Chapter 5
Seaweed Biomass Utilization Pathways in Microbes and Their Applications in the Production of Biofuels

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5.1 Introduction

Marine algae transform nearly 50 Gt of carbon dioxide each year from the atmosphere and convert it into biomass (Falkowski et al. 1998). Carbohydrates are the main components of the algal biomass, which function as storage of carbon and energy (Hehemann et al. 2010a, 2014). Carbohydrates also present 30–80% of the overall carbon content in algal biomass (Alderkamp et al. 2007). Microorganisms have the ability to convert a wide variety of carbohydrates produced by macroalgae in the ocean. Interestingly, most of the enzymes involved in the marine carbohydrates cycle are still unknown.

Macroalgae show numerous features of a potential feedstock that may help the increasing global requirement for energy. The farming of macroalgae does not require fertile land, freshwater, use of fertilizer, avoiding adversarial impacts on food supplies (Enquist-Newman et al. 2013). Additionally, algal polysaccharides are simpler to digest than terrestrial plant biomass (Horn et al. 2000a; Singh et al. 2017). Also, they are deficient or comprise minor quantities of lignin (Martone et al. 2009), the natural part that reduces cellulose degradation by cellulase. Macroalgae are classified into three main groups brown, red, and green. There are characteristic differences between each type in carbohydrate composition.

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In brown macroalgae, alginate, glucose, and mannitol are present in ample amount. Alginate is consumed by sea microbes (Mitulla et al. 2016). Alginate is one of the plentiful sea polysaccharide and is located in the cell walls of brown macroalgae (Smith 1981). Alginate is a polymer of two uronic acids, β-D-mannuronate (M), and α-L-guluronate (G). They are arranged in variable orders of poly β-D-mannuronate (polyM), poly α-L-guluronate (polyG), and the heteropolymer (polyMG) (Wong et al. 2000). Native alginate metabolism pathways have been reported in several microorganisms (Cao et al. 2007).

Microorganisms need several enzymes to decompose even the simplest polysaccharides into simple sugars. These enzymes involve hydrolytic glycoside hydrolases and lytic polysaccharide lyases (PL). Enzymes essential to deconstruct a variety of algal polysaccharides have been reported earlier (Hehemann et al. 2012b, c; Thomas et al. 2012). Alginate degrading microorganisms contain alginate lyases (Alys) which degrade the alginate via a β-elimination reaction (Wargacki et al. 2012). Alginate lyases from different microorganisms have been well explored (Thomas et al. 2012; Wong et al. 2000). Alginate lyases initiate the reaction by cleaving the polymer into longer oligosaccharides in an endo-mode of action. Oligoalginate lyases act in exo-mode from the ends of oligomers and polymers to convert them into monomer (Wong et al. 2000). Oligoalginate lyases eliminate unsaturated and saturated monomers from the nonreducing end of alginate polymers (Gimmestad et al. 2009; Suzuki et al. 2006).

Seaweed polysaccharides have been considered as cheap biomass to produce biodiesel, ethanol, and hydrogen (Beer et al. 2009; Chisti 2008; Giri and Pant 2019). Alginate has been projected as a sustainable source to produce ethanol (Wargacki et al. 2012; Zimmerman et al. 2013). The alginate degradation pathway has been transferred from the marine bacterium *Vibrio splendidus* 12B01 into an *Escherichia coli* and *Saccharomyces cerevisiae*. These hosts are further engineered to produce bioethanol from alginate (Wargacki et al. 2012).

This book chapter is focused on macroalgae-based biorefinery. It provides a background on macroalgae taxonomic classification, habitat environment, enzymes, and metabolic pathways involved in macroalgae polysaccharide catabolism. In addition, it is also focused on providing information on native and engineered microbial platforms for biofuel production from brown macroalgae.

5.2 Classification and Habitat of Macroalgae

Macroalgae are multicellular photosynthetic organisms made up of a leaf-like structure (Jung et al. 2013; Lobban et al. 1985). On the basis of pigment present in thallus, macroalgae are classified as green, brown, and red algae (Sze 1993) (Fig. 5.1). There are about 3050 and 1500 species of freshwater and seawater green macroalgae, respectively (Guiry 2014). Their composition is similar to land plants (Yu et al. 2002). More than 4000 species of red macroalgae and 2000 species of

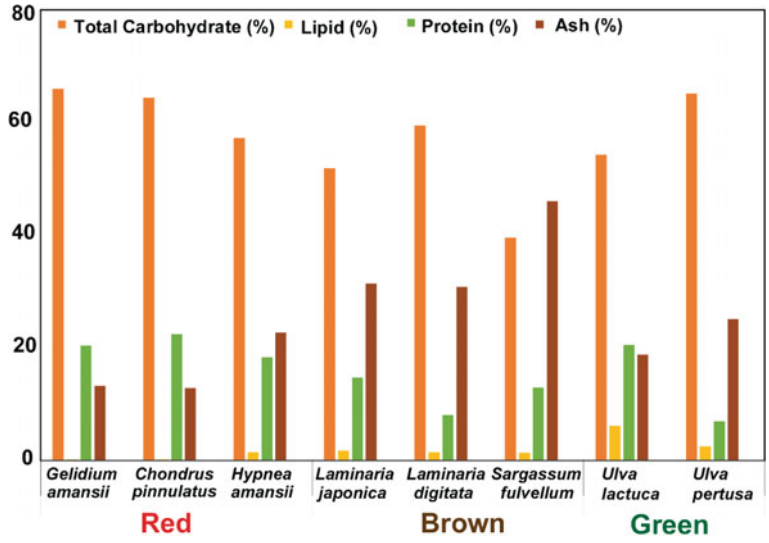

Fig. 5.1 Total composition of green, brown, and red algae

brown macroalgae are exist in seawater (Hoek et al. 1995). The brown color is derived from chlorophyll, β-carotene, and xanthophyll pigments (Sze 1993).

