

有害・有毒藻類ブルームを形成するプランクトンに対するリボンアオサとアナアオサ(アオサ目, 緑色植物門)の殺藻物質としての有効性

誌名	水産増殖 = The aquiculture
ISSN	03714217
著者	石橋, 郁人 北村, 等 Alamsjah, M.A. 藤田, 雄二
巻/号	54巻3号
掲載ページ	p. 325-334
発行年月	2006年9月

## The Effectiveness of *Ulva fasciata* and *U. pertusa* (Ulvales, Chlorophyta) as Algicidal Substances on Harmful Algal Bloom Species

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**Abstract:** The algicidal activity of fresh tissue, dry powder and methanol extracts of *Ulva fasciata* and *U. pertusa* were evaluated against harmful algal blooms (HAB) species. The results indicate that sporophyte of fresh tissue from *U. fasciata* and *U. pertusa* induced the growth inhibition and lethal effects on *Heterosigma akashiwo* and *Alexandrium catenella* higher than their gametophyte strains. The dry powder of sporophyte of *U. fasciata* and *U. pertusa* induced significantly high rate of reduced growth and cell death than gametophyte strains on *H. akashiwo* species. On the contrary, the dry powder of *Ulva* spp. had low effect on *A. catenella* species. Furthermore, the methanol extracts of sporophyte of *U. fasciata* and *U. pertusa* showed higher algicidal effects than their gametophyte strains on HABs. It was also demonstrated that microalgae respond differentially to the methanol extracts of *Ulva* spp. These assays were most effective against the cells of *Chattonella marina*, *H. akashiwo*, and were moderately effective against *Fibrocapsa japonica* and *Karenia mikimotoi* cells. Meanwhile, the methanol extracts were lowest effective against *A. catenella* cells. The difference of the response among these microalgae species tested suggests that algicidal effects of *Ulva* spp. could be utilized for selective control on HAB species.

**Key words:** *Ulva fasciata*; *Ulva pertusa*; Algicidal activity; Harmful algal bloom

Harmful algal blooms (HABs) are becoming a global threat to living resources, fishing, tourism and human health. HABs like, *Heterosigma akashiwo* (Raphidophyceae) and *Alexandrium catenella* (Dinophyceae) cells commonly occur in temperate coastal waters throughout the world. These HABs are a big threat to marine culture industry and human health (Okaichi 1997; Fukuyo et al. 2002). Although, various physical, biological and chemical methods are known to control HABs effectively (Cloern 1982; Suttle 1995; Anderson 1997; Choi et al. 1998; Bae et al. 1999), many are clearly unacceptable due to environmental side effects and poor benefit or cost ratios (Jeong et al. 2000).

Alternate, safe, biocontrol measures using

macroalgae have been exploited in recent years (Ono et al. 1993; Jeong et al. 2000; Nagayama et al. 2003; Wang et al. 2006). Jin and Dong (2003) also showed algicidal effects of *Ulva pertusa* on *H. akashiwo* and *A. tamarensis*. In an earlier study, of the 37 species screened for algicidal activity, *Ulva fasciata* and *U. pertusa* showed remarkable algicidal activity (Alamsjah et al. 2005). However, to date, only a limited research has been carried out on the comparative study of algicidal activity of *U. fasciata* and *U. pertusa* on HABs. Thus, in this study, we evaluated the effectiveness of the fresh tissue, the dry powder and the methanol extract of *U. fasciata* and *U. pertusa* as algicidal substances on HAB species.

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Received February 3, 2006; Accepted June 14, 2006.

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## Materials and methods

### *Preparation of thalli of U. fasciata and U. pertusa by indoor culture*

Methods described by Jin and Dong (2003) were used to evaluate the algicidal activity of sporophyte and gametophyte tissue of *U. fasciata* and *U. pertusa*. Samples were collected from sea coast area of Nagasaki city, Japan. Specific reproductive phase of the *Ulva* was identified as described by Seavey (2003). Twenty pieces of approximately  $2 \times 2$  cm<sup>2</sup> size of *Ulva* thallus tissue were cultured in Enriched Seawater Medium (EMS) (Provasoli 1966), in 500 ml flat bottom aeration flasks (pre-culture treatments) with medium change at every three days interval. These treatments were maintained by indoor culture for seven days at 20°C,  $40 \mu\text{E}/\text{m}^2/\text{s}$  using 40 watts fluorescent lamps (FL40SD, Toshiba, Japan) with 12L:12D cycle. In this study, the culture conditions of all experiments were the same as mentioned above. The pre-cultured tissues (twenty pieces) were further cultured for main culture in EMS for seven days, in 1000 ml flat bottom aeration flasks with medium change at every three days.

### *Axenic culture of U. fasciata and U. pertusa*

The tissues were harvested from main culture treatments,  $0.5 \times 0.5$  cm<sup>2</sup> size segments were washed with 0.02% liquid detergent (Joy, Procter & Gamble, Japan) in autoclaved sea water (ASW) for 1 minute. The segments were then dipped in 20 ml of ASW containing 2% of Isodine (Meiji, Japan) for 1 minute. Then, the segments were rinsed with ASW and were further treated in 100 ml of ASW containing 2% of antibiotic mixture (penicillin G 1 g, streptomycin sulfate 2 g, kanamycin 1 g, nystatin 0.025 g, neomycin 0.2 g in distilled water 100 ml; Lipperheide and Evans 1991) for 24 hours. Further, the tissues were rinsed in ASW and checked for axenicity in ZoBell 2216E agar medium (ZoBell 1946) over 5 days.

