Characterization of a renewable extracellular polysaccharide from defatted microalgae Dunaliella tertiolecta

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 highlights

► Extracellular polysaccharide (EPS) was obtained from defatted biomass of microalgae Dunaliella tertiolecta.
► Monosaccharide composition analysis confirmed that EPS consisted of glucose residues only.
► This exopolysaccharide was defined by FTIR, NMR and enzymatic cleavage analysis to be (1 → 4)-α-D-glucan.
► EPS was characterized as suitable substrate for biotechnological production of glucose and ethanol.

article info

Article history:
Received 1 September 2012
Received in revised form 12 November 2012
Accepted 19 November 2012
Available online 29 November 2012

Keywords:
Microalgae
Polysaccharide
Monosaccharide
FT-IR
NMR
Biorefinery

abstract

Extracellular polysaccharide (EPS) was isolated from defatted microalgae Dunaliella tertiolecta and defined as linear (1 → 4)-α-D-glucan based on monosaccharide composition, enzymatic and spectroscopic analyses. Optimization and characterization of acidic and enzymatic hydrolyses of EPS have been performed for its potential use as a renewable biorefinery material. The hydrolytic methods were improved to assess the effect of substrate specificity, reaction time, pH, ionic strength and temperature on efficiency of glucose production. EPS was effectively converted into glucose within one-step enzymatic or acidic hydrolysis under optimized conditions. Over 90% recovery of glucose was achieved for both hydrolytic approaches. High potential production of EPS and high yield conversion of this substrate to glucose may allow further exploration of microalgae D. tertiolecta as a potential biomass producer for biotechnological and industrial exploitation of bioethanol.

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1. Introduction

Because of the gradual depletion of fossil resources, exploring of alternative energy sources such as renewable biomass is critical issue of global energy security and climate change. It is a big challenge to reduce the exploitation of fossil fuel and to protect the climate through the reduction of greenhouse gases especially CO₂ released. In recent, the multi-stage screening and bioconversion of different biomasses to biofuel and other products including bioethanol based on the biorefinery concept of biomass in bioconversion is being considered as a more potentially powerful way to account sustainable bio-based economy (Chisti, 2008). These issues stimulated a demand for using human and animal harmless, eco-friendly and biodegradable materials (Octave and Thomas, 2009). Especially, the lignocellulosic feedstock biorefinery using cellulose-containing biomasses and wastes has significantly been focused much attention for the last decade operating large-scale biorefinery schemes (Duarte et al., 2007; Sanchez, 2009). Lignocellulosic feedstocks serves as the major source of polysaccharide in plants that finds wide applications not only in food but also in pharmaceutical or biomedical industries because of its biocompatibility, biodegradability, and non-toxicity. Particularly, isolation and identification of native fermentable biomaterial sources are required for determine its desired utility in the direct manufacturing of biofuels and bioethanol (Vertes et al., 2008; Zhang et al., 2008).

Since fossil fuels are becoming drained rapidly within couple of decades, the efficient process and bioconversion of polymeric
This alga is enclosed in a thin elastic plasma membrane, contains antiviral (Yim et al., 2004, 2005) activities. Furthermore, sulfo-Gyrodinium impudicum has recently been the subjects of structural study, and their biological functions have been also evaluated (Suarez et al., 2005). For example, sulfated EPS produced by the marine microalgae have been implicated as bioactive materials. In physiological aspect, the presence of EPS layer around the cell may be involved in the protection of cell to reduce the penetration of unnecessary ions through the cell surface. As it has been shown for cyanobacteria (Shaw et al., 2003) and proposed for cave inhabiting eukaryotic green microalgae like Dunaliella atacamensis (Azúa-Bustos et al., 2010), EPS are involved into capture and retention of ambient water in extreme environments. Microalgal EPS have recently been the subjects of structural study, and their biological functions have been also evaluated (Suarez et al., 2005). For example, sulfated EPS produced by the marine microalgae Gyrodinium impudicum strain KG03 has shown immunostimulating and antiviral (Yim et al., 2004, 2005) activities. Furthermore, sulfated EPS from a red microalgae Porphyridium sp. have shown, anti-inflammatory (Matsi et al., 2003) and antioxidant (Tannin-Spitz et al., 2005) activities. Previously we have studied the biomass production by microalgae Haematococcus lacustris (Park et al., 2011) and, surprisingly, EPS obtained and purified from this source had demonstrated immunostimulating activity. For these reasons, in addition to the importance for the human consumption as food ingredients, microalgae have attracted great interests also as the sources of biologically active materials including EPS.