The growth, pigments, and element structure of macroalgae are disturbed by habitat surroundings including nutrient, salinity, temperature, water motion, and light (Lobban et al. 1985). Light availability is a key contributing condition in the distribution of macroalgae (Choi et al. 2019; Patel et al. 2019). The specific pigment in macroalgae absorbs light with a particular wavelength (Guiry 2014; Pant et al. 2018).

5.3 Carbon Storage Potential of Macroalgae

Organic carbon is stored in photoautotrophic macroalgae by consuming CO_2 or HCO_3^- (Gao and McKinley 1994). The intake of HCO_3^- is preferred over CO_2 by macroalgae. The mass transfer rate of CO_2 in seawater is very slow (Giri and Pant 2019, 2020). A small number of macroalgae can directly use CO_2 as a substrate. Interconversion of CO_2 and HCO_3^- is catalyzed by RuBP carboxylase and carbonic anhydrase (Lobban et al. 1985).

Macroalgae can store carbon resources required for the production of biochemicals (Bhatia et al. 2019). The green and red macroalgae have a higher photosynthetic rate as compared to brown macroalgae. The 1 billion tons of carbon could sequestrate by macroalgae cultivation along with coastlines. Brown and red macroalgae are the most favorable species for biorefinery. *Laminaria japonica* and

Undaria pinnatifida are widely produced brown macroalgae. In red seaweed, Eucheuma spp.*, Kappaphycus alvarezii, and Gracilaria verrucosa constitute more than 40%.*

5.4 Composition of Macroalgae

The seaweed composition is significantly different from lignocellulosic biomass. Lignin is absent in macroalgae. Uranic acids, sugars, and sugar alcohol mannitol are more prevalent in seaweed (Table 5.1). Macroalgae are made up of 70–90% water per wet weight. They contain 10–50% alkali metals, 7–15% protein, and 1–5% lipids per dry weight (Ross et al. 2008). The 25–50, 30–60, and 30–50% carbohydrates are existing in green, red, and brown macroalgae, respectively (Becker 1994; Ross et al. 2008).

5.4.1 Green Macroalgae

The 1–4% starch and 0–6% lipids are the main polysaccharides in green macroalgae (Burton et al. 2009). *Ulva* and *Enteromorpha* sp. composed of ulvan and cellulose in their cell wall. Ulvan is comprised of D-glucuronic acid, xylose, rhamnose, and sulfate (Lahaye and Robic 2007).

Table 5.1 Carbohydrate and sugar composition of different algae

Class	Carbohydrate composition	Sugar composition
Red	Cellulose	Glucose
	Agarose	Galactose
	Carrageenan	
	Starch	
Brown	Laminarin	Glucose
	Mannitol	Fucose
	Alginate	Mannitol
	Fucoidan	Mannuronic acid
		Guluronic acid
Green	Cellulose	Glucose
	Ulvan	Mannose
	Starch	Uronic acid
	Mannan	Rhamnose
	Xyloglucan	Xylose
		Glucuronic acid

5.4.2 Red Macroalgae

The unique characteristic features of red macroalgae are floridean starch and floridoside. These carbohydrates are absent in green and brown macroalgae. Floridean starch is glucose homopolymer and accounts up to 70% of cell volume (Yu et al. 2002). Agar and carrageenan are the galactans and major polysaccharide constituents of red seaweed (McHugh 2003). Carrageenan contains a repeating unit of galactose and anhydrogalactose, with or without sulfate. Agar consists of interchanging β-D-galactose and α-L-galactose with limited sulfations (Jung et al. 2013; Lobban et al. 1985).

5.4.3 Brown Macroalgae

Alginate, laminarin, mannitol, and fucoidan are ample sugars in brown macroalgae (Table 5.2). Alginate is a major carbohydrate in brown macroalgae and is composed of β-1,4-D-mannuronate and α-1,4-L-guluronate residues (Davis et al. 2003; Usov et al. 2001). Alginate degradation is important for the efficient conversion of brown macroalgae. Laminarin is the main storage polysaccharide composed of a linear β-1,3-D-glucose chain with scattered branches of β-1,6-D-glucose. Mannitol is an alcohol polymer of mannose. Fucoidan is composed of fucose and sulfate. In addition, it also comprises galactose, xylose, glucuronic, and uronic acid (Li et al. 2008).

5.5 Pretreatment Technologies

The various pretreatment technologies have been used to release sugars from macroalgae comprising, physical, chemical, biological, or a combination of methods (Milledge et al. 2019). The physical structure of seaweeds can be disrupted by mechanical pretreatment. It enhances the hydrolysis of macroalgae to sugars for fermentation. Different mechanical treatments are used for macroalgae pretreatment

Table 5.2 Composition of brown algae

Brown algae	Alginate (%)	Laminarin (%)	Fucoidan (%)	Mannitol (%)
Laminaria digitata	16–45	0–18	2–4	4–22
Saccharina latissima	21–46	0–26	–	6–22
Laminaria hyperborean	22–35	0–24	2–4	6–18
Ascophyllum nodosum	15–30	0–10	5–10	5–10
Fucus vesiculoses	14–17	2–5	–	8–16

including a Hollander beating, size reduction by chopping, washing in freshwater to remove impurities, and sonication. Thermal pretreatment is used to release sugars from macroalgae. Bioethanol yields are increased when autoclave treatment used for brown, red, and green macroalgae. H_2SO_4 and NaOH are most commonly proposed for acid and alkali treatments. It causes swelling of macroalgae fibers and increases pore size to release sugars (Jagtap et al. 2013, 2014a). Ionic liquids and sodium chlorite have also been used as pretreatment methods. In biological pretreatment, enzymes or microbes are used for the conversion of macroalgae to sugars. Macroalgae degrading enzymes and commercial enzymes, such as Celluclast 1.5L, are most commonly used for the conversion of macroalgae. The widely used pretreatment method is hydrothermal treatment with acid or alkali (Kim et al. 2013). It releases inhibitors furfural, 5-hydroxymethylfurfural (5-HMF), and levulinic acid along with sugars (Martín et al. 2002). An eco-friendly gamma radiation method has been also reported as an effective pretreatment method (Yoon et al. 2012).

