Isolation and Characterization of Water Soluble Bioactive Substances from Marine Photosynthetic Microalga Tetraselmis Species

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ABSTRACT

Isolation, quantification and characterization of bioactive compounds from microalgae Tetraselmis species have been performed to elucidate the potential use as a bioactive material. Microalgae are a promising source of lipids for biofuel production and represent a diverse group of microscopic photosynthetic organisms and are able to convert solar energy into biomasses. The imposition of oxidative stresses by various environmental factors leads to the production of reactive oxygen species (ROS) in plant cells, including algal cell. Antioxidant activity of water soluble bioactive substances (WSBS) was determined by DPPH, ABTS, FRAP and similar SOD radical-scavenging assay. The content analysis of lipid, sugar, polyphenol and protein was performed to evaluate the potent usefulness of WSBS from the whole cells cultivated under the optimum conditions for the production of biodiesel. The yield of WSBS was determined to be 17% based on the total carbohydrate assay. The relative activity of WSBS toward FRAP and DPPH reagent was observed ranging from 13 to 55%, showing the significant anti-oxidative properties with wide range of temperatures from room temperature to 80°C. The outcomes of this study support utilization of WSBS from microalgae Tetraselmis species as a potential source of naturally occurring antioxidants.

Keywords: Tetraselmis, Polysaccharides, Antioxidant activity, Bioactive substances

Introduction

Algae are an essential part of marine ecosystem in bio-refinery aspect since they are involved in carbon dioxide recycling. In the cultivation of marine herbivores, marine microalgae are frequently used as food source (1). Genus Tetraselmis of microalgae are important bio-sources to use in aquaculture, as a food source marine fauna such as fishes, shrimps and shellfish. They are cultured on a large scale due to their pivotal role in driving marine food chain. Thus, it is vital to evaluate the dissemination of toxic compounds from the food chain, owing to possible role of these microalgal cells. Microalgae is of great for applications in many research fields, as such and in extract form, in the areas of human nutrition and feed in aquaculture. Some other applications are as bio fertilizers, anti-inflammatory, anti-allergic and analgesic agents and in treatment of effluents was recently reviewed (2).

The biochemical composition of algae is depending upon its cultivation conditions and external factors like temperature, light and nutrient composition of the culture medium (3). Being one of the major constituents of the algal biomass, proteins (up to 50% [w/w]) play an important role in algal bio-refined (4). As a food resource, they are great alternative protein source due to their abundance and strong amino acid profile (5). In replacing the current bio-fuel feedstock, microalgae are the most promising contenders. As they display a high degree of biodiversity therefore they are considered as an essential source for new product formation and other potential applications (6). In aquatic ecosystems, microalgae plays the primary role for the production of biomasses. Various lipid extraction methods are followed for the micro-algal biomass extraction. Due to their unique cell wall formation, microalgae are single cell microorganisms (7). Methods like mechanical, enzymatic, chemical, ultrasonication and microwave technology are used for the cell disintegration in microorganism biomass. In these methods, only a small amount of water is used in cell disruptions of algal biomass (8). Nowadays, Microwave-Assisted Extraction (MAE) technique is used to extract polysaccharides from plant materials due to its efficiency in the extraction process, as compared to conventional approaches. So, in this study we have used the MAE technique to obtain water soluble bioactive substances (WSBS) including polysaccharides from Tetraselmis species. Moreover, MAE method requires less time, and the yield and quality of WSBS extracted using this technique are similar to that produced by other conventional extraction. As described in previous studies, process variables in MAE such as microwave power, extraction time and weight of the sample have substantially affected the efficiency of whole process. In addition, it significantly increases the yield of polysaccharides with the

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right optimization of these parameters (9).

