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Growth Characteristics, Bio-chemical Composition and Antioxidant Activities of Benthic Diatom *Grammatophora marina* from Jeju Coast, Korea

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Benthic diatoms are known as a good food for shellfish in nature and in commercial hatchery of Jeju Island, Korea. *Grammatophora marina* is commonly found as dominant benthic micro-algae in Jeju coastal waters throughout the year. To know the best growth conditions of this species, culture was done in terms of three parameters; water temperature, salinity and nutrients. Each parameter was controlled by temperature of 15, 20 and 25°C; salinity of 25, 30 and 35 psu; and nutrient concentrations of 50, 100 and 200%. F/2 media was used with artificial seawater for the culture, which was continued for two weeks with L:D cycle 12:12 by using fluorescent light. Maximum specific growth rate was recorded 1.68 d^{-1} at temperature of 25°C with salinity of 35 psu and nutrient concentration of 200% on 6th day during the culture period. Maximum biomass was also observed 4.9×10^5 cells mL^{-1} in the same condition. This species may belong to the euryhaline and eutrophic habitat with warm condition. For nutritional aspects of this species, protein, lipid and carbohydrate were measured. The value of protein, lipid and carbohydrate was 4.96%, 15.82% and 5.65%, respectively. The antioxidant activities of 80% methanolic extract were 46.7%, 23.7% and 23.8% on DPPH (1,1-Diphenyl-2-picrylydrazy) radical, superoxide anion radical and hydrogen peroxide scavenging, respectively. Percentage metal chelating activity was 81.2%. Enzymatic extracts of Alcalase and Ultraflow showed remarkable scavenging activities on DPPH radical (86.5% and 57.2%, respectively), and superoxide anion scavenging activities were 45.3% and 41.4% from Kojizyme and Viscozyme extracts, respectively. Extract of Protomex revealed 24.8% activity on hydrogen peroxide and Neutase showed 30.8% on hydroxyl radical scavenging effects. Celluclast and Viscozyme extracts showed 33.2% and 32.1% activities on nitric oxide scavenging, respectively, while Alcalase showed 61.5% on metal chelating. This species contains higher lipids among the biochemical compounds and higher metal chelating activities from both 80% methanolic and enzymatic extracts.

Key Words: antioxidant activity, benthic diatom, enzymatic extracts, growth characteristics, mass-culture, methanolic extract

INTRODUCTION

Diatoms are indisputably the major component of many food webs, estimating their seasonal abundance, fluctuations and growth rate have been, and will be, an important component of marine science studies. Aquatic environments are subject to high temporal variability, with frequent reorganization of relative abundance and species composition of phytoplankton, as a result of interaction between physical, chemical and biological variables (Reynolds *et al.* 2000). Like all other marine organisms, diatom eco-physiology is influenced by water temperature, salinity, light intensity and nutrient concentrations (Tomas 1996; Thomas and Sommerfeld 1998; Thessen *et al.* 2005). Temperature is an important factor controlling the algal growth in natural environments (Lund 1949; Talling 1955) and growth response to temperature may be essential in regulating the predominance of phytoplankton species (Harris 1986). It is well known that salinity is an important abiotic factor affecting phytoplankton growth. Wide ranges of salinity and temperature may explain frequent appearance of phytoplankton throughout the year in the ocean (Hoshiai *et al.* 2003).

Jeju is a volcanic island of Korea which belongs to the subtropical region where the benthic diatoms are used as a live feed for shellfish in commercial hatcheries. The coastal water temperature and salinity of this island fluctuated widely and *Grammatophora marina* is being often found in a high abundance in this coastal water throughout the year (Affan and Lee 2004). However, microalgae

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are paid more attention as nutraceutical and health food in markets. Much effort has been expended on the search for new compounds of therapeutic potential, demonstrated in microalgae of all classes, possessing antibacterial, antifungal and anticancer. Extract of *Chlorella* sp. and *Spirulina* sp. are being proposed to be used with noodles, bread, green tea, beer and candy (Liang *et al.* 2004).

