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Host–parasite interactions and host species susceptibility of the marine oomycete parasite, *Olpidiopsis* sp., from Korea that infects red algae

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Abstract *Porphyra* farms in Korea occasionally suffer from *Olpidiopsis* infection. As *Porphyra* farming proceeds from October to March, this obligatory biotrophic parasite may need an alternative host to survive during other months of the year. To find a possible alternative summer host, we collected algae from Wando, Korea, where extensive *Porphyra* plantations are located, and discovered an oomycete assignable to the genus *Olpidiopsis* from *Heterosiphonia pulchra*. Host susceptibility tests showed that this oomycete could also infect *Heterosiphonia japonica, Dasya* sp., *Dasysiphonia chejuensis*, and also blades of *Porphyra tenera*. The minimum incubation time for this *Olpidiopsis* sp. to infect its hosts was approximately 4 h. Zoosporangia matured in 2 days and biflagellate zoospores were released. Free zoospores remained infective in seawater for up to 7 days. The infection of *Olpidiopsis* sp. to *H. japonica* was cell-type specific and extended rhizoid-like apical cells of determinate branches were preferentially infected. FITC-conjugated lectin staining showed specific binding of concanavalin A (ConA) to extended rhizoid-like apical cells. Attachment of *Olpidiopsis* sp. zoospores to the host cells was inhibited by α-mannosidase. Monosaccharide inhibition experiments showed that D(+)-mannose, complementary to the lectin ConA, could also block the infection, suggesting a lectin–carbohydrate interaction during host–parasite recognition.

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Introduction

Porphyra tenera Kjellman and *Porphyra yezoensis* Ueda are commercially important edible red algae in Asia. *P. yezoensis* contains high contents of vitamins, minerals, and antioxidant compounds (Tamura et al. 1998; Takahashi et al. 2000; Liu et al. 2007; Toyosaki and Iwabuchi 2009) and is therefore regarded as a low-energy food, which is rich in dietary fiber. Porphyran, a sulfated polysaccharide from *P. tenera* and *P. yezoensis*, has some physiological functionality such as the effect of improvement of the microflora and environment of the cecum (Kawadu et al. 1995), antitumor activity (Noda et al. 1990), antihypertensive and antihyperlipidemic effects (Ren et al. 1994), and macrophage stimulation activity (Yoshizawa et al. 1993, 1995), and has potential pharmaceutical, medicinal and research applications (Bhatia et al. 2008).

In *Porphyra* farming, the "red rot" and "chytrid blight" diseases caused by oomycetous fungi *Pythium porphyrae* Takahashi et Sasaki and *Olpidiopsis* spp., respectively, cause significant losses in crop production (e.g., Woo et al. 2002). The *Porphyra* diseases developed severely when the density of cultivation became larger (Japanese Society of Scientific Fisheries 1978; Ding and Ma 2005). Although the infected crop is harvested as well, its yield and quality are seriously lowered, and the sheet products are less lustrous, uneven, and discolored (Ding and Ma 2005).

There are more than 50 references in the peer-reviewed literature on the "red rot" disease of *Porphyra* (Gachon et al. 2010), whereas *Olpidiopsis* disease has received less

attention (e.g., Migita 1969, 1973; Song et al. 1993; Sekimoto et al. 2008, 2009; Strittmatter et al. 2009). During the "red rot," the motile fungal zoospores of *P. porphyrae* recognize and process host-specific signals by membrane bound receptors (Addepalli et al. 2002). To our knowledge, no information is available about the biochemical mechanisms involved in the process of host-specific infection by zoospores of *Olpidiopsis* spp.

To date, six non-oosporic *Olpidiopsis* species have been described as parasites of marine red algae (Aleem 1952; Feldmann and Feldmann 1955, 1967; Whittick and South 1972; Sekimoto et al. 2008, 2009). Comparisons of their morphology and host specificity are provided in Sekimoto et al. (2009). The molecular-phylogenetic data are available for *Olpidiopsis bostrychiae* Sekimoto, Klochkova, West, Beakes and Honda (Sekimoto et al. 2009) and *Olpidiopsis porphyrae* Sekimoto, Yokoo, Kawamura and Honda (Sekimoto et al. 2008), and taxonomy of remaining four species is based upon a few morphological features such as shape, size of the thallus, number of discharge tubes, plus host specificity (Sparrow 1960). Among these six red algae-infecting marine *Olpidiopsis* species, one is specific to *Porphyra* spp. and *Bangia* spp. (*O. porphyrae*) and *O. bostrychiae* infected the *Porphyra* conchocelis, although its primary host was *Bostrychia moritziana* (Sonder ex Kützing) Agardh.

In this study, we provide an overview of *Porphyra* infection by *Olpidiopsis* in Korea. Also, a *Porphyra*-infecting species of *Olpidiopsis* was isolated from *Heterosiphonia pulchra* (Okamura) Falkenberg, which is suggested to be a possible alternate summer host for this parasite. Cytochemical studies on the infection process suggested that host–parasite recognition might be mediated by a lectin–carbohydrate interaction.

