* spp., *Senedesmus* spp. and *Coccomyxa* spp. (Vanessa et al. 2012; Ambati et al. 2018). The accessory phycobiliproteins have been lost in Chlorophyta and replaced with chlorophyll *b*. In addition, the plastids of the *Chlorophyta* lineage contain the most diverse set of carotenoids. The CHLORO-1 pigment type comprises the basic set of carotenoids for the division; 9′-*cis*-neoxanthin, violaxanthin, lutein, zeaxanthin and β-carotene. Lutein serves as a precursor for the Prasinophyceae carotenoids; loroxanthin and its esters (PRASINO-2A), siphonaxanthin and its esters (PRASINO-2B), prasinoxanthin (PRASINO-3A) and uriolide, micromonol and micromonal (PRASINO-3B). The Trebouxiophyceae class contains CHLORO-1 plus vaucheriaxanthin esters (TREBOUX-1), while the Mesostigmatophyceae class contains CHLORO-1 plus *trans*-neoxanthin, siphonaxanthin and its ester derivatives (MESOTIG-1) (Jeffrey et al. 2011).

10.6.3 Secondary and Tertiary Endosymbiont Pigment Signatures

Eukaryotic algae descending from secondary endosymbiotic events between a primitive red algae cell and a heterotrophic host are assigned to three major supergroups: HACROBIA (Cryptophyceae and Haptophyceae), SAR (Heterokontophyceae, dinoflagellates and Chlorarachniophyceae) and euglenozoa. Microalgae within these lineages have undergone numerous gains and losses of genetic and plastid components, generating a high diversity of eukaryotic photosynthetic microalgae (Kim et al. 2014). The red algae lineage acquired their plastid through a series of secondary and tertiary endosymbiotic events. Their chemotaxonomic pigment types are defined based on the differences of chlorophyll *c* in the classes Prymnesiophyceae, Bacillariophyceae, Pelagophyceae and fucoxanthin and its derivatives within the Dinophyceae, Pelagophyceae, Dictyophyceae, Chrysophyceae, Raphidophyceae and Phaeophyceae macroalgae.

10.6.3.1 Cryptophyta

It has been proposed that Cryptophyceae and Haptophyceae monophyletic lineage defined as HACROBIA (Sakaguchi et al. 2009). However, further genome analysis has confirmed that Haptophyceae are a sister group of SAR and revealed that Cryptophyceae may represent an early diverging lineage with close relationship to plastid-lacking katablepharids with close phylogeny to Archaeplastida (Burki et al. 2012). Cryptophyceae are biflagellate flattened asymmetrical cells with a distinctive offset close to the atypical end. They are a common component of marine and freshwater phytoplankton communities. The cryptomonad pigment type CRYPT-1 comprises the definitive class-specific pigments chlorophyll *a*, chlorophyll c_2, α-carotene and the acetylene xanthophylls crocoxanthin and monadoxanthin and alloxanthin. The presence of phycobiliproteins within the thylakoid lumen gives *Chroomonas* spp. and *Rhodomonas* spp. their distinctive green and red colours, respectively.

10.6.3.2 Haptophyceae

Haptophyceae are composed of predominately marine algae known to form extensive blooms visible from space by satellite reflectance spectroscopy. They are classified by the presence of calcified scales (coccoliths) and a haptonema, although genetic analysis now also includes some strains which have lost their haptonema. Haptophyceae are divided into the classes Pavlovophyceae and Prymnesiophyceae of which commonly studied members include; *Isochrysis* spp., *Pavlova* spp. and *Emiliania huxleyi* (Andersen 2004).

Their pigment chemotaxonomy is defined based on the presence of derivatives of chlorophyll *c* and fucoxanthin resulting in 8 pigment types. HAPTO-1 and 2 are predominately associated with the class Pavlovophyceae. HAPTO-1 comprises the basic pigment signature of the group containing chlorophyll *a*, chlorophyll c_1 and c_2, fucoxanthin, diadinoxanthin, diatoxanthin and β-carotene, while the HAPTO-2 contains the additional chlorophyll c_2 *P. gyrans* pigment.

The pigments types HAPTO 3–8 are affiliated with the Prymnesiophyceae. They are defined by the variable distribution of six different chlorophyll *c* derivatives (Chl c_3, c_2, c_1, MV Chl c_3, Chl c_2-MGDG (18:4/14:0) and Chl c_2-MGDG (14:0/14:0)) and four fucoxanthin derivatives (4-ketofucoxanthin, 19′-butanoyloxyfucoxanthin, 19′-hexanoyloxyfucoxanthin and 4-keto-19′-hexanoyloxyfucoxanthin) (Zapata et al. 2004; Jeffrey et al. 2011).

10.6.3.3 Heterokontophyceae

Heterokontophyceae are a monophyletic group of microalgae comprising of 17 classes defined based on their morphology, chloroplast pigments, ultrastructural features and genomics. This large protist group contains heterotrophic and photoautotrophic microorganisms. The photoautotrophic lineage (Orchophyta) is comprised of the classes Bacillariophyceae (DIATO 1-3), Bolidophyceae (BOLIDO-1), Chrysophyceae (CHRYSO-1), Dictyochophyceae (DICTYO-1), Eustigmatophyceae (EUSTIG-1), Pelagophyceae (PELAGO-1), Raphidophyceae (RAHIDO-1), Synurophyceae (SYNURO-1) and Xanthophyceae (XANTHO-1) (Andersen 2004). As in the haptophyta lineage, the chemotaxonomy of Heterokontophyta is defined based on the presence of chlorophyll *c* and fucoxanthin derivatives with low levels of violaxanthin and zeaxanthin. The Eustigmatophyceae and Xanthophyceae classes lack fucoxanthin but contain vaucheriaxanthin and its esters.