Exogenous chemicals and endogenous metabolic processes in the human body or in the food system produce extremely dangerous reactive oxygen species (ROS) which have ability to react with a large variety of easily oxidisable cellular components (Fridovich 1995). Antioxidants can be involved with the oxidation process by scavenging free radicals, chelating catalytic metals and by acting as oxygen scavengers (Shahidi and Wanasundara 1992; Buyukokuroglu *et al.* 2001). Commercial antioxidant supplements such as BHA, BHT, α-tocopherol, propyl gallate (PG) and *tert*-butylhydroquinone (TBHQ) are using in order to reduce oxidative damages in the human body (Sherwin 1990; Gulcin *et al.* 2002). However, these materials have been suspected of being responsible for some side effects such as liver damage and carcinogenesis. Among the natural antioxidants, polyphenols in the human diet have been reported to contribute in decreasing of cardiovascular diseases and exerting a beneficial health effect (Muller *et al.* 1995; Hotta *et al.* 2002). High antioxidant action of c-phycocyanin from blue green algae *Arthospira maxima* was detected and this natural product is able to scavenge alkoxyl and hydroxyl radicals (Romay *et al.* 1998). The objectives of the present study were isolation, growth characteristics study, mass culture, and investigation of the bio-chemical composition and antioxidant properties (80% methanolic extract and enzymatic extracts) of the benthic diatom *Grammatophora marina*.

MATERIALS AND METHODS

Phytoplankton isolation

Benthic diatoms attached on papans (undulated plastic sheet) were collected from the abalone culture hatchery from NFRDI (National Fisheries Research and Development Institute) in Jeju. The attached diatoms were removed from the papan and diluted with the same seawater. The sample was again diluted and one mL was transferred to a S-R counting chamber. Single cell of the benthic diatoms was picked up from the counting chamber by using micropipette under an inverted microscope (Olympus IX71). The single cell was transferred into multi-well for subculture. Subculture of the isolated species was done with autoclaved seawater which was filtered through 0.45 mm millipore and enriched with F/2 nutrients media (Aquacenter Ltd. USA), trace metals and metasilicate anhydrous crystals (Na_2SiO_3). The isolation process was carried out until getting the mono-strain of the *Grammatophora marina*. For the identification of the cultured benthic diatom, samples were observed under the phase-contrast microscope (Zeiss Axioplan) at a magnification of X 400, and the identification was done as described by Shim 1994.

The mono-strain *Grammatophora marina* was again streaked on agar plate that was prepared with 2% agar (w/v) and 0.04% F/2 (v/v) media and autoclaved seawater. Mono-strain *Grammatophora marina* colony was transferred from the agar plate into the 250 mL flask which contained 100 mL of F/2 enriched culture media and antibiotics. Seven different doses of antibiotics solution (penicillin 100-250 units/mL, streptomycin 100-250 μg/mL and neomycin 200-500 μg/mL) at 25 units-penicillin/mL, 25 μg-streptomycin/mL and 50 μg-neomycin/mL doses were used (SIGMA P 4083). About 10 mL cultured *Grammatophora marina* sample was transferred from the antibiotic mixed media to a 250 mL flask having 100 mL media for the culture of this species and the cultured sample was again streaked on the bacto-agar media for the observation of the presence of bacteria. Finally axenic strain of *Grammatophora marina* was obtained for advanced study.

Growth characteristics study

To know the best growth conditions of *Grammatophora marina* the culture was done in terms of three parameters; water temperature, salinity and nutrient concentrations. Each parameter was controlled by temperature of 15, 20 and 25°C; salinity of 25, 30 and 35 psu; and nutrient concentrations of 50, 100 and 200%, and it was divided as low, medium and high temperature, salinity and nutrient concentrations, respectively. General dose of F/2 media (adding of 1 mL of A media with 7.75 liter water and 1 mL of B media with 7.75 liter water, trace element solution 13 μL/L and metasilicate anhydrous crystal 13 mg/L) was considered as 100% nutrient concentrations. The artificial seawater was enriched with F/2 media nutrients (Aquacenter Ltd. USA) which was used for the growth characteristics study and for mass culture of *Grammatophora marina*. Observation of the growth characteristics of this species was continued during the period of two weeks culture with fluorescent

light under the irradiance of 180 μE m^{-2} s^{-1} at 12:12 L:D photo cycle. Cell densities were approximately 20 cells mL^{-1} at the inoculation time in all experiment.