### *Microalgae species culture*

HAB species of *Chattonella marina* NIES-3,

*Heterosigma akashiwo* NIES-4, *Fibrocapsa japonica* NIES-462, *Alexandrium catenella* NIES-677, *Karenia mikimotoi* as *Gymnodinium mikimotoi* NIES-680 were obtained from the National Institute for Environmental Studies, Japan. All these microalgae species were cultured aseptically in Guillard's f/2 medium (Sigma, USA). Prior to the experiments, the microalgae species were sub-cultured for 7 days.

### *Inhibitory and lethal assays with fresh tissue of U. fasciata and U. pertusa*

In this study, inhibitory assays are assays of a substance estimated to inhibit the biological endpoint of interest (e.g. depressed growth on HAB species), whereas lethal assays are assays of a substance required to cause death on HAB species. Furthermore, Jeong et al. (2000) determined that algicidal activity means having the property in controlling or killing HAB species. Procedure and calculation of inhibitory and lethal assays were performed following the method reported by Jin and Dong (2003) with minor modification. The inhibitory assays were performed with mixed cultures of one strain of HAB species and one strain of *Ulva* spp. To analyze the concentration-dependent effects of fresh tissue of macroalgae on the growth inhibition of *H. akashiwo* and *A. catenella* species, different initial inoculation concentrations of fresh macroalgae tissue (0, 0.3, 0.6 and 1.2 g/l) were used. HABs and *Ulva* thalli were co-cultured in 100 ml conical glass flasks containing 40 ml f/2 medium. Exponentially growing *H. akashiwo* or *A. catenella* cells were inoculated with fresh tissue of macroalgae at the same time in medium at an initial cell density  $8 \times 10^3$  or  $8 \times 10^2$  cells/ml, respectively. These experiments lasted 7 days. At one day intervals, 1 ml of samples of every flask were collected and the microalgae cells were counted on a haemocytometer under an optical microscope (CKX41, Olympus, Japan) to determine the maximum growth of the microalgae. In this study, macroalgae materials gathered on the conical flask bottom, while collected of samples were performed without macroalgae materials. The calculations of cell number of microalgae were

counted for each single cell so that it prevented calculation for bundling of many cells of microalgae and mucus of macroalgae. After sampling, the flasks were replenished with 1 ml of fresh solution, which contained full f/2 ingredients. In addition to performing the lethal assays, *H. akashiwo* or *A. catenella* cells were inoculated with fresh tissue of macroalgae at the same time in 5 cm diameter of Petri dish containing 10 ml of f/2 medium with microalgae density  $3 \times 10^5$  or  $3 \times 10^4$  cells/ml, respectively. These experiments lasted 4 days. Controls were prepared by inoculating microalgae in the medium without addition of macroalgae.

#### *Inhibitory and lethal assays with dry powder of U. fasciata and U. pertusa*

Laboratory cultured *Ulva* tissue were dried at room temperature for 24 hours to complete dryness and ground into fine powder using dried mixer (T-351, Rong Tsong Iron, Taiwan) and sieved with 150  $\mu$ M mesh. To calculate inhibitory effect, different amounts (0, 0.03, 0.06 and 0.12 g/l) of dry powder of macroalgae were added to the 100 ml conical glass flasks containing 40 ml of f/2 medium with *H. akashiwo* or *A. catenella* cells at an initial cell density of  $8 \times 10^3$  or  $8 \times 10^2$  cells/ml, respectively. Microalgae cultures without addition of dry macroalgae powder served as controls. These experiments lasted 7 days. At one day interval, 1 ml of samples was collected from each flask and the microalgae cells were counted on a haemocytometer under an optical microscope to determine the maximum growth of the micro algae. After sampling, the flasks were replenished with 1 ml of fresh solution, which contained full f/2 ingredients. Besides, different amounts (0, 0.5, 1 and 1.5 g/l) of dry powder of macroalgae were added to the 5 cm diameter of Petri dish containing 10 ml of f/2 medium with *H. akashiwo* or *A. catenella* (cell density  $3 \times 10^5$  or  $3 \times 10^4$  cells/ml, respectively) to calculate lethal effect and these experiments lasted 4 hours.

#### *Lethal assays with methanol and water extracts of U. fasciata and U. pertusa*

Methods described by Jin et al. (1997) were

followed for extraction of methanol and water soluble fractions. Zero point one gram of the dried powder was treated with 5 ml of 99.8% methanol (Wako Pure Chemicals, Japan) for 24 hours at room temperature. The methanol extraction was repeated three times and the extracts were combined. The methanol soluble fractions were pooled and concentrated using a concentrator (TC 8, Taitec, Japan). After evaporating methanol completely from the remaining methanol-extracted tissue, 5 ml of distilled water was added to extract the water soluble fraction for 24 hours. Finally, all extracts were centrifuged through centrifuge (5417R, Eppendorf, Germany) at 10,000 rpm, 4°C, 30 minutes before use.

