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REVIEW

Platform construction of molecular breeding for utilization of brown macroalgae

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Brown macroalgae are characterized by a large size and high productivity without requiring arable land, fresh water, and fertilizer. Furthermore, since brown macroalgae contain little or no lignin, simple biorefinery processing can efficiently produce sugars from this material. Therefore, brown macroalgae have attracted attention as an alternative feedstock for bioethanol production. However, the utilization of biotechnologies previously developed for terrestrial biomass processing results in difficulties in the bioconversion of brown macroalgae. Recently, several studies have developed biotechnologies for using major carbohydrates of brown macroalgae, such as laminarin, mannitol, and alginate. This review focuses on these fermentation biotechnologies using natural or engineered microorganisms.

[**Key words:** Brown macroalgae; Bioethanol; Laminarin; Mannitol; Alginate]

Over the past hundred years, heavy consumption of fossil fuels has increased the carbon dioxide level in the atmosphere, contributing to environmental problems such as climate change and ocean acidification. Furthermore, in accordance with the population growth, the world energy demand is continuously increasing. Thus, the development of a renewable and clean source of bioenergy is essential for a sustainable energy future. Bioethanol has been produced from agricultural biomass such as corn, sugarcane, wheat, and sugar beet (1–3). Relative to fossil fuels, bioethanol is less toxic and its utilization produces fewer harmful environmental consequences (4). However, the use of biomass for energy production competes with its value as a foodstuff for humans and livestock. Although lignocellulosic biomass such as sugarcane bagasse, rice straw, and wheat straw has also been considered as an alternative feedstock for bioethanol production (5–7), the presence of lignin in lignocellulose biomass complicates the release of the polysaccharide in the conversion to bioethanol and results in inefficient bioethanol production. Therefore, using biomass with low lignin content is effective for the low-cost refining of bioethanol.

Macroalgae are a promising alternative biomass feedstock for bioethanol production because they contain little or no lignin; thus, simple biorefinery processes can be used to produce sugars (8). Macroalgae are plentiful in ocean ecosystems and can grow at rates that far exceed those of terrestrial plants, mainly because the ocean is not a water-limited environment (9). Commercial-scale cultivation of macroalgae for both food products and their biochemical constituents is already being carried out in many countries. Aquaculture-based world production of macroalgae was estimated to be approximately 15.1 million wet metric tons annually, based on 2006 data (Table 1) (10). Macroalgae production from aquaculture is concentrated in Asian countries and is mainly practiced by China, which accounted for 73% of the total value of macroalgae farming, based on the 2006 data. Macroalgae farming does not require arable land for cultivation and grows in salt water, avoiding competition for the fresh water resources required for field crop production. With these considerations, macroalgae appear well suited to be used in bioethanol production, circumventing adverse impacts on food supplies.

Macroalgae are classified according to their photosynthetic pigments, color schemes (e.g., green, red, and brown), and habitat (9,11). The exclusive economic zone of Japan is 4,479,358 km^2, the sixth-largest in scale in the world. Macroalgae present in Japan constitute more than 1000 species. Among them, brown macroalgae are characteristically large and have high productivity. For instance, *Undaria pinnatifida*, a kelp species native to the temperate shores of Japan, grows up to 3 m long (12). Furthermore, the two genera *Undaria* and *Laminaria* accounted for almost 50% of world production of macroalgae by aquaculture (13). Even so, increasing aquaculture-based macroalgal cultivation will be required for the practical use of macroalgal biofuel. Therefore, the technologies of brown macroalgae breeding for cost-effective and stable production are also required. Genetic manipulation techniques such as artificial selection, hybridization, and mutagenesis are valuable tools for brown macroalgae breeding (14). For example, Li et al. (15,16) succeeded to breed hybrids thorough hybridization of selected gametophyte clones from different *Laminaria* species. The hybrids of *Laminaria* species showed new properties such as enhanced tolerance to strong irradiance, high water temperature, and tissue rottenness.

In order to realize efficient bioethanol production from brown macroalgae, not only the technologies of brown macroalgae breeding but also biotechnologies for effective utilization of carbohydrate components of brown macroalgae are necessary. Nevertheless, the application of biotechnologies previously developed for terrestrial biomass processing results in inefficient

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TABLE 1. Top ten producers of cultured macroalgae and world total, plus monetary value of cultures (USD) in 2006.

Source (country)	Production (metric ton)	% of total	Value US$1000s	$/metric ton
World total	15075612	100.00	7187125	476.74
China	10867410	72.09	5240819	482.25
Philippines	1468905	9.74	173963	118.43
Indonesia	910636	6.04	127489	140.00
Republic of Korea	765595	5.08	269657	352.22
Japan	490062	3.25	1051361	2145.36
Korea DPRp	444300	2.95	244365	550.00
Chile	33586	0.22	52394	1560.00
Malaysia	30000	0.20	4500	150.00
Vietnam	30000	0.20	15000	500.00
Cambodia	16000	0.11	4000	250.00

bioconversion of brown macroalgae into bioethanol. Recently, several studies have developed biotechnologies for using major carbohydrates of brown macroalgae. In this review, we focus on these fermentation biotechnologies using natural or engineered microorganisms.

CARBOHYDRATES OF BROWN MACROALGAE

Brown macroalgae contain cellulose, hemicellulose, mannitol, laminarin, and alginate as major carbohydrates. Among the macroalgae, brown macroalgae are characterized by the high content of laminarin, mannitol, and alginate. Therefore, we have summarized the molecular breeding for utilization of laminarin, mannitol, and alginate in this review.

Laminarin is a β-1,3-linked glucose polymer with β-1,6 cross-link branches and contains 30 glucose residues on average (Fig. 1A). The higher degree of β-1,6 branching makes laminarin more soluble. It is used as a long-term storage compound produced in brown macroalgae and has seasonal variation ranging from 0% to 35% of the dry weight (17).