Polysaccharides, amongst one of main class of bioactive substances from fungi, algae, and higher plants, are found to be effective, nontoxic with a wide range of pharmacological activities, including broad antitumor and immunomodulatory effects. They have attracted lots of attention in the biochemical and medical areas for several decades. Eventually, most of microorganisms secrete WSBS including of Extracellular Polysaccharides (EPS). It helps them to grow and form colonies which are known as the Biofilms, either get released into the extracellular medium or remain attached to the cell surfaces (10). Polysaccharides may be heterogeneous as known as hetero polysaccharides or heteroglycans. Their composition is slightly different from the monosaccharides of repeating unit. To get released out, polysaccharides require pretreatment, for breaking down the complex cell wall, as they are embedded inside the cell wall. Therefore, various pretreatment methods are used for the conversion of the complex plant cell walls (11). Different type of polysaccharides plays a significant role in the biomedical field due to their powerful antioxidant, antitumor and immune stimulatory effects (12).

Antioxidants are free radical scavengers which block the chain initiated by high energy molecules and postpone the oxidation of other consequent reactions. Antioxidant activity is a brilliant topic to carry out an intensive investigations and research due to their increasing demand in industries like pharmaceutical and food. They also have role in commercial industries for the production of anti-aging products and fighting carcinogenic compounds to provide health benefits (13). Antioxidants are also important part of nutraceuticals products of many health benefits (14). Therefore, natural antioxidant isolated from marine algae, fungi and plants are also useful as a food resource and for the production health products and disease prevention. These properties are shown in vitro and had effective scavenging abilities (15). Therefore, we have focused on the isolation and characterization of WSBS from microalga Tetraselmis species to use as antioxidant materials for industrial applications.

Materials and Methods

Materials and chemicals

The microalgae Tetraselmis species KCTC 12432 BP (TS1) and KCTC 12236 BP (TS2) used in this study were collected from INHA University (InCheon, Korea). Sodium hydroxide (NaOH) and Hydroxy-benzhydrazid (PHABAH) used for the quantification of total carbohydrate and reducing sugar were obtained from Junsei Chemical Co. (Tokyo, Japan). Decanoic acid, gallic acid, and bovine serum albumin was obtained from Sigma Chemical Co. (St. Louis, MO, USA). DPPH (1,1-diphenyl-2-picryl-hydrazil), ABTS (2,2' -Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) di-ammonium salt), and 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) for FRAP (Ferric Reducing Antioxidant Power) reagent were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and used for antioxidant activities assays. Biochemical properties of WSBS were analyzed using analytical chromatography plate (TLC plate), which was purchased from Merck KGa Co. (Darmstadt, Germany).

Extraction of WSBS

Both microalga Tetraselmis species were used for the extraction, quantification analysis of WSBS including reducing sugar and polysaccharides. To extract WSBS, 2.5 g of dried algal biomass rinsed with 95% ethanol and air-dried prior to use was mixed with 25 mL of distilled water and placed in a microwave extraction apparatus (MAS- II Plus) purchased from Shanghai Sino Microwave Chemistry Technology Co. Ltd. (China). Extraction was carried out with different microwave power and extraction time. After extraction, insoluble biomasses of microalgae were removed by centrifugation (13,000 rpm, 5 min) as shown in Fig. 1. Then the supernatant was collected and filtered by using the 0.2-micrometer filter membrane. After that, pH of the supernatant was neutralized by using 1 N HCl. To remove insoluble materials in the supernatant, it was further centrifuged to collect the supernatant and further lyophilized. The dried WSBS was further then dissolved in distilled water. The yield of WSBS was calculated as follows: Yield = Dried WSBS/Biomass*100

Biochemical properties of WSBS

Chemical contents analysis of WSBS was performed by using different quantification methods provided by previous studies as following below. Analysis of total carbohydrates in the sample was determined based on phenol-sulfuric acid assay with some modification (16,17). A simple, rapid and reproducible method was used for the estimation of reducing sugars by using glucose as standard (18). The concentration of proteins in the WSBS was obtained by Bradford method (19) (Bio-Rad Lab. Inc., Hercules, CA, USA) using bovine serum albumin as the standard. Concentrations of lipids and polyphenol compounds were estimated as described in earlier studies (20).

TLC analysis

Chemical composition and basic properties of WSBS were analyzed by TLC assay. A chemical mixture consisting of N-propanol: NH₄OH: H₂O = 7: 1.5: 1.5 (v/v) was prepared and used as the developing solvent. WSBS separated on TLC plate were visualized under UV light at a wavelength