To test the effects of the methanol extracts on the microalgae, appropriate amounts of the methanol and water extracts of the macroalgae by indoor culture were added to one milliliter (cell density  $3 \times 10^4$  cells/ml) of *C. marina*, *H. akashiwo*, *F. japonica*, *A. catenella* and *K. mikimotoi* cultures to set the final concentration to 200, 100 and 50 mg/l for the methanol extracts and to 800 and 400 mg/l for the water extracts in a 24 well micro plate (Iwaki, Japan) for the activity assays. These experiments lasted 4 hours.

#### *Statistics*

All the experiments in this study were done separately in at least triplicate and tested by ANOVA test ( $p < 0.05$ ). Simple regression and correlation analysis was used to examine data for possible relationships and to obtain LC<sub>50</sub> (median lethal concentration) or EC<sub>50</sub> (median effective concentration) values (Nakai et al. 1999). For sigmoid curves, the y values (percentage) were converted by probit transformation using Microsoft Excel<sup>®</sup>.

## **Results**

#### *Effects of fresh tissue of U. fasciata and U. pertusa on H. akashiwo and A. catenella*

To evaluate the effects of fresh tissue of *Ulva* spp. on HABs, we co-cultivated a single species of HAB with different amounts of *Ulva* tissues

(Table 1). The maximum growth of HABs was measured to quantify the inhibitory effects of *Ulva* spp. In the present study, *U. fasciata* (sporophyte) tissue showed highest inhibitory effects on the growth *H. akashiwo* at 7 days after inoculation. To better distinguish the inhibitory effects of different *Ulva* strains we have evaluated the EC<sub>50</sub> values, and the 7 days EC<sub>50</sub> of fresh tissue of *U. fasciata* (sporophyte, female, male) and *U. pertusa* (sporophyte) were

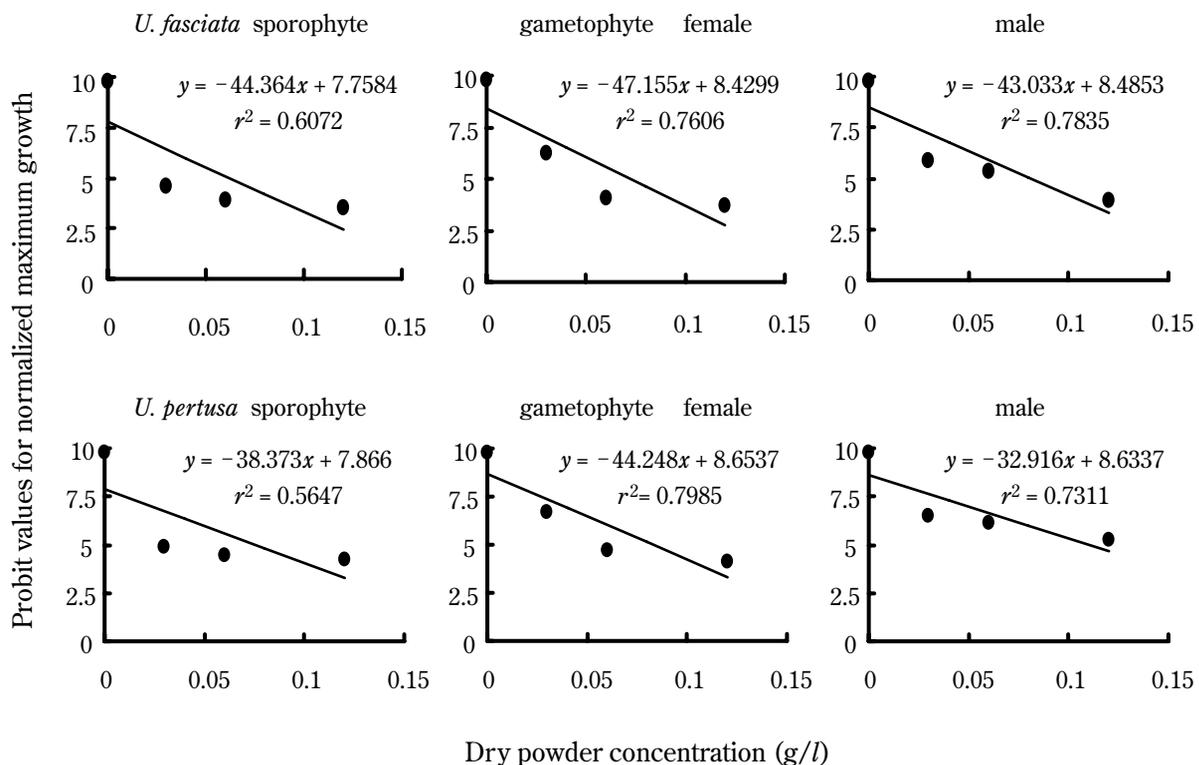
**Table 1.** Summary of inhibitory and lethal effects of the fresh tissue (g/l) of *U. fasciata* and *U. pertusa* strains on *H. akashiwo* NIES-4 and *A. catenella* NIES-677 species represented as 7 days EC<sub>50</sub> and 4 days LC<sub>50</sub> values

	<i>H. akashiwo</i> NIES-4		<i>A. catenella</i> NIES-677	
	EC <sub>50</sub>	LC <sub>50</sub>	EC <sub>50</sub>	LC <sub>50</sub>
<i>U. fasciata</i>				
Sporophyte	0.27	0.60	0.81	1.82
Gametophyte				
Female	0.53	0.60	1.10	1.98
Male	0.54	0.69	1.11	1.99
<i>U. pertusa</i>				
Sporophyte	0.53	0.60	1.08	1.85
Gametophyte				
Female	0.54	0.68	1.11	1.98
Male	0.90	1.77	1.55	3.47

0.27, 0.53, 0.54 and 0.53 g/l, respectively (Table 1). Our results indicate that the fresh tissue of *U. fasciata* (sporophyte) were more effective in inhibiting the growth of *H. akashiwo* cells.