Marine green microalgae of genus Dunaliella produce EPS of highly variable structure and composition depending on the species. For instance, HPLC analysis confirmed that EPS isolated from Dunaliella salina biomass consisted of four major constituent monosaccharides: galactose, glucose, xylose and fructose (Mishra and Jha, 2009). Among Dunaliella species, Dunaliella tertiolecta is very attractive because it is a unicellular halotolerant (halophilic) marine alga utilizing inorganic nutrients present in artificial sea water along with sunlight to produce biomass using CO₂ as a sole carbon source. Also, it can be easy to cultivate with a relatively high growth rate, high lipid content, and along with a high contents of biodegradable biomasses (Chen et al., 2011). The cell of this alga is enclosed in a thin elastic plasma membrane, contains cellular organelles typical for other microalgae, but is lack of rigid cell wall consisting of lipids, proteins, polysaccharides and other components. This fact support more effective isolation of extracellular products from the living cells of this microalga. As far as previous reports were devoted to production of extracellular biopolymers under variable conditions (Park et al., 2011), structural composition of extracellular biopolymer obtained from defatted microalgae D. tertiolecta has not been reported yet.

The structural analysis and utilization of biomasses which are generally composed of various polysaccharides are not studied well in the production of bioethanol. In general, the dilute sulfuric acid hydrolysis under moderate reaction conditions was employed to be a reliable and easily performed low cost method for quantitative conversion of hemicelluloses and α-amylase to monomeric sugars, providing high effectiveness of the overall hydrolysis process. However, no report about the polysaccharide composition of microalgae biomasses of D. tertiolecta and monosaccharide production from this source has been published yet. Moreover, it is the lack of reports devoted to bio-ethanol production from defatted algal biomasses.

The objective of this study is to elucidate microalgae D. tertiolecta for its potential use for the production of fermentable monomeric sugar based on structural analysis of its extracellular polysaccharide. The present study reports the optimization of enzymatic and chemical hydrolyses of this polysaccharide up to glucose, a fermentable material for the potential bio-ethanol production.

2. Methods

2.1. Microalgal strain and cultivation

Among many kinds of microalgae, D. tertiolecta (UTEX LB 999), a green halophilic alga that accumulates β-carotene, was selected and obtained from the University of Texas at Austin (UTEX), since the structural analysis and utilization of biomasses are not studied well. In order to establish the optimum condition for the microalgal culture, cells were cultured under various conditions tested previously in our laboratory. 5 L artificial sea water plus f/2 medium for 7 days at 25 °C, with 0.1 Vm aeriation with compressed air containing 2% CO₂, under 150 μE/(m²·s) with fluorescent light irradiation was used as a small scale, and furthermore cells were cultured in a large scale (70 L) of plate type photobioreactor for the production of high level of biomass for 28 days under the same condition described above. The culture flask was illuminated with external lights that were automatically turned on/off to simulate a circadian cycle, without controlling the pH. Each type of light was previously evaluated for their effect on algal growth rate. If necessary, initial light intensities of 100, 200, and 350 μE/(m²·s) were attained using one, two, and four fluorescent lights, respectively, to address the effect of light intensity on the production of biomass and changing the structural component of cells.