5.6 Enzymes for Seaweed Conversion and Mechanism of Action

5.6.1 Laminarinase and Fucoidanases

Laminarinase and fucoidanases belong to glycoside hydrolyase (Badur et al. 2020; Becker et al. 2017, 2020). They break β-1,3-glycosidic linkages and β-1,6-glycosidic linkages of laminarin in one step or two steps reactions. Laminarinase is functional on the β-1,3-linked substrates including curdlan, laminarin, and lichenan. The glycoside bonds between sulfated fucose residues are cleaved by fucoidanases (Kusaykin et al. 2015).

5.6.2 Agarase and Carrageenanase

Agarase catalyzes the hydrolysis of polysaccharide agar. Agarases are categorized as α-agarase or β-agarase depending on the cleavage pattern. The cleavage of α-1,3 linkages by α-agarase produces agarooligosaccharides associated with agarobiose. β-agarases cleaves β-1,4 linkages to generate neoagarooligosaccharides linked to neoagarobiose (Fu and Kim 2010; Hehemann et al. 2012a, b; Pluvinage et al. 2013).

Carrageenan is a sulfated carbohydrate composed of galactose and 3, 6-anhydrogalactose. It is categorized by the number and the location of sulfated esters including κ-, ι- and λ-carrageenan. Carrageenases are endohydrolases that cleave the internal β-(1-4) linkages of carrageenans to generate the

oligocarrageenans (Chauhan and Saxena 2016). Carrageenases are evolved and adapted to degrade the different forms of carrageen (Hettle et al. 2019; Pluvinage et al. 2013).

5.6.3 Alginate Lyases

Alginate lyase is categorized based on the conversion of polyM, polyG, or polyMG regions of alginate (Fig. 5.2). They belong to the 23 protein families of polysaccharide lyases (Murata et al. 2008). Alginate lyases belong to the PL5 to PL7, PL14 to PL15, and PL17 to PL18 families (Yamasaki et al. 2005). PL5 and PL7 alginate lyases act on polymers in endolytic fashion and exolytic fashion to generate smaller oligomers and monomers (Thomas et al. 2013).

Few bacteria can produce alginate, and alginate lyase for alginate degradation including *Pseudomonas aeruginosa* and *Azotobacter vinelandii* (Ertesvåg 2015; Gimmestad et al. 2009). They can be secreted outside, bound to the membrane, or intracellular. Several bacteria use a number of alginate lyases to degrade alginate polymer (Neumann et al. 2015). *Saccharophagus degradans* 2–40 has 13 predicted genes encoding alginate lyase (Hutcheson et al. 2011). *Alteromonadales Zobellia galactanivorans* also processes multiple alginate lyases (Zhu et al. 2017).

V. splendidus 12B01 represents the most abundant *vibrio* of temperate waters. Several alginate lyases within a single *vibrio* have been assessed which provide a keen understanding of how *vibrio* can proficiently use alginate as its main carbon source and potentially inform the strategy for creating new organisms efficient of making biofuels from alginate.

Alginate lyases belong to *vibrio* showed an optimal pH 7.5–8.5, an optimal temperature 20–25 °C, and an optimal NaCl 400–1000 mM (Badur et al. 2015). AlyB, AlyD, and AlyE were reported to have signal peptides and different substrate specificity.

5.6.4 Oligoalginate Lyases

The oligoalginate lyases from *V. splendidus* 12B01 alginate degradation pathway belong to PL15 (OalA) and PL17 (OalB, and OalC) families (Wargacki et al. 2012). Their functional characterization has been reported earlier (Jagtap et al. 2014b) (Fig. 5.2). These enzymes have complementary functions regarding substrate scope and physiological adaptations. They also showed different kinetic rates of alginate degradation.