To further evaluate the lethal effects of the fresh tissue on HABs, we investigated the mortality of HABs in presence of fresh tissue. The 4 days LC<sub>50</sub> value induced by fresh tissue of *U. fasciata* (sporophyte) on *H. akashiwo* was very close to those of *U. fasciata* (female) and *U. pertusa* (sporophyte) (Table 1), suggesting that these tissues have influenced similar amounts of algicidal activity.

Interestingly, higher amounts of tissue were required to induce significant inhibitory effects on the growth of *A. catenella* cells. Furthermore, *A. catenella* cells were comparatively less responsive to the tissues from other *Ulva* strains at all concentrations tested in this study. The 7 days EC<sub>50</sub> of fresh tissue of *U. fasciata* (sporophyte) was 0.81 g/l (Table 1), indicating that fresh tissue of *U. fasciata* (sporophyte) is highly effective. In the present study, although the growth of *A. catenella* was significantly ( $p < 0.05$ ) lowered by all concentrations



**Fig. 1.** The inhibitory effects of dry powder of the *U. fasciata* and *U. pertusa* on the growth of *H. akashiwo* NIES-4 at 7th days. Data are the mean of probit values from at least three independent assays,  $P < 0.05$ .

of fresh tissue of macroalgae strains, *U. fasciata* (sporophyte) tissues were most effective in inhibiting *A. catenella* cells.

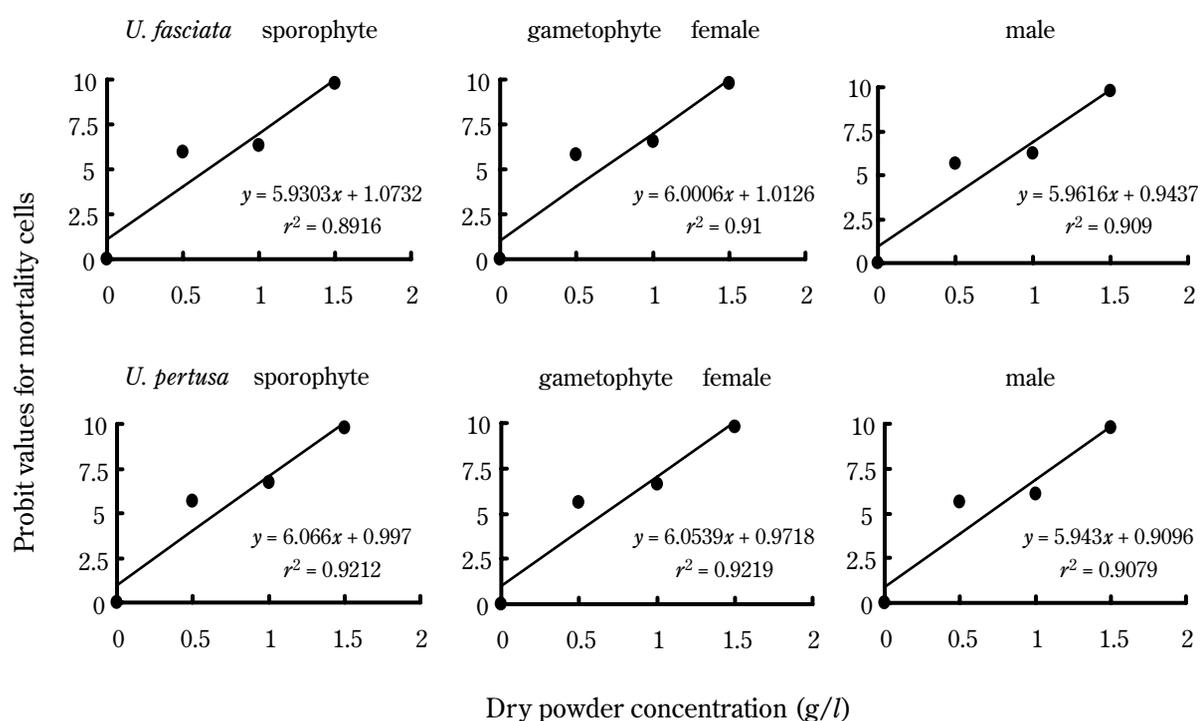
The 4 days LC<sub>50</sub> value for *A. catenella* of fresh tissue of *U. fasciata* (sporophyte) was 1.82 g/l, while the 4 days LC<sub>50</sub> values of fresh tissue of *U. fasciata* (female, male) and *U. pertusa* (sporophyte) were 1.98, 1.99 and 1.85 g/l, respectively (Table 1). Thus it also showed that the lethal effects of fresh tissue of *U. fasciata* and *U. pertusa* had a very similar toxic concentration to cause death response of *A. catenella* cells.

