The phaeophyte *Hizikia fusiformis* extracts suppress rhizoid and blade formation in seaweeds

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Abstract

Background: Plants, including marine algae, produce allelochemicals that influence the growth, survival, and reproduction of other organisms.
Questions: To identify natural algicidal or antifouling allelochemicals, we screened 18 common seaweed extracts for suppression of rhizoid and blade production in a convenient *Porphyra suborbiculata* monospore assay.
Species study and data description: Addition of extract from the most potent phaeophyte, *Hizikia fusiformis*, suppressed rhizoid formation, rhizoid number, rhizoid length, blade formation, and blade length.
Study site and dates: Seaweed thalli for methanol extraction were collected on the coast of Korea from October 2012 to July 2015.
Methods: Extracts were tested using the *P. suborbiculata* monospore assay system.
Results: The 50 % suppression doses were 15 μg ml^{-1} for rhizoid formation, 2.4 μg ml^{-1} for rhizoid number, 13 μg ml^{-1} for rhizoid length, 6 μg ml^{-1} for blade formation, and 11 μg ml^{-1} for blade length. The *H. fusiformis* extract also suppressed rhizoid and blade production in leafy green (*Ulva pertusa*) and brown (*Undaria pinnatifida* and *Ecklonia cava*) seaweed spores, as well as suppressing diatom settlement.
Conclusions: The allelochemicals that suppressed or eliminated competing seaweed species may be efficacious for new seaweed control technologies, including the development of antifouling or algicidal agents based on natural products.
Key words: Allelochemicals, *Hizikia fusiformis*, monospore, rhizoid, suppression.

El extracto de la feofita *Hizikia fusiformis* suprime la formación de hojas y rizoides en algas marinas

Resumen

Antecedentes: Las plantas, incluyendo las algas marinas, producen aleloquímicos que influyen en el crecimiento, sobrevivencia y reproducción de otros organismos.
Pregunta: Para identificar aleloquímicos algicidas o antiincrustantes naturales, se seleccionaron 18 extractos de algas marinas comunes para la supresión de la producción de rizoides y láminas foliares, con un ensayo de monoesporas de *Porphyra suborbiculata*.
Especie en estudio y descripción de datos: Adición de extracto del faeofito más potente, *Hizikia fusiformis*, suprimiendo formación, número y longitud de rizóide, formación y longitud de la hoja.
Sitio de estudio y fechas: Los talos de las algas para la extracción con metanol fueron colectados en la costa de Corea de octubre de 2012 a julio de 2015.
Métodos: Los extractos fueron probados usando un sistema ensayo de monosporas de *P. suborbiculata*.
Resultados: Las dosis de supresión del 50 % fueron de 15 μg ml-1 para la formación de rizoides, 2,4 μg ml^{-1} para el número rizóide, 13 μg ml^{-1} para la longitud rizóide, 6 μg ml^{-1} para la formación de la hoja y de 11 μg ml^{-1} para la longitud de la hoja. El extracto de *H. fusiformis* también suprimió la producción de rizóides y láminas foliares en esporas de algas verdes (*Ulva pertusa*) y marrón (*Undaria pinnatifida* y *Ecklonia cava*), así como suprimiendo el establecimiento de diatomeas.
Conclusiones: Los aleloquímicos que suprimieron o eliminaron las especies de algas competidoras pueden ser eficaces para nuevas tecnologías de control de algas marinas, incluyendo el desarrollo de agentes anti producción de hojas o algicidas a base de productos naturales.
Palabras clave: Aleloquímicos, *Hizikia fusiformis*, monoesporas, rizoides, supresión.

