Chapter 6

Absorption and Transport of Inorganic Carbon in Kelps with Emphasis on *Saccharina japonica*

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Additional information is available at the end of the chapter

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Abstract

Due to the low CO_2 concentration in seawater, macroalgae including *Saccharina japonica* have developed mechanisms for using the abundant external pool of HCO_3^- as an exogenous inorganic carbon (C_i) source. Otherwise, the high photosynthetic efficiency of some macroalgae indicates that they might possess CO_2 concentrating mechanisms (CCMs) to elevate CO_2 concentration intracellularly around the active site of ribulose-1, 5-bisphosphate carboxylase/oxygenase (RuBisCo). As the photosynthetic modes of macroalgae are diverse (C3, C4 or a combination of C3 and C4 pathway), CCMs in different carbon fixation pathways should vary correspondingly. However, both in C3 and C4 pathways, carbonic anhydrase (CA) plays a key role by supplying either CO_2 to RuBisCO or HCO_3^- to PEPC. Over the past decade, although CA activities have been detected in a number of macroalgae, genes of CA family, expression levels of CA genes under different CO_2 concentrations, as well as subcellular location of each CA have been rarely reported. Based on analysis the reported high-throughput sequencing data of *S. japonica*, 12 CAs of *S. japonica* (*SjCA*) genes were obtained. Neighbor-Joining (NJ) phylogenetic tree of SjCAs constructed using Mega6.0 and the subcellular location prediction of each CA by WoLFPSORT are also conducted in this article.

Keywords: Macroalgae, Inorganic carbon uptake, C3 and C4 metabolism, Carbonic anhydrase, *Saccharina japonica*

1. Introduction

Kelps demonstrate high photosynthetic rates. According to the reports, productivity of large brown algae (e.g., *Macrocystis*, *Laminaria*, *Ecklonia*, *Sargassum*) ranges from 1000 to 3400 g $m^{-2}yr^{-1}C$ or about 3300 to 11,300 g $m^{-2}yr^{-1}$ dry weight, and red algae show a similar range of produc-

tion. Cultivated macroalgae can yield even higher values. The projected yield of cultivated *Laminaria japonica* on an annualized basis is equivalent to 1300 t ha^{-1} fresh weight or 6.5 times the maximum projected yield for sugarcane, the most productive of land plants under cultivation. In general, 45% yield of the dry weight of plants is accounted by carbon, which is assimilated in plant through Calvin cycle. The high productivities of kelps indicate their higher photosynthetic efficiency than C4 terrestrial plants [1].

The enzyme ribulose-1, 5-bisphosphate carboxylase/oxygenase (RuBisCo) is crucial in CO_2 assimilation. This bifunctional enzyme could catalyse the initial steps of photosynthetic carbon reduction and photorespiratory carbon oxidation cycles by combining CO_2 and O_2 with ribulose-1, 5-bisphosphate (RuBP) [2, 3]. RuBP carboxylation determines the net photosynthetic efficiency of photoautotrophs [4]. However, RuBisCo has a surprisingly low affinity for CO_2 and the oxygenase activity is intrinsic to RuBisCo. For kelps, the enzymatic efficiency of RuBisCo is also limited by the low concentration and diffusion coefficient of CO_2 in seawater [5]. At a natural pH of about 8, the major part of the dissolved inorganic carbon (DIC) is in the form of bicarbonate (HCO_3^-), and only about 12 μM is present as dissolved CO_2 [6], which is much lower than the half-saturation constant (Ks) of RuBisCo for CO_2 ranges from 30 μM to 60 μM in marine macroalgae [7, 8]. To support photosynthesis and growth, seaweeds require an exogenous inorganic carbon (C_i), while only CO_2 and HCO_3^- can be used as a CO_2 source for photosynthesis. Due to the low CO_2 concentration in seawater, it is not surprising that most seaweed have developed mechanisms for using the abundant external pool of HCO_3^- as an exogenous C_i source [9–11]. And it seems likely that those macrophytes that are able to use HCO_3^- would possess advantages compared with that rely solely on diffusive CO_2 entry. Here the question is how C_i is absorbed, transported to supply high CO_2 concentration around RuBisCo in kelps since unlike CO_2, HCO_3^- cannot diffuse through the lipid bilayer of the plasma membrane [12] and the produced or absorbed CO_2 are readily leaked out due to the high CO_2 permeability of cytomembrane. Otherwise, different models of photosynthesis such as C3, C4 and CAM might employ different CCMs in kelps. Thus, this review mainly focuses on the mechanisms of C_i absorption, transportation and concentration mechanisms of multicellular marine algae, including representatives of Chlorophyceae, Rhodophyceae and Phaeophyceae with different photosynthetic types.

2. Photosynthetic modes of macroalgae

As with terrestrial angiosperms where a single family may possess species with divergent photosynthetic modes [13], the marine macroalgal divisions also exhibit diversity. The photosynthetic carbon fixation pathways of marine macrophytic algae generally follow that of C3 plants [14]. However, for certain genera, a number of studies have shown photosynthesis to possess C4-like photosynthetic characteristics, including the high phosphoenolpyruvate carboxykinase (PEPCK) activity with low phosphoenolpyruvate carboxylase (PEPC) activity, little photorespiration and the labelling of malate and aspartate as an early product of carbon

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fixation. Based on this, it has been suggested that these macroalgae are of the C4 type, or a combination of C3 and C4, type [15–17], although Kremer and Küppers [18] had contradicted the decision whether a species is a C4 plant or not based only on chromatographic and enzymatic analysis. In recent decades, our understanding of the possible metabolic pathways of macroalgae has been extended with using the available sequencing resources and molecular technologies and applying molecular approaches. Reiskind et al. [19] reported that a limited C4-like system in the green alga *Udotea* with the high PEPCK activity and low PEPC activity was a novel characteristic. Whereafter, Reiskind and Bowes [20] found that when PEPCK activity was inhibited *in vivo* with 3-mercaptopicolinic acid, thallus photosynthesis was decreased by 70% and the labelling of early photosynthetic products such as malate and aspartate was reduced by 66% and thus provided new evidences for the existence of C4 acid metabolism in this green alga. In contrast to *Udotea*, *Codium*, a macroalga closely related to *Udotea*, exhibits gas exchange characteristics that resemble terrestrial C3 plants, and neither C4 acids nor PEPCK plays a part in photosynthesis [19]. This demonstrates the diversity of photosynthetic mechanisms in the Chlorophyta. *Ulva*, a common green seaweed, was previously reported as a typical C3 plant based on some biochemical evidences that 3-phosphoglyceric acid (3-PGA) was the main primary product formed photosynthetically and a high RuBPcase/PEPcase ratio was found in it [21], while, recently, it was reported that *Ulva* possessed rather comprehensive carbon fixation pathways including C3, C4 and CAM mechanisms because key genes of enzymes involved in these photosynthetic modes were got from the expressed sequence tag (EST) using Kyoto encyclopedia of genes and genomes (KEGG) [22]. Recently, C4-like carbon fixation pathway was also found in representatives of Rhodophyceae and Phaeophyceae based on the analysis of ESTs or transcriptomes. In red algae, Fan et al. [23] speculated that the sporophyte of *Pyropia haitanensis* most likely possesses a C4-like carbon fixation pathway since genes of the key enzymes in the PCK-type C4 carbon-fixation pathway were abundantly transcribed. Wang et al. [24] assumed that a C4-like carbon-fixation pathway might play a special role in fixing inorganic CO_2 in *Porphyra yezoensis* with the evidence that except pyruvate-phosphate dikinase all genes involved in C4-pathway were discovered from the transcriptome. Xu et al. [25] had reported that PEPCK, an important enzyme in carbon fixation in C4 plants, had very high activity in the sporophyte of *L. japonica*. Besides, haploid gametophytes and diploid sporophytes of some marine macroalgae with dimorphic life cycles might even employ different photosynthetic mode. Wang et al. [24] found that both the RuBisCo content and the initial carboxylase activity were notably higher in gametophytes than in the sporophytes of four seaweed species — *P. yezoensis*, *P. haitanensis*, *Bangia fuscopurpurea* (Rhodophyte) and *L. japonica* (Phaeophyceae). They assumed that in the sporophyte of these algae, the major carbon fixation pathway may be a C4-like carbon fixation pathway, and thus a high abundance of RuBisCo would not be necessary for the sporophytes. And for *L. japonica*, the higher RuBisCo content and activity in gametophyte was corresponding to the lower photosynthetic rate, which implied there might be a greater difference between sporophytes and gametophytes of this alga in their photosynthetic mode. Conclusively, the existence of C4-like pathway in macroalgae has been verified using more evidence, while the distribution between C3 and C4 pathways was unknown during growth of macroalgae with comprehensive carbon fixation pathways including C3 and C4.

