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Massive Prevalence of Viral DNA in *Ectocarpus* (Phaeophyceae, Ectocarpales) from Two Habitats in the North Atlantic and South Pacific

D. G. Müller[a,*], R. Westermeier[b], J. Morales[b], G. Garcia Reina[c], E. del Campo[c], J. A. Correa[d] and E. Rometsch[a]

[a] Fakultät für Biologie, Universität Konstanz, D-78457 Konstanz, Federal Republic of Germany
[b] Facultad de Pesquerías, Campus Puerto Montt, Universidad Austral, Puerto Montt, Chile
[c] Instituto de Algología Aplicada, Universidad de Las Palmas de Gran Canaria, Telde, Gran Canaria, Spain
[d] Departamento de Ecología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile

* Corresponding author

Ectocarpus, a cosmopolitan genus of filamentous marine brown algae, contains two species, *E. siliculosus* and *E. fasciculatus*. Both species are subject to virus infections, which either destroy the host's sporangia or persist in a latent state without visible symptoms. We used PCR amplification of a viral gene fragment to monitor the infection status of *Ectocarpus* samples from Gran Canaria Island, North Atlantic, and southern Chile over 26 months.

At both sites, we found persistently high levels of pathogen prevalence: 40–100% of the *Ectocarpus* specimens from Chile and 55–100% from Gran Canaria contained viral DNA. No evidence for seasonal variation could be detected. We conclude that vertical transmission of viral DNA through mitotic zoospores of the host is the key mechanism for the persistence of the pathogen. The PCR amplification characteristics of samples from Gran Canaria indicate that two different virus genotypes coexist in the same host population.

Introduction

The two brown algal species *Ectocarpus siliculosus* (Dillwyn) Lyngbye and *E. fasciculatus* Harvey are well known cosmopolitan inhabitants of coasts in cold and warm temperate climate zones. Since the end of the last century numerous reports described specimens with abnormal sterile sporangia from many parts of the world (Sauvageau 1896, Cardinal 1964, Asensi 1974). Silva (1957) established the new species *Ectocarpus dimorphus* Silva to accomodate such individuals. In 1974 Clitheroe and Evans detected virus like particles in malfunctioning sporangia of *Ectocarpus*. Subsequently Müller *et al.* (1990) isolated an icosahedric DNA virus from *E. siliculosus* and demonstrated that free virions are able to infect spores or gametes of their host. Bräutigam *et al.* (1995) developed a polymerase chain reaction (PCR) protocol, which allows the detection of viral DNA in *Ectocarpus*. With this technique Sengco *et al.* (1996) showed, that in a sample of 98 isolates from our *Ectocarpus* culture collection nearly 50% contained latent virus DNA. This result suggests that natural *Ectocarpus* populations are subject to much higher virus prevalence than hitherto judged from morphologically visible infection symptoms.

We describe here the level and temporal fluctuation of virus prevalence in two natural *Ectocarpus* populations determined by PCR detection of viral DNA.

Materials and Methods

Two habitats with perennial populations of *Ectocarpus* were selected as collection sites. 1) A platform of volcanic rock 100 × 20 m at mid-intertidal level outside of Muelle de Taliarte, Telde, Gran Canaria, Spain (28°00′ N, 15°22′ W). In this habitat *Ectocarpus* grows as an epiphyte on *Fucus spiralis* Linnaeus, and on rocks, shells or limpets in tide pools. 2) An experimental mariculture area located in the estuary of the Maullín river near Puerto Montt, Chile (41°40′ S, 73°45′ W). In this sandy habitat *Ectocarpus* grows epiphytically on *Gracilaria chilensis* Bird, McLachlan *et* Oliveira and other substrata in the mid-intertidal zone.

At each site 30 tufts of *Ectocarpus* (approx. 5 mg dw each) were collected at bi-monthly intervals from August 1995 to October 1997. Samples were blotted dry with filter paper and sealed in polyethylene bags with 1 g of dehydrated silica gel (Fluka blue 85340). Subsamples were fixed in acetocarmine and mounted in Karo® syrup as permanent slides for microscopic examination.

The DNA was extracted from 0.5–1 mg of the dry samples and subjected to PCR amplification and detection of a fragment of the viral gene gp-1, following the protocol described by Sengco *et al.* (1996). In some analyses the stringency of the PCR reaction was reduced by lowering the annealing temperature from 64 °C (standard condition) to 55 °C.

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Results

Due to the great variability of *Ectocarpus* (Russell 1966) identification of our field material to species level was not possible. All *Ectocarpus* specimens examined from both study sites were sporophytes with plurilocular sporangia. Unilocular sporangia were present in 3 out of 91 samples from the Chilean site, and were completely absent in samples from Gran Canaria. Specimens with visible symptoms of virus infection were rather rare in the Gran Canaria *Ectocarpus* (1 out of 123 samples), and more frequent in the Chilean habitat (15 out of 91 samples).

Our standard PCR amplification method revealed that between 40 and 100% of the *Ectocarpus* samples from the Chilean study site contained viral DNA. Temporal fluctuations in the infection level were irregular and showed no evidence for seasonality (Fig. 1). The detection of viral DNA in *Ectocarpus* samples from the Gran Canaria site proved to be more complex. Analyses with the standard PCR protocol gave low infection levels ranging from zero to 20% (Fig. 2). However, under less stringent conditions the proportion of samples with viral DNA in the Canarian *Ectocarpus* increased strongly to values between 55 and 100%. Fluctuations were irregular, and a clear seasonal trend could not be detected (Fig. 2).