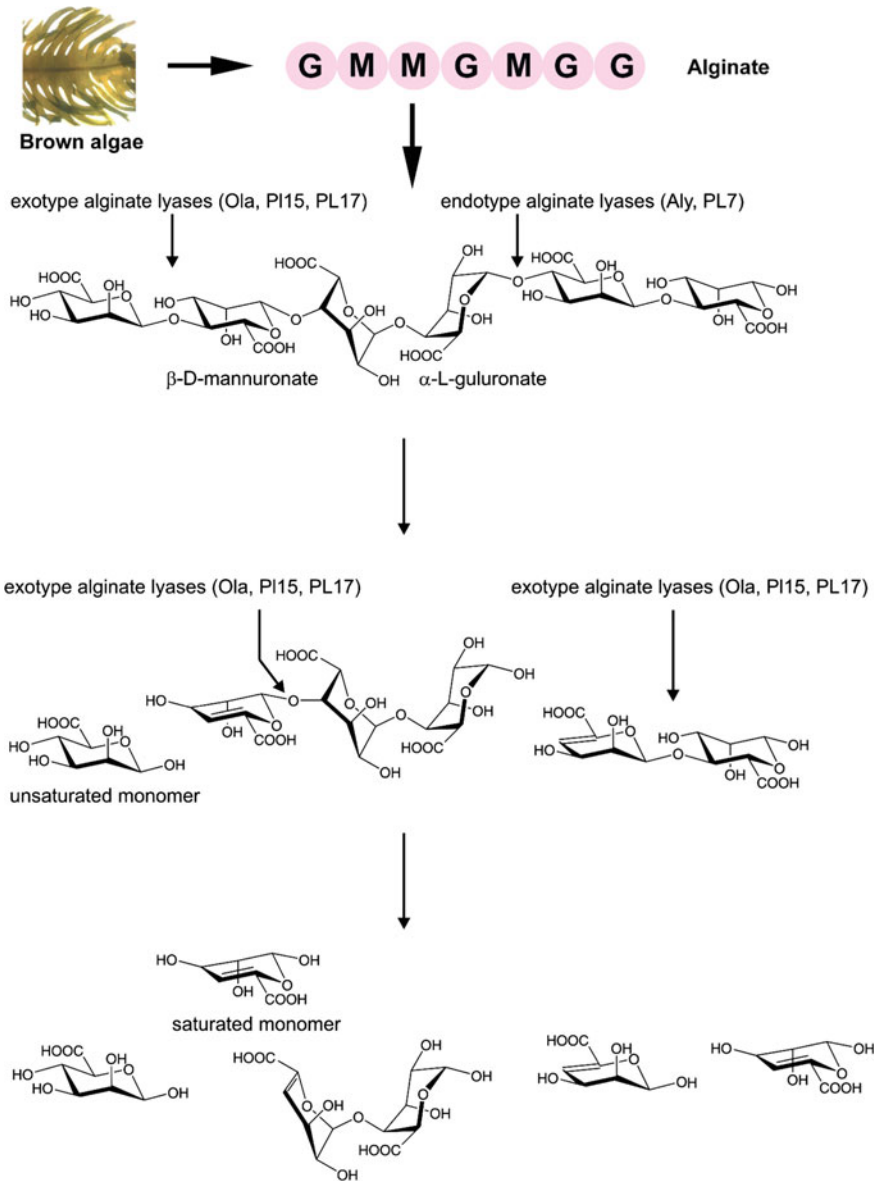

Fig. 5.2 Alginate lyase catalyzed degradation of alginate. Exolytic oligoalginate lyases (PL15 and PL 18) degrades alginate via a β-elimination reaction to monosaccharides in an exolytic manner

5.6.5 Complementary Oligoalginate Lyases

The harmonizing substrate choice was observed for oligoalginate lyases from *V. splendidus*. In addition, complementary role is also observed in optimum temperature and optimum pH adaptations. In the coastal marine environment, bacteria are exposed to high temperature and pH fluctuations. Thus, the temperature range covered with three Oal's increase enzymatic activity at different temperatures. Altogether, the broad substrate scope and the physiological adaptations explained the presence of multiple oligoalginate lyases in 12B01.

5.6.6 Mechanism of Action

Carbohydrate active enzymes use diverse mechanisms to degrade agars, alginate, and ulvan polysaccharides from red, brown, and green macroalgae, respectively (Hehemann et al. 2014). β-agarase is fitted to GH16, GH50, GH85, GH118 families. It uses two catalytic glutamate residues for hydrolysis of agar. α-agarase is a part of families GH96 and GH117. It uses histidine as a catalytic acid and aspartate as a base for catalysis. Brown macroalgae are digested by polysaccharide lyase which belongs to families PL7, PL14, PL15, and PL17. Alginate lyases can either have endo- or exo-acting specificity on alginate polymer (Wong et al. 2000). The mechanism of alginate degradation has been previously suggested (Linker et al. 1960), which includes a positive residue that steadies the negative charge on the carboxyl group, residue operating as a general base abstracts the proton from C-5 of the sugar ring, and acid residue protonates the glycosidic bond oxygen. Ulvan lyases belong to family GH105. The catalytic hydrolysis happens through the addition of water to unsaturated bond for a cleavage of glycosidic bond.

5.7 Alginate Metabolism in *V. splendidus*

In *V. splendidus* 12B01, alginate lyase gradually converts alginate polymer into oligomers with chain length of 2–6 m. Oligoalginate lyases rapidly convert these smaller oligomers into the monomers. These monomers naturally rearrange into 4-deoxy-L-erythro-5-hexoseulose uronic acid (DEH). Consequently, DEH reductase (DEHR) reduces DEH into 2-keto-3-deoxygluconate (KDG), a shared metabolite that enters into the Entner-Doudoroff (ED) pathway. KDG finally resulted in yielding pyruvate and glyceraldehyde-3-phosphate through the activities of the KDG kinase (KDGK) and KDG-6-phosphate aldolase (Eda) and finally produced precursor for the biofuel synthesis (Wargacki et al. 2012).

The capability of alginate degradation is investigated in diverse closely related marine *Vibrionaceae* bacteria (Hehemann et al. 2016). These bacteria can degrade

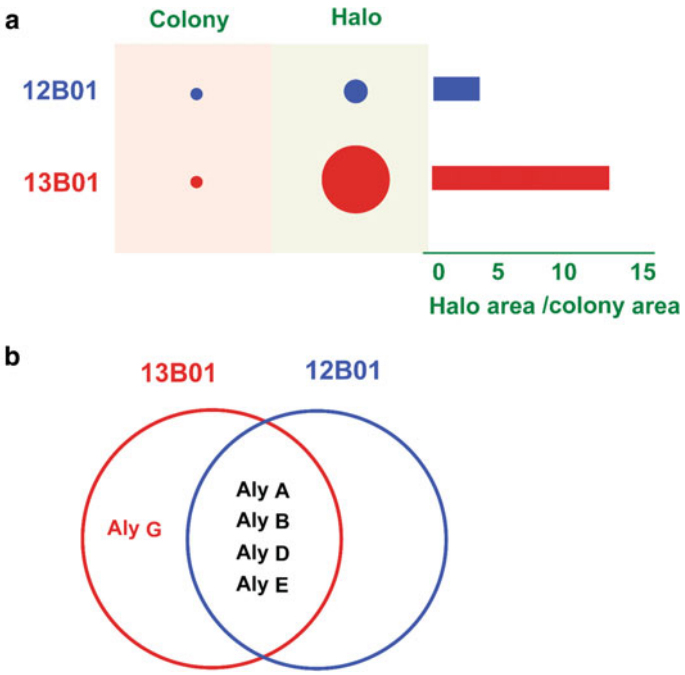

Fig. 5.3 Comparison of secreted alginate lyase activity among closely related *Vibrio splendidus* strains. **a** Phylogenetic comparison of closely related *V. splendidus* strains. The dark halos indicative of alginate digested by secreted alginate lyase. **b** Venn diagram analysis of the alginate lyases from *V. splendidus* 13B01 and *V. splendidus* 12B01