In C3 and C4 metabolisms, CO_2 is the substrate of RuBisCo and assimilated through the Calvin cycle. In this cycle, CO_2, catalysed with RuBisCo, combines with RuBP to form two molecules of 3-PGA. PGA is reduced to triose. RuBisCo, a bifunctional enzyme, may catalyse the combination of RuBP and CO_2 for photosynthetic carbon reduction or may combine with O_2 for C_2 photorespiration [3]. The ratio of CO_2 to O_2 around RuBisCo is a major factor for the enzyme to choose the photosynthetic carbon reduction or C_2 photorespiration carbon oxidation [26]. The low CO_2 concentration around RuBisCo may not only impose restrictions on photosynthesis but also cause permanent light injuries to photosynthetic organelle [27–29]. The speciation of DIC (C_i) is pH dependent. Above pH 4.5, the proportion occurring as CO_2 (aq) decreases and HCO_3^- increases, while above pH 8.3, the bicarbonate equivalence point, the equilibrium begins to shift towards carbonate (CO_3^{2-}). In the upper layer of the oceans, HCO_3^- ions predominate, and the dissolved CO_2 represents only about 1% of the total dissolved carbon with a concentration of about 21 μM [30]. The *K*m (CO_2) value of RuBisCO is significantly higher than this, having been reported as being as high as 200 μM in some cyanobacteria [31]. To survive under the selective pressure of low CO_2 concentration, high permeability of CO_2 for plasma membrane and low affinity of CO_2 for RuBisCo, many algae, including macroalgae living in the subtidal zone, have evolved with inorganic CCM that allows them to overcome this potentially limiting shortage of CO_2 [9, 32–36]. So, the productivity of most macroalgae is not currently considered limited by DIC. Unlike terrestrial C4 plants possessing Kranz anatomy to prevent futile recycling of CO_2 by segregating the initial carboxylation and decarboxylation reactions in different cells, macroalgae concentrate CO_2 internally, which is mediated by C_i transporters at the plasma membrane or chloroplast envelope and CA. As for carboxylases are different between C3 and C4 metabolism, C_i acquisition, transportation and concentration mechanisms might be diverse.

Based on a series of reports on the presence of CCM in blue-green algae and *Chlamydomonas* (*Chlamydomonas reinhardtii*) and some other microalgae [37–40], Badger [41] reported that the CCM of algae possess at least three functional elements: (1) the transportation of the C_i dissolved in seawater into cells in the form of CO_2 and/or HCO_3^-; (2) the accumulation of the C_i in cells in the form of HCO_3^-, forming pools of the dissolved C_i and (3) the delivery of CO_2 to the periphery of RuBisCo from such pools.

3. Inorganic carbon absorption mechanisms of macroalgae

The methods of CO_2 and/or HCO_3^- absorption of macroalgae cells (Figure 1) include the following: (1) non-CCM macroalgae (that do not possess or use CCM) rely exclusively on diffusive uptake of CO_2, (2) CCM macroalgae uptake of C_i, as CO_2 and/or HCO_3^- via mechanisms of the external carbonic anhydrase (CA_{ext}) mechanism, the anion exchange (AE) transport mechanism, the plasma membrane associated with H^+-ATPase mechanism and passive transport of CO_2 by diffusion. In the first mechanism, HCO_3^- in the periplasmic space is converted to CO_2 at the presence of CA_{ext}, an enzyme that is located in the cell wall in the

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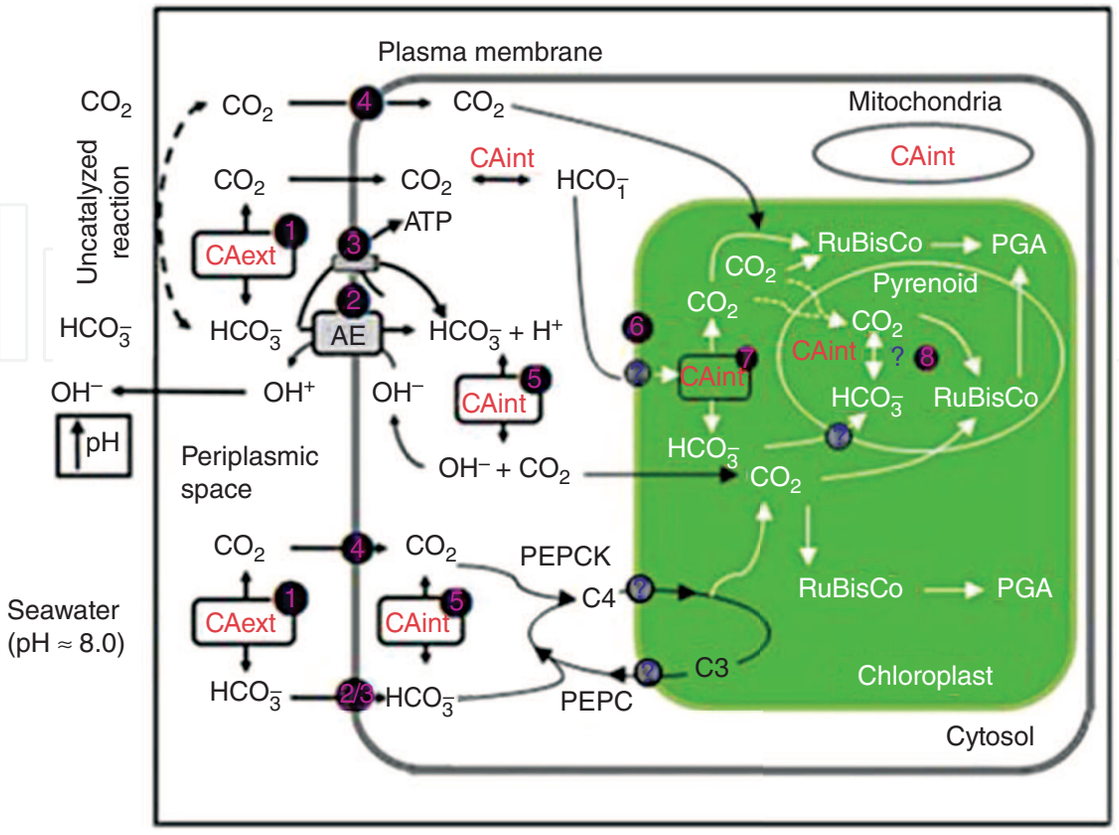

Figure 1. A schematic diagram on the photosynthetic carbon physiology of some macroalgae revised from [45].

majority of seaweeds and could be inhibited by the membrane impermeable acetazolamide (AZ), and then the resulting CO_2 is readily taken into the cell by passive diffusion. This seems to be the most prevalent for HCO_3^- utilization among seaweeds [42, 43], but it may be non-functional under high pH (>9.00) [44, 45]. The AE transport mechanism is HCO_3^- direct uptake through the AE protein in plasma membrane [11, 43, 46–48], which is 4,4'-diisothiocyanostilbene-2,2'-disulfonate (DIDS) sensitive. This operates equally well at pH 8.4 and 9.4 [44, 45]. H^+-ATPase mechanism refers to a plasma membrane associated H^+-ATPase pump that extrudes the excess cellular H^+ to the outside of the plasma membrane facilitating a H^+/HCO_3^- co-transportation or enhancement of the external uncatalysed dehydration of HCO_3^- to CO_2 in the periplasmic space [49]. However, this has only been reported in some Laminariales such as *S. latissima* and *L. digitata*. Along with the uptake of CO_2 and/or HCO_3^-, the internal charge balance (OH^-/H^+) will be absolutely changed. To maintain intracellular ion balance, macroalgae employ diverse strategies. In AE mechanism, the active transport of HCO_3^- into the cell might result in an outward flux of OH^- [50–53, 45] as this mechanism is involved in a one-for-one exchange of anions across the plasma membrane. The OH^- efflux can increase H^+ in the cell [52]. To maintain the intracellular OH^-/H^+ balance, H^+ extrusion might be required. In macroalgae possessing H^+-ATPase mechanism, their plasma membrane associated with H^+-

ATPase pump might extrude excess cellular H^+ to the outside of the plasma membrane, while in macroalgae that do not have H^+-ATPase pump in their plasma membrane, the regulation of intracellular ion balance might be related to a high activity of internal carbonic anhydrase (CA_{int}), including the CA in cytoplasm, chloroplast stroma, thylakoid lumen and mitochondria [45].