10.6.3.4 Dinoflagellates

Dinoflagellates are a diverse group of microalgae occurring in a wide range of habitats including open ocean, marine sediments, freshwater and in symbiotic relationships with corals. They have both photoautotrophic, heterotrophic or mixotrophic lifestyles and can form cyst's under suboptimal conditions. The majority of dinoflagellates are characterised by their theca cell wall and "whirling" swimming motility. They classified into four phylogenetic orders comprising the Prorocentrales, Dinophysiales, Peridiniales and the Gymnodiniales (Tomas 1997). Species such as *Dinophysis* and *Alexandrium* biosynthesise potent biotoxins and responsible for harmful algal bloom events while others are responsible for oceanic bioluminescence (*Pyrocystis* spp. and *Gonyaulax* spp.) (Knaust et al. 1998; Dees et al. 2017). Their LHC is composed of chlorophyll *a*/*c* and the primary light-harvesting xanthophyll pigment, peridinin (DINO-1) (Jeffrey et al. 2011). Some species of dinoflagellates have acquired additional accessory pigments through tertiary endosymbiotic events between a haptophyta (*Karlodinium* spp., DINO-2), a diatom (*Durinskia* spp., DINO-3), a Cryptophyta (*Dinophysis* spp., DINO-4) or a Chlorophyta (*Lepidodinium* spp., DINO-5) (Figs. 10.2 and 10.3).

10.6.3.5 Chlorarachniophyceae and Euglenozoa

The Chlorarachniophyceae are marine amoeboid organisms who have acquired their plastids from green algae and contain a pigment signature similar to PRASINO-2A. Euglenoids are flagellate microalgae found in eutrophic aquatic environments and marine sediments. They are classified within the most basal eukaryotic supergroup, Excavata, which originated from a secondary endosymbiotic event between a heterotrophic protist and a Chlorophyta (Keeling 2013). Their

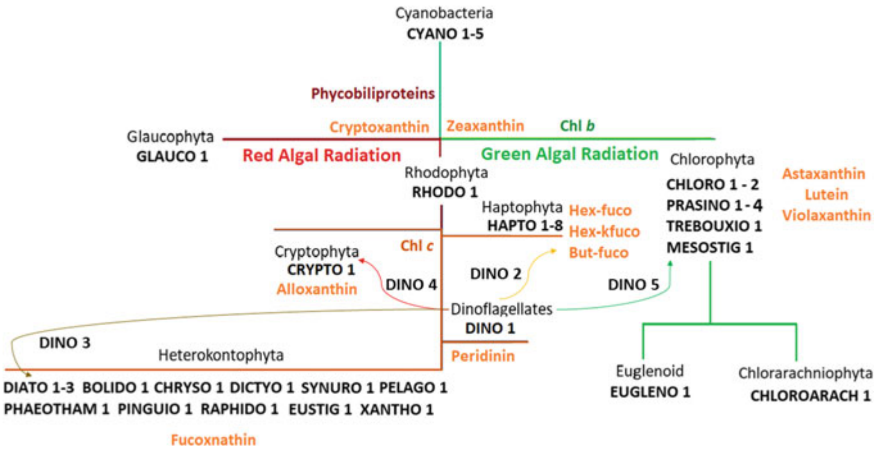

Fig. 10.3 Chemotaxonomic distribution of microalgal pigment types and their high-value pigments

pigment signature reflects their complex evolutionary history comprising of both Chromophyceae pigments (diadinoxanthin, diatoxanthin and heteroxanthin) and green algal pigments (chlorophyll *b*, 9′-*cis*-neoxanthin, loroxanthin and siphonaxanthin). They also contain species-specific xanthophylls such as eutreptiellanone associated with *Eutreptiella gymnastica* and hexadehydro-β,β-caroten-3-ol and octadehydro-β,β-carotene associated with *Euglena viridis* (Fiksdahl et al. 1984; Fiksdahl and Liaaen-Jensen 1988). *Euglena sanguinea* is capable of biosynthesising the secondary keto-carotenoids adonirubin (3%), diesters of (3S, 3′R)-adonixanthin (13%) and diesters of (3S, 3′S)-astaxanthin (75%); however, the presence of ichthyotoxins limits the commercial development of these species (Grung and Liaaen-Jensen 1993; Triemer et al. 2003).

10.7 Conclusions

Microalgal pigment chemotaxonomy provides a key diagnostic tool as part of a polyphasic taxonomic approach in the characterisation of new isolates. In particular, plastid pigment types can facilitate the identification of pico-algae which lack molecular data and can be notoriously difficult to identify by microscopy alone. Advances in HPLC techniques have increased the resolution of chemotaxonomic pigment pairs and shone a light on the diversity of pigments in microalgae. These include the distribution in the Chl-*c* and fucoxanthin derivatives within red algal radiation and lutein derivatives within the green algae radiation. The application of LC–MS to pigments analysis continues to lead to the identification of new carotenoids and chlorophylls. Similarly, molecular tools are providing new insight into carotenoid biosynthesis which could lead to improved strains for the industrial production of high-value carotenoids.

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