extracellular alginate to variable degrees of oligomers. *V. splendidus* 13B01 has significantly greater secreted alginate lyase activity than *V. splendidus* 12B01 (Badur et al. 2015). Genomic comparison discovered dissimilarities between alginate lyases in *V. splendidus* strains (Fig. 5.3). Both *V. splendidus* strains possessed four alginate lyases (PL7A, PL7B, PL7D, and PL7E) involved in alginate metabolism. Interestingly, PL7G alginate lyase is present in *V. splendidus* 13B01 only, but not in 12B01. The key role of PL7G alginate lyase for fast extracellular alginate degradation is investigated using a combination of different approaches (Ahmet et al. 2017).

5.8 Alginate Degradation Pathway in *Sphingomonas* sp. A1

Sphingomonas genus bacteria are gram negative, yellow colored, rod shaped, and aerobic in nature. *Sphingomonas* sp. A1 has superchannel or pit on the cell surface to import alginate without degradation. Mouth like pit is formed by *Sphingomonas* sp. A1 cells when grown on alginate plus medium (Hashimoto et al.

2010; White et al. 1996). The alginate operon is involved in alginate incorporation by strain A1 cells (Fig. 5.5). The five genes operon is assembled in the genome encodes for the ATP-binding proteins (AlgS/AlgS), transmembrane domains (AlgM1 and AlgM2), and two alginate-binding proteins (AlgQ1 and AlgQ2). AlgQ1 and AlgQ2 are inducibly expressed in the periplasm of strain A1. ABC transporter is constituted of AlgS as an ATPase and AlgM1 and AlgM2 as a permease. AlgQ1-AlgQ2 opened widely after alginate binding to release alginate. The AlgM1 and AlgM2 inducibly activated the periplasmic entrance. Subsequently, AlgS is found to be incorporating the alginate to the cytoplasm (Hashimoto et al. 2010).

Sphingomonas sp. A1 produced the three cytoplasmic endolytic alginate lyases including A1-I, A1-II, and A1-III. The cleavage of N terminal peptide of the precursor protein (Po) leads to synthesis of A1-I. It displayed the affinity for both polyM and polyG. A1-I includes N terminal A1-III and C terminal A1-II. They are processed to generate A1-III and A1-II for polyG and polyM. Alginate is degraded by A1-I, A1-II, and A1-III to unsaturated oligomers. These saturated and unsaturated oligosaccharides are converted to monosaccharides by oligoalginate lyases A1-IV (Hashimoto et al. 2000). The unsaturated uronic acid is spontaneously converted to DEH, which further reduced to 2-keto-3-deoxy-D-gluconate and metabolized into pyruvate (Fig. 5.4). Bioethanol has been produced by the engineering of the strain A1 metabolism (Takeda et al. 2011).

5.9 Brown Macroalgae Degradation Pathway in Engineered *E. coli*

The rapid engineering of native organisms has bottlenecks for the production and optimization of desired product and minimization of byproducts. These efforts were also prevented by a scarcity of tools for genetic engineering and the absence of sturdiness under modern fermentation conditions (Alper and Stephanopoulos 2009). *E. coli* is engineered for alginate catabolism (Fig. 5.5). The secreatable Aly system is engineered to allow effective and fast conversion of alginate (Wargacki et al. 2012). Alginate polymer converted into oligomers by several alginate lyases. Oligomers are transferred through the outer-membrane porins (KDGMN) into the periplasmic place. Subsequently, periplasmic alginate lyases (AlyABCD) converted these oligomers with a degree of polymerization (DP) larger than 3 into dimer, trimer, and tetramers. Oligomers are transported into the cytoplasm via oligoalginate transporters (ToaABC). Oligomers are further converted into unsaturated monomers by oligoalginate lyase (OalABC). These monomers naturally reorganized into DEH. Subsequently, DEHR was reported to reduce DEH into KDG, which entered the ED pathway (Fig. 5.5). KDG finally produced pyruvate and glyceraldehyde-3-phosphate through the actions of the KDGK and KDG-6-phosphate aldolase (Eda) (Wargacki et al. 2012).