The extent to which marine macroalgae are able to acquire HCO_3^- for photosynthesis varies among taxa and/or species, and the special strategies by which the alga acquire C_i is closely related to habitat including pH and depth, conferring as adaptation advantage to the alga [9, 33, 36, 54–56]. Cornwall et al. [57] reported when light is low, CCM activity of macroalgae is reduced in favour of diffusive CO_2 uptake and the proportion of non-CCM (diffusive uptake of CO_2) species increased with depth. Otherwise, pH might also control C_i use by macroalgae. In *U. lactuca*, the CA_{ext}-mediated mechanism is the main method of HCO_3^- utilization under normal pH conditions, whereas when they were grown at high pH, direct uptake of HCO_3^- via a DIDS-sensitive mechanism can be induced [44]. Similar HCO_3^- utilizing mechanisms were found in another green macroalgae *Enteromorpha intestinalis* [54]. For the red alga *Gracilaria gaditana*, the HCO_3^- use is also carried out by the two DIC uptake mechanisms, in which the indirect use of HCO_3^- by an external CA activity being the main pathway and the potential contribution to HCO_3^- acquisition by the DIDS-sensitive AE mechanism was higher after culturing at a high pH [58]. However, these two mechanisms do not occur simultaneously, and the DIDS-sensitive mechanism is induced only under high pH. *Solieria filiformis*, another red marine macroalgae, in which the general form of C_i transported across the plasma membrane is CO_2, but HCO_3^- acquisition takes place simultaneously between CA_{ext} mechanism and direct uptake [59]. CA_{ext} mechanism is also the main pathway for DIC acquisition for the species of Phaeophyta. *S. latissima* mainly uses CA_{ext} mechanism for HCO_3^- absorption, since when AZ is used to treat *S. latissima*, its photosynthetic efficiency drops by 80% [11]. Otherwise, *S. latissima* also has a H^+-ATPase mechanism, of which the proton pump may support the antiport of H^+/HCO_3^- or the discharge of H^+, creating an acid environment in the periplasmic space and causing the dehydration of HCO_3^- into CO_2 with CA to quickly diffuse into cells [49]. *S*imilar to *S. latissima*, *L. digitata* also has a CA_{ext} mechanism of absorbing HCO_3^- and a P-H^+-ATPase mechanism [49]. Gametophytes of *Ectocarpus siliculosus* utilize the CA_{ext} mechanism and the HCO_3^- transport protein [60] on the cell membrane to absorb HCO_3^-. *Macrocystis pyrifera* utilizes the CA_{ext} mechanism and the AE protein mechanism to absorb HCO_3^-, in which the main mechanism of HCO_3^- uptake is via AE protein and CA_{ext} contributes little [45]. For *Sargassum henslowianum*, like most seaweed, the main Ci acquisition strategy is also CA_{ext} metabolism, since its photosynthetic O_2 evolution could be drastically depressed by AZ at pH 8.1 (i.e., the normal seawater pH value) and at pH 9.0. And direct uptake for HCO_3^- via DIDS-sensitive AE protein mechanism was unlikely to be present in C_i acquisition of this kelp, because the photosynthesis in either blade or receptacle tissue of this alga was not affected by DIDS [61]. For *Hizikia fusiformis*, CA_{ext}+ diffusive uptake of CO_2 could support its metabolic

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requirements sufficiently since there is no known other active C_i transport mechanisms [62]. For *S. japonica*, Yue et al. [63] found that the C_i absorption of the CA_{ext} mechanism in its juvenile sporophytes accounts for 75% of the total C_i absorption in algae cells, whereas free CO_2 absorption accounts for 25% only.

Thus, the CA_{ext} mechanism plays an important role in the CCM macroalgae absorption and the utilization of the relatively abundant HCO_3^- in seawater.

4. C_i transition process in CCMs of macroalgae

C_i acquisition mechanisms are extensively studied and well-known in microalgae [44, 38]. For instance, regardless of the C_i form (CO_2 or HCO_3^-) taken up by the microalga *C. reinhardtii*, HCO_3^- is the primary form accumulated into the cell to prevent CO_2 leakage [38]. In macroalgae, most C_i use processes are speculated based on some biochemical evidence. For C3 photosynthesis, the CO_2 that entered the cytoplasm is transformed into HCO_3^- under the catalytic action of CA in the cytoplasm and stored in the cytoplasm [38] to maintain the equilibrium of different forms of C_i and to regulate the pH value of the cytoplasm [26, 38]. The HCO_3^- in the cytoplasm enters the chloroplast stroma via the C_i transport protein on the chloroplast membrane, and the CO_2 in the cytoplasm directly enters the stroma via the chloroplast membrane. In diatom *Phaeodactylum tricornutum*, genes with homology to bicarbonate transporters from SLC4 and SLC6 families, two HCO_3^- transporters studied thoroughly in human, were got from its genome and one of these SLC4-type HCO_3^- transporters has recently been confirmed to function as a Na^+-dependent HCO_3^- transporter on the outer membrane [64, 65]. However, the molecular nature of HCO_3^- transporters of macroalgae is unknown now, and their similarity to those found in diatoms is uncertain. The transportation of C_i from the cytoplasm to the chloroplast is the major C_i flux in the cell and the primary driving force for the CCM. This flux drives the accumulation of C_i in the chloroplast stroma and generates a CO_2 deficit in the cytoplasm, inducing CO_2 influx into the cell. Given that the pH value of the chloroplast stroma is closer to 8, the stroma C_i is mostly enriched in the form of HCO_3^-, forming C_i pools [66]. In macroalgae, which have pyrenoids, HCO_3^- is putatively carried into the thylakoid by the C_i transport protein on the thylakoid membrane, forming CO_2 in the thylakoid space under the catalytic action of thylakoid CA [67, 68]. The thylakoid membrane partially sinks into the pyrenoids [69], where the diffused CO_2 is quickly fixed by the RuBisCo in the pyrenoids. The diffused CO_2 from the thylakoid space outside the pyrenoids or the unfixed CO_2 leaked from the pyrenoids is transformed into HCO_3^- under the action of CA in the starch sheath on the periphery of the pyrenoid, thus increasing the number of HCO_3^- pools in the matrix [70]. For macroalgae without pyrenoids, such as *L. japonica*, HCO_3^- entered the chloroplast stroma after being dehydrated under the action of chloroplast stroma CA and provided CO_2 for the RuBisCo in the matrix (Figure 1).

For C4 photosynthesis, CA is required to convert CO_2 to HCO_3^- in the cytosol, and thus supply PEPC with substrate. HCO_3^- will be fixed into malate. For non-PEPC algae with PEPCK, the CO_2 entering the cytoplasm will be directly fixed in the form of four-carbon acid [71]. The produced four-carbon acid may be transported into the mitochondria, forming pyruvate after decarboxylation and CO_2 release, which is fixed in the form of carbohydrate in the Calvin cycle. In fact, the presence of CA in C4 plants has been suggested to accelerate the rate of photosynthesis in C4 plants 10^4-fold over what it would be if this enzyme were absent [72].

In conclusion, CA (CA_{ext}+CA_{int}) is essential for the reversible HCO_3^-–CO_2 conversion both in the cell and in the periplasm. They participate in photosynthesis by supplying either CO_2 to RuBisCO or HCO_3^- to PEPC for C4 type.

