Comparison of two DNA viruses infecting the marine brown algae *Ectocarpus siliculosus* and *E. fasciculatus*

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The marine brown algal genus *Ectocarpus* contains two species, *E. siliculosus* and *E. fasciculatus*. Field populations of both species include plants with infection symptoms caused by DNA viruses. We have established clonal cultures from infected and normal host plants and investigated the properties of the endogenous viruses. Both host species contain virus particles with a hexagonal cross-section and a diameter of ca. 150 nm. The genomes of both virus types consist of double-stranded DNA, approximately 320 kb in size. Restriction digestion with *SfiI* revealed differences between the two virus genomes. However, PCR experiments suggest that at least one gene, which encodes a major capsid protein, is quite similar in both virus species. In cross-infection experiments the *E. siliculosus* virus did not initiate an infection cycle in *E. fasciculatus*. In contrast, the *E. fasciculatus* virus infected *E. siliculosus* zoospores. The resulting plants showed aberrant symptoms and produced virus particles which were not infectious. We conclude that the two *Ectocarpus* species are hosts for different, but closely related viruses.

Introduction

Virus infections are common in filamentous brown algae. These viruses are latently present in all cells of the infected plant, but produce pathological symptoms only in reproductive organs such as sporangia and gametangia (Müller et al., 1990). Mature virions are released into the surrounding sea water and infect free-swimming spores or gametes, while somatic cells of the adult plant are protected against infection by their solid cell wall.

Infections are usually restricted to the same algal species. *Ectocarpus siliculosus* virus, however, can also infect the species *Feldmannia simplex*, but cannot produce infective progeny virions in this host. In contrast, the same virus can complete the entire infection cycle in the related species *Kuckuckia kylmii* (Müller, 1992; Müller & Parodi, 1993; Müller et al., 1995).

*Ectocarpus siliculosus* virus, EsV, is presently the best known example of this novel group of plant viruses. EsV particles are characterized by hexagonal multilayered coats enclosing a circular double-stranded DNA genome 320 kb in size (Lanka et al., 1993). The genome contains numerous single-stranded regions (Klein et al., 1994) where DNA molecules tend to break, yielding linear unit length products as well as a broad spectrum of DNA fragments in the 10–60 kb range (Lanka et al., 1993). Genetic analysis of EsV has been initiated with the identification of an open reading frame encoding a major capsid protein, glycoprotein-1 (gp-1; Klein et al., 1995).

We have previously noted that a gp-1 related gene appears to occur in a virus derived from *Ectocarpus fasciculatus*, a sister species of *E. siliculosus* (Sengco et al., 1996). Both *Ectocarpus* species carry viruses of similar size and shape (Müller & Stache, 1992). It is therefore of interest to determine possible similarities and differences between the viruses isolated from these two species, and more importantly, whether species-specific differences affect cross-species infectivities. We report here that EsV cannot infect *E. fasciculatus*, while EfasV, the *E. fasciculatus* virus, initiates an abortive infection cycle in *E. siliculosus*.

Methods

- **Cells and viruses.** Healthy and virus-infected specimens of *Ectocarpus siliculosus* were collected at Kaikoura, New Zealand in 1988 (Müller et al., 1990). The virus-producing strain NZVicZ14 used for our experiments is a diploid hybrid sporophyte created by mating a male gamete of an infected gametophyte from Kaikoura with a healthy female gamete from Victoria, Australia. We used another isolate from Kaikoura (NZ4a3, Fig. 1a) as a healthy recipient culture for infection experiments. Two sporophyte cultures of *Ectocarpus fasciculatus* were derived from material collected in 1993 near Roscoff (Brittany, France; Parodi & Müller, 1994). One isolate is normal (Ros 31-Z7-G12; Fig. 1c), while the second culture (Ros 32-1-v3; Fig. 1d) expresses infection symptoms.
Fig. 1. Normal and infected Ectocarpus plants. (a, b) E. siliculosus sporophytes: normal (a), and with infection symptoms (b) caused by EsV. (c, d) E. fasciculatus sporophytes: normal (c), and with infection symptoms caused by EfAsV (d). (e, f) Aberrant infection symptoms (inflated filament sections, arrows) developing on E. siliculosus sporophytes after infection with EfAsV. The normalized filament (asterisk) produced functional sporangia and gave rise to a stable symptom-free subclone. Scale bar, 100 μm.