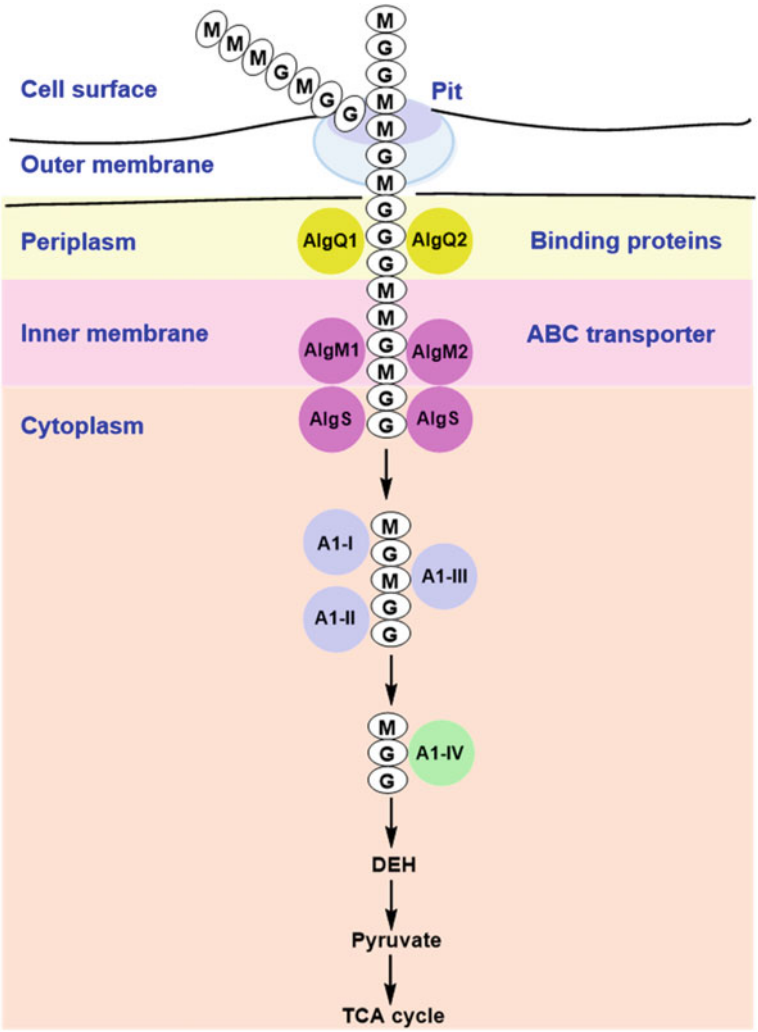

Fig. 5.4 Alginate uptake and degradation system in *Sphingomonas* sp. strain A1. G, L-guluronate; M, D-mannuronate; alginate lyases (A1-I, A1-II, and A1-III); AlgS, AlgM1, and AlgM2, ABC transporter genes for alginate import; AlgQ1 and AlgQ2, alginate-binding proteins; A1-IV, oligoalginate lyase

The alginate metabolic pathway was constructed using a fosmid library of arbitrary DNA pieces using the genomic DNA of *V. splendidus*. The 40-kbp fragment is composed of everything necessary for alginate transport and metabolism. The expression of supplementary genes encoding KDGMN, ToaC, and Alys

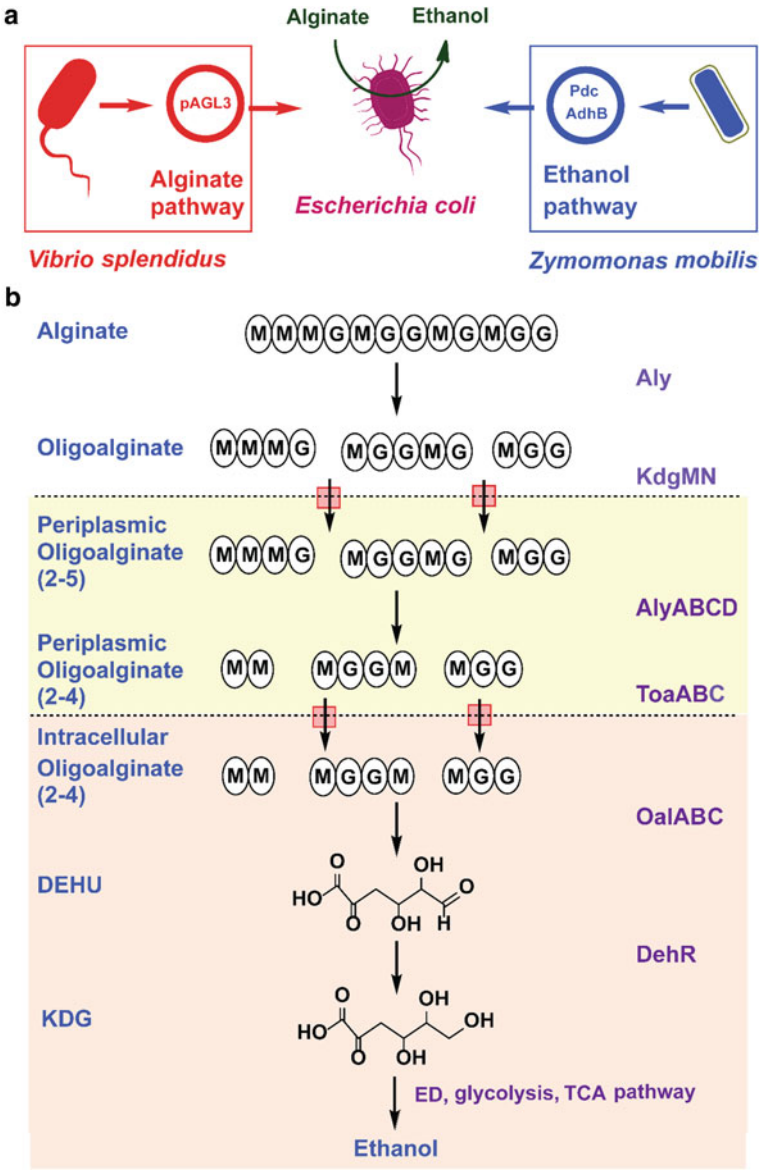

Fig. 5.5 *Escherichia coli* platform for the production of ethanol from macroalgae. **a** The fosmid pALG1 contained the genes for alginate metabolism from *Sphingomonas* sp. strain Al, and homoethanol pathway consisting *Zymomonasmobilis* pyruvate decarboxylase (Pdc) and alcohol dehydrogenase B (AdhB). **b** Alginate is degraded into oligomers by an alginate lyase. The oligomers relocated through the outer-membrane porins (KDGMN) into the periplasm. Oligomers with a degree of polymerization (DP) more than 3 are degraded into smaller oligomers by periplasmic alginate lyases (AlyABCD). These oligomers are transported into the cytosol via oligoalginate transporters (ToaABC). Oligoalginate lyases (OalABC) then degrade oligomers into monomer units (DEH). DEH is converted by DEH reductase (DEHR) to KDG, which enters the ED pathway

enhanced the alginate utilization by engineered *E. coli* strain. The heterologous pathway containing of pyruvate decarboxylase (Pdc) and alcohol dehydrogenase B (AdhB) from *Zymomonas mobilis* transferred into the engineered *E. coli*. *Saccharina japonica* (kombu) and brown macroalgae used as a fermentation substrate. *E. coli* can naturally produce ethanol by assimilating mannitol and glucose. A wild type *E. coli* without the engineered alginate assimilation pathway has been reported to produce $\sim 10\ gL^{-1}$ ethanol after 150 h. An engineered microbial platform produced ethanol at a final titer of ~4.7% v/v and over 80% of the highest speculative yield from macroalgae fermentation (Wargacki et al. 2012).