Materials and methods

Blades of *Porphyra tenera* and *P. yezoensis* infected with *Olpidiopsis* species (hereafter, *Olpidiopsis* sp. 1) were collected from a commercial plantation in Seochon (Seochonsuhyup Co., Korea) on 3 February 2010.

Host–parasite culture

Olpidiopsis-infected alga, *Heterosiphonia pulchra*, was collected from Wando, Korea on 17 May, 2006. The plants of *H. pulchra* infected with this *Olpidiopsis* isolate (strain code KNU-M-omc1; hereafter, *Olpidiopsis* sp. 2) were pre-incubated in IMR medium (Kim et al. 2006) half-diluted with original collected seawater for 1 week. Thereafter, plants were transferred to pure IMR medium and treated with GeO_2 (final concentration 1 mg L^{-1}) for 2 weeks to eliminate diatoms. Cultures of *H. pulchra* with *Olpidiopsis* sp. 2 were constantly maintained for over 4 years without any special treatment.

Another stable host–parasite culture was established using *H. japonica* Yendo collected from Wando, Korea. It has been used for cytochemical studies because in *H. japonica* this *Olpidiopsis* sp. 2 provided numerous zoosporangia and zoospore release.

Host susceptibility experiment

Other host algae tested for infection with *Olpidiopsis* sp. 2 are given in Table 1. Except for *Porphyra* spp., algal isolates were grown in IMR medium at 15°C under 15 μmol photons m^{-2} s^{-1} irradiance (16L, 8D). Blades of *Porphyra* spp. were grown in MGM (Modified Grund Medium) with constant aeration at 10°C under 30 μmol photons m^{-2} s^{-1} (16L, 8D). Stock solution of MGM consisted of Na_2EDTA 3.72 g, $FeSO_4{\cdot}7H_2O$ 0.28 g, $Na_2HPO_4{\cdot}12H_2O$ 10.74 g, $NaNO_3$ 42.5 g, $MnCl_2{\cdot}4H_2O$ 0.019 g, Vitamin B_{12} 1 mg, ddH_2O 1 L, and was diluted to a concentration of 1 mL L^{-1} in filtered autoclaved seawater with salinity of 30 psu. Conchocelis and monospores, released from the juvenile blades, were cultured in MGM at 20°C under 30 μmol photons m^{-2} s^{-1} (16L: 8D).

Host susceptibility experiment of *Olpidiopsis* sp. 2 with red algae was performed as follows; to maintain a combination of host and parasite, *H. japonica* thalli infected with *Olpidiopsis* sp. 2 were added to cultures containing another potential host alga (Table 1) in 90×15-mm Petri dishes, and samples were examined daily with Olympus BX-50 microscope. For time-lapse video-microscopy, the infected algal thalli were placed on a glass slide and a coverslip was lowered and sealed with VALAP (1:1:1; vaseline/lanolin/paraffin) melted on a hot plate at 70°C. The slide preparations were examined microscopically under the oil immersion×20 objective lens and recorded on a Digital Imaging Time-Lapse Recorder (DITRS, TCS Korea, Korea).

Application of fluorescent probes

Fluorescein isothiocyanate (FITC)-conjugated lectins (Table 2; Fluorescent Lectin Kit-2100, Vector laboratories, USA) were diluted in PBS buffer (Kim et al. 2006) and added to *H. japonica* in IMR medium to give a final concentration of approximately 10 μg mL^{-1}. After incubation for 30 min at room temperature, the unbound lectin was removed by three washes in IMR medium for 3 min each and materials were examined with confocal laser scanning microscope (Fluoview, v. 2.0.28, Olympus). Fluorescence was detected with the BA510IF and BA510-540 filter set (Ex. λ=490 nm, Em. λ=525 nm). No auto-

Table 1 Host susceptibility experiment using *Olpidiopsis* sp. 2 and red algae

Species	Infection
Bostrychia moritziana (♀, 4314, MAD)	−
Antithamnion densum (tetrasporophyte, KOR)	−
A. densum (♀, KOR)	−
Antithamnion glanduliferum (♂, Utex)	−
Antithamnion nipponicum (♀, KOR)	−
Aglaothamnion byssoides (♂, Utex)	−
A. byssoides (♀, FRA)	−
A. byssoides (♂, NOR)	−
A. byssoides (♂, FRA)	−
Heterosiphonia japonica (KOR)	+
Heterosiphonia pulchra[a] (KOR)	+
Dasya sp. (3446, AUS)	+
Dasya sessilis (KOR)	−
Dasysiphonia chejuensis (KOR)	+
Porphyra pulchella (conchocelis, AUS)	−
Porphyra tenera (blades; wild, red, long-brown types, KOR)[b]	+
P. tenera (conchocelis; wild, red, long-brown, green types, KOR)	−
Porphyra yezoensis (blades, KOR)	−
Neodilsea yendoana (KOR)	−
Spyridia elongata (KOR)	−

MAD Madagascar, *AUS* Australia, *FRA* France, *NOR* Norway, *KOR* South Korea

[a] Original host

[b] ddH_2O-pre-incubated blades

fluorescence of the material was observed in this setting. Sugar specificity of the lectin–ligand interactions was assayed as described by Kim and Fritz (1993).

