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Algicidal activity of the brown seaweed, *Ecklonia cava* against red tide microalgae

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Abstract

The algicidal activity of brown seaweed *Ecklonia cava* was examined against the red tide microalgae *Cochlodinium polykrikoides, Heterosigma akashiwo, Alexandrium minutum* and *A. lusitanricum*. *E. cava in* conditioned PES media (ECPE) and seawater (ECSE) extracts showed strong algicidal activity against *C. polykrikoides* and *H. akashiwo*, however, weak activity against *A. minutum* and *A. lusitanricum.* Moreover, ECPE and ECSE are equally effective as algicidal agents. Results also revealed that *E. cava* required a temperature of 25ºC or above for its potent algicidal activity against *C. polykrikoides* and *H. akashiwo*, and that maximum algicidal activity was attained after 24 hrs. Both the results indicated a potential algicidal activity of *E. cava* and suggested that this brown alga can be used as an environmental-friendly and economically viable potential tool for reducing and controlling the harmful algal blooms caused by *C. polykrikoides* and *H. akashiwo* in the coastal aquaculture area of Korea and Japan.

INTRODUCTION

The red tide phytoplankton known as harmful algal blooms (HABs) is reported to occur in world-wide with warning for public health and fisheries industries [1]. HABs now occur annually in many coastal waters due to eutrophication. HABs disrupt marine ecosystem, causing catastrophic mass mortality of fish in open water and in coastal fish farms through depleting oxygen, clogging the gills of fish, generating damaging reactive oxygen substance (ROS) and producing toxin [2], [3]. Dense growth of red tide algae cause severe problems, such as hindering boat traffic, blocking approaches, obstructing wash processes, creating unattractive foul-smelling loads [4]. Moreover, red tide can cause illness and death in human and in fish, shellfish and seabirds [5]. Losses to the fisheries industry by HABs have been estimated at US$ 1 million per year for South Korea alone and more than US$ I billion per year for Japan [6]. Over the last three decades, the occurrence of the red tide has been increased. Several methods to control red tide have been developed, such as physical agents e.g. Clay minerals [7], aminoclay [5], dredge sediment [8], yellow loses [9], [10]; chemical agents e.g. Copper sulphate [11], hydrogen peroxide [12] and biological agents e.g., bacteria [13], virus [14], planktonic cialiate [15] and heterotrophic dinoflagellate [16]. But these methods have some disadvantages like difficulty of application, high cost, water quality deterioration, adverse effect to the other organisms and toxic effect to the human. Although this method seems effective in short duration, they may have potentially dangerous environmental consequences. A promising alternative approach is to use macroalgae for controlling of microalgae. Macroalgae are distributed widely and are indigenous to the marine environment. Abundant seaweed species is considered as an easy, low cost and environmentally benign potential algicidal source [17]. *E. cava*, a brown seaweed, widely distributed in the coast of Japan and Korea, contains a variety of compound, such as phlorotannins, fucoidan, alginate, playing diverse biological and ecological role [18], [19]. In this work, the algicidal activity of *E. cava* was evaluated against four red tide microalgae.

MATERIALS AND METHODS

A. Collection of seaweed sample

The brown seaweed *E. cava* Kjellman was collected from the Kijang, (35°12´49´´N, 129°13´28´´E), coast of Busan, South Korea by scuba diving from July to November, 2011. After collection, instantly kept in a seawater tank and then transfer to an aquarium tank (200 L) with a semi closed circulating and filtering system. Flow-through seawater (3 L min^{-1}) was supplied to the tank and the temperature and light was maintained at 20±1°C temperature and 12h light: 12h dark cycle, respectively. Mature thallus blade were cut into 1 cm^2 long piece, washed with filtered autoclaved sea water and sonicated 1 min for removal of epiphyte.

B. Preparation of E. cava conditioned PES media (ECPE) and seawater (ECSE) extracts

For each 1 g of seaweed sample, 25 ml of PES media [20] or autoclaved sea water was added. Algal samples were conditioned for 24 hrs at 10°C, 15°C, 20°C, 25°C, 30°C temperature at 60 µmol m^{-2} s^{-1} light. After removing the algal biomass, the conditioned seaweed extracts were stored at +4°C. The conditioned seaweed extracts were filtered again through 0.20 µm pore size Cellulose Acetate syringe filters (DISMIC-25CS, Toyo Kaisha, Japan) before use.