5. Carbonic anhydrase

CAs are metalloenzymes that catalyse the reversible interconversion of CO_2 and HCO_3^- [73]. They are encoded by six evolutionary divergent gene families and the corresponding enzymes are designated as α, β, γ, δ, ε and ζ-CA [39]. These six types of CAs share no sequence similarity in their primary amino acid sequences and seem to have evolved independently [26, 74]. In macroalgae, almost all known CAs belong to α, β and γ classes, with the β class predominating [26, 39]. The δ, ε and ζ classes of CA are found only in some diatoms [75], bacteria [76] and marine protists [77, 78]. The active site of CA contains a zinc ion (Zn^{2+}), which plays a critical role in the catalytic activity of the enzyme. The ζ and γ classes of CAs represent exceptions to this rule since they can use cadmium (ζ), iron (γ) or cobalt (γ) as cofactors [79–81]. CA plays an important role in photosynthesis by supplying either CO_2 to RuBPCO or HCO_3^- to PEPC. They also participate in some other physiological reactions such as respiration, pH homeostasis, ion transport and catalysis of key steps in the pathways for the biosynthesis of physiologically important metabolites [41]. The CA synthesis in the cytoplasm [82] is located in the periplasmic space, mitochondria, chloroplast stroma and chloroplast thylakoid lumen, carboxysome and pyrenoid [66, 70, 83, 84]. Different subcellular localizations make different CA functions in CCM. Periplasmic CA (CA_{ext}) can catalyse the conversion of HCO_3^- into CO_2 to promote the diffusion of CO_2 at the cell surface across the plasma membrane [85, 86]. Therefore, CA_{ext} has been postulated to be part of the CCM in most macroalgae. The cytoplasm CA stores Ci in the form of HCO_3^- to avoid leakage of CO_2 and to regulate the pH value of cytoplasm by maintaining the equilibrium of different forms of Ci, which is important for algal CCM [39]. CAs on the chloroplast membrane and in the stroma mainly provide CO_2 for RuBisCo [26, 38, 87]. In cyanobacteria, CAs in the carboxysomal shell function to convert accumulated HCO_3^- into CO_2 and pass it to RuBisCo inside the cytoxysome [88]. CA in the thylakoid lumen was proposed to function to create an efficient CO_2 supply to RuBisCo by taking advantage of the acidity of the lumenal compartment [69]. Stromal CA is also thought to operate by converting leaking CO_2 into HCO_3^- [70]. Recently, data provided by various genome sequencing studies have revealed the multiplicity of CA isoforms in algae. For

example, in the model microalga *C. reinhardtii*, there are at least 12 genes that encode CA isoforms, including three α, six β and three γ or γ-like CAs [39]. For marine diatom, nine and thirteen CA sequences were found in the genomes of *P. tricornutum* and *Thalassiosira pseudonana*, respectively [89]. *P. tricornutum* contains two β-CA genes, five α and two γ CA genes, whereas *T. pseudonana* has three α-, five γ-, four δ- and one ζ-CA genes [89]. As for macroalgae, CA genes have only been reported in few species. Six full-length CA of *P. haitanensis* (PhCA) genes were reported, which include two α-CAs, three β-CAs and one γ-CA [90]. Besides, one β-CA and one α-CA were reported in *P. yezoensis* [91] and *S. japonica* [92, 93]. Otherwise, although the activity of CA_{ext} and CA_{int} has been detected in many macroalgae, the subcellular localization and functions of CA_{ext} and CA_{int} remain unclear [71, 93].

Conclusively, CAs, including CA_{ext} and CA_{int} (Figure 1), play an important role in the transportation or concentration process of the C_i. And as for C3 and C4 metablisms have different carboxylase, CAs might play different roles in CCMs of macroalgae with different photosynthetic mode. Thus, isolating of the *CA* genes, studies on their expression levels in different CO_2 concentrations, in different life phase, and under different environmental stress, as well as studies on subcellular locations of CAs should be conducted in macroalgae to help reveal their Ci assimilation processes.

6. Studies of *S. japonica* CCM

S. japonica is an economically important brown seaweed. It has been cultivated extensively for food and industrial alginate in East Asia, such as in China, Japan and South Korea. China is by far the largest producer, and in 2009, its production in China rose sharply to 4.14×10^9 kg wet weight [94], accounting for approximately 80% of the global production, over several decades. This has been attributed to both its large-scale farming and high kelp yield per unit area. Production of this kelp in China under natural conditions is within the range of 3,300 to 11,300 g dry matter $m^{-2}\cdot year^{-1}$, whereas that under artificial conditions is higher [1]. For example, its production during the 7-month cultivation is 15,000 g dry matter m^{-2} area (equivalent to 150 t per ha), which is 2.8 times higher than the maximum productivity of sugarcane in the United States (fresh weight about 95 t per ha·year) [1], which indicates that *S. japonica* has higher photosynthetic efficiency than sugarcane and other C4 plants. In fact, the photosynthetic efficiency of macroalgae (e.g., kelp) is 6%–8%, which is 1.8%–2.2% higher than that of land plants [95]. In seawater, the dominant species of Ci is HCO_3^- [11]. Since there is a fairly high photosynthetic rate in these kelps [34], a CCM involving an efficient HCO_3^- utilization mechanism is expected to exist. Indeed, 75% of the total C_i absorption in the juvenile sporophytes of this kelp is via the CA_{ext} mechanism [63], whereas CO_2 diffusion accounts for 25% only. By analysis of genome annotation data of *S. japonica* [96], all the essential genes related to C3-pathway (23 unigenes) were discovered (Table 1), which provided the unequivocal molecular evidence that there existed C3-pathway in *S. japonica*. Otherwise, 16 enzyme-encoding unigenes involved in C4-pathway were found, covering almost all enzymes needed

for C4-carbon fixation except the malic enzyme (Table 1). The results helped us to understand the carbon fixation process of this species.

Photosynthesis modes	Enzyme names	Unigenes
C3-pathway		23
	Glyceraldehyde-3-phosphate dehydrogenase (phosphorylating) (GAPDH)	4
	Transketolase	1
	Phosphoribulokinase	2
	Phosphoglycerate kinase (PGK)	5
	Fructose-1,6-bisphosphatase (FBPase)	1
	Sedoheptulose-bisphosphatase (SBPase)	3
	Fructose-bisphosphate aldolase	1
	Ribulose-phosphate 3-epimerase	2
	Triose-phosphate isomerase (TIM)	1
	Ribose-5-phosphate isomerase	1
	Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo), small	1
	Ribulose-1,5-bisphosphate carboxylase/oxygenase(RuBisCo), large	1
C4-pathway		16
	Malate dehydrogenase	4
	Aspartate aminotransferase (AST)	4
	Pyruvate kinase	4
	Phosphoenolpyruvate carboxylase (PEPC)	1
	Phosphoenolpyruvate carboxykinase (PEPCK)	1
	Pyruvate phosphate dikinase	1
	Arginine/alanine aminopeptidase	1
Total		39

Table 1. Statistics of C3/C4-pathway related enzymes of *S. japonica*.

