Antialgal Activity of Glycoglycerolipids Derived from a Green Macroalgae *Ulva prolifera* on Six Species of Red Tide Microalgae

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Antialgal Activity of Glycoglycerolipids Derived from a Green Macroalgae *Ulva prolifera* on Six Species of Red Tide Microalgae

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Abstract. 1-O-palmitoyl -3-O-β-D-galactopyranosyl glycerol, 1-O-octadecanoic acid-3-O-β-D- galactopyranosyl glycerol, and 1-O-palmitoyl-2-O-oleoyl-3-O-β-D-galactopyranosyl glycerol were isolated from *Ulva prolifera* for the first time in our previous research. There are growth inhibiton of these three glycoglycerolipids against six species of red tide microalgae (*Amphidinium carterae*, *Heterosigma akashiwo*, *Karenia mikimotoi*, *Phaeocystis globosa*, *Prorocentrum donghaiense*, and *Skeletonema costatum*) investigated. Results showed that they have selective antialgal activity against six species of red tide microalgae, and antialgal activities against test red tide microalgae obviously enhanced with the increase of concentration of glycoglycerolipids. Among them, 1-O-octadecanoic acid-3-O-β-D-galactopyranosyl glycerol and 1-O-palmitoyl-2-O-oleoyl-3-O-β-D-galactopyranosyl glycerol exhibited more extensive antialgal activities, and the growth of *Phaeocystis globosa*, *Prorocentrum donghaiense*, and *Skeletonema costatum* (or *Karenia mikimotoi*, and *Skeletonema costatum*) was inhibited by these two glycoglycerolipids. Further, $EC_{50\text{-}96h}$ values of these three glycoglycerolipids for six red tide microalgae were obtained for the first time. By analyzing and comparing $EC_{50\text{-}96h}$ values, it has been determined that 1-O-octadecanoic acid-3-O-β-D- galactopyranosyl glycerol and 1-O-palmitoyl-2-O-oleoyl-3-O-β-D-galactopyranosyl glycerol showed the superior application potential than potassium dichromate as a characteristic antialgal agent against *Phaeocystis globosa*; More importantly, 1-O-octadecanoic acid-3-O-β-D- galactopyranosyl glycerol exhibited the superior application potential than potassium dichromate and other reported compounds as a characteristic antialgal agent against *Prorocentrum donghaiense*.

1. Introduction

Green algae *Ulva prolifera* has a wide distribution in Chinese coastal waters, and its natural biomass is very abundant even excessive. Researchers have found a lot of solvent extracts from *Ulva prolifera* has anti-oxidant [1], anti-tumor [2], anti-inflammatory [3], anti-bacteria [4], anti-hepatic injury activity [5], antialgl [6], and other biological activities [7, 8].

Allelopathic effects of *Ulva prolifera* on growth of *Karenia mikimotoi* was studied in early time [9]. Also, allelopathic effects of *Ulva prolifera* on *Isochrysis galbana* [10], *Heterosigma akashiwo* [11], *Karenia mikimotoi* and *Alexandrium tamarense* [12] and *Skeletonema costatum* [12, 13] were found. Sun et al. (2010) reported growth inhibition of solvent extracts from *Ulva prolifera* for *Amphidinium*

carterae, *Karenia mikimotoi*, *Alexandrium tamarense* and *Skeletonema costatum* [14]. The antialgal principles of *Ulva prolifera* against *Heterosigma akashiwo* were found to be fatty acids [15]. Sun et al. (2014) showed that antialgal effect of *Ulva prolifera* on the growth of *Karenia mikimotoi*, *Alexandrium tamarense* and *Skeletonema costatum* were probably resulted by lactone and coumarins compounds [16]. Recently, three glycoglycerolipids (1-*O*-palmitoyl -3-*O*-β-D-galactopyranosyl glycerol, 1-*O*-octadecanoic acid-3-*O*-β-D- galactopyranosyl glycerol, and 1-*O*-palmitoyl-2-*O*-oleoyl-3-*O*-β-D-galactopyranosyl glycerol) with antialgal activities against some red tide microalgae from *Ulva prolifera* were isolated for the first time [17].