For the estimation of standing crop, one mL sample was collected from the each experimental cultured flask at every two days interval and fixed with Logul's iodine solution. These fixed samples were diluted and cells were directly counted by using a S-R counting chamber under the observation of inverted microscope at X 400. Growth rate is calculated as doubling time (t_d) and specific growth rate (μ). Increasing of cell density per unit time was used for specific growth rate (μ) calculation of this study based on Pirt (1975).

$$\mu\ (\text{day}^{-1}) = \frac{\text{In}\ (X_1/X_0)}{t_1 - t_0}$$

Where the X_0 and X_1 are the quantitative expression of the cell density at the beginning time (t_0) to the end time (t_1) between the selected time intervals during the incubation. For each sample, duplicate counting was done and the mean value was plotted for growth curve.

Mass culture of phytoplankton

The mass culture of this species was done with the same F/2 media in a 10-litter bottle and in a glass tank with the papan which was used for the abalone culture in the shellfish hatchery at the maximum growth condition. *Grammatophora marina* cells were separated from the media by centrifuging 10 minutes at 560 g (Hanil 17R) and the precipitated cells were transferred in the petri dish. The sample content petri dish was kept in the deep freeze for 24 hours at -70°C and freeze-drying was done at temperature -50°C with 5 m Torr.

Bio-chemical composition of freeze- dried *Grammatophora marina*

Total protein, lipid and carbohydrate were measured by following the methods of BCA protein assay kit 23227 (Pierce USA), Taylor (1995), and Bligh and Dyer (1959), respectively.

Preparation of 80% methanolic extract

Freeze dried *Grammatophora marina* sample was ground in to a fine powder. Powdered sample (5 g) was immersed in 80% methanol (1000 mL) and placed in a shaking incubator for 24 h at 25°C. The macerated mixture was filtered and methanol extract was collected and concentrated. All antioxidant activities of *Grammatophora marina* extracts were compared with commercial antioxidants (BHT and α- tocopherol) dissolved in methanol.

Materials of enzymatic extracts

Viscozyme L, Celluclast 1.5 L FG, AMG 300 L, Termamyl 120 L, Ultraflow L, Protamex, Kojizyme 500 MG, Neutrase 0.8 L, Flavourzyme 500 MG and Alcalase 2.4L FG were used from Novo Co. (Novozyme Nordisk, Bagsvaerd Denmark). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), dimethyl sulfoxide (DMSO), butylated hydroxytoluene (BHT), xanthine, xanthine oxidase from butter milk, nitro blue tetrazolium salt (NBT), butylated hydroxyanisole (BHA), α-tocopherol and Folin-Ciocalteu reagent were used from Sigma Co. (USA). 2,2-Azino-bis (3-ethylbenzthiazolin)-6-sulfonic acid (ABTS) and peroxidase were purchased from Fluka Co. All the other chemicals used were analytical grade supplied by Fluka or Sigma Co.

Preparation of enzymatic extracts

Freeze dried *Grammatophora marina* was ground into a fine powder and 1 g was mixed with 100 mL of distilled water. The optimum pH of the each reaction mixtures were adjusted with 1M HCl/NaOH. Optimum pH and temperature conditions for the respective enzymes used were similar to those reported by Heo *et al.*(2003). In this test, five carbohydrate degrading enzymes (Viscozyme, Celluclast, AMG, Termamyl and Ultraflow) and five proteases (Protamex, Kojizyme, Neutrase, Flavourzyme and Alcalase) were used. Enzymes were added at the dosage (enzyme/ substrate ratio) of 1% and the mixtures were kept in the shaking incubators at optimal conditions for 24 h. Resultant mixtures were filtered and the enzymes activity of hydrolysates was inactivated by heating at 100°C for 10 min. Thereafter, the pH of the each hydrolysate was adjusted to pH 7 with 1M HCl/NaOH.