Stock cultures of all four strains were maintained on 1% agar plates with culture medium. Liquid cultures were established by placing fragments of plants from agar into sterile culture medium prepared by supplementing autoclaved natural sea water or a commercial salt mixture (Wimex, Krefeld, Germany) with the PES enrichment of Starr & Zeikus (1993). Cultures were illuminated with fluorescent light at 12 μmol/m²/s (400–700 nm) for 14 h per day at 12 or 18 °C.

For infection experiments mature virus-producing (Fig. 1b, d) and healthy recipient cultures (Fig. 1a, c) were stored in darkness at 4 °C overnight. Spore release and discharge of virus particles were induced the next morning by exposure to light and a rise in temperature to 18 °C. Swimming spores and virus-producing Ectocarpus filaments were then co-incubated in a drop (0.4 ml) of culture medium placed in the centre of a plastic Petri dish. After 1 h the Ectocarpus plants were removed, fresh
culture medium was added and the Petri dish incubated at 18 °C. After 15–20 days the developing sporophytes became fertile and were examined for the presence of infection symptoms under a stereo microscope at 63× magnification. Electron microscopical techniques have been described by Parodi & Müller (1994).

**Viral DNA.** Virus particles were isolated from bulk cultures of infected plants following the procedures described by Lanka et al. (1993) and Klein et al. (1995). Virus particles were included in agarose blocks and incubated with proteinase K. Treatment of EsV preparations was performed as previously described (Lanka et al., 1993), whereas a more extensive proteinase treatment was necessary for the release of DNA from EfV particles (2 mg/ml proteinase K; 90–96 h at 50 °C). After proteolysis, the agarose blocks were washed four times with 50 mM-Tris–HCl, 5 mM-EDTA (pH 8) at 50 °C. Agarose blocks were then incubated with the restriction enzyme SfiI (60 U) overnight. The DNA fragments were electrophoresed in 1% agarose and visualized by ethidium bromide staining. Pulsed field gel electrophoresis of intact viral DNA was carried out under the following conditions: 1% agarose, 50 mM-Tris-borate, 5 mM-EDTA (pH 8); interval times 0–3–17 s; field angle 120°; voltage gradient 6 V/cm; running time 26–40 h.

For the detection of EsV specific DNA we used the PCR procedure as described by Sengco et al. (1995), amplifying a fragment of the gene coding for the capsid glycoprotein gp-1.

**Results**

**Infection experiments**

All attempts to infect spores of *E. fasciculatus* with EsV failed. In contrast, 10–20% of the plants developing from spores of *E. siliculosus* infected with EfV showed pathological symptoms (Fig. 1e, f). Infected plants formed hyaline lateral vesicles and inflated filament segments with impaired cross-wall formation. These symptoms are similar to those observed after infection of *E. siliculosus* plants by their endogenous virus, EsV (Fig. 1b). *E. siliculosus* sporophytes infected by EfV grew well, and maintained their infection symptoms. However, no virus particles were released, and infection experiments with such plants as virus donors were unsuccessful. Occasionally, infected filament segments lost their pathological symptoms and formed intact pluri- and unilocular sporangia (Fig. 1f). One subclone from such a normalized fragment was isolated and maintained its symptom-free status. In addition, we have followed the development of 20 *E. siliculosus* plants originating from an originally infected unilocular sporangium. All plants were female gametophytes with normal gametangia free from infection symptoms. We conclude that EfV initiates an abortive infection process in the *E. siliculosus* host.

**Virus particles**

Electron microscopical investigation of *E. siliculosus* plants infected with EfV (Fig. 1e, f) revealed the presence of irregularly distributed particles in the catenate vesicular cells. We found empty capsids with open or complete coats side by side with particles containing cores of varying electron density (Fig. 2c). In contrast, EsV and EfV particles are complete and densely packed in cells of their natural host species (Fig. 2a, b).