Glycoglycerolipids are important components of cell membrances and play an important role in cellular biology. A number of glycoglycerolipids has been already isolated from higher plants [18, 19], freshwater and marine microalgae [20, 21] and marine macroalgae [22-33]. Glycoglycerolipids derived from microalgae and marine macroalgae display various bioactivities, such as anti-tumor [34], anti-HIV [35], anti-bacteria [25, 34], anti-infection [36], influenza virus-neutralizing [36] and other biological activities [26, 30]. There is currently considerable interest in glycoglycerolipids as a source of biologically active substances. However, these are very few studies that antialgal activity of glycoglycerolipids against red tide microalgae [37]. In our previous research, we only studied antialgal activities of these three glycoglycerolipids against *Alexandrium tamarense*, *Heterosigma akashiwo*, *Karenia mikimotoi*, *Prorocentrum donghaiense* and *Skeletonema costatum* at the concentration of 28.8 μg/mL [17], but failed to analyze quantitative relationship between the inhibition of algal growth and the concentration of glycoglycerolipids and determine half effective concentration (EC_{50}). Thus, this paper will be carried out to study antialgal activity of these three glycoglycerolipids on *Amphidinium carterae*, *Heterosigma akashiwo*, *Karenia mikimotoi*, *Phaeocystis globosa*, *Prorocentrum donghaiense* and *Skeletonema costatum*, to determine a quantitative relationship between the inhibition of algal growth and the concentration of each glycoglycerolipid and to obtain important parameters (EC_{50}) for future practical HAB control. Antialgal activities of these three glycoglycerolipids on six red tide microalgae (*Amphidinium carterae*, *Heterosigma akashiwo*, *Karenia mikimotoi*, *Phaeocystis globosa*, *Prorocentrum donghaiense*, and *Skeletonema costatum*) previously has not been reported.

2. Materials and Methods

2.1. HAB algae and Compound Samples

Red tide microalgae (*Amphidinium carterae*, *Heterosigma akashiwo*, *Karenia mikimotoi*, *Phaeocystis globosa*, *Prorocentrum donghaiense*, and *Skeletonema costatum*) were provided by the Microalga Research Laboratory of the Ocean University of China, Qingdao, China. All nine microalgae were cultured aseptically in Erlenmeyer flasks with f/2 medium. Cultures were incubated at 20°C and illuminated with fluorescent lamps in a 16/8 dark/light cycle, at an irradiance level of 60 μmol photons m^{-2} s^{-1}. All the flasks containing microalgae were shaken twice at a set time every day to prevent wall growth. Microalgae were cultured to exponential phase before subsequent inoculation.

Three glycoglycerolipids samples were prepared as the method reported by Sun et al. [17]. Each glycoglycerolipid sample was dissolved in methanol at a concentration of 5 mg/mL and filtered through a 0.22 micron syringe filter as a stock solution.

2.2. Seawater for Experiments

Seawater was obtained from the coast of Lianyungang. Aged natural seawater was filtered with cotton sheets, boiled, cooled, and filtered through glass fiber papers (Whatman GF/C, 0.22 μm poresize) to eliminate organic particles and debris of organisms. The pH and salinity of the seawater were adjusted to 8.0 and 30 ppt, respectively. This seawater was used for culture seawater of microalgae in all experiments of this study.

2.3. Antialgal Activity Assays

To determine antialgal ability of three glycoglycerolipids, 10 μL of each glycoglycerolipid sample was added to teat glass containing 0.5 mL of algal inoculant and 4.5 mL of culture medium (initial

concentration of glycoglycerolipid sample in suspensions of microalgae: 0.4, 2, 10, and 50 μg/mL), growth inhibition of algal species in six red tide microalgae (*Amphidinium carterae*, *Heterosigma akashiwo*, *Karenia mikimotoi*, *Phaeocystis globosa*, *Prorocentrum donghaiense*, and *Skeletonema costatum*) was measured. Controls received the same volume of methanol. Potassium dichromate was used as a positive control. There were four replicates for every treatment used in this experiment, and the culture conditions were the same as mentioned above. This experiment lasted for 4 days. $EC_{50\text{-}96h}$ was calculated as the method reported by Marklund and Marklund [38]. Cells of microalgae were counted by hemocytometer, while morphology of red tide microalgae was observed under an Olympus optical microscope.

2.4. Data Process And Statistical Analysis

All the data of the growth assays in this study were analyzed by ANOVA and Tukey's test.