Determination of antioxidant activities

DPPH radical scavenging assay: Free radical scavenging activity of the different fractions of *Grammatophora marina* was determined as follows according to the modified method of Brand-Williams (1995).

Superoxide anion scavenging assay: according to the method described by Nagai *et al.* (2003).

Hydrogen peroxide scavenging assay: according to the method of Muller *et al.* (1995).

Hydroxyl radical scavenging assay: according to Chung *et al.* 1997.

Nitric oxide radical inhibition assay: Nitric oxide rad-

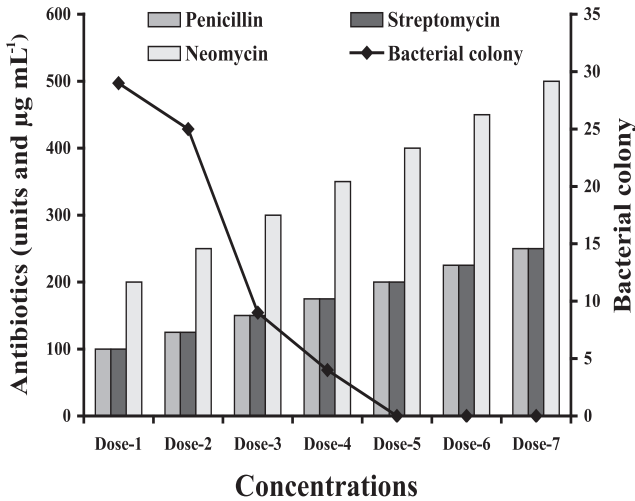

Fig. 1. Reduction of bacterial colonies with increasing of the antibiotics concentrations and Dose 1; Penicillin 100 units/mL, Streptomycin 100 μg/mL and Neomycin 200 μg/mL, and each dose increased by 25 units of Penicillin, 25 μg of Streptomycin and 150 μg of Neomycin per mL, respectively.

ical inhibition was determined according to the method reported by Garrat (1964).

Metal chelating assay: according to the method by Decker and Welch (1990).

RESULTS

Axenic strains

For getting the bacteria free strain, various doses of antibiotics were used and absolutely bacteria free mono-strain was found above a dose of 200 units-penicillin/mL, 200 μg-Streptomycin/mL and 400 μg-Neomycin/mL solution, while the concentration of antibiotics above 250 units-penicillin/mL, 250 μg-streptomycin/mL and 500 μg-neomycin/mL solution were found to be lethal for *Grammatophora marina* (Fig. 1).

Growth characteristics

Growth characteristic experiments were done to find out the best growth condition for mass culture. *Grammatophora marina* was found to grow well in all experiments during the culture period. At the temperature of 15°C with several salinities and various nutrient concentrations; the maximum specific growth rate was observed to vary from 0.77 to 1.09 d^{-1} with the average of 0.94 d^{-1} (Fig. 2A), and the maximum (4.44×10^5 cells mL^{-1}) standing crop was found at the maximum specific growth rate of 1.00 d^{-1} on 12th day after inoculation of the sample in a media which contained salinity of 30 psu and 100% nutrient concentrations (Fig. 2A and 3A).