Blocking of infection with monosaccharides and enzyme

Healthy plants of *H. japonica* were pre-incubated with *Olpidiopsis* sp. 2 zoospores in IMR medium containing 0.05 M of each carbohydrate (Table 3) for 6 h. Thereafter, plants were washed with a brush, put in new IMR medium, and checked under the microscope over time to see if fungal zoospores were able to attach and germinate while being pre-incubated in carbohydrate-containing seawater.

Same experimental procedures were used for the inhibition with α-mannosidase (Table 4; M-7257, Sigma, ~20 units mg^{-1} protein), except that samples were pre-incubated for 4 h. α-mannosidase was used in concentrations 0.1, 0.2, 0.4, and 0.8 units mg^{-1}. Each experiment was repeated seven to ten times, and the percentages in control (without monosaccharides and enzyme) and averages were calculated.

Table 2 Results of FITC-conjugated lectin staining

Lectin	Specificity	Staining result		
		Olpidiopsis sp. 2 zoospores	*Heterosiphonia japonica*	
			Extended rhizoid-like apical cell	Other cell types
Concanavalin A	α-D-Glucose α-D-Mannose	−	+	−
Dolichos biflorus agglutinin	α-*N*-Acetyl-galactosamine	−	−	−
Peanut agglutinin	β-D-Galactose D-Galactose-β-galactosamine	−	−	−
Ricinus communis agglutinin	β-D-Galactose	−	−	−
Soybean agglutinin	*N*-Acetyl-D-galactosamine	−	−	−
Ulex europaeus agglutinin	L-Fucose	−	−	−
Wheat germ agglutinin	*N*-Acetyl-D-glucosamine	−	−	−

Table 3 Percentage of *Heterosiphonia japonica* cells infected with *Olpidiopsis* sp. 2 developed in seawater containing various monosaccharides (0.05 M)

Carbohydrate	Percentage of infected cells (%)	
	Normal apical cells	Extended rhizoid-like apical cells
Control[a]	1.7±1.2	16.9±3.4
D(+)-Galactose	2.1±1.7	14.5±2.8
D(+)-Glucose	2.9±2.1	16.0±3.7
L(−)-Fucose	1.8±1.2	16.4±4.2
N-Acetyl-D-galactosamine	1.0±0.8	11.0±3.7
N-Acetyl-D-glucosamine	1.6±1.2	16.6±4.1
L(−)-Fructose	2.0±1.7	17.2±3.2
D(+)-Mannose	0	0

[a] IMR medium that did not contain sugars

Results

The economic damage caused by the outbreak of *Olpidiopsis* disease (Fig. 1) was evaluated in a commercial plantation of *Porphyra* farm in Seochon (Seochonsuhyup Co.), western coast of Korea. In Korea, commercial plantations use *P. tenera* and *P. yezoensis*, which are either grown separately or mixed together on the same net. The industrialized farm in Seochon used floating-net method (Fig. 1a, c) in the area of 3,000 ha and daily harvest continued from early January to late March. The farm produced about 9,000–12,000 t (fresh weight) of *Porphyra* spp. every year and the daily production from January to February was about 150 t. *Olpidiopsis* disease usually started from late November to early December when young blades of *P. tenera* and *P. yezoensis* began to grow and the seawater temperature still remained above 10°C in that area. The recent outbreak of *Olpidiopsis* disease was reported for the first time on 17 December 2009. As stated by farmers, it started after several days of heavy rain. The outbreak lasted for over 2 months and devastated the farm. Approximately one third of the plantation area was infected by February and the economic loss was estimated at US $3–4 million. In addition to the production loss, the price of one bag containing 80–100 kg of raw seaweeds (Fig. 1b) also dropped from US $150 to $40–60 due to quality reduction. Although the seawater temperature was below 0°C during collecting time in February, most nets still had lot of infected *Porphyra* with zoosporangia of *Olpidiopsis* sp. 1 (Fig. 1d–i).

Table 4 Percentage of attachment of *Olpidiopsis* sp. 2 zoospores to *Heterosiphonia japonica* thalli in IMR medium containing enzyme α-mannosidase

Control[a]	Concentration (units mg^{-1})[b]			
	0.1	0.2	0.4	0.8
100	91.6±5.8	41.6±3.8	25.1±2.7	2.5±0.8

[a] IMR medium that did not contain α-mannosidase. Values obtained from the control group served as 100% and other values were calculated from it

[b] Samples were pre-incubated in α-mannosidase-containing IMR medium for 4 h

Establishment of stable host–parasite dual culture and host susceptibility experiment

Host–parasite dual cultures were always very difficult to maintain in case of *Porphyra* because whole blade degenerated within several days of infection. Therefore, it was necessary to find other host algae to establish a stable dual culture for the infection experiment (Fig. 2).