2.2. Growth rate evaluation

All experiments were conducted with the seed culture during its exponential growing phase to evaluate the growth rate of D. tertiolecta. The algal cells were cultured at 25 °C under a continuous light intensity of 15 μE/m²/s using white fluorescent lamps, established as the optimum culture condition. The algal cell numbers and the average cell size were measured with a Coulter counter (Model Z2, Coulter Electronics, Inc., Hialeah, FL, USA) following the instructors manual. Data from the Coulter counter were collected by AccuComp software and then exported to an Excel spreadsheet to calculate the cell numbers and size distribution as well as average cell size.
2.3. Isolation and analysis of EPS

To obtain extracellular polysaccharide (EPS), the lipid extraction was performed by the method of Bligh and Dyer (1959), and the defatted biomass was weighted after freeze drying (lyophilizer II-Shin Co., Korea). Briefly, cells were collected from the culture broth by centrifugation at 10,000 rpm for 30 min and washed twice with 10 times volume of distilled water. Cells were lyophilized and lipids were extracted twice with 10 times volume of extracting solvent (chloroform:methanol = 1:2, v/v) from the dried cells. Cells obtained from the solvent extracting process were used as crude defatted biomass. Proteins were removed from the defatted dried biomass by the washing with 0.1 M NaOH solution to obtain partially pure EPS. Obtained purified product was analyzed for its carbon, hydrogen and nitrogen contents using an EL III Universal CHNOS Elemental Analyzer (Elementar Analysensysteme GmbH, Germany). Total amount of carbohydrates in EPS was determined by the method of Bligh and Dyer (1959), and the concentration of protein in the defatted biomass was determined by Bradford method (Bradford, 1976). Ash content was determined as the quantity of mineral matter which remains as incombustible residue of the EPS. Burning EPS to ashes was carried out at 700 °C, and is completed when the cool residue was nearly white. The ash quantity in EPS was expressed to dry matter. 

2.4. Monosaccharide composition analysis

Monosaccharide composition of EPS was determined by high performance anion-exchange chromatography (HPAEC) of its hydrolysate obtained at strong acidic conditions using a Bio-LC system (Dionex, USA) equipped with a pulsed amperometric detector (PAD). Briefly, EPS was resuspended in 1 mL of distilled water and then mixed with an equal volume of 4.0 M trifluoroacetic acid (TFA). Samples were stand for 6 h at 100 °C to allow the acid-hydrolysis, filtered through 0.45 μm syringe filter and vacuum dried using a Speed-Vac (Biotron, Korea). Residual acid was removed by repeated vacuum drying, and dried sample was analyzed on a CarboPac PA-1 column (Dionex, USA), following conditions for monosaccharide analysis by HPAEC-PAD as following the instructor’s instruction.

2.5. FT-IR spectroscopy

Absorption FT-IR spectra (400–4000 cm⁻¹) of EPS and potato amyllose (Fluka, Germany) were recorded in KBr pellets by Nicolet 6700 FT-IR spectrometer (Nicolet Analytical Instruments, USA); 64 scans were accumulated with a spectral resolution of 4.0 cm⁻¹. The spectra were smoothed and baseline was corrected using Omnic 8.0 (Nicolet Analytical Instruments, USA) software. The positions of overlapped bands were estimated by the second derivative algorithm. Then the processed spectra were converted into the CSV format and exported to Origin 6.0 software (Microcal Origin, USA) for preparation of graphs.

2.6. NMR spectroscopy

The 1H and 13C NMR spectra of purified EPS were recorded on Bruker Avance III TM 500 MHz in D2O solutions. Working frequencies were 499.8 MHz for 1H and 125.7 MHz for 13C, respectively. Correlation spectroscopy 1H, 1H COSY and 1H, 13C HSQC were applied for signals assignment.

2.7. Dilute acid hydrolysis of EPS

For the optimization of acidic hydrolysis, various amounts of EPS (0.1–1 g) and 0.5–1.5% sulfuric acid solution (20–100 mL) were mixed at 50–90 °C up to 24 h. Undigested solids was removed from the solution by centrifugation and kept frozen for analysis and processing. The collected hydrolysates were examined by TLC or HPAEC-PAD as described above. Hydrolysis of EPS was defined as the relative content of glucose in dried hydrolysate.