The marine ecosystem is a community of living and non-living organisms, including plants, animals, rocks, sediment, and seawater, with severe competition, cooperation, and regulation occurring between them. For competition, some seaweeds produce allelopathic substances—biochemicals that influence the growth, survival, and reproduction of other organisms—that facilitate growth of the producing organism (Whittaker & Feeny 1971). Allelochemicals are either beneficial (positive allelopathy) or detrimental (negative allelopathy) to the target organisms and play an important role in plant defense against herbivory (Stamp 2003). Allelochemicals that suppress or eliminate competing plant species have received special attention due to their potential as natural herbicides in agriculture (Vyvyan 2002). This focus has shifted attention to alternative seaweed control technologies, such as antifouling or algicidal agent development based on selective natural products. For example, the coralline alga *Lithophyllum yessoense* produces an algal spore lytic C17 fatty acid (Luyen *et al*. 2009), and the red seaweed *Ceramium rubrum* has anti-germination activity in *Sargassum muticum*, *Enteromorpha intestinalis*, and *Ulva lactuca* (Hellio *et al*. 2002). The brown seaweed *Dictyota dichotoma* inhibits the growth of the harmful algal blooming dinoflagellate *Ostreopsis* cf. *ovata* in co-cultures, cultured medium filtrate, and dry powder assays (Accoroni *et al*. 2015). The green seaweed *U. lactuca* inhibits the growth of several harmful algal bloom species via allelopathy (Tang & Gobler 2011). For the development of environmentally friendly algicidal or antifouling products, natural compounds from plant and animal sources are the best replacements for traditional harmful metals. Many seaweeds, and other invertebrates, are relatively free from fouling organisms, as they produce a diverse array of secondary metabolites with antibacterial (Hellio *et al*. 2001), antialgal (Hellio *et al*. 2002), and antifungal (Kubanek *et al*. 2003) activities (Almeida & Vasconcelos 2015).

The red seaweed *Porphyra suborbiculata* Kjellman is a common wild seaweed that uses a discoidal holdfast to grow on rocks in the higher intertidal zone (Aye-Mon-Sein *et al*. 2003). Monospores (blade archeospores) from juvenile blades can be produced year-round by adjusting culture conditions in the laboratory. Most monospores germinate to produce new juvenile blades, which themselves produce monospores under axenic culture conditions (Choi *et al*. 2002, 2005). Thus, monospores of *P. suborbiculata* are used as a bioassay for rhizoid and blade formation. To search for natural algicidal or anti–seaweed fouling products, we prepared 18 common seaweed extracts and screened for suppression of rhizoid and blade formation using the convenient monospore assay in the laboratory scale. Lead extracts were further tested against other seaweed species spores and optimized for treatment concentions, and the effects during culturing with the most potent repressor, *Hizikia fusiformis* (Harvey) Okamura, were also measured.

Materials and methods

Collection and extraction of seaweed. Seaweed thalli collected from common 18 different species on the coast of Korea from October 2012 to July 2015 were dried for 3–7 days at room temperature. Thalli were then ground to a powder for 5 min using a coffee grinder. For each 20 g sample, 1 l methanol was used for extraction at room temperature for 24 h. For a stock solution of each methanol-soluble fraction, 1 ml dimethyl sulfoxide (DMSO) was added to every 40 mg dried extract.

Spore collection. To obtain monospores, juvenile blades of *Porphyra suborbiculata*, collected from Cheongsapo (35° 9' 46.47" N, 129° 11' 43.76" E), Busan, Korea, were sonicated (28 kHz) twice for 1 min in autoclaved seawater, and immersed in 1 % betadine for 2 min to eliminate epiphytes (Jin *et al*. 1997). For each 24-well plate, five excised tissue pieces (each 5 × 5 mm^2) were cultured in 1 ml Provasoli's enriched seawater (PES) (Provasoli 1968). The blades were incubated at 18 °C with 40 μmol m^{-2} s^{-1} light intensity on a 12 h light: 12 h dark cycle to obtain monospores. Spores from *Ulva pertusa*, *Undaria pinnatifida*, and *Ecklonia cava* were obtained from each matured thallus that had been washed five times in autoclaved seawater, sonicated twice for 1 min, and dried by pressing between sheets of paper towel. Thalli were pretreated at 4 °C for 1 day to maximize spore release (Fletcher 1989). Spore release was induced by placing the thalli in PES at 20 °C with 80 μmol m^{-2} s^{-1} light intensity for 1 day.