Considering that *Olpidiopsis* spp. infecting *Porphyra* blades are obligatory parasites there should be some other host algae to survive during summer time when *Porphyra* blades are almost unavailable. In 2006, after extensive search around *Porphyra* farms in Wando where many *Porphyra* plantations are located, we collected *H. pulchra* infected with *Olpidiopsis* sp. 2 (Fig. 2a–c).

The original host, *H. pulchra* (Fig. 2a), was first isolated as 2-cm shoots that grew relatively fast and sustained normal morphology (Fig. 2b) despite the presence of the oomycete developing mainly in the apical cells of determinate branches (Fig. 2c). Infection was rarely observed in the main polysiphonous axis, supporting cell between the axis, monosiphonous lateral branches, or apical cells of indeterminate branches. Infected host cells often swelled two to four times their original diameter (Fig. 2d–g), even when containing only one parasite thallus. The pathogen was identified as *Olpidiopsis* because of its holocarpic, endobiotic nature and morphology of thalli and zoospores. Zoospores were kidney shaped, 3–4 μm in size, and had two laterally inserted heterokont flagella (Fig. 3a(1 and 2)). Released

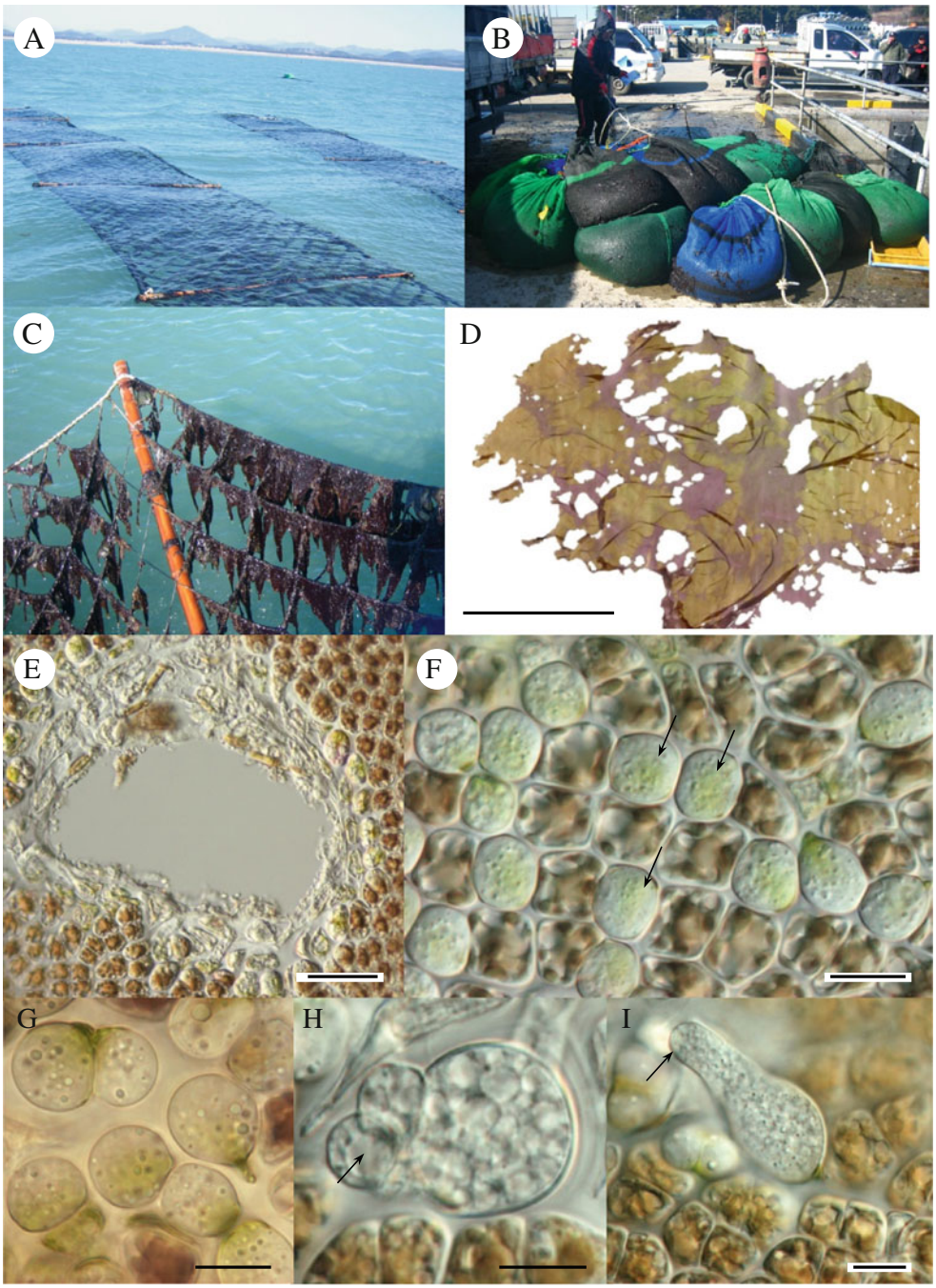

Fig. 1 Development and complication of *Olpidiopsis* disease in *Porphyra* spp. from Seochon (Korea). *Scale bars*=10 cm (**d**), 100 μm (**e**), and 20 μm (**f**–**i**). **a** Floating nets with *Porphyra* spp. **b** Harvested *Porphyra* is packed into bags (80–100 kg wet weight) and loaded on a truck for transportation for sell or storage. **c** Cultivation net with infected *Porphyra.* **d** Infected discolored blade with numerous holes (**e** enlarged image). **f**–**i** Different stages of *Olpidiopsis* sp. 1 zoosporangia development inside *Porphyra* cells. **f** Approximately 12-h-old zoosporangia (*arrows*). **g** Mature zoosporangia before development of discharge tubes. **h**, **i** A number of small vesicles develop inside mature zoosporangium to push out liberation tube for the discharge of zoospores (*arrows*)

zoospores were swimming actively for several hours after liberation from zoosporangia and then discarded or retracted the flagella (Fig. 3b). When zoospore infecting ability was tested over time, they remained infective for up to 7 days after liberation in seawater (data not shown).

We have maintained dual cultures of *H. pulchra* and *Olpidiopsis* sp. 2 for over 4 years, and neither the host nor the parasite died. Moreover, during 4 years that this *Olpidiopsis* sp. 2 has been in dual culture with this host, its ability to infect other potential dasyaceaen host algae has remained consistently high. After liberation of zoospores from mature zoosporangia, wound repair occurs in cells of *H. pulchra* and the uninfected cells grow through adjacent infected cells with empty parasitic zoosporangia (Fig. 4a, b). Usually, two to five developing zoosporangia were seen in a single 2- to 3-cm algal thallus, localizing in remote cells, but free-swimming zoospores were not readily seen, unless just released. Sexual reproduction and resting spores were not observed.