Considering CAs play key roles in CCMs of macroalgae, it is important to determine the numbers and characterizations of CA genes of *S. japonica*. Herein, based on unigene sequences [96], the high-throughput sequencing data of *S. japonica* [97, 98] and *S. latissima* [99], as well as combined with the preparatory work of our group [92, 93], 12 CAs of *S. japonica* (*SjCA*) genes were obtained. Among them, we have cloned the full-length complementary DNA (cDNA) sequences of SjαCA1, SjβCA1 and SjβCA2 using rapid amplification of cDNA ends, which are 2804 [94], 1291 and 1261 nucleotides, respectively. The encoded proteins were 290, 314 and 307 amino acids. For further analysis the gene subtypes of CAs, a phylogenetic tree was constructed

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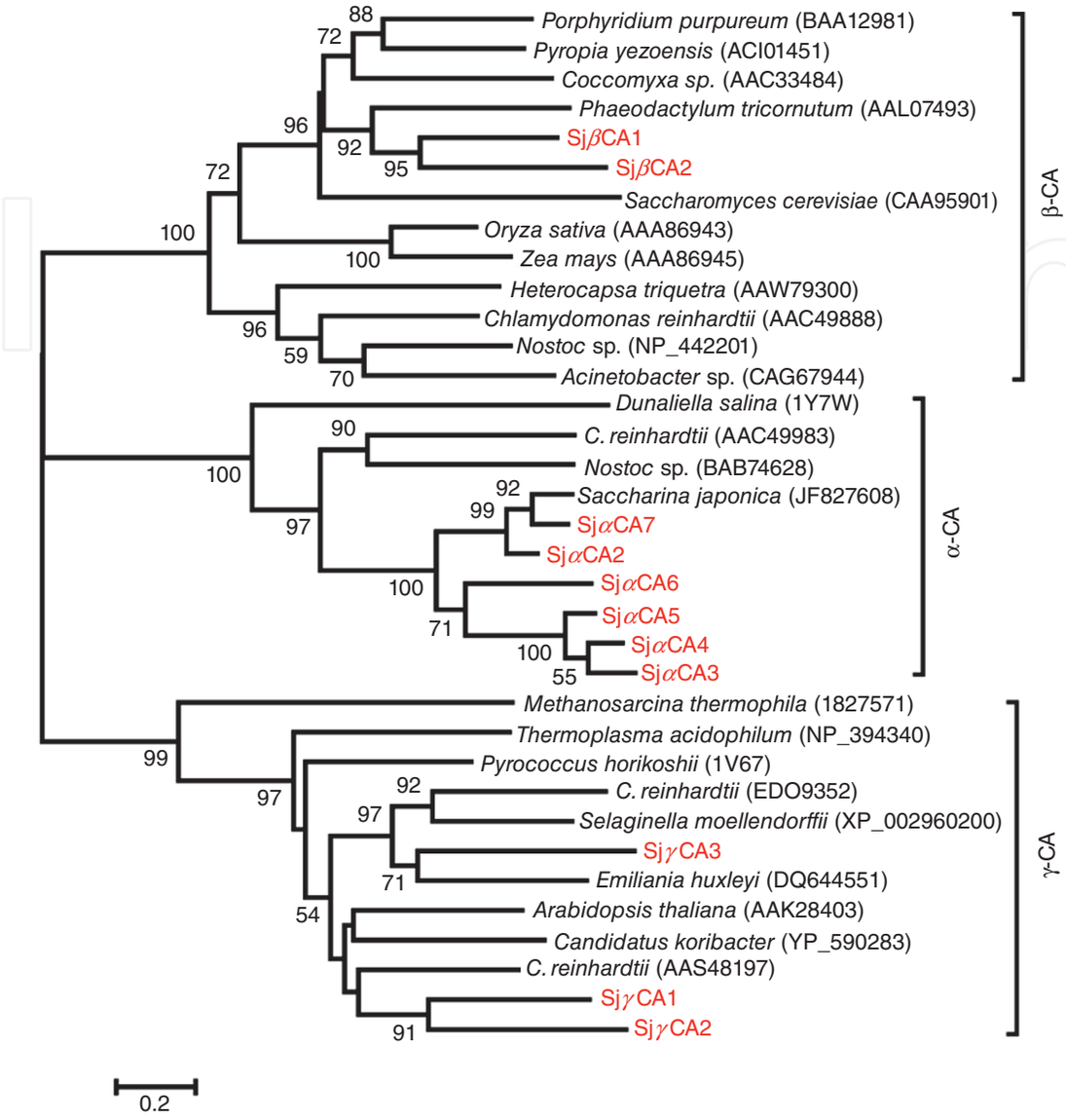

Figure 2. Phylogenetic tree constructed using SjCA amino acid sequences.

by using the neighbour-joining algorithm of the MEGA6.0 software [100] with Poisson correction and pairwise deletion parameters. A total of 1000 bootstrap replicates were performed. On the basis of conserved motifs and phylogenetic tree analysis (Figure 2), the *SjCA*s were divided into three CA classes: from SjαCA1 to SjαCA7 are α-CA; SjβCA1 and SjβCA2 are β-CA; SjγCA1, SjγCA2 and SjγCA3 are γ-CA. Among them, only one α-CA (SjαCA1) has been localized in the chloroplast and thylakoid membrane of the gametocytes of *S. japonica* under immunogold electron microscopy [93]. To get a general idea of functions of each SjCA, herein, the subcellular localizations of SjCAs were predicted using WoLFPSORT (http://www.genscript.com/wolf-psort.html). Based on the predicted results (Table 2), SjαCA2 might be an external CA and exist in periplasmic space, SjαCA3; SjαCA4, SjαCA6, SjαCA7 and SjγCA1 might be cytoplasmic CA; SjαCA5, SjβCA2 and SjγCA2 might present in mito-

chondria; SjβCA1 and SjγCA3 might exist in chloroplasts. However, most of the SjCAs' subcellular localizations are predicted, which need to be verified by further studies. Otherwise, sporophyte and gametophyte of this kelp might employ different carbon fixation process since the content and activity of RuBisCo enzyme in gametophyte are significantly higher than those in sporophyte implying they may have different types of photosynthetic metabolism [24]. As for CA might play different role in CCMs of C3 and C4 pathway, full-length cDNA as well as DNA sequences of each SjCA should be cloned from sporophytes and gametophytes of this kelp in the future studies. CA gene expression levels under different CO_2 concentrations and the subcellular location of each CA should also be conducted to help reveal C_i assimilation process of *S japonica*.

Enzyme	Gene ID[a]	AA no.	Full length (Y/N)	Subcellular location prediction
SjαCA1	JF827608	290	Y	Chloroplast and thylakoid membrane [93]
SjαCA2	SJ07762	205	N	Secreted
SjαCA3	SJ07765	160	N	Cytoplasmic
SjαCA4	SJ13238	151	N	Cytoplasmic
SjαCA5	SJ13240	294	N	Mitochondrial inner membrane
SjαCA6	SJ18135	257	N	Cytoplasmic
SjαCA7	SJ18141	189	N	Cytoplasmic
SjβCA1	SJ12311	314	Y	Chloroplast thylakoid membrane
SjβCA2	SJ17783	307	Y	Mitochondrial
SjγCA1	SJ07587	305	N	Cytoplasmic
SjγCA2	SJ22175	161	N	Mitochondrial
SjγCA3	SJ21158	246	N	Chloroplast

Abbreviation: *AA,* amino acid.
[a] JF827608 is the NCBI gene accession number; 'SJ' in the table stands for the gene IDs for *S. japonica.*

Table 2. Prediction of subcellular locations of SjCAs.

The completion of the CCM modelling of sporophyte and gametophyte in *S. japonica* will give a solid foundation for further exploring its highly efficient photosynthetic mechanism. In addition, conducting studies on the inorganic carbon metabolism of macroalgae is of positive significance on developing the biomass energy from kelp and other algae and slowing down seawater acidification and global warming.

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References

[1] Gao K, McKinley KR. Use of macroalgae for marine biomass production and CO_2 remediation: a review. *Journal of Applied Phycology*. 1994; 6(1): 45–60. DOI: 10.1007/BF02185904

[2] Berry JA, Osmond CB, Lorimer G. Fixation of CO_2 during photorespiration. *Plant Physiology*. 1978; 62: 954–967.

[3] Mizohata E, Matsumura H, Okano Y. Crystal structure of activated ribulose-1,5-bisphosphate carboxylase/oxygenase from green alga *Chlamydomonas reinhardtii* complexed with 2-carboxyarabinitol-1,5-bisphosphate. *Journal of Molecular Biology*. 2002; 316: 679–691. DOI: 10.1006/jmbi.2001.5381

[4] Jensen GR, Bahr TJ. Ribulose 1,5-bisphosphate carboxylase-oxygenase. *Annual Review of Plant Physiology*. 1977; 28: 379–400. DOI: 10.1146/annurev.bi.52.070183.002451

[5] Reinfelder JR. Carbon concentrating mechanisms in eukaryotic marine phytoplankton. *Annual Review of Marine Science*. 2011; 3: 291–315. DOI: 10.1146/annurev-marine-120709-142720

[6] Stumm W, Morgan JJ. Aquatic Chemistry: Chemical Equilibria and Rates in Natural Waters. 3rd ed. New York: Wiley; 1996. 1022 p.