Monospore culture and bioassay. Spore germination assays (for rhizoid and blade formation) were performed by adding approximately 100–200 spores to a 200 μl aliquot of PES in a 96-well plate, which was placed in the dark at 18 °C for 1 day. After non-settled spores were removed by centrifugation (1,500 rpm, 15 min) in an inverted position, 200 μl fresh PES was added to each well with 1 μl extract (from a 10-fold diluted stock solution; 20 μg ml^{-1} final concentration). DMSO inhibited spore germination by a minimum of 0.5 % (data not shown). Spore cultures were placed at 18 °C and 80 μmol m^{-2} s^{-1} light intensity on a 12L:12D cycle for 1 week to facilitate spore development (Cho *et al*. 2001). After 1 week, rhizoid formation (number of spores that produced rhizoids per total spores tested), number of rhizoids per rhizoid-holding spore, rhizoid length, blade formation (number of spores that produced blades per total spores tested), and blade length were measured using a microscope (200×). The relative rate (%) of rhizoid or blade formation was determined by the following formula: (S/T) × 100, where S = number of spores that produced rhizoids or blades, and T = total spores tested.

Diatom attachment. The microalgae *Navicula annexa* KMMCC-902 and *Nitzschia pungens* KMMCC-803 were obtained from the Korean Marine Microalgae Culture Center. Diatoms were cultured in F/2 media at 20 °C with 50 μmol m^{-2} s^{-1} light intensity on a 12L:12D cycle with shaking at 50 rpm (Guillard & Ryther 1962). The effect of *H. fusiformis* extract on diatom attachment was investigated in a 12-well plate containing 3.75 ml autoclaved seawater and 1 ml diatom cell suspension (~1 × 10^4 cells ml^{-1}). A glass slide (2.5 × 1.5 cm^2) was immersed in a well and 25 μl stock extract was added to a final 100 μg ml^{-1} culture and mixed. The plate was incubated in a growth chamber under fluorescent light (50 μmol m^{-2} s^{-1}) for 12 h. The cell suspension was then discarded, and loosely attached cells were washed with 5 ml sterile seawater. The attached diatom cells were counted using a microscope (200×).

Statistical analysis. Various concentrations of the *Hizikia fusiformis* extract were added to the monospore culture to determine suppression activity. The suppression dose 100 (SD_{100}) is expressed as the concentration of *H. fusiformis* extract required to prevent monospores from rhizoid and blade production after 7 days of culture. The suppression dose 50 (SD50) is the concentration of H. fusiformis extract required to limit rhizoid and blade production in 50 % of monospores after 7 days. The experiments were repeated at least three times. Mean differences between extract and control assays were compared using Student's *t*-test.

Results

To search for allelopathic agents in seaweed, common seaweed extracts were tested for their ability to suppress rhizoid and blade production in a *Porphyra suborbiculata* monospore assay. The 18 seaweed species tested included green seaweed (*Codium fragile*, *Monostroma nitidum*, *Ulva linza*, *Ulva pertusa*), brown seaweed (*Ecklonia cava*, *Eisenia bicyclis*, *Hizikia fusiformis*, *Ishige sinicola*, *Saccharina japonica*, *Sargassum fulvellum*, *Sargassum hemiphyllum*, *Sargassum hornei*, *Sargassum thunbergii*, *Scytosiphon lomentaria*, *Undaria pinnatifida*), and red seaweed (*Chondrus ocellatus*, *Corallina pilulifera*, *Pachymeniopsis elliptica*). Among the seaweed extracts tested at 20 μg ml^{-1}, *H. fusiformis*, *U. pertusa*, *S. hemiphyllum*, and *M. nitidum* exhibited more than 50 % inhibition of rhizoid formation compared with the control PES (Table 1). *Hizikia fusiformis* had the strongest suppression, with 8 % of monospores producing only rhizoids compared with 41 % with PES ($P < 0.05$). Rhizoid numbers per rhizoid-holding spore ranged from 1.0 to 1.6. In *H. fusiformis*, *S. hornei*, *S. thunbergii*, and *P. elliptica*, rhizoid length was suppressed significantly, to less than half. *Hizikia fusiformis* had the strongest inhibition, with average rhizoid lengths of 8.7 μm compared with 19.5 μm for the control ($P < 0.05$). Regarding germinated spores, *H. fusiformis*, *E. cava*, and *I. sinicola* extracts reduced blade formation to less than half. *Hizikia fusiformis* suppressed blade formation the most, with 1 % of monospores germinating to juvenile blades in 1 week compared with 14 % for the control ($P < 0.05$). *Hizikia fusiformis* extracts reduced blade growth significantly, to an average of 0.7 μm compared with 7.1 μm for the control ($P < 0.05$). Thus, the *H. fusiformis* extract was selected for further evaluation.