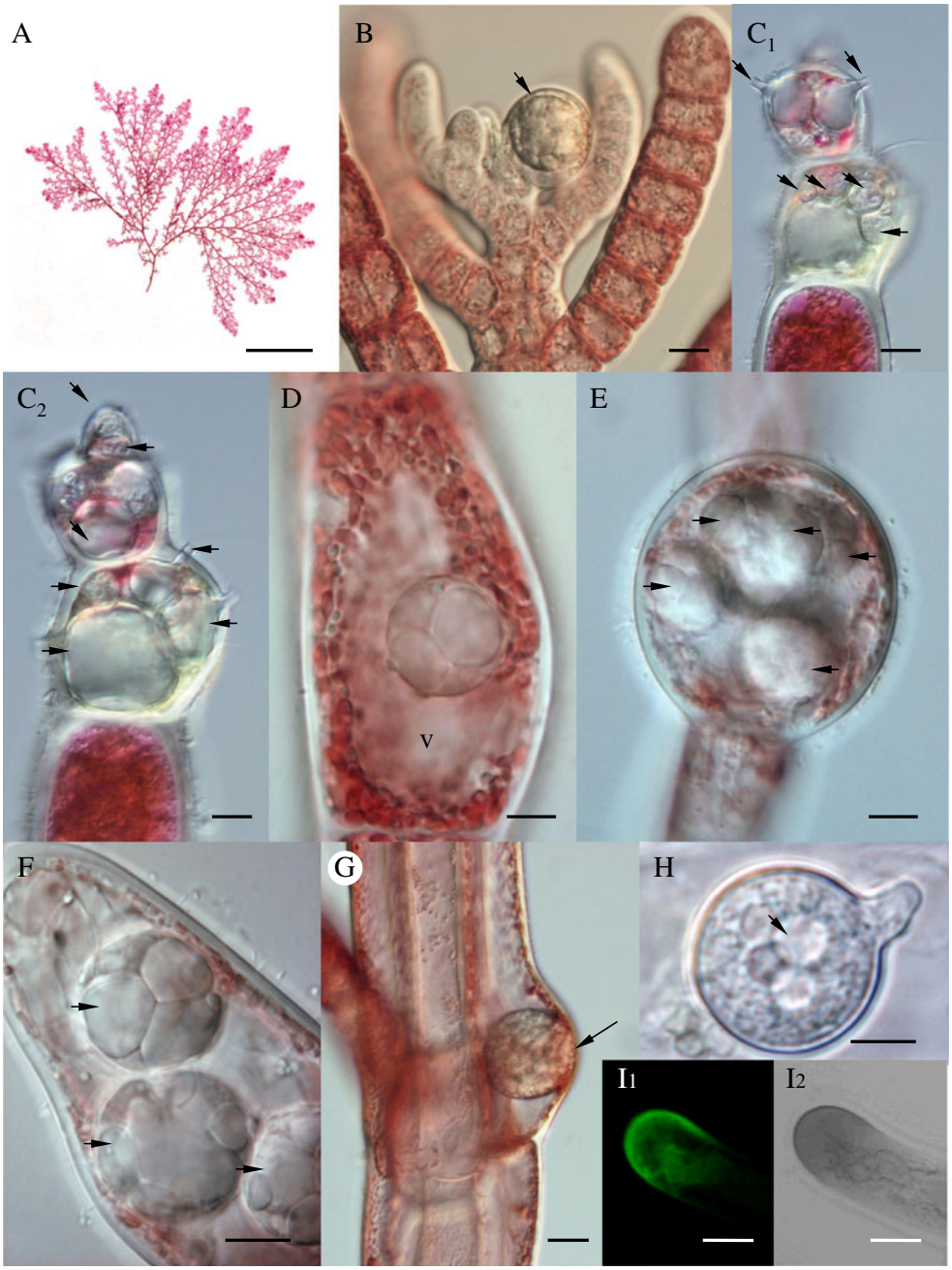

Fig. 2 General process of *Olpidiopsis* sp. 2 infection in dasyaceaen host algae. *Scale bars*=1 cm (**a**), 10 μm (**b**, **d**–**i**), and 20 μm (**c** *1* and *2*). **a**–**h** Infection in *Heterosiphonia pulchra*. **a** Host plant, *H. pulchra*. **b** *Olpidiopsis* zoosporangium developing inside apical cell of indeterminate branch (*arrow*). **c** *1* and *2* Through-focus images of same infected cells with at least 13 empty fungal zoosporangia in field-collected plants of *H. pulchra* (*arrows*). **d**–**g** *Olpidiopsis* infection often caused host cell swelling. **d**–**f** The infected host cells are still alive even with several parasitic thalli inside (*arrows*, *v* vacuole). The host cells collapse approximately 1 day after the beginning of infection. **g** Infection of polysiphonous axis was rare, 0.5–1% of the total number of infected cells (*arrow*). **h** A number of small vesicles or one large vesicle developed inside mature zoosporangium to push out liberation tube for the discharge of zoospores (*arrow*). **i** FITC-conjugated ConA staining in extended rhizoid-like apical cell of *Heterosiphonia japonica*. **i***1* FITC image. **i***2* Transmitted image

Host susceptibility experiment was carried out using 19 other isolates of red algae (Table 1). Among them, *H. japonica*, *Dasya* sp., and *Dasysiphonia chejuensis* Lee and West were infected with this *Olpidiopsis* sp. 2. Some infected plants displayed pathomorphological changes of growth and shape (Fig. 4c, d), including cell necrosis, hypoplasia, and hyperplasia.