#### Effects of dry powder of *U. fasciata* and *U. pertusa* on *H. akashiwo* and *A. catenella*

In the inhibitory assays, we did not find dead cells of microalgae. The cell number of microalgae in control always increased, meanwhile the growth percentage of cell number of microalgae in treatment inclined to decrease during experiment. At 0.03 g/l concentration, dry powder of *U. fasciata* (sporophyte) showed inhibitory effect on the growth more than 50% (<5 for probit values) of *H. akashiwo*, whereas the other macroalgae strains showed less than 50% inhibitory effects (Fig. 1). However, at 0.12 g/l, the

growth of *H. akashiwo* cells were depressed more than 80% (<4.15 for probit values) by dry powder of *U. fasciata* (sporophyte) as well as by *U. fasciata* (female and male). Even at the highest concentration, no lethal effects were observed. In the present study, the growth of *H. akashiwo* cells were significantly ( $r^2 = 0.61$   $p < 0.05$ ) lowered by dry powder of *U. fasciata* (sporophyte). Meanwhile, *U. fasciata* (female, male) and *U. pertusa* (sporophyte, female, male) were significantly ( $p < 0.05$ ) effective in inhibiting the growth of *H. akashiwo* ( $r^2 = 0.76, 0.78, 0.56, 0.80, 0.73$ , respectively). The 7 days EC<sub>50</sub> concentration of dry powder of *U. fasciata* (sporophyte, female, male) and *U. pertusa* (sporophyte, female, male) on *H. akashiwo* cells were 0.06, 0.07, 0.08, 0.07, 0.08, 0.11 g/l, respectively.

In the lethal assays, the dry powder of *U. fasciata* and *U. pertusa* showed a very significant algicidal activity and more than 50% of the *H. akashiwo* cells lost their motility within 30 minutes after dry powder were added. Furthermore, the cells that were not dead within 30 minutes after the addition of the dry powder, showed decreased motility and eventually the cells lost motility. Almost all of the



**Fig. 2.** The mortality of *H. akashiwo* NIES-4 cells by dry powder of *U. fasciata* and *U. pertusa* at 4 hours. Data are the mean of probit values from at least three independent assays,  $P < 0.05$ .

non-motile cells become round, expanded and then burst. In the present study, the dry powder of *U. fasciata* (sporophyte) at 0.5 g/l induced more than 80% ( $<4.15$  for probit values) *H. akashiwo* cells death (Fig. 2). The dry powder of *U. fasciata* (sporophyte, female, male) and *U. pertusa* (sporophyte, female, male) were significantly ( $p < 0.05$ ) correlated with mortality of *H. akashiwo* cells ( $r^2 = 0.89, 0.91, 0.91, 0.92, 0.92, 0.91$ , respectively). Besides that, the 4 h  $LC_{50}$  values of dry powder of *U. fasciata* (sporophyte, female, male) and *U. pertusa* (sporophyte, female, male) on *H. akashiwo* cells death were 0.66, 0.66, 0.68, 0.66, 0.67, 0.69 g/l, respectively.

The growth of *A. catenella* cells were inhibited 42.74% (as same as 5.18 for probit values) by dry powder of *U. fasciata* (sporophyte) at 0.12 g/l (Fig. 3). *U. fasciata* (female, male) and *U. pertusa* (sporophyte, female, male) inhibited less than 40% ( $> 4.75$  for probit values) the growth of *A. catenella*, that is 36.23, 37.38, 35.48, 34.18, 29.29% (5.35, 5.32, 5.37, 5.41, 5.54 for probit values, respectively).

Similar trends were observed on the lethal effects of the dried powders on the *A. catenella* cells (Fig. 4). In the present study, the dry

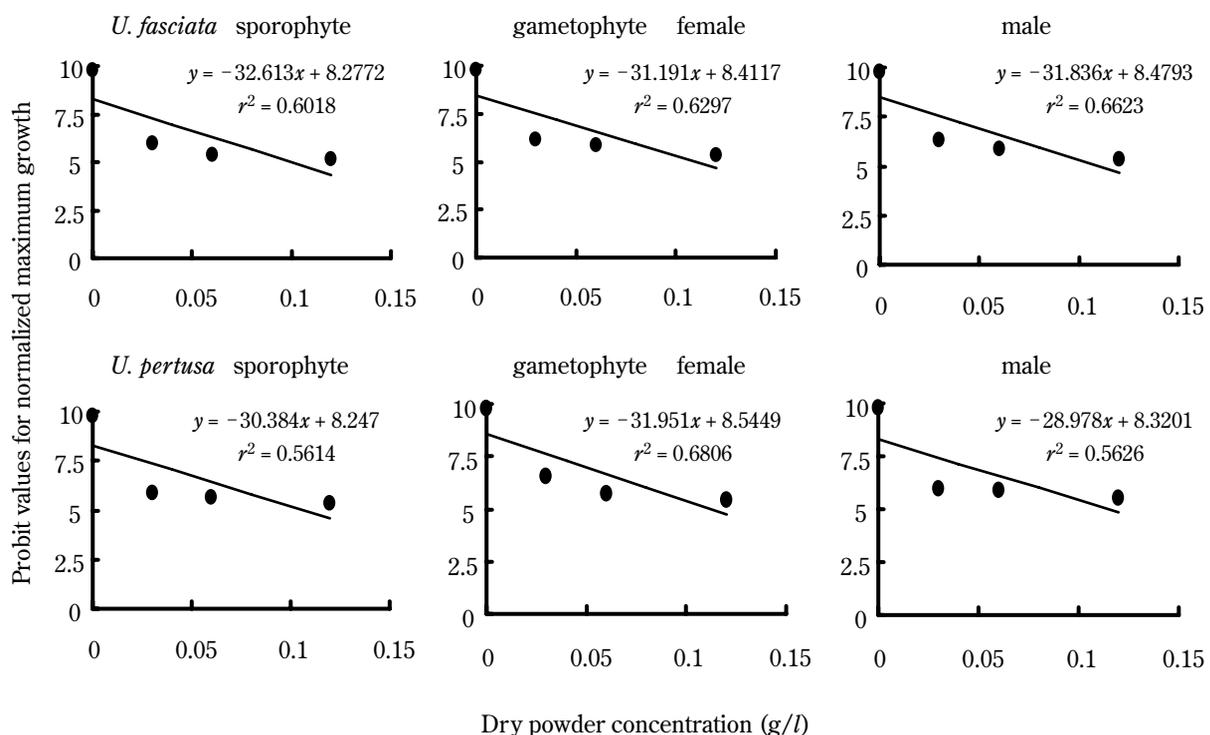
powder of macroalgae showed almost similar effect on *A. catenella* cells death. At 1.5 g/l, the dry powder of *U. fasciata* (sporophyte, female, male) and *U. pertusa* (sporophyte, female, male) on mortality of *A. catenella* cells were 8.51, 6.67, 6.31, 7, 6.33 and 5.67% (3.63, 3.50, 3.47, 3.52, 3.47 and 3.42 for probit values, respectively).