Table 1. Comparison of the ability of seaweed extracts (20 µg ml^{-1}) to inhibit *Porphyra suborbiculata* monospores' rhizoid and blade production. [a]Seaweed number 1, *Chondrus ocellatus*; 2, *Codium fragile*; 3, *Corallina pilulifera*; 4, *Ecklonia cava*; 5, *Eisenia bicyclis*; 6, *Enteromorpha linza*; 7, *Hizikia fusiformis*; 8, *Ishige sinicola*; 9, *Monostroma nitidum*; 10, *Pachymeniopsis elliptica*; 11, *Saccharina japonica*; 12, *Sargassum fulvellum*; 13, *Sargassum hemiphyllum*; 14, *Sargassum horneri*; 15, *Sargassum thunbergii*; 16, *Scytosiphon lomentaria*; 17, *Ulva pertusa*; 18, *Undaria pinnatifida*; PES, Provasoli's enriched seawater. [b]Values are expressed as means ± SE ($n > 3$). *$P < 0.05$ *vs*. PES control.

Seaweed number[a]	Rhizoid formation (%)[b]	No. of rhizoids / rhizoid-holding spore[b]	Rhizoid length (µm)[b]	Blade formation (%)[b]	Blade length (µm)[b]
1	30 ± 15	1.0 ± 0.0	19.9 ± 6.8	8 ± 2	1.7 ± 0.5
2	50 ± 6	1.1 ± 0.0	19.9 ± 5.9	11 ± 1	8.9 ± 1.2
3	34 ± 6	1.3 ± 0.1	18.4 ± 1.2	7 ± 2	7.9 ± 1.4
4	49 ± 8	1.6 ± 0.2	26.8 ± 3.2	5 ± 5	2.1 ± 2.1
5	35 ± 3	1.0 ± 0.0	16.4 ± 1.2	11 ± 3	7.7 ± 1.9
6	25 ± 9	1.0 ± 0.0	11.9 ± 2.6*	15 ± 11	3.5 ± 1.9
7	8 ± 3*	1.0 ± 0.0	8.7 ± 3.2*	1 ± 1*	0.7 ± 0.7*
8	42 ± 9	1.2 ± 0.4	20.0 ± 6.7	6 ± 1*	13.7 ± 1.2
9	16 ± 1*	1.1 ± 0.0	20.0 ± 6.7	16 ± 5	8.1 ± 1.4
10	26 ± 4*	1.0 ± 0.0	9.7 ± 5.9*	16 ± 3	8.9 ± 1.7
11	24 ± 7*	1.1 ± 0.1	19.9 ± 6.7	10 ± 4	6.6 ± 1.5
12	35 ± 1	1.2 ± 0.1	16.7 ± 0.1	8 ± 3	10.3 ± 0.5
13	13 ± 4*	1.0 ± 0.2	19.8 ± 4.1	11 ± 4	8.2 ± 2.5
14	43 ± 1	1.1 ± 0.0	8.9 ± 1.8*	15 ± 2	9.2 ± 0.6
15	36 ± 4	1.0 ± 0.0	9.4 ± 0.2*	20 ± 3	5.4 ± 1.5
16	43 ± 4	1.0 ± 0.2	17.3 ± 11.1	9 ± 5	1.2 ± 0.8
17	12 ± 4*	1.3 ± 0.0	20.0 ± 6.7	17 ± 0	7.9 ± 1.7
18	35 ± 16	1.0 ± 0.0	18.3 ± 4.9	7 ± 3	7.3 ± 1.2
PES	41 ± 5	1.1 ± 0.0	19.5 ± 2.1	14 ± 4	7.1 ± 0.4

Various concentrations of the *H. fusiformis* extract were added to the monospore culture to determine suppression activity. The suppression dose 100 (SD_{100}) and suppression dose 50 (SD_{50}) values reflecting the amount of *H. fusiformis* extract required to suppress rhizoid formation (in terms of number of spores with rhizoids per total spores tested) were 114 and 15 μg ml^{-1},

Table 2. Effects of *Hizikia fusiformis* extract on the production of rhizoids and blades from *Ulva pertusa*, *Undaria pinnatifida*, *Ecklonia cava*, and *Porphyra suborbiculata* spores. Spores were cultured in the extract (100 µg ml^{-1}) for 1 week.