2.8. Enzymatic hydrolysis of EPS

The enzymatic hydrolysis of EPS with an enzyme mixture consisting of α-amylase (EC 3.2.1.1) and α-glucosidase (EC 3.2.1.20) (Sigma, St. Louis, USA) was tested in comparing with artificial substrate pNP-glucoside (Sigma, St. Louis, USA). The enzyme activity of α-glucosidase towards pNP-glucoside was determined by photometry at 405 nm (absorption of releasing free p-nitrophenol (pNP), molar absorption coefficient 18,400). One unit of α-glucosidase activity was defined as 1 nmol of pNP released per minute. To complete the conversion of EPS to glucose, α-amylase and α-glucosidase (10 U/each) known as an endo- and exo-type enzymes was mixed with defatted dried algal biomass (0.5 g) in 50 mM sodium phosphate buffer (pH 6.8) and incubated at 37 °C for 0–48 h. The sample was then boiled for 10 min at 90 °C to quench the reaction. Centrifugation at 9000g for 15 min was performed to separate the solid residue and the supernatant. The release of soluble sugars (basically glucose) formed in preliminary hydrolysis experiments was determined by reducing sugar assay (Smogyi, 1952) and corrected by blank tests on substrate and enzymes. The enzymatic digestibility was defined as a relative amount of EPS digested to glucose. For the statistical analysis, data from three independent experiments were expressed as the means ± S.D and were considered to have statistical significances at P < 0.05.

3. Results and discussion

3.1. Isolation procedures

Besides polysaccharides, algal biomass contains many other compounds, so it was important to isolate and purify EPS by removing of ballast ones and then it would be possible to characterize its structure and biological activities. Lipids and other hydrophobic molecules were separated from microalgal biomass by extraction in chloroform:methanol mixture (1:2, v/v) from the dried cells yielding defatted material (crude EPS). Defatted biomass was obtained from the total microalgal biomass with the yield of 69.4%. Regarding cell growth and EPS accumulation responding to environmental changes, the results suggest that significant changes of EPS production were dependent on the cell growth rate and amount of biomass. Structurally diverse and some unique properties of polysaccharides of microalga are regarded as a renewable resource of biotechnologically important materials, some of which may useful for special applications. Investigation of cell growth and accumulation of EPS responding to environmental changes is considerable point. Therefore the production of EPS was investigated under different stress conditions including salinity in the range 0–2.0 M under the same light intensity. As results, we found that the synthesis of exo-cellular carbohydrates in total defatted cells was found increasing slightly with salt concentration. Although EPS production and its variation under salt concentration and light intensity have not been reported, we have accounted 0.5 M salt concentration in which of sea water. In addition, no significant changes of the composition of polysaccharides in EPS have observed (data not shown), which is the major point of
the structure of EPS isolated from the defatted cells. To evaluate further fermentation of EPS, defatted biomass was treated by sonication (Power Sonic 405, Whashin Co., Korea) at 50 °C for 30 min to disperse all dried component in distilled water. Soluble proteins and other ballast substances were then removed by centrifugation at 13,000 rpm for 30 min. Since it was difficult to separate protein and polysaccharides possibly due to the complexation between them, alkali reagent (0.1 M NaOH) was used to remove most of proteins from EPS as the next purification step. The insoluble part was then further twice rinsed with ethanol and lyophilized to powder, yielded as the purified EPS; its composition is summarized in Table 1. There was a minor discrepancy between nitrogen content and low amount of proteins, or total carbohydrates of EPS, upon testing several times. Based on the results of elemental analysis, if most of N originates from proteins, it should be about 17% of proteins. These results suggest that may be Bradford method is not suitable in this case. Subsequently, carbohydrates predominate in this preparation, while protein was found to be the minor component or impurity. The undefined impurities could be small molecules, may be phenolics, bonded tightly to the EPS.