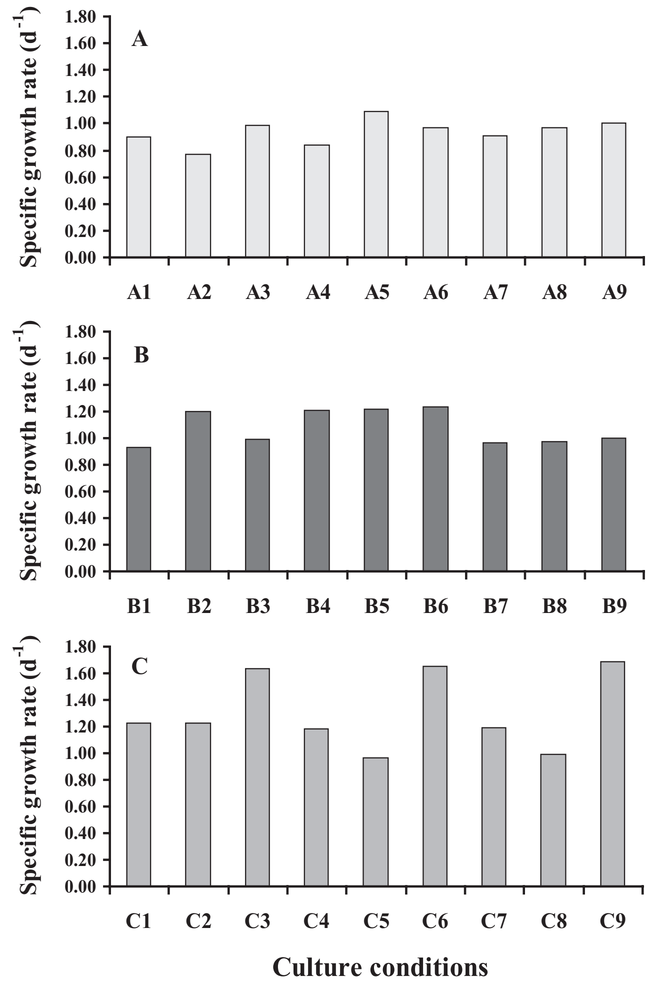

Fig. 2. Specific growth rate (μ) of *Grammatophora marina* at temperature of 15, 20 and 25°C with nutrient concentrations of 50%, 100% and 200% and with the salinity of 25, 30 and 35 psu. Culture conditions A1 (25 psu and 50% nutrient concentrations), A2 (25 psu and 100% nutrient concentrations), A3 (25 psu and 200% nutrient concentrations), A4 (30 psu and 50% nutrient concentrations), A5 (30 psu and 100% nutrient concentrations), A6 (30 psu and 200% nutrient concentrations), A7 (35 psu and 50% nutrient concentrations), A8 (35 psu and 100% nutrient concentrations) and A9 (35 psu and 200% nutrient concentrations) are at 15°C water temperature, and conditions of B at 20°C and C at 25°C are same regarding salinity and nutrient concentrations.

At the water temperature of 20°C, the maximum specific growth rate was found to be varied from 0.93 to 1.24 d^{-1} with the average of 1.08 d^{-1} among several salinities and nutrient concentrations (Fig. 2B), and the best specific growth rate (1.24 d^{-1}) and highest standing crop (4.01×10^5 cells mL^{-1}) were found on 10th day after inoculation of the samples into the media which contained salinities of 30 psu and 100% nutrient concentrations, respectively

Table 1. Comparison of antioxidants activities of *Grammatophora marina* crude extract by 80% MeOH and commercial antioxidants

Antioxidants	DPPH· activity %	Superoxide anion scavenging activity %	H_2O_2 Scavenging activity %	Hydroxyl radical activity %	NO· scavenging activity %	Metal chelating activity %
Extract	46.7 ± 1.50	23.7 ± 0.89	23.8 ± 0.85	35.2 ± 1.3	20.2 ± 0.2	81.2 ± 1.32
α-Tocopherol	69.6 ± 1.07	32.60 ± 1.65	73.90 ± 1.90	79.5 ± 2.2	43.4 ± 1.7	9.78 ± 0.96
BHT (Butylated hydroxytoluene)	71.75 ± 1.34	64.15 ± 1.80	67.25 ± 2.11	76.6 ± 2.8	56.3 ± 19	10.77 ± 1.93

Sample concentration is 2 mg/mL and M ± SE of determinations was made in triplicate