	Rhizoid formation (%)	No. of rhizoids / rhizoid-holding spore	Rhizoid length (µm)	Blade formation (%)	Blade length (µm)
Spores of *U. pertusa* in the extract	4 ± 0*	1.6 ± 0.2	11.8 ± 2.4*	5 ± 2*	22.8 ± 1.7*
Control	54 ± 3	2.0 ± 0.1	23.0 ± 0.7	20 ± 0	28.6 ± 0.7
Spores of *U. pinnatifida* in the extract	13 ± 0*	1.3 ± 0.1	5.3 ± 0.7	0 ± 0*	19.9 ± 0.8
Control	39 ± 3	1.5 ± 0.1	6.6 ± 0.6	4 ± 0	21.4 ± 0.6
Spores of *E. cava* in the extract	1 ± 1*	1.0 ± 0.0*	2.9 ± 0.4*	2 ± 0*	15.0 ± 1.8
Control	36 ± 3	1.6 ± 0.0	4.3 ± 0.1	55 ± 5	16.3 ± 1.1
Spores of *P. suborbiculata* in the extract	1 ± 0*	0.5 ± 0.0*	3.0 ± 0.3*	1 ± 0*	3.2 ± 0.1*
Control	30 ± 2	1.3 ± 0.1	17.5 ± 0.3	12 ± 1	12.2 ± 0.6