[7] Kerby NW, Raven JA. Transport and fixation of inorganic carbon by marine algae. *Advances in Botanical Research*. 1985; 2: 71–123.

[8] Cook CM, Colman B. Some characteristics of photosynthetic inorganic carbon uptake of a marine macrophytic red alga. *Plant, Cell and Environment*. 1987; 10: 275–278. DOI: 10.1111/1365-3040.ep11602301

[9] Maberly SC. Exogenous sources of inorganic carbon for photosynthesis by marine macroalgae. *Journal of Phycology*. 1990; 26(3): 439–449. DOI: 10.1111/j.0022-3646.1990.00439.x

[10] Larsson C, Axelsson L. Bicarbonate uptake and utilization in marine macroalgae. *European Journal of Phycology*. 1999; 34: 79–86. DOI: 10.1080/09670269910001736112

[11] Axelsson L, Mercado JM, Figueroa FL. Utilization of HCO_3^- at high pH by the brown macroalga *Laminaria saccharina*. *European Journal of Phycology*. 2000; 35(1): 53–59.

[12] Gutknecht JM, Bisson A, Tosteson FC. Diffusion of carbon dioxide through lipid bilayer membranes. Effects of carbonic anhydrase, bicarbonate, and unstirred layers. *Journal of General Physiology*. 1977; 69: 779–794. DOI: 10.1085/jgp.69.6.779

[13] Edwards GE, Huber SC. The C4 pathway. In: Hatch MD, Boardman NK, editors. The Biochemistry of Plants. A Comprehensive Treatise. Vol. 8, Photosynthesis. New York: Academic Press; 1981. p. 237–281.

[14] Kremer BP. Aspects of carbon metabolism in marine macroalgae. *Oceanography and Marine Biology: An Annual Review*. 1981; 19: 41–94.

[15] Johsi GV, Karekar MD, Gowda CA. Photosynthetic carbon metabolism and carboxylating enzymes in algae and mangrove under saline conditions. *Photosynthetica*. 1974; 8: 51–52.

[16] Karekar MD, Joshi GV. Photosynthetic carbon metabolism in marine algae. *Botanica Marina*. 1973; 16(4): 216–220. DOI: 10.1515/botm.1973.16.4.216

[17] Patil BA, Joshi GV. Photosynthetic studies in *Ulva lactuca*. *Botanica Marina*. 1970; 13(2): 111–115. DOI: 10.1515/botm.1970.13.2.111

[18] Kremer BP, Küppers U. Carboxylating enzymes and pathway of photosynthetic carbon assimilation in different marine algae—Evidence for the C4-pathway? *Planta*. 1977; 133(2): 191–196. DOI: 10.1007/BF00391918

[19] Reiskind JB, Seamon PT, Bowes G. Alternative methods of photosynthetic carbon assimilation in marine macroalgae. *Plant Physiology*. 1988; 87(3): 686–692. DOI: 10.1104/pp.87.3.686

[20] Reiskind JB, Bowes G. The role of phosphoenolpyruvate carboxykinase in a marine macroalga with C4-like photosynthetic characteristics. *Proceedings of the National Academy of Sciences of the United States of America*. 1991; 88(7): 2883–2887. DOI: 10.1073/pnas.88.7.2883

[21] Beer S, Israel A. Photosynthesis of *Ulva* sp III. O_2 effects, carboxylase activities, and the CO_2 incorporation pattern. *Plant Physiology*. 1986; 81(3): 937–938. DOI: 10.1104/pp.81.3.937

[22] Niu J, Hu H, Hu S. Analysis of expressed sequence tags from the *Ulva prolifera* (Chlorophyta). *Chinese Journal of Oceanology and Limnology*. 2010; 28: 26–36. DOI: 10.1007/s00343-010-9120-4

[23] Fan XL, Fang YJ, Hu SN. Generation and analysis of 5318 expressed sequence tags from the filamentous sporophyte of *Porphyra haitanensis* (Rhodophyta). *Journal of Phycology*. 2007; 43(6): 1287–1294. DOI: 10.1111/j.1529-8817.2007.00415.x

[24] Wang C, Fan X, Wang G. Differential expression of RuBisCo in sporophytes and gametophytes of some marine macroalgae. *PloS One*. 2011; 6(1): e16351. DOI: 10.1371/journal.pone.0016351

[25] Xu ZM, Yao NY, Li JZ. Studies on the activity of PEPck in *L. japonica*. *Marine Science*. 1991; 2: 41–45.

[26] Moroney JV, Bartlett SG, Samuelsson G. Carbonic anhydrases in plants and algae. *Plant, Cell & Environment*. 2001; 24(2): 141–153. DOI: 10.1111/j.1365-3040.2001.00669.x

[27] Krause GH. Photoinhibition of photosynthesis. An evaluation of damaging and protective mechanisms. *Physiologia Plantarum*. 1988; 74(3): 566–574. DOI: 10.1111/j.1399-3054.1988.tb02020.x

[28] Hanelt D, Huppertz K, Nultsch W. Photoinhibition of photosynthesis and its recovery in red algae. *Botanica Acta*. 1992; 105(4): 278–284. DOI: 10.1111/j.1438-8677.1992.tb00299.x

[29] Hanelt D, Huppertz K, Nultsch W. Daily course of photosynthesis and photoinhibition in marine macroalgae investigated in the laboratory and field. *Marine Ecology Progress Series*. 1993; 97(1): 31–37. DOI: 10.3354/meps097031

[30] Roleda MY, Hurd CL. Seaweed responses to ocean acidification//Seaweed Biology. *Springer Berlin Heidelberg*. 2012; 219: 407–431. DOI: 10.1007/978-3-642-28451-9_19

[31] Moroney J V, Somanchi A. How do algae concentrate CO_2 to increase the efficiency of photosynthetic carbon fixation? *Plant Physiology*. 1999, 119(1): 9–16. DOI: 10.1104/pp.119.1.9

[32] Cook CM, Lanaras T, Colman B. Evidence for bicarbonate transport in species of red and brown macrophytic marine algae. *Journal of Experimental Botany*. 1986; 37(7): 977–984. DOI: 10.1093/jxb/37.7.977

[33] Surif MB, Raven JA. Exogenous inorganic carbon sources for photosynthesis in seawater by members of the Fucales and the Laminariales (Phaeophyta): ecological and taxonomic implications. *Oecologia*. 1989; 78(1): 97–105. DOI: 10.1007/BF00377203

[34] Axelsson L, Uusitalo J, Ryberg H. Mechanisms for concentrating and storage of inorganic carbon in marine macroalgae. Seaweed Cellular Biotechnology, Physiology and Intensive Cultivation. COST-48. Universidad de las Palmas de Gran Canaria, España. 1991; 185–198.

[35] Johnston AM, Maberly SC, Raven JA. The acquisition of inorganic carbon by four red macroalgae. *Oecologia*. 92(3): 317–326. DOI: 10.1007/BF00317457

[36] Mercado JM, Gordillo FJL, Figueroa FL. External carbonic anhydrase and affinity for inorganic carbon in intertidal macroalgae. *Journal of Experimental Marine Biology and Ecology*. 1998; 221(2): 209–220. DOI: 10.1016/S0022-0981(97)00127-5

[37] Badger MR, Hanson D, Price GD. Evolution and diversity of CO_2 concentrating mechanisms in cyanobacteria. *Functional Plant Biology*. 2002; 29(3): 161–173. DOI: 10.1071/PP01213

[38] Spalding MH. Microalgal carbon-dioxide-concentrating mechanisms: *Chlamydomonas* inorganic carbon transporters. *Journal of Experimental Botany*. 2008; 59(7): 1463–1473. DOI: 10.1093/jxb/erm128

[39] Moroney JV, Ma YB, Frey WD. The carbonic anhydrase isoforms of *Chlamydomonas reinhardtii*: intracellular location, expression, and physiological roles. *Photosynthesis Research*. 2011; 109(1–3): 133–149. DOI: 10.1007/s11120-011-9635-3

[40] Price GD. Inorganic carbon transporters of the cyanobacterial CO_2 concentrating mechanism. *Photosynthesis Research*. 2011; 109(1–3): 47–57. DOI: 10.1007/s11120-010-9608-y