#### Effects of methanol and water extracts of *U. fasciata* and *U. pertusa* on HAB species

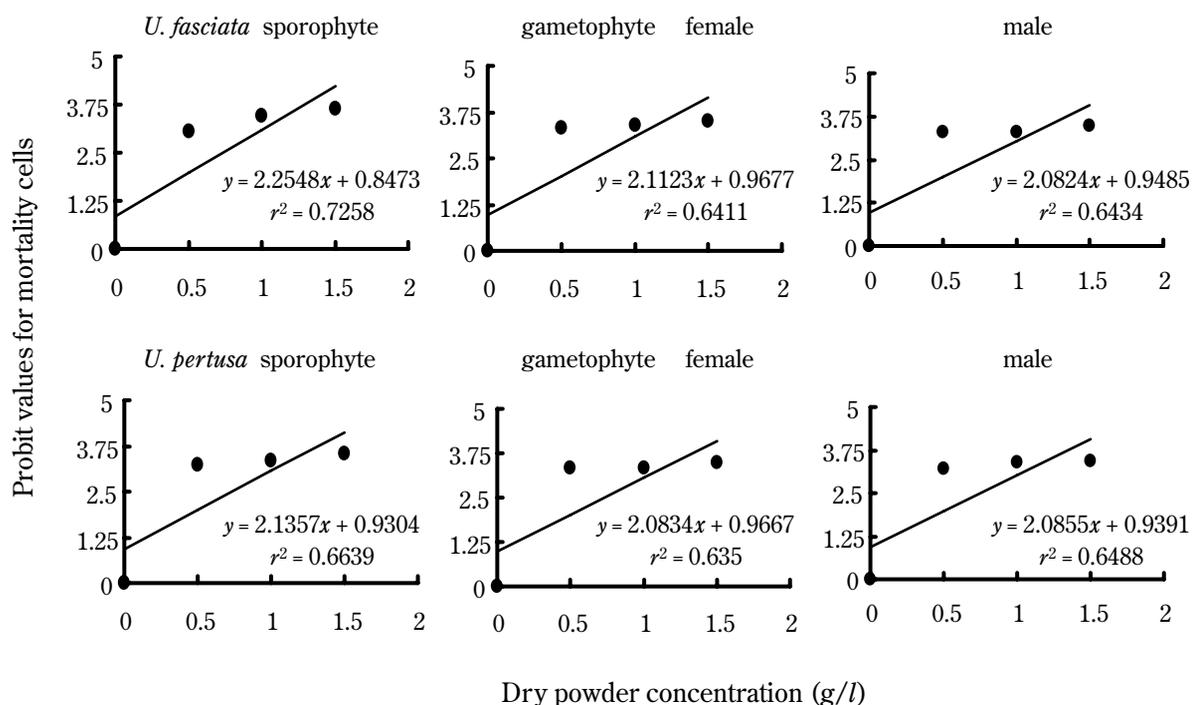
The growth of HAB species were not influenced by methanol up to 2% concentration. Thus, for all the lethal assays with methanol extracts from the *Ulva* spp., respective HAB cells grown in 2% methanol, not exceeding 2% of the total culture volume served as the experimental controls.

The lethal responses of the methanol extracts of *Ulva* spp. at different concentrations were tested on *H. akashiwo*, *A. catenella*, *C. marina*, *F. japonica* and *K. mikimotoi* (Table 2). However, in this study, water extracts did not show any significant ( $p > 0.05$ ) effects on microalgae species (data not shown).

The lethal effects were concentration dependent and at 200 mg/l concentration, all the microalgae were killed over 80%. The extracts



**Fig. 3.** The inhibitory effects of dry powder of the *U. fasciata* and *U. pertusa* on the growth of *A. catenella* NIES-677 at 7th days. Data are the mean of probit values from at least three independent assays,  $P < 0.05$ .