5.10 Brown Macroalgae Utilization Pathway in Engineered Yeast

S. cerevisiae has been engineered to convert brown seaweed into ethanol (Enquist-Newman et al. 2013) (Fig. 5.6). The four major modifications were applied, involving the rebuilding of a bacterial alginate catabolic pathway, DEHU transporter integration, down regulation of an innate mannitol catabolic pathway, and redox balance conservation.

In the first modification, the multiple enzymes in DEHU catabolism are overexpressed and efficient genes were chromosomally incorporated into both DEHU transporter selection strain and ethanol making strains. In the second modification, RNA-seq and cDNA library-based approaches were used to find a gene coding the DEHU transporter. The resulting strain was able to cultivate on DEHU as the only sugar substrate. In the third modification, microarrays were performed for the evaluation of *S. cerevisiae* strains cultivated in glucose, raffinose, and mannitol. The native mannitol 2-dehydrogenases and mannitol transporters were overexpressed. *S. cerevisiae* growth on mannitol was optimized by using different gene combinations. In the last modification, the genes in alginate and mannitol degradation were chromosomally incorporated into a *S. cerevisiae*. The cofactor selection of DEHR was examined to upkeep higher ethanol production from brown seaweed (Enquist-Newman et al. 2013).

S. cerevisiae wild type strains Lalvin and Pasteur Red produced $\sim 10\ gL^{-1}$ ethanol during growth on $30\ gL^{-1}$ mannitol. Ethanol fermentation was performed by mimicking the sugars present in brown macrolagae. The 1:2 molar ratio of DEHU:mannitol at 6.5% (w/v) and 9.8% (w/v) overall sugars were used in fermentation experiments using engineered strains. Ethanol was competently formed in both cases, accomplishing titers of 4.6% (v/v) ($36.2\ gL^{-1}$) and 83% of the highest theoretic yield from consuming sugars was reported (Enquist-Newman et al. 2013).

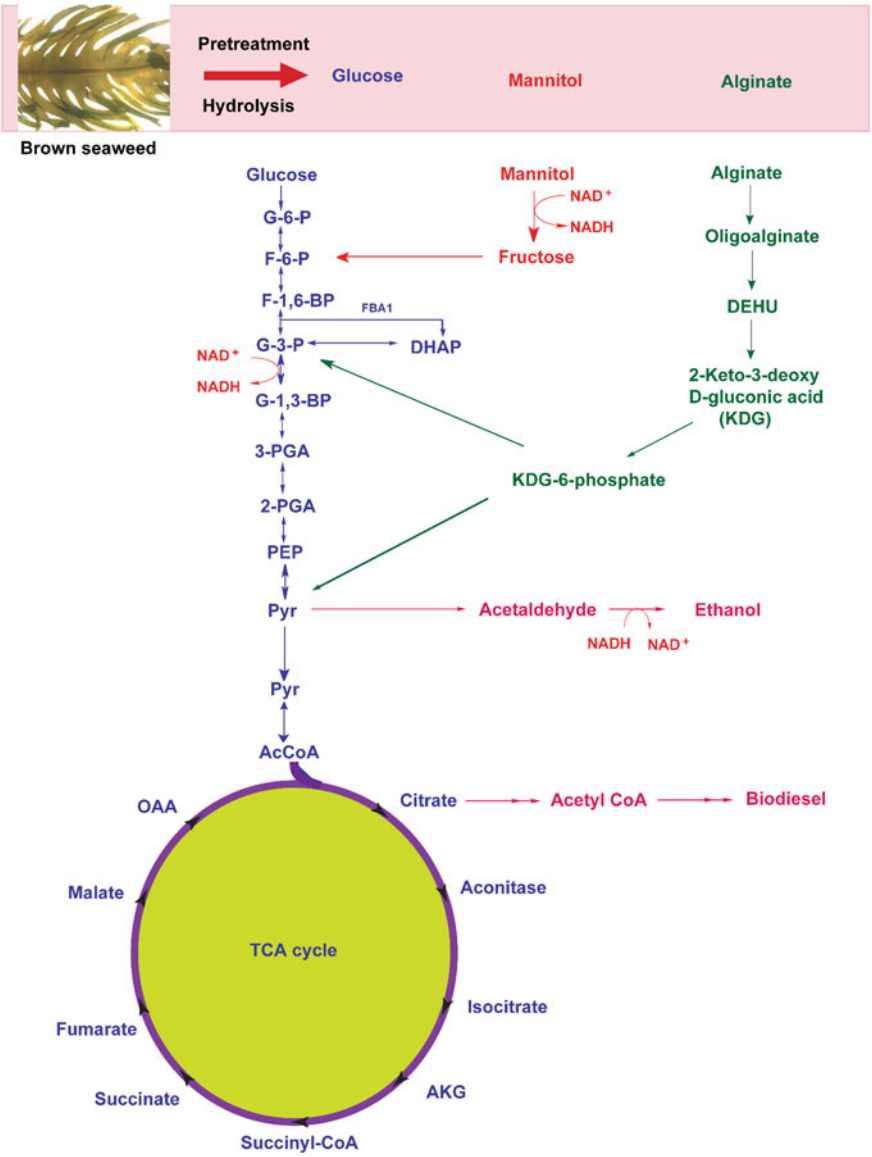

Fig. 5.6 *Saccharomyces cerevisiae* platform that can co-metabolize alginate, mannitol, and glucose to produce ethanol. The expression of heterologous alginate and optimization of endogenous mannitol pathways are essential to accomplish high ethanol production. Alginate is depolymerized into monomers by action of alginate lyases. DEHU is transported into the cytoplasm and converted into 2-keto-3-deoxy-D-gluconate (KDG) by DEHU reductase (DEHR). KDG is converted to the glycolytic intermediates