Porphyra tenera from Korea showed good infection in blades pre-incubated in ddH_2O for 15–30 s. However, the conchocelis filaments were not infected with this *Olpidiopsis* sp. 2. In infection-susceptible algae, when healthy plants were mixed with thalli already infected with *Olpidiopsis* sp. 2, it usually took about 2–4 h to initiate infection. Fungal zoosporangia appeared variable in size ranging from 10 to 100 μm in diameter. Zoospores were liberated from the zoosporangium through a liberation tube that pushed through the thick host cell wall (Fig. 2h).

In *H. pulchra*, the infection rate was low and zoosporangia developed in much slower pace than in other host algae. Therefore, *H. japonica* was chosen as host species for cytochemical studies, because it showed strong infection in short time and plants survived long enough to perform inhibition experiments.

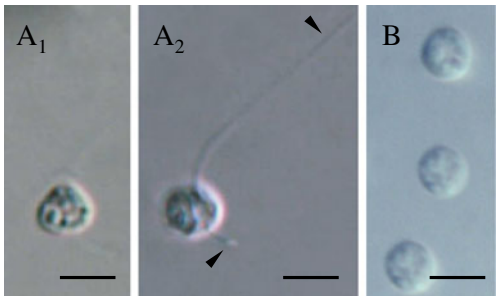

Fig. 3 Zoospores of *Olpidiopsis* sp. 2 from *Heterosiphonia pulchra*. *Scale bars*=3 μm. **a** *1* and *2* Through-focus images of same motile biflagellate zoospore. *Arrowheads* point to lateral inserted two unequal flagella. **b** Non-motile zoospores after flagella were discarded or retracted

Cytochemical studies and inhibition experiment

Olpidiopsis sp. 2 infection to *H. japonica* was cell-type specific (Table 3; Fig. 5). The extended rhizoid-like cells showed almost ten times higher infection rate (16.9%) than that of normal apical cells (1.7%). Other parts of the thallus were infected seldom (<1%).

To test for the surface chemical difference among the cell types, FITC-lectin staining was performed (Table 2; Fig. 2i). *Olpidiopsis* sp. 2 zoospores were not labeled with any of FITC-conjugated lectins. However, concanavalin A (ConA), which is specific to α-D-glucose and α-D-mannose, bound strongly to the surface of extended rhizoid-like apical cells of *H. japonica* (Fig. 2i). Although ConA also bound to the round shaped normal apical cells, the staining was much weaker than that of extended rhizoid-like cells. Other parts of *H. japonica* thallus were not stained with any of the FITC-lectins.