**Fig. 4.** The mortality of *A. catenella* NIES-677 cells by dry powder of *U. fasciata* and *U. pertusa* at 4 hours. Data are the mean of probit values from at least three independent assays,  $P < 0.05$ .

**Table 2.** Summary of lethal effects of the methanol extracts of *U. fasciata* and *U. pertusa* strains on HAB species represented as the 4 h  $LC_{50}$  (mg/l) values

	<i>U. fasciata</i>			<i>U. pertusa</i>		
	Sporophyte	Gametophyte		Sporophyte	Gametophyte	
		Female	Male		Female	Male
<i>C. marina</i> NIES-3	80.1	98.7	248.0	89.9	173.8	963.7
<i>H. akashiwo</i> NIES-4	97.6	151.7	442.2	134.3	192.2	477.3
<i>F. japonica</i> NIES-462	115.5	197.5	360.1	180.3	263.7	1180.8
<i>A. catenella</i> NIES-677	957.7	1889.5	2748.2	1315.1	2012.3	16273.4
<i>K. mikimotoi</i> *	321.3	374.1	690.2	352.9	556.1	987.8

\* as *G. mikimotoi* NIES-680.

from sporophytes of *U. fasciata* were most effective and had very low  $LC_{50}$  value against *C. marina* (4 h  $LC_{50}$ : 80.1 mg/l) and *H. akashiwo* (4 h  $LC_{50}$ : 97.6 mg/l), respectively.

Interestingly, the cells of *A. catenella* were resistant to the extracts from *Ulva* spp. and had very high  $LC_{50}$  values (Table 2) in comparison to other microalgae tested. The extracts from the sporophyte of *U. fasciata* were moderately effective against *F. japonica* and *K. mikimotoi*.

In summary, our results clearly demonstrated that the methanol extracts from the sporophytes of *U. fasciata* and *U. pertusa* were most effective against HABs. Furthermore, in the present study, methanol extracts of *Ulva* spp. showed differential responses to different

HABs tested. It is noteworthy that the methanol extracts from the sporophytes of *U. fasciata* and *U. pertusa* were most effective (in terms of  $LC_{50}$  values) against *C. marina* and *H. akashiwo* cells.

## Discussion

In the present study, the  $EC_{50}$  and  $LC_{50}$  values of the fresh tissue of *U. fasciata* and *U. pertusa* showed almost similar algicidal activity. Suggesting that these two *Ulva* species have similar composition and concentration of the algicidal compounds. Furthermore, we performed experiments of fresh tissue of macroalgae in axenic culture to preclude effects of bacteria in order to investigate the inhibitory

or lethal effects of *Ulva* spp. on *H. akashiwo* and *A. catenella* species. Similar trends were observed on the inhibitory effects of fresh tissue of *U. pertusa* on *H. akashiwo* (cell density  $3 \times 10^4$  cells/ml), whereas the growth reduction varied between 42 and 100% in batch co-cultures, between 28 and 100% in semi-continuous cultures, and between 21 and 100% in isolated co-cultures (Nan et al. 2004). These results may indicate that a continuous algicidal supply from the fresh tissue is essential to effectively inhibit the growth of HAB species. Imai (2002) and Imai et al. (2002) also proposed a new prevention strategy of red tide by co-culturing of *Ulva* sp. and/or *Gelidium* sp. in cage culture of red sea bream or yellowtail. Many algicidal bacteria will be continually released from the surface of macroalgae to seawater, and contribute to reduce cell densities of phytoplankton, including harmful species by algicidal activity. Based on these results, the existence of *Ulva* spp. in aquaculture may support to mitigate HAB occurrences. It may suggest that the natural products of *Ulva* spp. and algicidal bacteria are releasing algicidal substances on HAB species simultaneously.

Jin and Dong (2003) determined that initial algicidal concentrations of dry powder of *Ulva* sp. on culture media of HAB species were easier to release than algicidal concentrations of fresh tissue. The inhibitory and lethal substances may be more efficiently released into the culture medium at a higher concentration. Jeong et al. (2000) reported that the *H. akashiwo* (cell density  $8 \times 10^3$  cells/ml) treated with dry powder of red algae *Corallina pilulifera* (6.6 g/l) were immobilized and ruptured within 1 day. Jin and Dong (2003) also demonstrated that the dry powder of non sexual strain of *U. pertusa* (2.4 g/l) killed all cells of *H. akashiwo* (cell density  $2 \times 10^5$  cells/ml) within 1 day. Interestingly, in the present study, dry powder of *U. fasciata* (sporophyte) (1.5 g/l) killed all cells of *H. akashiwo* (cell density  $3 \times 10^5$  cells/ml) less than 1 hour. Taken together, our results strongly indicate that the dry powder of *Ulva* spp. is more effective against *H. akashiwo* cells even at short exposure times.