C. Microalgae and culture condition

The axenic strains of *Cochlidinium polykrikoides*, was collected from Professor CH Kim Laboratory, Department of Marine Bio-material and Aquaculture, Pukyong National University, Busan 608-737, South Korea and *Heterosigma akashiwo* (CCMP 452), *Alexamdrium minutum* (CCMP 113) and *A. lusitanricum* (CCMP1888) were obtained from the Provasoli-Guillard Centre for the Culture of Marine Phyto-plankton (CCMP). Stock culture of the red tide microalgae were maintained axenically in PES media. The microalgae were transferred in to fresh media in every week and maintained at 40 µmol m^{-2} s^{-1} light. , 14:10 hrs light:dark condition at 20°C in a illuminated multi-room incubator (VS1203PF-LN, Vision Scientific Co. Ltd., Kjeonggi-do, Korea). All cultured samples were shaken twice a day to prevent algal growth on the glass wall.*D. Determination of algicidal activity*

For screening of algicidal seaweed extracts, approximately 10000 cells of 4 microalgae species were inoculated in 2 ml of ECPE or ECSE per 13 ml glass culture (Kimble glass Inc, USA) at a range of temperature condition. After 6, 12 and 24 hrs, the remaining cells were counted under a microscope (OLYMPUS, SZ3060, No 8B01435, Japan) with a Sedgewick Rafter cell counter. Algicidal activity was calculated using following formula of Kim et al. [4].

$$\% \text{ Algicidal Activity} = \left(1 - \frac{Tt}{Ct}\right) \times 100$$

Where, T (Treatment), C (Control) cell densities and t is the inoculation time

D. Determination of time effect

To determine the time effect, ECPE and ECSE at 25°C were used. After 6, 12 and 24 hrs, remaining cells were counted and expressed as % of algicidal activity.

E. Statistical Analysis

All data are presented as the mean ± S.E.M. The results shown in each figure are representative of at least three independent experiments.

RESULTS AND DISCUSSION

Firstly the algicidal activity of *E. cava* was determined in conditioned PES media (ECPE) and seawater (ECSE) extracts at different temperature. Almost 100% algicidal activity was observed in ECPE and ECSE extracts at 25ºC and 30ºC against *C. polykrikoides* and *H. akashiwo*. In *A. minutum and A. lusitanricum* algicidal activity was below or nearly 20% (Figure 1 & 2). However, there was no algicidal activity difference between ECPE and ECSE.

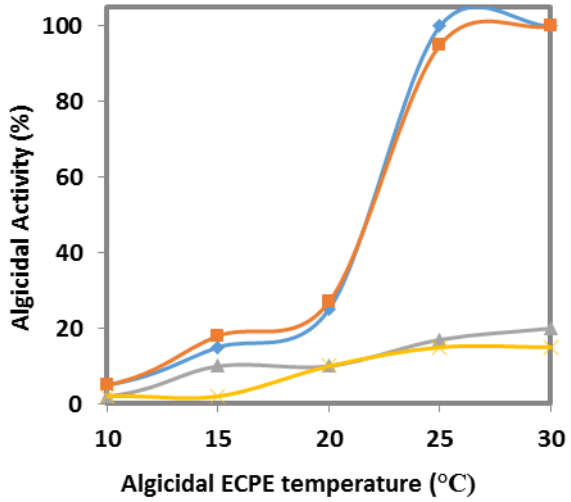

Fig.1. Algicidal activity of ECPE for *C. polykrikoides* (◊), *H. akashiwo* (■), *A. minutum* (▲) and *A. lusitunricum* (×). Algal culture conditions were maintained at 40 µmol m^{-2} s^{-1} light. , 14:10 hrs light:dark condition at different temperature. Data are the means from at least three independent experiments.

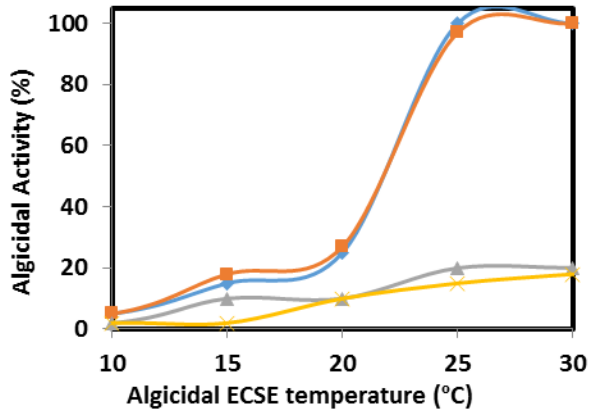

Fig. 2. Algicidal activity of ECSE for *C. polykrikoides* (◊), *H. akashiwo* (■), *A. minutum* (▲) and *A. lusitunricum* (×). Algal culture conditions were maintained at 40 µmol m^{-2} s^{-1} light. , 14:10 hrs light:dark condition at different temperature. Data are the means from at least three independent experiments.

Then the time effects was determined in ECPE and ECSE at 25ºC for *C. polykrikoides* and *H. akashiwo*, *A. minutum and A. lusitanricum*. After 24 hrs, 100% algicidal activity was observed against *C. polykrikoides* and *H. akashiwo*, and it was below or nearly 20% for *A. minutum and A. lusitanricum*. (Figure 3 & 4).