3. Results

3.1. Antialgal Activity of Three Glycoglycerolipids Against Six Species of Red Tide Microalgae

A significant increase of antialgal activity was observed at the concentration range (0.4~50 μg/mL) of glycoglycerolipid (Fig. 1).

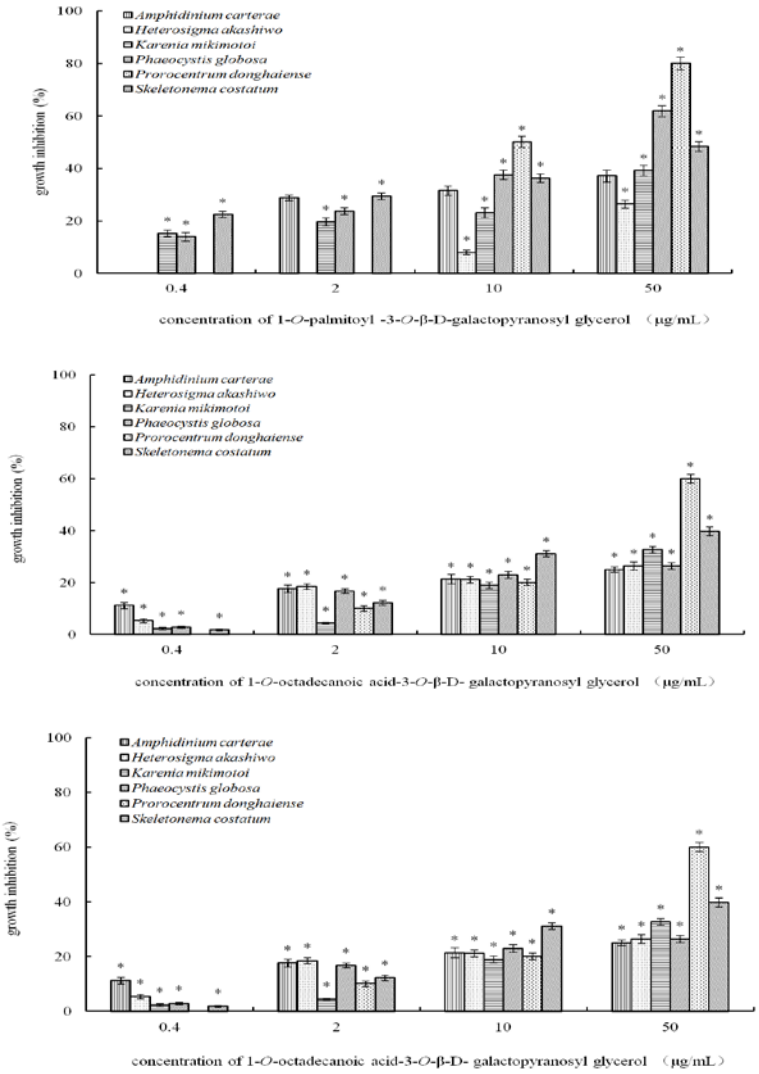

Figure 1. Antialgal activity of three glycoglycerolipids against the growth of six species of red tide microalgae. Data represent average values (n=4)

At 50 μg/mL, 1-*O*-palmitoyl -3-*O*-β-D-galactopyranosyl glycerol only significantly (p<0.05) inhibited the growth of *Prorocentrum donghaiense*. 1-*O*-octadecanoic acid-3-*O*-β-D- galactopyranosyl glycerol stronger (p<0.05) inhibited the growth of *Phaeocystis globosa* and *Prorocentrum donghaiense*. 1-*O*-palmitoyl-2-*O*-oleoyl-3-*O*-β-D-galactopyranosyl glycerol showed stronger (p<0.05) antialgal activity against others five red tide microalgae besides *Amphidinium carterae*. Among three glycoglycerolipids, 1-*O*-palmitoyl-2-*O*-oleoyl-3-*O*-β-D-galactopyranosyl glycerol exhibited more extensive antialgal activity.