When the infection experiment was performed in seawater containing 0.05 M of D(+)-mannose, the infection of *H. japonica* with *Olpidiopsis* sp. 2 dropped to zero in both cell types (Table 3). Attachment of fungal zoospores to *H. japonica* thalli was also inhibited by 8.4–97.5% in IMR medium containing different concentrations of α-mannosidase (Table 4), an enzyme involved in the cleavage of α-D-mannose.

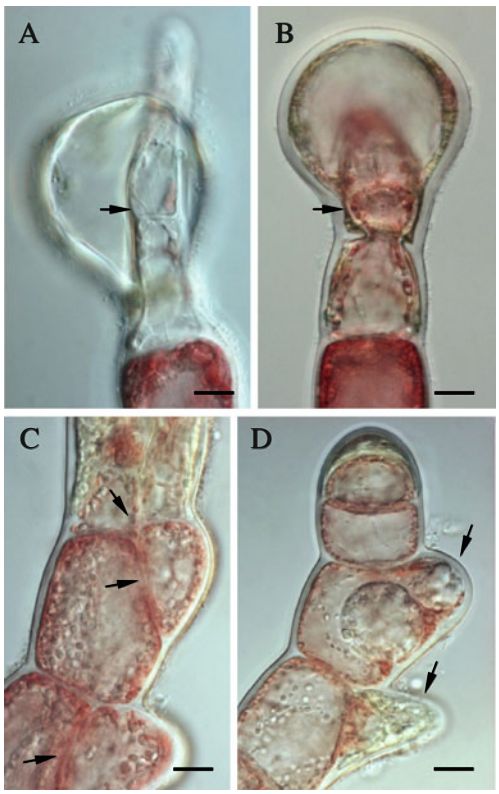

Fig. 4 General process of *Olpidiopsis* sp. 2 infection and complication of the disease in dasyaceaen host algae. *Scale bars*=10 μm. **a**, **b** Woundhealing occurs in some dasyaceaen algae after the release of zoospores, and adjacent cells grow through the empty fungal zoosporangia (*arrows*). **a** Extended rhizoid-like cell. **b** Normal apical cell of determinate branch. **c**, **d** Pathomorphological changes of growth and shape in the infected plants of *Dasysiphonia chejuensis*. **c** *Arrows* point to cross walls that developed in a monosiphonous lateral branch. **d** Monosiphonous branch developing lateral cells (*arrows*)

Discussion

The "chytrid blight," green spot, diatom felt, "red rot," and "white blight" are dominant diseases of commercially cultivated *Porphyra* spp. in Korea (Cho and Chang 1986). In Korea, the *Porphyra* cultivation begins in October and finishes in March when the seawater temperature is relatively low. However, the outbreaks of fungal infections occur regularly, despite of preventive measures such as net freezing at −20°C and net immersion into an organic acid at a pH of about 2. Most recent estimates by Ministry of Marine Affairs and Fisheries of Korea show that, on average, 10% of the annual production can be lost due to the pathogens. Reductions of 15–20% and 20–30% in yield and value, respectively, due to the *Olpidiopsis* spp. and *Pythium* spp. have been reported for Korea before (Park et al. 2000) while in early years (1983–1985) the occurrences of diseased laver was estimated at 17.2–77.5% (Cho and Chang 1986). The estimates indicate that the cultivation methods and disease control have become more successful during last two decades.

In modern cultivation of *Porphyra*, the use of nets pretreated by freezing at −20°C before hanging up on the cultivation frame is one of the main methods attempting to prevent the "red rot" and *Olpidiopsis* disease (e.g. Fujita and Migita 1980; Ding and Ma 2005). However, the complications of fungal diseases were still found on the cells in fronds from the nets frozen at −20°C for 20 days, suggesting that the method could not kill the fungi completely, but could inhibit the disease outbreak and spread in high temperature seasons (Ding and Ma 2005).