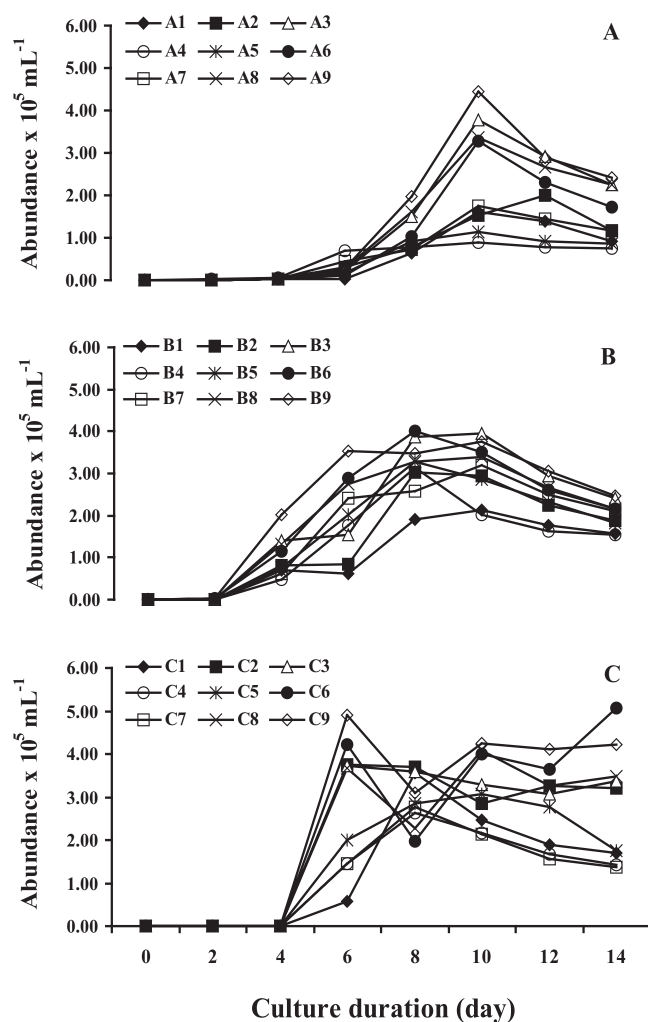

Fig. 3. Growth curves of *Grammatophora marina* at different salinities and nutrient concentrations (culture conditions are same as in Fig. 2).

(Fig. 3B).

The best specific growth rate (1.68 d^{-1}) was found at the temperature of 25°C with salinity of 35 psu and nutrient concentration of 200% on 6th day during the culture period among the all culture conditions (Fig. 2C). Maximum biomass was also observed 4.9×10^5 cells mL^{-1} in the same condition (Fig. 3C). In the bottle culture the highest cell abundance was 6.65×10^5 cells mL^{-1} on 12th day of culture period. In the culture tanks this species was found to grow well on the papans with strong attachment by producing thick mats. The cell density varied from 6.25 to 7.86×10^5 cells/surf.cm^2 of the papans. The cell abundance was found to be higher near the surface area than the bottom of the papans.

Biochemical composition

For obtaining the nutritional status of this species, biochemical composition such as protein, lipid and carbohydrate was measured and their values were 4.96%, 15.82% and 5.65% of one gram of dry weight sample, respectively. The remaining percentage comprised of ashes and waters.

Antioxidant activities

The antioxidant activities of 80% methanolic extract of this species were 46.7%, 23.7%, 23.8%, 35.2% and 20.2% on DPPH radical, superoxide anion scavenging, hydrogen peroxide scavenging, hydroxyl radical and nitric oxide radical scavenging, respectively (Table 1). Percentage metal chelating activity of the same species was 81.2% (Table 1). For the extraction of more antioxidative compounds, *Grammatophora marina* was enzymatically hydrolyzed by carbohydrases and proteases and potential antioxidant activities were evaluated. Enzymatic extracts of Alcalase, Ultraflow and Nutrase showed higher activities (86.5%, 57.2% and 53.1%, respectively) on DPPH radical scavenging, and Kojizyme and Viscozyme showed 45.3% and 41.4% on superoxide anion scavenging. In addition Protomex (24.8%) was effective on hydrogen peroxide scavenging and Neutrase revealed 30.8% on hydroxyl radical scavenging. Further, on nitric oxide scavenging Celluclast (33.2%) and Viscozyme (32.1%) exhibited higher activities and Alcalase showed 61.5% on metal chelating (Table 2).