And Growth inhibition of 1-*O*-octadecanoic acid-3-*O*-β-D- galactopyranosyl glycerol for *Prorocentrum donghaiense* at the concentration of 2 μg/mL was more than 50%; growth inhibition of 1-*O*-palmitoyl-2-*O*-oleoyl-3-*O*-β-D-galactopyranosyl glycerol for *Prorocentrum donghaiense* at the concentration of 10 μg/mL was 50%. Three glycoglycerolipids don't show significantly growth inhibition for *Amphidinium carterae*. Results showed that different algal species had variable sensitivity to same compounds. Among six red tide microalgae, *Prorocentrum donghaiense* was more sensitivity to three glycoglycerolipids; and *Amphidinium carterae* was most insensitive to three glycoglycerolipids. These considerable differences in antialgal activity of glycoglycerolipids against red tide microalgae may be due to species-specific differences.

3.2. $EC_{50\text{-}96h}$ of Three Glycoglycerolipids for Six Red Tide Microalgae

$EC_{50\text{-}96h}$ of potassium dichromate and three glycoglycerolipids for six red tide microalgae is shown in Table 1. As indicated in the literature [39], the grading standards for the toxicity of the algae growth inhibition experiment: $EC_{50\text{-}96h}$ < 1 μg/mL is extremely high toxic; 1~10 μg/mL is high toxic; 10~100 μg/mL is medium toxicity; > 100 μg/mL is low toxicity. Thus, 1-*O*-palmitoyl -3-*O*-β-D-galactopyranosyl glycerol was medium toxicity for *Heterosigma akashiwo, Phaeocystis globosa* and *Prorocentrum donghaiense*. It showed low toxicity for other three red tide microalgae; 1-*O*-octadecanoic acid-3-*O*-β-D- galactopyranosyl glycerol was high toxic for *Prorocentrum donghaiense*, and its $EC_{50\text{-}96h}$ for *Phaeocystis globosa* and *Prorocentrum donghaiense* was significantly (p<0.05) less than that of potassium dichromate. It exhibited medium toxicity for *Phaeocystis globosa* and *Skeletonema costatum*, and low toxicity for *Karenia mikimotoi*; 1-*O*-palmitoyl-2-*O*-oleoyl-3-*O*-β-D-galactopyranosyl glycerol showed medium toxicity for *Karenia mikimotoi*, *Phaeocystis globosa*, *Prorocentrum donghaiense* and *Skeletonema costatum*, and low toxicity for *Amphidinium carterae* and *Heterosigma akashiwo*. And its $EC_{50\text{-}96h}$ for *Phaeocystis globosa* was less than that of potassium dichromate. Results showed 1-*O*-octadecanoic acid-3-*O*-β-D- galactopyranosyl glycerol (or 1-*O*-palmitoyl-2-*O*-oleoyl-3-*O*-β-D-galactopyranosyl glycerol) possessed good application potential as a characteristic antialgal agent against *Phaeocystis globosa* and *Prorocentrum donghaiense* (or *Prorocentrum donghaiense*).

Table 1. EC_{50} (μg/mL) of potassium dichromate and three glycoglycerolipids against several red tide microalgae.

	Potassium dichromate	1	2	3
A. carterae	3.90	–	–	753
H.akashiwo	36.1	80.0	–	186
K.mikimotoi	16.2	156	133	41.0
P. globosa	38.3	55.4	14.0	24.5
P. donghaiense	5.00	37.0	2.28	10.0
S. costatum	2.70	989	66.1	10.3

Note: "–" no calculated.

4. Discussions

Three glycoglycerolipids isolated from *Ulva prolifera* were found to be 1-*O*-palmitoyl -3-*O*-β-D-galactopyranosyl glycerol, 1-*O*-octadecanoic acid-3-*O*-β-D- galactopyranosyl glycerol and 1-*O*-palmitoyl-2-*O*-oleoyl-3-*O*-β-D-galactopyranosyl glycerol, two of which, 1-*O*-palmitoyl -3-*O*-β-D-galactopyranosyl glycerol and 1-*O*-palmitoyl-2-*O*-oleoyl-3-*O*-β-D-galactopyranosyl glycerol, were the