### 3.2. Acidic hydrolysis and monosaccharide composition

To evaluate the structure of EPS and its availability for the chemical treatment, series of preliminary hydrolytic experiments were carried out under variable conditions, i.e. sulfuric acid concentration (0.5–1.5%), reaction temperature (50–90 °C) and reaction time (0–60 min). The aim of these experiments was obtaining of the optimal conditions for complete hydrolysis of EPS. In varying preliminary tests with acid hydrolysis we defined that only a single monosaccharide component was observed in TLC analysis (data not shown). In addition to this, glucose was recovered as the major monosaccharide after dilute-acid hydrolysis of EPS, but this process depended on the reaction conditions applied in the experiments. In general, the increase in severity (i.e., at higher acid concentration, temperature and duration) enhanced the whole hydrolytic process but also intensified substantially the secondary degradation of monomeric sugars thereby decreasing the final glucose yield. Selectivity of acidic hydrolysis should therefore assure the maximal glucose concentration in the hydrolysate. Evidently, no other sugars except glucose were formed during acidic hydrolysis of EPS (data not shown). High values of glucose recovery were achieved varying the reaction conditions. Obviously, the temperature of 80 °C provides more preserving conditions for acidic hydrolysis. Maximal glucose recovery (about 90% of total glucose) was observed when EPS was hydrolyzed at 80 °C for 60 min in 1.0% H2SO4. As can be seen, the effective chemical conversion of EPS into glucose is influenced mainly by acid concentration and by the ratio temperature/acid concentration. Some of algal EPS are heteropolymers consisting mainly of galactose, glucose, xylose, some deoxy sugars and uronic acids defined in a separate works (unpublished data). Also, the major monosaccharides obtained after total acidic hydrolysis of EPS from D. salina were galactose, xylose and glucose (Mishra and Jha, 2009). By contrast, EPS from D. tertiolecta described here is quite different from those obtained from other Dunaliella species. Evidently, our results are quite remarkable, since they confirmed that EPS of D. tertiolecta is glucan. This difference may be explained by the fact that Dunaliella species and other algae are sensitive to the environmental conditions supporting photosynthetic assimilation of carbon dioxide and its utilization as energy source.

### 3.3. Structural assignments by enzymatic hydrolysis

Prior to define optimal conditions for the production of glucose as by-product, purified EPS was partial digested with an \textit{endo}-type \(\alpha\)-amylase and used as initial source for the kinetic study on \textit{exo}-type enzyme \(\alpha\)-glucosidase. Enzymatic hydrolyses of EPS by enzyme mixture were performed under variable temperature, pH and ionic strength affecting enzyme activity. As the most abundant constituent, EPS can be used as the source of fibers or fermentable sugars (glucose) for bio-ethanol production. Removal of impurities such as lipids or proteins is assumed to increase the accessibility and digestibility of EPS and thus leads to greater glucose production. Prior to assess this effect, model synthetic substrate \(p\)-nitrophenyl glucoside was hydrolyzed with commercial \(\alpha\)-glucosidase (10 U/g
of EPS added) to optimize the reaction conditions. Enzyme activity and reaction yield were expressed from the time-dependent releasing of pNP (Fig. 1a). Optimal conditions were estimated to be pH 6.0 and 37 °C, respectively. Increasing of ionic strength by an addition of up to 100 mM of the salt (NaCl or KCl) did not inhibit enzyme activity, but even enhanced it by 20% in comparison with the untreated control reaction (data not shown). As can be seen from Fig. 1b, the enzymatic digestibility of EPS assessed by reducing sugar assay was significantly improved over the untreated control samples dependently on reaction time and substrate concentration (Fig. 1b). It is known that α-amylase (EC 3.2.1.1) is endo-type enzyme, so the end products of its action on (1→4)-α-glucan are α-glucooligosaccharides (dextrins) of varying length and, in the case of D-6 branched glucan, also branched oligosaccharides α-limit dextrins (van der Maarel et al., 2002). Also, α-glucosidase (EC 3.2.1.20) known as an exo-type amylolytic enzyme was used to complete conversion of α-glucan into glucose, acting on the external glucose residues of α-glucan or its fragments and produce only glucose. About 89% (w/w) of EPS was converted to glucose (2.67 g of glucose per 3.0 g of EPS) after 24 h of enzymatic hydrolysis with excess amount of enzyme. This amount corresponded closely to the total carbohydrates (see Table 1), so the glucan was completely hydrolyzed by this enzyme. No other remarkable amounts of monosaccharide were observed from TLC (data not shown) or HPAEC-PAD (Fig. 2) analyses of the enzymatic hydrolysates. These results were in agreement with those obtained by the same methods for the acidic hydrolysates and suggest that, like in the case of acidic hydrolysis, EPS was completely converted into glucose by the combinational treatment with α-amylose and α-glucosidase.