[41] Badger M. The roles of carbonic anhydrases in photosynthetic CO_2 concentrating mechanisms. *Photosynthesis Research*. 2003; 77(2–3): 83–94. DOI: 10.1023/A:1025821717773

[42] Israel A, Hophy M. Growth, photosynthetic properties and RuBisCo activities and amounts of marine macroalgae grown under current and elevated seawater CO_2 concentrations. *Global Change Biology*. 2002; 8(9): 831–840. DOI: 10.1046/j.1365-2486.2002.00518.x

[43] Fernández PA, Roleda MY, Hurd CL. Effects of ocean acidification on the photosynthetic performance, carbonic anhydrase activity and growth of the giant kelp *Macrocystis pyrifera*. *Photosynthesis Research*. 2015; 124: 293–304. DOI: 10.1007/s11120-015-0138-5

[44] Axelsson L, Ryberg H, Beer S. Two modes of bicarbonate utilization in the marine green macroalga *Ulva lactuca*. *Plant, Cell & Environment*. 1995; 18(4): 439–445. DOI: 10.1111/j.1365-3040.1995.tb00378.x

[45] Fernández PA, Hurd CL, Roleda MY. Bicarbonate uptake via an anion exchange protein is the main mechanism of inorganic carbon acquisition by the giant kelp *Macrocystis pyrifera* (Laminariales, Phaeophyceae) under variable pH. *Journal of Phycology*. 2014; 50(6): 998–1008. DOI: 10.1111/jpy.12247

[46] Drechsler Z, Sharkia R, Cabantchik ZI. Bicarbonate uptake in the marine macroalga *Ulva* sp. is inhibited by classica probes of anion exchange by red blood cells. *Planta*. 1993; 191: 34–40. DOI: 10.1007/BF00240893

[47] Axelsson L, Larsson C, Ryberg H. Affinity, capacity and oxygen sensitivity of two different mechanisms for bicarbonate utilization in *Ulva lactuca* L.(Chlorophyta). *Plant, Cell & Environment*. 1999; 22(8): 969–978. DOI: 10.1046/j.1365-3040.1999.00470.x

[48] Giordano M, Beardall J, Raven JA. CO_2 concentrating mechanisms in algae: mechanisms, environmental modulation, and evolution. *Annual Review of Plant Biology*. 2005; 56: 99–131. DOI: 10.1146/annurev.arplant.56.032604.144052

[49] Klenell M, Snoeijs P, Pedersén M. Active carbon uptake in *Laminaria digitata* and *L. saccharina* (Phaeophyta) is driven by a proton pump in the plasma membrane. *Hydrobiologia*. 2004; 514(1–3): 41–53. DOI: 10.1023/B:hydr.0000018205.80186.3e

[50] Smith RG, Bidwell RGS. Mechanism of photosynthetic carbon dioxide uptake by the red macroalga, *Chondrus crispus*. *Plant Physiology*. 1989; 89(1): 93–99. DOI: 10.1104/pp.89.1.93

[51] Choo K, Snoeijs P, Pedersén M. Uptake of inorganic carbon by *Cladophora glomerata* (Chlorophyta) from the Baltic Sea. *Journal of Phycology*. 2002; 38(3): 493–502. DOI: 10.1046/j.1529-8817.2002.01083.x

[52] Beer S. Mechanisms of inorganic carbon acquisition in marine microalgae (with special reference to the Chlorophyta). *Progress in Phycological Research*. 1994; 10: 179–207.

[53] Beer S. Photosynthetic utilization of inorganic carbon in *Ulva*. *Scientia Marina*. 1996; 60(Supl. 1): 125–128.

[54] Larsson C, Axelsson L, Ryberg H. Photosynthetic carbon utilization by *Enteromorpha intestinalis* (Chlorophyta) from a Swedish rockpool. *European Journal of Phycology*. 1997; 32(1): 49–54. DOI: 10.1080/09541449710001719365

[55] Snoeijs P, Klenell M, Choo K. Strategies for carbon acquisition in the red marine macroalga *Coccotylus truncatus* from the Baltic Sea. *Marine Biology*. 2002; 140(3): 435–444. DOI: 10.1007/s00227-001-0729-x

[56] Murru M, Sandgren CD. Habitat matters for inorganic carbon acquisition in 38 species of red macroalgae (Rhodophyta) from Puget Sound, Washington, USA. *Journal of Phycology*. 2004; 40(5): 837–845. DOI: 10.1111/j.1529-8817.2004.03182.x

[57] Cornwall CE, Revill AT, Hurd CL. High prevalence of diffusive uptake of CO_2 by macroalgae in a temperate subtidal ecosystem. *Photosynthesis Research*. 2015; 124(2): 181–190. DOI: 10.1007/s11120-015-0114-0

[58] Andría JR, Pérez-Lloréns JL, Vergara JJ. Mechanisms of inorganic carbon acquisition in *Gracilaria gaditana* nom. prov.(Rhodophyta). *Planta*. 1999; 208(4): 564–573. DOI: 10.1007/s004250050594

[59] Gómez-Pinchetti JL, Ramazanov Z, García-Reina G. Effect of inhibitors of carbonic anhydrase activity on photosynthesis in the red alga *Soliera filiformis* (Gigartinales: Rhodophyta). *Marine Biology*. 1992; 114(2): 335–339. DOI: 10.1007/BF00349536

[60] Gravot A, Dittami SM, Rousvoal S. Diurnal oscillations of metabolite abundances and gene analysis provide new insights into central metabolic processes of the brown alga *Ectocarpus siliculosus*. *New Phytologist*. 2010; 188(1): 98–110. DOI: 10.1111/j.1469-8137.2010.03400.x

[61] Zou D, Gao K, Chen W. Photosynthetic carbon acquisition in *Sargassum henslowianum* (Fucales, Phaeophyta), with special reference to the comparison between the vegetative

and reproductive tissues. *Photosynthesis Research*. 2011; 107(2): 159–168. DOI: 10.1007/s11120-010-9612-2

[62] Zou DH, Gao KS, Xia JR. Photosynthetic utilization of inorganic carbon in the economic brown alga, *Hizikia fusiforme* (Sargassaceae) from the South China Sea. *Journal of Phycology*. 2003; 36: 1095–1100. DOI: 10.1111/j.0022-3646.2003.03-038.x

[63] Yue GF, Wang JX, Wang JF. Inorganic carbon acquisition by juvenile sporophyte of Laminarials (*L. japonica* ×*L. longissima*). *Oceanologia et Limnologia Sinica*. 2001; 32(6): 647–652.

[64] Nakajima K, Tanaka A, Matsuda Y. SLC4 family transporters in a marine diatom directly pump bicarbonate from seawater. *Proceedings of the National Academy of Sciences of the United States of America*. 2013; 110(5): 1767–1772. DOI: 10.1073/pnas.1216234110

[65] Hopkinson BM. A chloroplast pump model for the CO_2 concentrating mechanism in the diatom *Phaeodactylum tricornutum*. *Photosynthesis Research*. 2014; 121(2–3): 223–233. DOI: 10.1007/s11120-013-9954-7

[66] Karlsson J, Clarke AK, Chen ZY. A novel α-type carbonic anhydrase associated with the thylakoid membrane in *Chlamydomonas reinhardtii* is required for growth at ambient CO_2. *EMBO Journal*. 1998; 17(5): 1208–1216. DOI: 10.1093/emboj/17.5.1208

[67] Karlsson J, Hiltonen T, Husic HD. Intracellular carbonic anhydrase of *Chlamydomonas reinhardtii*. *Plant Physiology*. 1995; 109(2): 533–539. DOI: 10.1104/pp.109.2.533

[68] Hanson DT, Franklin LA, Samuelsson G. The *Chlamydomonas reinhardtii* cia3 mutant lacking a thylakoid lumen-localized carbonic anhydrase is limited by CO_2 supply to RuBisCo and not photosystem II function in vivo. *Plant Physiology*. 2003; 132(4): 2267–2275. DOI: 10.1104/pp.103.023481