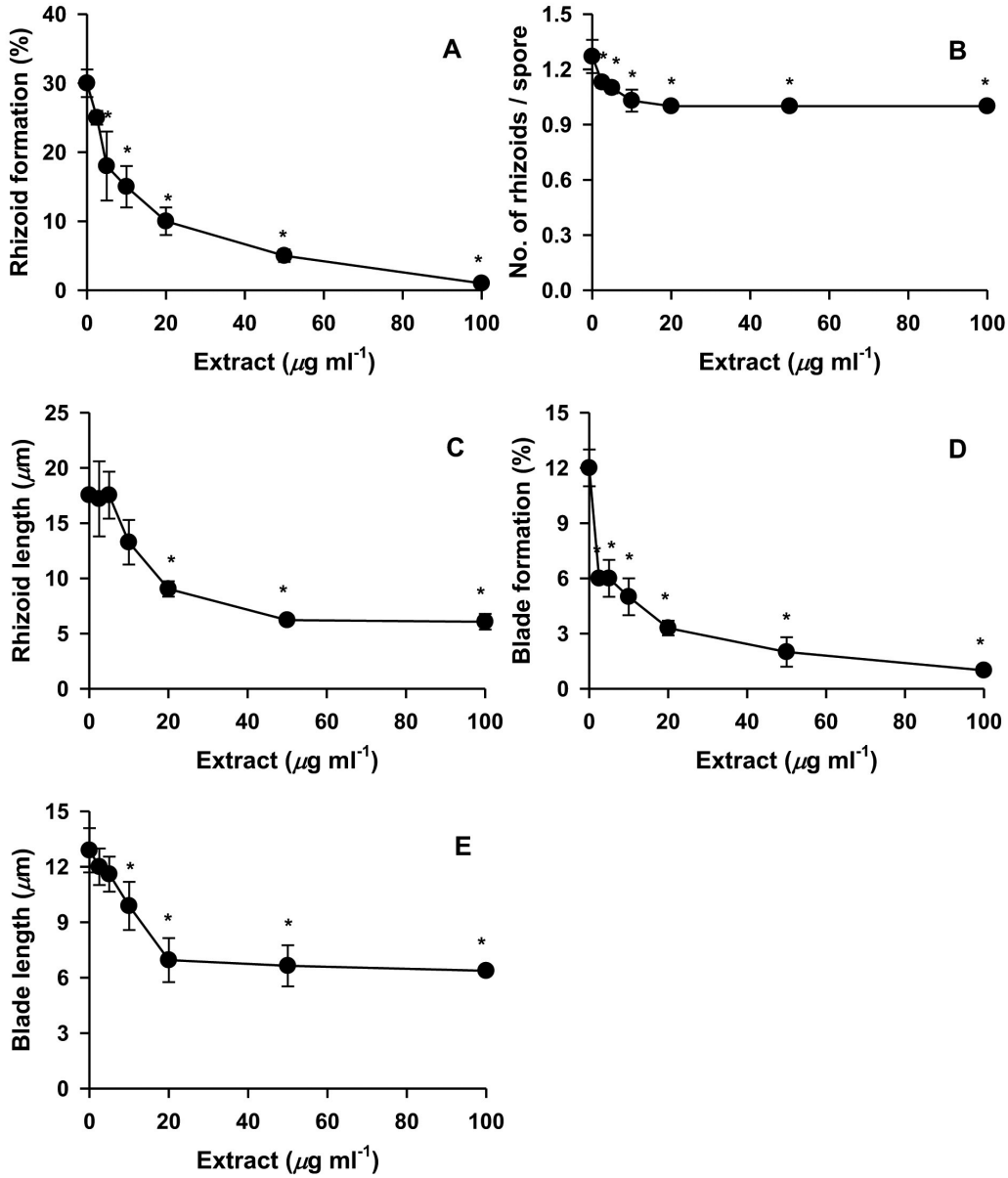

Figure 1. Effects of different concentrations of *Hizikia fusiformis* extract on the production of rhizoids and blades from *Porphyra suborbiculata* monospores. Suppression activities were measured using rhizoid formation (% of spores with rhizoids / total spores tested; A), number of rhizoids / rhizoid-holding spore (B), rhizoid length (C), blade formation (% of spores with blades / total spores tested; D), and blade length (E). *$P < 0.05$ *vs*. PES control.

respectively, in the monospore culture (Figure 1A). The SD_{100} and SD_{50} values of the extract required to suppress rhizoid number were 20 and 2.4 μg ml^{-1}, respectively (Figure 1B). Most suppressed rhizoids grew at least 6 μm in 7 days, and the SD_{100} and SD_{50} values reflecting the suppression of rhizoid length were 88 and 13 μg ml^{-1}, respectively (Figure 1C). The SD_{100} and SD_{50} values for suppression of blade formation (number of spores with blades per total spores tested) were 145 and 6 μg ml^{-1}, respectively, where blades grew an average of 6 μm in 1 week (Figure 1D). The SD_{100} and SD_{50} values for blade length suppression were 100 and 11 μg ml^{-1}, respectively (Figure 1E). Next, growth of *P. suborbiculata* monospores was observed upon treatment with 100 μg ml-1 (approximate SD_{100} against all parameters) *H. fusiformis* extract for 10 days. The *H. fusiformis* extract almost completely suppressed rhizoid formation (Figure 2A), rhizoid numbers per rhizoid-holding spore (Figure 2B), rhizoid length (Figure 2C), blade formation (Figure 2D), and blade length (Figure 2E) in the monospore assay. However, after 10 days of culture, monospores began to increase rhizoid formation, rhizoid numbers, and rhizoid length.

The suppression effects of the *H. fusiformis* extract were also evaluated using spores from other common seaweed species, including *Ulva pertusa*, *Undaria pinnatifida*, and *Ecklonia*