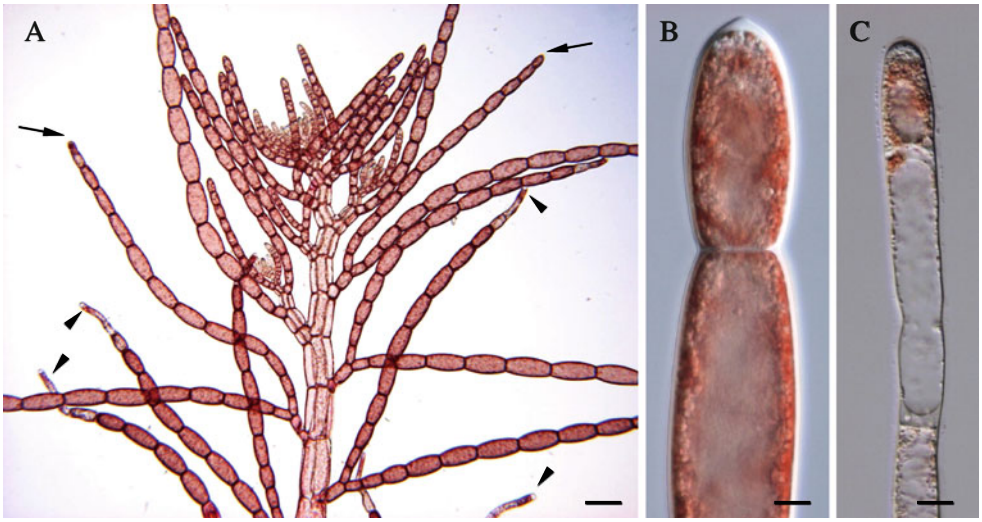

Fig. 5 Cell-type specific to infection of *Olpidiopsis* sp. 2. *Scale bars*=50 μm (**a**) and 10 μm (**b**, **c**). **a** *Heterosiphonia japonica* plant with normal apical cells (*arrows*, **b**) and extended rhizoid-like apical cells (*arrowheads*, **c**)

The farmers in Korea use this method to make the crops more resistant to disease and environmental factors and faster-growing (personal communication). Our results suggest that oomycete migration from another algal host species could also be an additional cause of infection, if such infected plant grew near *Porphyra* farm.

Song et al. (1993) stated that the occurrence of the diseased laver in Korean farms was indirectly associated with seawater temperature. They estimated that the salinity of seawater in the farming area was 27.87–30.52‰. Considering that normal salinity of seawater is 35‰, the reduction of salinity by 12.8–20.4% was most likely an additional cause of infection, which in combination with warm temperature resulted in disease outbreak. Ding and Ma (2005) also stated that high temperature, low salinity, absence of free-changing tides and inadequate air-drying were the main factors causing the disease. In China, rot of *P. yezoensis* happened in large areas in years of high temperature, rich rain and gentle breeze from the south (Ding and Ma 2005). As stated by farmers, *Olpidiopsis* infection in Seochon plantation in Korea also happened following several days with rains, when the salinity dropped and the outside temperature increased.

Although *Olpidiopsis* infection had been recorded in Korea before, the pathogen was not commented on (Cho and Chang 1986). Currently, six non-oosporic *Olpidiopsis* species are known as parasites of marine red algae (Sekimoto et al. 2009). Akiyama (1977) reported *Olpidiopsis* infection on the sporophytes of *Undaria pinnatifida* (Harvey) Suringar. Because the genus *Olpidiopsis* has a simple morphology, species taxonomy is based upon a few morphological features and host specificity. *Olpidiopsis* sp. 2 described in this study has not yet been identified. It had narrow host specificity and tended to generally prefer dasyaceaen algae, and was capable of infecting *P. tenera* blades pre-incubated in distilled water. Moreover, even in dasyaceaen algae the infection was cell-type specific.

Our experimental results suggested involvement of lectin–carbohydrate interactions during attachment of *Olpidiopsis* sp. 2 zoospores to the host algal cells. A mannose-specific receptor seems to be located on the surface of *Olpidiopsis* zoospores, and complementary carbohydrate-binding moiety is on the cell surface of host algae. Further studies are necessary to isolate a specific lectin from the surface of *Olpidiopsis* zoospores.

Several studies have been done on the behavior of another *Porphyra*-infecting oomycete, *P. porphyrae*, when the host plant is absent. For example, *P. porphyrae* oospores are produced in *Porphyra* thalli throughout the farming period (Arasaki 1947, 1956, 1962; Arasaki et al. 1968) and remain in seawater after it decays (Arasaki 1962). Zoospores derived from oversummering oospores caused “red rot” disease on healthy thalli under laboratory conditions (Arasaki 1962; Fujita 1978). Also, oospores were viable for at least 2 months in sterile seawater at room temperature of 14°C to 29°C (Yokoo et al. 1999). Thus, this pathogen may oversummer primarily as oospores in the thallus tissues and continue to survive in seafloor sediments after decay of the thallus. No studies have been conducted on survival of the marine *Olpidiopsis* in natural environmental conditions.

We found that after the release from zoosporangium, free zoospores of *Olpidiopsis* sp. 2 remained infective in seawater for only 7 days. Stable dual culture of *Olpidiopsis* with *Porphyra* is not achievable because the blades degenerate within several days of infection. Moreover, all marine *Olpidiopsis* species reported to date and also our strain were non-oosporic. In such cases, parasites require a long-term host to survive for a time period when one potential host, e.g. *Porphyra* blades, are almost absent in

the field. It is noteworthy that *Olpidiopsis* sp. 2 grew in dual laboratory cultures with *H. pulchra* for over 4 years without any special treatment and remained infective against other host algae. Studies on the host range of another marine *Olpidiopsis* species, *O. bostrychiae*, also revealed that the patterns of infection were not specific to taxonomic groups as it infected *Bostrychia* spp., *Porphyra* spp., and *H. japonica* (West et al. 2006; Sekimoto et al. 2009), but was able to grow in dual culture only with *B. moritziana* (isolate 4439) (West et al. 2006). Therefore, *Olpidiopsis* species may use different algae as short-term and long-term hosts to ensure their survival when some hosts are unavailable.

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