same compounds that had been isolated from *Gracilaria lemaneiformis* [37]. Other researchers have also reported that brown algae such as *Laminaria japonica* [40, 41], *Ecklonia kurome* [40], *Ectocarpus fasciculatus* [42], *Sargassum hemiphyllum* var. Chinense [23], *Sargassum carpophyllum* [24], *Ishige okamurai* [25], *Fucus vesiculosus* [43], *Sargassum horneri* [26], *Sargassum wightii* in India's coast [25], *Sargassum thunbergii* [27, 33], *Zonaria diesingiana* [44], *Sargassum fusiforme* [30] in Dalian, *Sargassum fulvellum* [31], *Sargassum muticum* [32] and *Sargassum pallidum* [41]; red algae such as *Chondiria dasyphylla* [45], *Gracilaria verrucoca* [40], *Eucheuma muricatum* [40], *Hypnea charoides* [46], *Laurencia majuscula* [46], *Chondria armata* [47], *Laurencia tristicha*[29], *Chondria crasscaulis* [29, 48], *Ahnfeltia tobuchiensis* [41] and *Porphyra haitanensis* [48]; green algae such as *Codium iyengarii* [22], *Caulerpa racemosa* [50], *Tydemania expeditionis* [51] and *Ulva fenestrata* [41] contain glycoglycerolipids. Glycoglycerolipids derived from marine macroalgae have been reported to have a few biological effects. Monogalactosyl diacylglycerol (MGDG) from brown algae *Sargassum carpophyllum* is shown activity causing morphological anbormality of *Pyricularia oryzae* mycelia and weak anti- tumor activity [34]. Digalactosyl diacylglycerol (DGDG) derived from brown algae *Sargassum horneri* showed inhibitory effect on the proliferation of CacO-2 cells *in vitro* [26]. Sulfoquinovosyl diacylglyccerol (SQDG) isolated from red algae *Gigartina tenella* inhibits DNA-polimerase and HIV-reverse transcriptase [34]. Sulfoquinovosyl diacylglyccerol (SQDG) derived from *Sargassum wightii* was active against *Xanthomonas oryzae* pv. oryzae which causes bacterial blight of rice [25]. Two glucopyranosyldiacylglycerols obtained from brown algae *Sargassum fulvellum* showed fibrinolytic activities in the reaction system of pro-u-PA and plasminogen *in vitro* [31]. In this work, we proposed new biologically active glycoglycerolipids isolated from *Ulva prolifera* for antialgal agent (Fig. 1). 1-*O*-octadecanoic acid-3-*O*-β-D- galactopyranosyl glycerol (or 1-*O*-palmitoyl-2-*O*-oleoyl-3-*O*-β-D-galactopyranosyl glycerol) possessed good application potential as a characteristic antialgal agent against *Phaeocystis globosa* and *Prorocentrum donghaiense* (or *Prorocentrum donghaiense*) (Table 1). Among them, 1-*O*-palmitoyl-2-*O*-oleoyl-3-*O*-β-D-galactopyranosyl glycerol exhibited more effective antialgal activity against tested red tide microalgae than others two compounds; and antialgal effects of 1-*O*-palmitoyl -3-*O*-β-D-galactopyranosyl glycerol on tested red tide microalgae were more stronger than that of 1-*O*-octadecanoic acid-3-*O*-β-D- galactopyranosyl glycerol (Fig. 1 and Table 1). These three glycoglycerolipids derived from *Ulva prolifera* are monogalactosyl diacylglycerols. Their structures showed that there are different in the composition of fatty acid segments [17]. Thus, we think that the inhibitory effects of three glycoglycerolipids correlated with their fatty acid segments. The unsaturated fatty acid had higher inhibitory activity than the saturated fatty acid did. The antialgal actions of the saturated fatty acids were related to the carbon chain length. The longer the carbon chain length was, the less the inhibitive effect was. These results are supported by these experiments by [52], Yin [29], Luo and Zeng [53]. Zhang et al. showed that the inhibitory effect of fatty acids correlated with their chemical structures, the more unsaturated linkages in fatty acid, the stronger the algal growth inhibited; the shorter the carbon chain of fatty acid, the better the algal growth inhibited [54].

Sugar chain of glycolipids can interaction with protein receptors, but also signaling molecules. Glycolipids are the main group of the outer cell membrane, cells provide structural stability and strength. Therefore, when the outer edge of the glycolipid compounds adhesion of algal cells, glycolipids can effectively and cytoplasmic membrane integration, the destruction of the cell membrane of algal cells, thereby affecting algal cell growth regulation, apoptosis, and ultimately affect the growth and proliferation of cells. Further research on the precise mechanism and mode of action of the antialgal glycoglycerolipids.

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