[69] Raven JA. CO_2-concentrating mechanisms: a direct role for thylakoid lumen acidification? *Plant, Cell & Environment*. 1997; 20(2): 147–154. DOI: 10.1046/j.1365-3040.1997.d01-67.x

[70] Mitra M, Lato SM, Ynalvez RA. Identification of a new chloroplast carbonic anhydrase in *Chlamydomonas reinhardtii*. *Plant Physiology*. 2004; 135(1): 173–182. DOI: 10.1104/pp.103.037283

[71] Sültemeyer D. Carbonic anhydrase in eukaryotic algae: characterization, regulation, and possible function during photosynthesis. *Canadian Journal of Botany*. 1998; 76(6): 962–972. DOI: 10.1139/b98-082

[72] Badger MR, Price GD. The role of carbonic anhydrase in photosynthesis. *Annual Review of Plant Biology*. 1994; 45(1): 369–392. DOI: 10.1146/annurev.pp.45.060194.002101

[73] Badger MR, Price GD. The CO_2 concentrating mechanism in cyanobacteria and microalgae. *Physiologia Plantarum*. 1992; 84(4): 606–615. DOI: 10.1034/j.1399-3054.1992.840416.x

[74] Hewett-Emmett D, Tashian RE. Functional diversity, conservation, and convergence in the evolution of the α-, β-, and γ-carbonic anhydrase gene families. *Molecular Phylogenetics and Evolution*. 1996; 5(1): 50–77. DOI: 10.1006/mpev.1996.0006

[75] Roberts SB, Lane TW, Morel FMM. Carbonic anhydrase in the marine diatom *Thalassiosira weissflogii* (Bacillariophyceae). *Journal of Phycology*. 1997; 33: 845–850. DOI: 10.1111/j.0022-3646.1997.00845.x

[76] So AK, Espie GS, Williams EB.A novel evolutionary lineage of carbonic anhydrase (epsilon class) is a component of the carboxysome shell. Journal of Bacteriology. 2004; 186: 623–630. DOI: 10.1128/JB.186.3.623-630.2004

[77] Lane TW, Morel FMM. Regulation of carbonic anhydrase expression by zinc, cobalt, and carbon dioxide in the marine diatom *Thalassiosira weissflogii*. *Plant Physiology*. 2000; 123: 345–352. DOI: 10.1104/pp.123.1.345

[78] Park H, Song B, Morel FMM. Diversity of the cadmium-containing carbonic anhydrase in marine diatoms and natural waters. *Environmental Microbiology*. 2007; 9(2): 403–413. DOI: 10.1111/j.1462-2920.2006.01151.x

[79] Lane TW, Saito MA, George GN. Biochemistry: a cadmium enzyme from a marine diatom. *Nature*. 2005; 435(7038): 42. DOI: 10.1038/435042a

[80] Xu Y, Feng L, Jeffrey PD. Structure and metal exchange in the cadmium carbonic anhydrase of marine diatoms. *Nature*. 2008; 452(7183): 56–61. DOI: 10.1038/nature06636

[81] Ferry JG. The γ class of carbonic anhydrases. *Biochimica et Biophysica Acta*. 2010; 1804(2): 374–381. DOI: 10.1016/j.bbapap.2009.08.026

[82] Coleman JR, Grossman AR. Biosynthesis of carbonic anhydrase in *Chlamydomonas reinhardtii* during adaptation to low CO_2. *Proceedings of the National Academy of Sciences of the United States of America*. 1984; 81(19): 6049–6053. DOI: 10.1073/pnas.81.19.6049

[83] Fujiwara S, Fukuzawa H, Tachiki A. Structure and differential expression of two genes encoding carbonic anhydrase in *Chlamydomonas reinhardtii*. *Proceedings of the National Academy of Sciences of the United States of America*. 1990; 87(24): 9779–9783. DOI: 10.1073/pnas.87.24.9779

[84] Eriksson M, Karlsson J, Ramazanov Z. Discovery of an algal mitochondrial carbonic anhydrase: molecular cloning and characterization of a low-CO_2-induced polypeptide in *Chlamydomonas reinhardtii*. *Proceedings of the National Academy of Sciences of the United States of America*. 1996; 93(21): 12031–12034. DOI: 10.1073/pnas.93.21.12031

[85] Moroney JV, Husic HD, Tolbert NE. Effect of carbonic anhydrase inhibitors on inorganic carbon accumulation by *Chlamydomonas reinhardtii*. *Plant Physiology*. 1985; 79(1): 177–183. DOI: 10.1104/pp.79.1.177

[86] Van K, Spalding MH. Periplasmic carbonic anhydrase structural gene (Cah1) mutant in *Chlamydomonas reinhardtii*. *Plant Physiology*. 1999; 120(3): 757–764. DOI: 10.1104/pp.120.3.757

[87] Lucas WJ. Photosynthetic assimilation of exogenous HCO_3^- by aquatic plants. *Annual Review of Plant Physiology*. 1983; 34(1): 71–104. DOI: 10.1146/annurev.pp.34.060183.000443

[88] Peña KL, Castel SE, de Araujo C. Structural basis of the oxidative activation of the carboxysomal γ-carbonic anhydrase, CcmM. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107(6): 2455–2460. DOI: 10.1073/pnas.0910866107

[89] Tachibana M, Allen AE, Kikutani S. Localization of putative carbonic anhydrases in two marine diatoms, *Phaeodactylum tricornutum* and *Thalassiosira pseudonana*. *Photosynthesis Research*. 2011; 109(1–3): 205–221. DOI: 10.1007/s11120-011-9634-4

[90] Chen C, Dai Z, Xu Y. Cloning, expression, and characterization of carbonic anhydrase genes from *Pyropia haitanensis* (Bangiales, Rhodophyta). *Journal of Applied Phycology*. DOI: 10.1007/s10811-015-0646-x

[91] Zhang BY, Yang F, Wang GC. Cloning and quantitative analysis of the carbonic anhydrase gene from *Porphyra yezoensis*. *Journal of Phycology*. 2010; 46: 290–296. DOI: 10.1111/j.1529-8817.2009.00801.x

[92] Yu Z, Bi YH, Zhou ZG. Cloning and characterization of carbonic anhydrase (CA) gene from *Laminaria japonica* gametophytes. *Journal of Fisheries of China*. 2011; 35: 1343–1353.

[93] Ye RX, Yu Z, Shi WW. Characterization of α-type carbonic anhydrase (CA) gene and subcellular localization of α-CA in the gametophytes of *Saccharina japonica*. *Journal of Applied Phycology*. 2014; 26: 881–890. DOI: 10.1007/s10811-013-0221-2

[94] The FAO yearbook of Fishery and Aquaculture Statistics: Aquaculture Production. Rome: Food and Agriculture Organization of the United Nations; 2009. 221 p.

[95] Ross AB, Jones JM, Kubacki ML. Classification of macroalgae as fuel and its thermochemical behaviour. *Bioresource Technology*. 2008; 99(14): 6494–6504. DOI: 10.1016/j.biortech.2007.11.036

[96] Ye N, Zhang X, Miao M. *Saccharina* genomes provide novel insight into kelp biology. *Nature Communications*. 2015; 6: 6986. DOI: 10.1038/ncomms7986

[97] Deng YY, Yao JT, Wang XL. Transcriptome sequencing and comparative analysis of *Saccharina japonica* (Laminariales, Phaeophyceae) under blue light induction. *PLoS One*. 2012; 7(6): e39704. DOI: 10.1371/journal.pone.0039704

[98] Wang WJ, Wang FJ, Sun XT. Comparison of transcriptome under red and blue light culture of *Saccharina japonica* (Phaeophyceae). *Planta*. 2013; 237(4): 1123–1133. DOI: 10.1007/s00425-012-1831-7

[99] Heinrich S, Valentin K, Frickenhaus S. Transcriptomic analysis of acclimation to temperature and light stress in *Saccharina latissima* (Phaeophyceae). *PloS One*. 2012; 7(8): e44342. DOI: 10.1371/journal.pone.0044342

[100] Tamura K, Stecher G, Peterson D. MEGA6: molecular evolutionary genetics analysis Version 6.0. *Molecular Biology and Evolution*. 2013; 30: 2725–2729. DOI: 10.1093/molbev/mst197

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