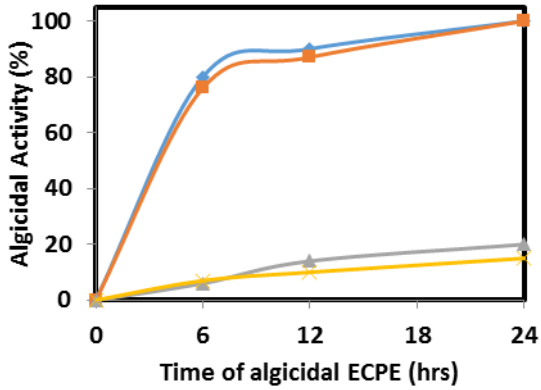

Fig. 3. Time effect of algicidal ECPE for *C. polykrikoides* (◊), *H. akashiwo* (■), *A. minutum* (▲) and *A. lusitunricum* (×). Algal culture conditions were maintained at 40 µmol m^{-2} s^{-1} light. , 14:10 hrs light:dark condition at 25°C temperature. Data are the means from at least three independent experiments.

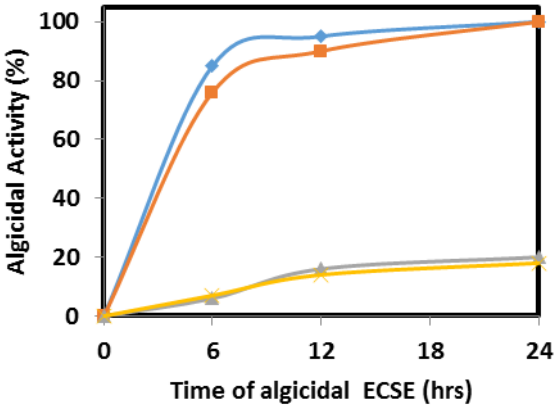

Fig. 4. Time effect of algicidal sea water media for *C. polykrikoides* (◊), *H. akashiwo* (■), *A. minutum* (▲) and *A. lusitunricum* (×). Algal culture conditions were maintained at 40 µmol m^{-2} s^{-1} light. , 14:10 hrs light:dark condition at 25°C temperature. Data are the means from at least three independent experiments.

Management of HABs has focused on the use of algicides for controlling and suppression. The most widely used strategy is to spray the clay materials, algicidal or microbial enzymes, etc. But the mechanisms are problematic due to requirement of huge amounts of materials, which are costly and significantly change the nature of environment and also harm the mid water and bottom dwelling organisms. Another approach is to use the chemical agent, such as copper sulphate, hydrogen peroxide etc. which are effective in controlling bloom within a short period, however, they are potentially dangerous for the aquatic ecosystem. In the search for HABs control agents, that are efficient and environmental-friendly, more attention is being directed to explore natural resources like allelopathic substances releasing aquatic organisms. Macroalgae and microalgae have long been maintained an antagonistic relationship in both natural and experimental aquatic ecosystems [21]. Hogetsu et al. [22] surmised that macrophytes released allelochemicals that inhibit algal growth. Several bioactive substances from seaweed have been extracted and purified successfully for practically HABs control and management [1], [23], [24]. Nagayama et al. [24] found that phlorotannins, specially phlorofucofuroeckol-A, a pentamer of phloroglucinol, from the brown seaweed *E. kurome* was very effective in killing or inhibiting the swimming of red tide dinoflagellates. *E. cava* also contains the phlorotannin phlorofucofuroeckol-A. Choudhury *et al*. [25] revealed that the

powder of boiled *E. cava* release both phlorotannins, dieckol and phlorofucofuroeckol-A in the water and suggested that in a comparatively higher temperature, *E. cava* may release phlorotannins to the sea water or in PES media which have algicidal activity. In natural condition, red tide usually occurs in the summer when the environmental temperature remains relatively higher than 25 °C. So, creating *E. cava* forest in the red tide affected coastal area may control the HABs by reducing the nutrients sufficiently for microalgal growth and releasing phlorotannins in the sea water. Since *E. cava* is abundant in the coast of Korea and Japan, creating the *E. cava* forest in these areas may be very cheap and easy method for controlling red tide in the fertile and eutrophic coastal areas of these two countries.

CONCLUSION

The results demonstrated that the brown seaweed *E. cava* exhibted potential algicidal activity in conditioned PES media and sea water extracts against two red tide microalgae *C. polykrikoides* and *H. akashiwo*. Therefore, *E. cava* may be an efficient and environmentally benign HABs control agent in the coastal aquaculture areas of Korea and Japan.

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