Mannitol is also the carbon storage compound of brown macroalgae and represents up to 20–30% of the dry weight depending on the species, harvesting season, and habitat (18) (Fig. 1B). This polyol is produced from fructose-6-phosphate via the enzymatic reaction of a mannitol-1-phosphate dehydrogenase and mannitol-1-phosphatase (19). Mannitol acts as not only a carbon storage but also as an osmoprotectant and antioxidant in brown macroalgae.

Alginate, one of major components of brown macroalgae, is a linear polysaccharide composed of α-L-guluronic acid and its C5-epimer β-D-mannuronic acid (Fig. 2A). Alginate has 1,4-glycosidic bonds between the pyranose rings of the two monosaccharide components (Fig. 2B) (20). In the cases of *Ascophyllum nodosum* and *Laminaria digitata*, the alginate contents are 22–30% and 25–44% on a dry weight basis, respectively (21). The biological function and physiological properties of alginate in brown macroalgae are thought to be similar to those of cellulose in terrestrial plants (20). The sequence of alginate is variable but consists of three distinct regions: a mannuronic acid-rich region (polyM), a guluronic acid-rich region (polyG), and a random region (polyMG). A higher ratio of polyM increases the viscosity and gel strength of the polymer, and *O*-acetylation of polyM decreases the efficiency of degradation within the cell (22). The relation between structure and function varies according to species and tissues from the same algae. For instance, in *Laminaria hyperborean*, stipe and holdfast have a very high content of polyG, providing high mechanical rigidity. In contrast, the leaves of the same macroalgae have a low content of polyG, providing a flexible texture (20). Annual industrial production of alginate is estimated to be approximately 30,000 metric tons, mainly from the genera *Laminaria* and *Macrocystis* of brown macroalgae (23). Alginate is widely used across various industries in food, textiles, cosmetics, and bioplastics (24). More recently, alginate has been utilized in high-value biomedical applications including wound management, anti-adhesion, drug-delivery systems, and tissue encapsulation for regenerative therapy (25). However, alginate is not only of interest as a polymer; the degradation products of alginate are useful for various applications. Oligosaccharides are known to have various physiological properties such as radical scavenging activity toward superoxide radicals, enhancing the cytokine secretion from human macrophages, and growth of human keratinocytes and endothelial cells (26–31).

ETHANOL PRODUCTION FROM LAMINARIN

Laminarinase such as β-1,3-glucanase and β-1,6-glucanase is required to convert laminarin into glucose for ethanol production. Several studies have demonstrated ethanol production from laminarin by microorganisms including bacterium, yeast, and fungus (32–38). Horn et al. (34) demonstrated that *Pacchysolen tannophilus*, *Kluyveromyces marxianus*, and *Pichia angophorae* can produce ethanol from laminarin directly. Among them, *P. angophorae* produces ethanol from both laminarin and mannitol. Salt tolerance of microorganisms used for fermentation is an advantageous feature for bioethanol production from brown macroalgae. However, the growth of *P. angophorae* is inferior to that of *Saccharomyces cerevisiae* BY4742 strain under saline conditions (39). Motone et al. (37) identified a novel laminarinase, Gly5M, using focused proteomic analysis from marine bacterium *Saccharophagus degradans*. Furthermore, Gly5M was successfully displayed on the cell surface of *S. cerevisiae* using the yeast cell surface display system (Fig. 3). This system is a groundbreaking method for the construction of whole-cell biocatalysts and enables simultaneous saccharification and fermentation of the substrate, a cost-effective process that provides enhanced rates, yields, and concentration of ethanol with less capital investment (40). Gly5M-displaying *S. cerevisiae* showed a laminarin-degrading activity and mainly generated gentiobiose, which is a disaccharide of glucose with a β-1,6-bond. *Aspergillus aculeatus* β-glucosidase (BG)-displaying *S. cerevisiae* was also constructed for further conversion of gentiobiose into glucose. The co-culture system of Gly5M and BG-displaying yeasts produced 5.2 g/L of ethanol from 20 g/L of laminarin directly (a conversion efficiency of 46%).

ETHANOL PRODUCTION FROM MANNITOL

For ethanol production from mannitol, the conversion of mannitol into D-fructose by mannitol-2-dehydrogenase is necessary, and then D-fructose further enters central carbon metabolism. Several researches have achieved ethanol production from mannitol by using bacteria and yeasts (34,35,39,41,42). Horn et al. (35) demonstrated that *Zymobacter palmae* produced approximately 10 g/L of ethanol from a synthetic mannitol medium containing 38 g/L of mannitol with a yield of 0.38 g ethanol (per gram of mannitol). Wild-type *Escherichia coli* has a native metabolic pathway of mannitol. Kim et al. (41) demonstrated that *E. coli* KO11 (ATCC11303) produced 25.8 g/L ethanol from a provided 75 g/L of mannitol.

Ota et al. (42) screened six yeast strains (*Saccharomyces paradoxus* NBRC 0259, *Kuraishia capsulata* NBRC 0721, *K. capsulata* NBRC 0974, *Ogataea glucozyma* NBRC 1472, *Ogataea minuta* NBRC 1473, and *Debaryomyces hansenii* NBRC 0794) through ethanol fermentation tests from a medium containing either glucose or mannitol. Among them, *S. paradoxus* NBRC 0259 was selected based on its ethanol productivity from mannitol. This strain produced over 40 g/L of ethanol from 100 g/L of mannitol.

Chujo et al. (39) found that wild-type *S. cerevisiae* BY4742 acquired the ability to assimilate mannitol during prolonged

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