**Comparison of the two viral genomes**

To learn more about the relationship between EsV and EfV we performed pulse field gel electrophoresis and showed
that both Ectocarpus viruses contain dsDNA with a size of ca. 320 kb (Fig. 3). We subjected the DNA of the two Ectocarpus viruses to restriction digestion with Sfil and obtained different fragment patterns as shown in Fig. 4. This result suggests differences in the nucleotide sequences of the two viral genomes.

**Detection of viral DNA in host plants**

We have previously used PCR amplification of a segment of the EsV gene gp-1 as a diagnostic tool to detect virus infection in Ectocarpus plants. This technique has shown that a PCR signal obtained with EsV gp-1 specific primers, can be detected not only in total nucleic acid extracts from EsV infected plants, but also in EfasV infected hosts (Sengco et al., 1996). This result indicates that at least the sequences encoding the capsid protein gp-1 are similar in both virus species (Fig. 5, lanes 2 and 4). This PCR reaction could therefore be used to follow the fate of virus infections in Ectocarpus plants. We found that *E. siliculosus* plants with symptoms caused by EfasV contained at least a portion of the viral genome, as expected from our light and electron microscopical observations (Fig. 5, lane 7). The PCR technique also confirmed that EsV did not enter the host *E. fasciculatus* (Fig. 5, lane 6). Furthermore, a symptom-free subclone of *E. siliculosus*, which had lost the infection symptoms caused by EfasV, still contained viral DNA, and produced the weak amplification signal shown in Fig. 5, lane 8. This shows that regression of symptoms is possible even though viral DNA is still present. The weak intensity of this signal might indicate that not all cells of this clone carried the EfasV genome. In contrast, we found that passage through an unilocular sporangium resulted in the elimination of the gp-1 coding EfasV DNA in daughter gametophytes of *E. siliculosus* (not shown).

**Discussion**

The two species of Ectocarpus are natural hosts for viruses which are indistinguishable by morphological criteria. The viral genomes consist of dsDNA of similar size. They also share common nucleotide sequences such as the primer-binding sites in their genes coding for the gp-1 capsid protein.
Nevertheless, our infection experiments show that the two Ectocarpus viruses are separate entities with subtle, but clear differences in host specificity. EsV could not infect spores of *E. fasciculatus*, while EfasV was able to enter and propagate in *E. siliculosus*, although with morphological and physiological deficiencies. In addition, the restriction nuclease SfiI yielded different DNA fragment patterns, which reflect differences in the nucleotide sequences of the two viral genomes. Expression of symptoms by the foreign virus EfasV in *E. siliculosus*, although still latently present as confirmed by our PCR experiments, can be completely inhibited by the host.

We conclude from our experiments that the viruses EsV and EfasV possess genomes of similar size, and share at least one coding region. However, they differ in as yet unidentified genes or DNA regions giving rise to incompatibility effects ranging from failure of infection to the production of defective, non-infectious hybrid virions.

The EsV genome enters unicellular spores or gametes of its host, and is found in a temperate condition in all somatic cells of the developing plants (Kuhlenkamp & Müller, 1994), which suggests an intimate association of the viral DNA with the host’s nuclear genome. This conclusion is confirmed by the observation that meiosis in infected diploid sporophytes of *E. siliculosus* can create virus-free progeny in a Mendelian segregation pattern (Müller, 1991; Bräutigam et al., 1995). In our present study, unilocular sporangia of *E. siliculosus*, which normally are the site of meiosis in brown algae, eliminated the foreign EfasV genome. However, since in this case the unilocular sporangia were formed on haploid sporophytes, the mechanism of this elimination process is difficult to understand.

The immunity of one Ectocarpus species to infection by a virus from the sister species is in agreement with findings on other ectocarpalean viruses. In most cases the host range of viruses is restricted to one algal species. Interspecific or intergeneric infections are rare and may represent dead ends for the virus genomes. Nevertheless, it remains an interesting possibility that occasional extra-specific virus contacts permit non-sexual gene transfer between ectocarpoid species and genera.

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References


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