3.4. FTIR analysis

As it was mentioned above, according to TLC and HPAEC-PAD analyses of acidic and enzymatic hydrolysates, the EPS isolated from defatted biomass of *D. tertiolecta* consist of only glucose units, i.e. it should be glucan. However, it is still unclear about its configuration. To obtain more structural information, EPS was analyzed by FTIR spectroscopy. This non-invasive method is able to detect the presence of various functional groups and compounds (primary amine group, amide bonds, aromatics, alkyl groups, etc.) in algal materials (Jebsen et al., 2012; Laurens and Wolfrum, 2011) and is also sensitive to structure of plant and algal polysaccharides (Pereira et al., 2009). The FTIR spectrum of EPS was compared with the spectrum of potato amyllose (Fig. 3); band assignment is summarized in Table 2. Highly overlapped IR bands in the region of 950–1200 cm⁻¹ (CO and CC stretching vibrations in carbohydrates) predominates in the both spectra. By contrast, less pronounced IR bands at 1649 cm⁻¹ and 1536 cm⁻¹ were found only for EPS. These bands were assigned to amide I and amide II vibrations, respectively, which are typical for proteins (Kong and Yu, 2007). The former band is overlapped with the band of in-plane bending vibration of water found in the spectrum of amyllose at 1641 cm⁻¹. Comparing the regions of sugar and protein vibrations mentioned above it is evident that polysaccharide is the major component of EPS, while protein is present only as the impurity. The IR bands in the region of 800–950 cm⁻¹ are very sensitive to anomeric configuration of glucose (Zhabankov et al., 1995). The band at 860–862 cm⁻¹ was found in both spectra indicating α-configuration of glucose units. By contrast, the marked bands at 890–900 and 822 cm⁻¹, which are characteristic respectively for β-α-glycosidic bonds (Gutiérrez et al., 1996) and (1→3)-α-α-glycosidic bonds (Wang et al., 2007), were absent in these spectra. Weak shoulder near 895 cm⁻¹ found in both spectra probably arose from the COH deformation. Analysis of other structurally sensitive IR bands of polysaccharide vibrations also identified EPS to be amylese-like α-glucan. Several IR bands of EPS at 1154, 1081, 1022, 928, 862, 765, 709, 612, 578 and 532 cm⁻¹ were identical to those of amylase, as the previous study demonstrated (Cael et al., 1975). EPS is thus structurally similar to amyllose, but is different from other polysaccharides used in bioetanol production, i.e. heteroxylan from other microalgae (Simas et al., 2004), and cyclic (1→2)-β-D-glucan from other microalgae (Suarez et al., 2005).

3.5. NMR analysis

To confirm the FTIR results about the structure of EPS, this polysaccharide was also analyzed by NMR spectroscopy. 1H and 13C NMR spectra of EPS dissolved in D2O were measured. Correlation 1H, 1H, COSY and 1H, 13C HMQC experiments were used for better interpretation of proton and carbon signals (Table 3). The single H1 proton signal at δ 5.35 (J = 4.0 Hz) and corresponding C1 signal at δ 99.6 indicate α-anomeric configuration of the polysaccharide. No proton signal near δ 5.00 indicates the absence of
branching at O-6 (Nilsson et al., 1996). The downfield shifted C4 signal at δ 76.8 confirmed glycosylation at O-4 position. Some weak proton signals and corresponding COSY cross peaks were assigned to terminal α-D-glucopyranose residues (Fig. 4 left). The 1H NMR spectrum of EPS also contains weak signals of aromatic and aliphatic protons that could be arise from protein impurity. The HMQC spectrum (Fig. 4 right) contained only six signals of 1,4-linked α-glucopyranose units typical for amylose (Falk and Stanek, 1997). Therefore, NMR data confirmed that EPS is linear (1→4)-α-D-glucan structurally similar to amylose.