Table 2. Comparison of antioxidant activities of enzymatic extract of *Grammatophora marina* with commercial antioxidants

Grammatophora marina Enzymatic extract	DPPH˙ activity %	Superoxide anion scavenging activity %	H_2O_2 Scavenging activity %	Hydroxyl radical activity %	NO˙ scavenging activity %	Metal chelating activity %
Viscozyme	47.3 ± 1.8	41.4 ± 1.2	14.1 ± 0.8	24.7 ± 0.9	32 ± 1.1	42 ± 1.1
Celluclast	41.2 ± 1.7	25.6 ± 0.8	15.5 ± 0.7	10.6 ± 0.2	33 ± 1.1	40 ± 1.2
AMG	52.9 ± 1.8	39.1 ± 1.1	17.6 ± 1.1	14.1 ± 0.3	29 ± 0.8	39 ± 1.1
Termamyl	44.3 ± 1.1	33.2 ± 1.2	17.3 ± 0.9	10.5 ± 0.3	28 ± 0.9	41 ± 1.2
Ultraflow	57.2 ± 1.9	39.3 ± 1.3	14.6 ± 0.7	19.1 ± 0.7	29 ± 0.9	32 ± 1.2
Protamex	48.2 ± 1.1	34.7 ± 1.1	24.8 ± 1.2	20.6 ± 0.9	23 ± 0.7	45 ± 1.6
Alcalase	86.5 ± 2.2	40.3 ± 1.5	16.2 ± 0.7	24.3 ± 1.1	22 ± 0.6	61 ± 2.2
Flavourzyme	43.7 ± 0.9	30.3 ± 1.3	19.8 ± 0.9	17.9 ± 1.1	12 ± 0.2	41 ± 1.8
Neutrase	53.1 ± 1.8	26.7 ± 1.1	20.8 ± 1.1	30.8 ± 1.3	28 ± 0.8	21 ± 1.2
Kojizyme	52.7 ± 1.7	45.3 ± 1.6	17.2 ± 0.6	27.9 ± 1.2	29 ± 0.7	38 ± 1.1
α-Tocopherol	69.6 ± 1.07	32.60 ± 1.65	73.90 ± 1.90	79.5 ± 2.2	43.4 ± 1.7	9.78 ± 0.96
BHT (Butylated hydroxytoluene)	71.75 ± 1.34	64.15 ± 1.80	67.25 ± 2.11	76.6 ± 2.8	56.3 ± 19	10.77 ± 1.93

Sample concentration is 2 mg/mL and M ± SE of determinations was made in triplicate

DISCUSSION

Axenic species

Bacteria were found to grow more with this species in the culture media and it was decomposing the phytoplankton cell and it was suspected that bacteria might play a role for the determination of antioxidants by providing its own cells compounds. For obtaining the axenic species antibiotics were used and the doses of antibiotics was lower than the dosages (Gentamycin 0.05 mg/L, Penicillin-G 16 mg/mL and Streptomycin 0.8 mg/mL) used by Kotaki *et al.* (2000) who used antibiotics for the axenic culture of domoic acid producing Nitzschia sp. The dosages of antibiotics may be dependent on the bacterial load or the presence of bacterial species.

Growth characteristics

Grammatophora marina was found to grow well in all the experimental conditions with wide range of temperature and salinity. The growth pattern of this species seems to be eurythermal and euryhaline with eutrophic habitat as the best specific growth was found to be occurred in a condition with medium temperature, higher salinity and maximum nutrient concentrations. *Grammatophora marina* was abundant in the Jeju coast at moderate temperature and salinity with available nutrient concentrations (Affan and Lee 2004). Furthermore, mass culture in a bottle is better than in a tank culture, as the bottle culture can protect bacterial contamination easily.

Bio-chemical compositions

In this species the lipid content was found to be higher than protein and carbohydrate. The percent carbohydrate of *Grammatophora marina* was similar with the *Skeletonema coastatum* (4.9%), and the lipid content (15.82%) was nearly similar with *Nitzschia closterium*, *Cylindrotheca fusiformis* and *Skeletonema coastatum* (18 to 20%) and the protein content (4.96%) of this species was very low compared with *Nitzschia closterium*, *Cylindrotheca fusiformis* (16 to 38%) (Brown and Jeffreyi 1995). *Grammatophora marina* was found as a fast growing benthic diatom with strong attachment to the substrate. It also makes nice mats on the substrate during growth. This single species mats on papans can be used as a live feed for abalone in the shellfish aquaculture as good energy source because of high lipid value.