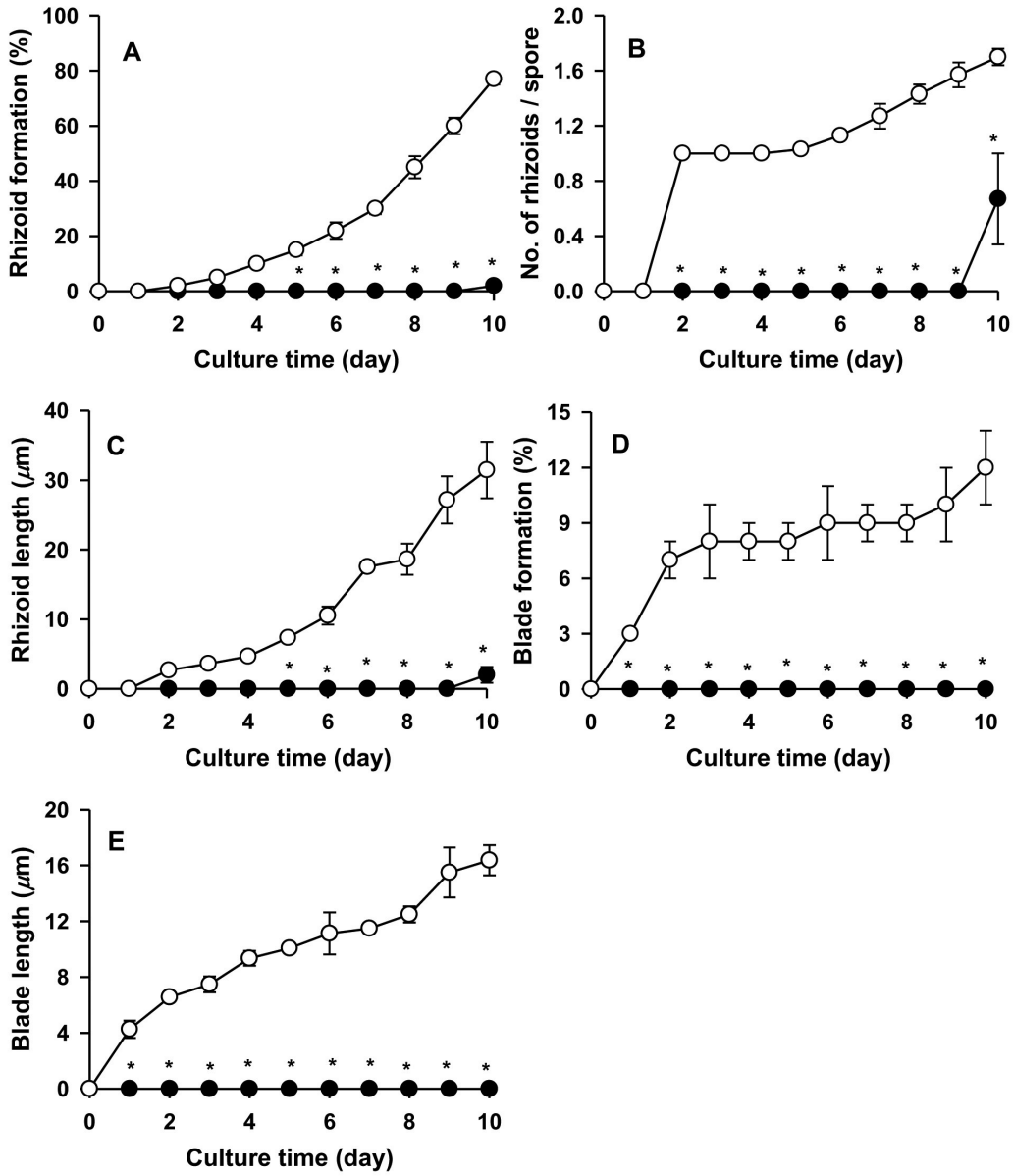

Figure 2. Effects of *Hizikia fusiformis* extract on the production of rhizoids and blades from *Porphyra suborbiculata* monospores. Suppression was measured by rhizoid formation (% of spores with rhizoids / total spores tested; A), number of rhizoids / rhizoid-holding spore B), rhizoid length C), blade formation (% of spores with blades / total spores tested; D), and blade length E). ●, *H. fusiformis* extract (100 μg ml^{-1}). ○, PES control. *$P < 0.05$ *vs.* control.

Table 3. Effects of *Hizikia fusiformis* extract on the settlement of diatoms *Navicula annexa* and *Nitzschia pungens*. Diatoms were cultured in extract (100 μg ml^{-1}) for 12 h. Values are expressed as means ± SE ($n > 3$). *$P < 0.05$ *vs.* control.

	No. of settled diatoms	Relative value against control
Navicula annexa	379 ± 111	86 %
Control	443 ± 76	
Nitzschia pungens	303 ± 67*	38 %
Control	790 ± 43	

cava. The extract significantly suppressed the rhizoid formation of *U. pertusa*, *U. pinnatifida*, and *E. cava* spores. This extract caused suppression to 7 % (relative % against each control; 4/54 spores), 33 % (13/39 spores), and 3 % (1/36 spores), respectively, which were comparable to 3 % from *P. suborbiculata* spores (Table 2). *Hizikia fusiformis* extract also reduced rhizoid length (51 %), blade formation (25 %), and blade length (80 %) of *U. pertusa*, compared with control. The extract also suppressed rhizoid numbers (63 %), rhizoid length (67 %), and blade forma-

tion (4 %) from *E. cava* spores. Taken together, the *H. fusiformis* extract suppressed rhizoid and blade production from leafy green (*U. pertusa*), brown (*U. pinnatifida* and *E. cava*), and red (*P. suborbiculata*) seaweed spores. The methanol extract of *H. fusiformis* also inhibited the attachment of the fouling diatoms *Navicula annexa* and *N. pungens* by 86 % and 38 %, respectively (Table 3). The crude extract (100 μg ml^{-1}; approximate SD_{100} against seaweed parameters) significantly suppressed settlement of *N. pungens*.