5.11 Products

Multiple microorganisms have been explored for the making of numerous value-enhanced target products from lignocellulosic sugars (Jagtap and Rao 2018a, b; Jagtap et al. 2019). The composition of lignocellulosic biomass and algal biomass is different (Bhatia et al. 2020). Macroalgal biomass has been explored for the manufacture of biogas, ethanol, and biodiesel. The different pretreatment methods are used including conversion using microbes, hydrothermal liquefaction (HTL), and pyrolysis. These technologies do not require macroalgae degrading enzymes, thus, they are not included here. The high carbohydrate content of macroalgae makes it a suitable substrate to produce biofuels including ethanol, 2,3-butanadiol, and 2,5-furandicarboxylic acid.

5.11.1 Bioethanol

Brown macroalgae is an ideal substrate for ethanol production. It is made up of a high percentage of carbohydrate and can be easily mass cultured using existing agriculture technologies. The certain microorganisms readily utilize mannitol and laminarin. Mannitol and laminarin extract from *L. hyperborea* were used to produce bioethanol (Horn et al. 2000a, b). *Pichia angophorae* concurrently consumed both mannitol and laminaran to produce ethanol. This yeast achieved the highest yield of 0.43 g ethanol g substrate. The enzymatic saccharification extract of *Saccharina latissimi* was fermented to ethanol and resulted in 0.45% (v/v) ethanol yield (Adams et al. 2009). *Pichia* yeast fermented mannitol, laminarin, and glucose into ethanol with a yield 0.43 g ethanol g substrate (Horn et al. 2000b). The extract of *Laminaria digitata* was fermented by *P. angophorae* and accomplished ethanol yield to 0.89% (v/v). An engineered *E. coli* KO11 metabolized mannitol and glucose. The concurrent saccharification and fermentation of the acid lysates of *S. japonica* resulted in a final ethanol yield of up to 29 gL^{-1} (Takeda et al. 2011).

E. coli platform was constructed to produce ethanol. The resulting strain BAL1611 made 37 gL^{-1} of ethanol or titer of ~4.7% v/v of ethanol from *S. japonica* extracts. Yeast platform is also developed for the production of ethanol. The concurrent consumption of DEHU and mannitol resulted in ethanol titers of 4.6% (v/v) using *S. cerevisiae* (Enquist-Newman et al. 2013).

5.11.2 Biobutanol

The acetone-butanol fermentation by *Clostridium* sp. resulted in biobutanol production from macroalgae (Kudahettige-Nilsson et al. 2015). *Clostridium* sp. can efficiently produce biofuels, and organic acids using various substrates. Contrarily,

this bacterium did not efficiently consume some glucose-based polysaccharides, resulted in slow reaction and productivity (Kudahettige-Nilsson et al. 2015).

Macroalgae can also be used as a substrate for the production of valuable chemicals. 2,3-butanediol and acetoin were produced using brown macroalgae. An engineered *E.coli* strain yielded 0.43 g/g of 2,3 butanediol (Mazumdar et al. 2013) The synthetic 2,3 butanediol pathway from *Enterobactor aerogenes* KCTC 2190 was integrated in *E.coli* strain. A commercial enzyme mixture was used for the *S. japonica* lysates pretreatment which was later fermented with engineered *E. coli* for 2,3-butanol production.

5.11.3 2,5-Furandicarboxylic Acid

2,5-furandicarboxylic acid (FDCA) can be a possible a precursor for polyethylene terephthalate (PET), nylons, and jet fuels production (Yoshikuni et al. 2016). Alginate degrading enzymes have been used to make DEH, which can subsequently convert into FDCA.

5.11.4 Methane

Sugars from macroalgae are converted to acetate, CO_2, and H_2 by microbial processes like acidogenesis and acetogenesis (Milledge et al. 2019; Giri et al. 2020; Sharma et al. 2020). Thereafter, it converted to methane and CO_2 by methanogens. Mechanically pretreated macroalgae such as *U. lactuca, Laminaria* spp., and *L. digitata* were incubated with cattle manure in bottles with rubber stoppers for 3–4 weeks. Methane yields were in the range of 150–330 mL g^{-1} volatile solids. The 34% higher methane yield was observed when washed and dried *U. lactuca* used as compared to that of unwashed and wilted (Milledge et al. 2019). Methane yield was 143–244 mL g^{-1} volatile solids in thermally pretreated macroalgae. Thermochemical pretreatment of *Ulva* spp., *F. vesiculosus*, and *L. digitata* enhanced methane yields by 2.5-fold as compared to untreated macroalgae (Milledge et al. 2019).

5.12 Conclusion

Macroalgae have many environmental and economic benefits and can be bulk cultured with current agricultural knowledge to generate biochemical and biofuels. The exploration of varying composition of carbohydrate in macroalgae is the future research needs to maximize their utilization and applications. The degradation of carbohydrates to release sugar requires specific enzymes and conversion of these

sugars into fuels and chemicals needs specialized metabolic pathways. These specific metabolic enzymes and pathways are not widely distributed in native microorganisms. These microorganisms are unable to depolymerize polysaccharides because of the absence of assimilation pathways. Few studies described the agarose and alginate metabolism in some microorganisms. However, more research is required to identify possible metabolism pathways of different polysaccharides for maximum utilization of the algal polysaccharides. Hence, the identification and characterization of unique polysaccharide converting enzymes and pathways are required for the development of efficient seaweed biomass degrading microbes. Metabolic engineering and synthetic biology approaches can become a new direction of research for the development of fermenting microorganisms that transform seaweed biomass to biofuels.

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