Antioxidant activities

Plants have numerous antioxidant defense systems and because of that they are not susceptible to damage by ROS. Recently the interest about the antioxidants from the natural resources like plant has been increasing because of their reducing power on ROS, since they produce polyphenolics, and nitrogen containing compounds, phytosterols, carotenoids and chlorophyll derivatives. In the present study, antioxidant potentiality was determined by using different kinds of antioxidant assays from the 80% methanolic extract and different enzymatic extracts of *Grammatophora marina*. DPPH free radical scavenging assay is widely used to evaluate antioxidant activities. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a sta-

ble diamagnetic molecule (Soares *et al.* 1997). In cells, free radicals are continuously produced either as by-products of metabolism or deliberately as in phagocytes (Cheeseman and Slater 1993). In this study 80% methanolic extract and Alcalase extract of *Grammatophora marina* showed high activities when compared to commercial antioxidants (α-Tocopherol and BHT). Effects of superoxide anion can be exaggerated as it produces other kinds of cell damaging free radicals and oxidizing agents (Liu and Ng 2000). It is a precursor to active free radicals that have potential of reacting with biological macromolecules and thereby inducing tissue damage (Halliwell and Gutterridge 1989). The superoxide anion scavenging effects of enzymatic extracts of Kojizyme, Viscozyme, and Alcalase are even higher than that of α-Tocopherol. Thus it can be suggested that the enzymatic extracts may exert a better function in superoxide radical scavenging. Hydroxyl radical is the most reactive oxygen species among all ROS because of its strong affinity for reaction with various biomolecules. It can abstract the hydrogen atoms from phospholipid membranes and can do peroxidic reactions with lipids (Kitada *et al.* 1979). Nitric oxide is a gaseous free radical which has important functions for the physiological and pathological conditions, like renal injuries. The hydroxyl radical and nitric oxide scavenging ability of both 80% methanol extract and enzymatic extracts is found to be very little compared to commercial antioxidants. Ferrozine can make complexes with ferrous ions and ferrous ion can initiate lipid peroxidation by the Fenton reaction as well as accelerating peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals (Halliwell 1991). The obtaining results about the metal chelating activity of 80% metahnolic extract suggests that this species has the high ability for the iron binding than enzymatic extracts.

With 80% methanol both hydrophilic and hydrophobic bioactive compounds that contain antioxidant properties may extract a relatively poor yield and for getting a higher yield of antioxidant compounds enzymatic extracts were also done. According to aforementioned results except in a few cases enzymatic extracts have shown relatively higher activities than the extracts of 80% methanol as algae contains great amount of highly viscous polysaccharides in large extents (Mabeau and Kloareg 1987). Both insoluble and soluble fiber together with the other cell wall material acts as a physical barrier for the extraction of desired bioactive materials. Enzymatic hydrolysis of cells has informed significant yields of desired compounds and convenient industrial approaches in extraction and purification (Chiang *et al.* 1999; Nagai and Suzuki 2000). In addition, enzymatic extracts possess innovative advantages and characters over conventional extraction procedures. Breakdown of barriers can enhance the extraction of desired bioactive materials in the tissues and cells. Also, the breakdown/releasing of high molecular weight polysaccharides and proteins themselves can contribute to enhance the antioxidative activities (Ramos and Xiong 2002).

Cultures of diatoms for biotechnological interest are still at the early stage of development, except for the aquaculture (commercial exploitation). Development will depend on the utilization sectors and with less restriction in cultivation, like low cost and sustainable technique. In conclusion, the percentage of antioxidant activity on DPPH and the metal chelating activity were found to be higher from the 80% methanolic extract. Further, after hydrolyzed with carbohydrases and proteases activities of DPPH, superoxide anion and nitric oxide was increased. Therefore, further studies are very important for the isolation and purification concerning biochemical compounds that responsible for the antioxidants activity of this species.

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