Detailed analysis of EPS isolated from defatted biomass of D. tertiolecta thus revealed some general features important for

### Table 2

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| 1H, 1H COSY (left) and 1H, 13C HMQC (right) spectra of EPS from microalgae Dunaliella tertiolecta. | Fig. 4. | 1H, 1H COSY (left) and 1H, 13C HMQC (right) spectra of EPS from microalgae Dunaliella tertiolecta. |
its possible use in industry. Although numerous bacterial and microalgal polysaccharides are regarded as abundant bio-renewable sources and potentially available in various research fields, the major composition of EPS obtained from those microorganism are too complex to be used as a bioactive substance or sources in complete bio-refinery. Numerous effects regarding the stress conditions on the production of lipid and carbohydrates (as starch) contents of microalgae have been reported in many literature, relevant pathways at molecular level have not been fully documented. Although some of transcribed genes and their partial pathway information are requisite for understanding and successful genetic manipulations in microalgae, these efforts have been hampered due to the lacking of genomic information on microalgal lipids and carbohydrate metabolisms. Therefore we believe that more reliable creation of these transcriptomes enables researchers to focus on the species of organisms of direct interest on lipid and carbohydrate metabolisms. In aspects of molecular level, sequences in relevant lipid and carbohydrate metabolisms can be screened, reconstructed and demonstrated the capability of using transcriptomic data to identify pathways of interest and potential targets for metabolic engineering in microalgae. However, variations of conditions such as salinity, light intensity, nitrogen deprivation are known to potentially influence the production and accumulation of lipids, proteins and carbohydrates in microalgae. Subsequently, genomic and transcriptomic information used to increase the expression of genes and maximize the related to these processes are obviously useful. Further study on genomic and transcriptomic analysis for cell culture on the production and accumulation of lipids, proteins and carbohydrates in microalgae under limited culture condition is ongoing. Unlike it has been reported earlier for EPS originated from other industrial microalgae (Mishra and Jha, 2009; Park et al., 2011), the EPS produced by D. tertiolecta was identified to be simple glucan, and thus it can be used in production of glucose and then bio-ethanol. It seems likely that, however, in addition to this glucans, many more other polysaccharide structures, unusual and unique in some cases, responsible for the various biological activities will be uncovered as more studies are performed. Certainly, further analyses of this D. tertiolecta EPS should be done to get better understanding of its exact structure, molecular mass and conformation. However, even this first report is sufficient to define its composition and anomeric structure based on results of TLC and HPAEC-PAD analyses of the hydrolysates as well as FTIR and NMR spectroscopy of the purified sample. Finally, we can conclude that this research has significant commercial potential to increase net ethanol production through the utilization of microalgal defatted biomass.

4. Conclusion

According to FTIR and correlation NMR analyses, the structure of EPS from microalga D. tertiolecta biomass was determined to be linear (1 → 4)-α-D-glucan similar to amyloge. Optimal reaction conditions for acidic and enzymatic hydrolysis of EPS yielding mainly glucose were established. These results strongly suggest that this EPS can be a promising renewable polymeric material, since most of microalgal EPS are known as multi-sugar compounds that are not suitable for producing homogenous monosaccharide convertible to bio-ethanol. Conclusively, EPS of D. tertiolecta is identified as a homopolysaccharide consisting of glucose, promising candidate for industrial exploitation for the source of bio-refinery.

Author disclosure statement

No competing financial interests exist.

Acknowledgements

This work was supported partly by the project “Technology development for bioenergy production from marine biomasses” from the program funded by Ministry of Land, Transport and Maritime Affairs of Korean Government, and by the project No. CEZ: MSM6046137305 of the Ministry of Education of the Czech Republic. Authors are thankful for financial support by these projects.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2012.11.077.

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