Discussion

In marine ecosystems, some seaweeds are covered heavily by epiphytes, while others in the same habitat have defense mechanisms that prevent colonization. Such epiphyte-free seaweeds may synthesize secondary metabolites that protect against epibiont colonization (Águila-Ramírez *et al*. 2012). To search for such allelopathic agents in seaweed, common seaweed species extracts were tested for their ability to suppress rhizoid and blade production in a monospore assay. The monospores are easily maintained under laboratory conditions and reproduces throughout the year (Choi *et al*. 2005). They germinate to produce new juvenile blades, which can then produce additional monospores about every 20 days. It allows the monospores to be conveniently used as a target organism in a preliminary screening system for detecting allelopathic or antifouling compounds in the seawater condition. Even though this monospore assay system has a limit that is not done under natural marine environment, the bioassay provides a valuable early indication of the allelopathic or antifouling action efficacy prior to field testing.

Among the seaweed species tested, *Hizikia fusiformis* showed the strongest suppression activities. *H. fusiformis* is an edible and abundant aquaculturable brown seaweed. The amount of *H. fusiformis* produced by farming in 2013 amounted to 13,000 t (wet weight), with an additional 2,000 t (wet weight) collected from natural populations in Korea (Korea Fisheries Association 2014). In our previous study (Choi *et al*. 2005), methanol extracts of the seaweed at high concentration (200 μg ml^{-1}) lysed monospores of *Porphyra yezoensis*. Additionally, the methanol extract of *H. fusiformis* also suppressed tissue growth (49 %), spore settlement (59 %), and zygote formation (49 %) of the green seaweed *Enteromorpha prolifera* (Cho *et al*. 2001). The *H. fusiformis* extract almost completely suppressed rhizoid formation, rhizoid numbers, rhizoid length, blade formation, and blade length at the early stages of the *P. suborbiculata* monospore assay. However, after 10 days of culture, monospores began to increase rhizoid formation, rhizoid numbers, and rhizoid length. Thus, epigenetic adaptation of rhizoids resulting in the induction of alternative pathways, enzymes, or detoxification may overcome the extract treatment (Gonzales & Widholm 1985). The suppression effects of the *H. fusiformis* extract were also demonstrated against rhizoid formation, rhizoid numbers, rhizoid length, blade formation, and blade length from leafy green (*Ulva pertusa*), brown (*Undaria pinnatifida* and *E. cava*), and red (*P. suborbiculata*) seaweed spores. Mature *U. pertusa* has a wide blade and forms extensive mats in shallow coastal waters due to its fast rate of growth and high reproductive capacity (Han *et al*. 2003). This seaweed is a main component of green tides and fouling coverage (Sidharthan *et al*. 2004), and contributes to higher trophic levels. *Undaria pinnatifida* is an invasive species, competing with native kelp species occupying shallow sublittoral and infralittoral zones (Farrell & Fletcher 2006). *Ecklonia cava* is a marine forest species commonly used to develop artificial seaweed forests (Hayashida 1984). Thus, by suppressing fast-growing and massive leafy seaweeds, *H. fusiformis* or its extract may provide negative allelophatic activity against diverse fouling seaweeds. In the early stages of biofouling, single-cell diatoms sense the surface, settle, and form a biofilm (Costerton *et al*. 1995). The inhibition of biofilm is assumed to lead to the inhibition of adhesion and subsequent fouling stages (Hellio *et al*. 2001). The methanol extract of *H. fusiformis* inhibited the attachment of the fouling diatoms *N. annexa* and *N. pungens*, and possibly contains allelochemicals that suppress or eliminate soft marine foulants. Such allelochemicals may act as a selective natural herbicide for seaweed control technologies, including antifouling or algicidal agent development. The suppressing compound from *H. fusiformis* was soluble in methanol, chloroform, acetonitrile, and dimethylsulfoxide. Isolation of the active compound presumed as a moderately polar phenolic compound is now in progress.

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