The methanol extracts of *U. fasciata* and *U. pertusa* at 50 mg/l showed the lethal response on a part of *C. marina*, *H. akashiwo*, *F. japonica*, *A. catenella* and *K. mikimotoi* within 4 hours after the addition of the extracts. In a similar study, Jeong et al. (2000) reported that the methanol extracts (100 mg/l) of *U. pertusa* showed growth inhibition of the red tide *Cochlodinium polykrikoides* at an initial cell density of  $5 \times 10^2$  cells/ml for eight days. The differences in our results may be attributed to the different responses of HAB species and the lower concentrations of *U. fasciata* and *U. pertusa* extracts used in the evaluation of the inhibitory and lethal effects on the HABs. However, our studies along with others (Jeong et al. 2000) confirmed that methanol is one of the effective solvent for extraction of inhibitory and lethal substances from *Ulva* spp. and the hydrophobic components are an integral part of the activity. Alamsjah et al. (2005) showed that algicidal compounds from methanol extract of *U. fasciata* were determined as C16 and C18 polyunsaturated fatty acids. In the present study, algicidal activity of sporophyte of *U. fasciata* and *U. pertusa* were higher than gametophyte strains on HAB species. This may suggest that quantity of algicidal compounds of sporophyte of *U. fasciata* and *U. pertusa* are probably higher than gametophyte strains. Algicidal substances of *Ulva* spp. were extracted completely by methanol solvent so that the water extract of *Ulva* spp., as a continuation extraction from methanol extract, did not have significant algicidal effect on HAB species. Although fatty acids are hydrophobic substances with a very limited solubility in water, Parrish (1988) suggested that  $100 \mu\text{g/l}$  would be a conservative estimate for the solubility of various lipid classes in distilled water at 20°C. Kattner et al. (1983) also studying the lipid concentration during a spring phytoplankton bloom in the northern North Sea in Europe estimated that fatty acid constituted about 3% of total dissolved organic matter. The total fatty acid concentration amounted to about  $1.15 \mu\text{mol/l}$  before the bloom and increased to a maximum of  $5 \mu\text{mol/l}$  during a bloom. Meanwhile, Ikawa

(2004) determined that polyunsaturated fatty acids which released under natural bloom conditions may indeed be natural inhibitors. It suggests that the correlation of algicidal sources from fresh tissue, dry powder and methanol extracts of *Ulva* spp. to cause growth inhibition or lethal effects on HAB species.

Microalgae species showed different responses when coexisting with fresh tissue, dry powder and methanol extracts of *U. fasciata* and *U. pertusa*. For example, *A. catenella* cells were not killed completely at all concentration tested. We also found that algicidal activity of *U. fasciata* and *U. pertusa* had no effect on the culture of *Chaetoceros gracilis*, *Nitzschia* sp., *Isochrysis tahitiana* and *Tetraselmis succia* (data not shown). The LC<sub>50</sub> value obtained after treatment with the methanol extracts and the microscopic observations leading to death of the cells further supported these differential responses of *Ulva* spp. extracts on different HABs. Kakisawa et al. (1988) also showed that allelochemical of octadecatetraenoic acid from the brown algae *Cladosiphon okamuranus* have different responses against microalgae. Sun et al. (2004) mentioned that the *H. akashiwo* lack cell walls and the cytoplasm is only surrounded by the cell membrane, which may be the main reason for the immediate lyses of this species under the action of lethal substances, while *Alexandrium* sp. cells are covered by hard shells. The results indicate that cell wall plays an important role in keeping the integrity of the cell membrane.

In conclusion, our study strongly suggests the possible applications of natural products from *Ulva* spp. on HABs. Inhibitory and lethal substances of *Ulva* spp. on HAB species could be utilized for alternative prevention, control and mitigation of harmful algal bloom impacts. The world-wide distribution of *U. fasciata* and *U. pertusa* will also give an opportunity to evaluate the genetic variability of *Ulva* spp. for their toxic effects on HABs. Further research on the precise identification and mode of action of the algicidal substances from *Ulva* spp. to cause death of HAB species may shed new light on the algicidal activity and application of *Ulva* spp.

as effective control agents.

## Acknowledgments

We are grateful to Dr. Srinivasa Rao Uppalapati (Plant Biology Division, Samuel Roberts Noble Foundation, USA) for valuable advice in the correction of the manuscript. This study was financially supported by Nagasaki Prefecture Collaboration of Regional Entities for Advancement of Technological Excellence, JST.

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## 有害・有毒藻類ブルームを形成するプランクトンに対する リボンアオサとアナアオサ（アオサ目，緑色植物門）の 殺藻物質としての有効性

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有害・有毒藻類ブルームを形成する植物プランクトンに対するリボンアオサとアナアオサの生葉体，乾燥粉末及びメタノール抽出物の殺藻活性を測定した。これらアオサ類の孢子体及び配偶体の生葉体は *Heterosigma akashiwo* と *Alexandrium catenella* に対して生育抑制と致死効果を示した。アオサ類孢子体の乾燥粉末は生育抑制と殺藻効果において *H. akashiwo* には高い活性を示したが，*A. catenella* には活性が低かった。アオサ類のメタノール抽出物の殺藻活性は，植物プランクトン5種類のうち *Chattonella marina* と *H. akashiwo* に対して最も高く，次いで *Fibrocapsa japonica*，*Karenia mikimotoi* であり，*A. catenella* に対しては低かった。乾燥粉末とメタノール抽出物では，アオサ類の配偶体よりも孢子体からのものの殺藻活性が高かった。このようなアオサ類の殺藻効果は有害・有毒藻類ブルームを形成する植物プランクトンの抑制に利用できる可能性が示唆された。