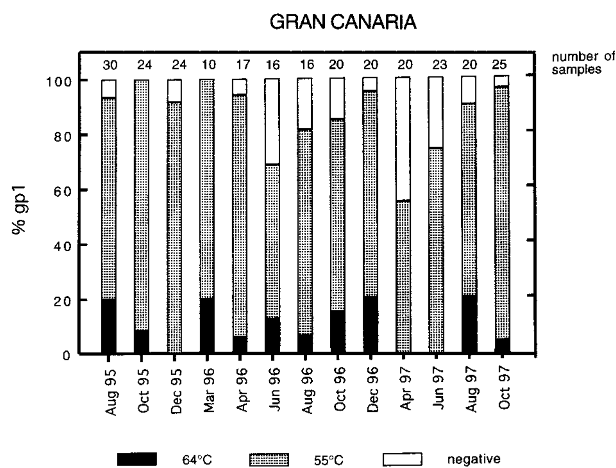

Fig. 2. PCR detection of viral DNA in *Ectocarpus* from the study site at Gran Canaria over 26 months. Total length of bars represents 100% of sample size. Black and stippled areas represent percentages of samples containing viral DNA detected at two different stringency levels (PCR annealing temperatures as indicated).

Discussion

Laboratory cultures of *Ectocarpus siliculosus* and *E. fasciculatus* are subject to infections by two different virus types (Müller *et al.* 1996). These two pathogens are species-specific, but closely related. Sengco *et al.* (1996) confirmed that the viruses of both *Ectocarpus* species respond in the same positive manner to PCR amplification of the virus-specific gene fragment gp-1. Now our study confirms and extends the findings of Sengco *et al.* (1996) to natural populations of *Ectocarpus*: 1) The prevalence of viral DNA in *Ectocarpus* is exceedingly high and ranges between 40 and 100%. 2) In contrast, between less than 1 up to a few percent of *Ectocarpus* specimens exhibit morphological infection symptoms with the full expression of virion formation. This discrepancy can be explained by either a high degree of latency in the *Ectocarpus* virus, or the inactivation of the pathogen by partial elimination of viral DNA.

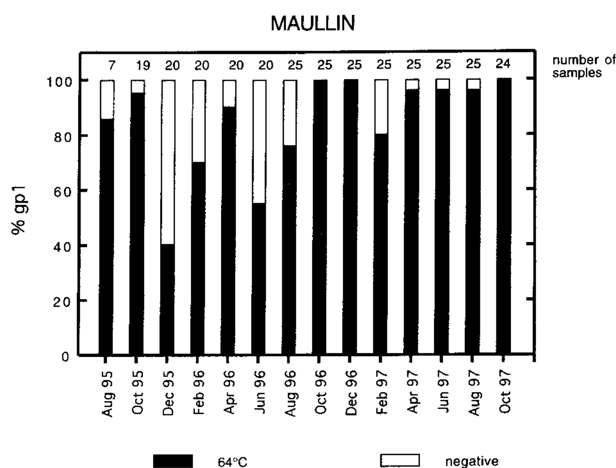

Fig. 1. PCR detection of viral DNA in *Ectocarpus* from the study site at Maullín, Chile over 26 months. Total length of bars represents 100% of sample size. Black areas show percentage of samples containing viral DNA obtained under standard conditions.

Unilocular sporangia are the site of meiosis in brown algae, and laboratory cultures of infected *Ectocarpus* sporophytes can produce virus free progeny by meiotic segregation (Müller 1991, Bräutigam *et al.* 1995). The relevance of his mechanism is negligible, since unilocular sporangia are completely lacking in the Canarian, and only rarely found in the Chilean *Ectocarpus*. Likewise, infections of new hosts must be rare events, since only low numbers of virions can be expected from the few individuals with visible symptoms in both study sites. Consequently, vertical transmission via mitotic spores from plurilocular sporangia of the hosts remains the only plausible mechanism to maintain the high prevalence of virus infections in the field populations of *Ectocarpus*. Samples from the Gran Canaria site responded differentially to reduction of the annealing temperature from 64 to 55 °C in our PCR protocol. This implies the coexistence of virus genotypes with nucleotide sequence differences in their gp-1 genes.

Presently, all attempts to explain the emergence and persistence of a viral pandemy system in a marine cosmopolitan host like *Ectocarpus* must remain speculative. Since the *Ectocarpus* virus DNA is integrated in the nuclear genome of its host (Delaroque *et al.* 1999) it can be expected to interfere with mei-

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otic pairing of chromosomes. This fits with the experience that laboratory cultures of *Ectocarpus*, *Hincksia* and *Feldmannia* rarely form unilocular meiotic sporangia, which in addition tend to abort or produce moribund progeny. This effect can lead to the elimination of sexuality, which is replaced by vegetative reproduction with mitotic spores, providing a favourable survival strategy for opportunistic colonists like *Ectocarpus* and related genera. Further studies on the interaction between host and virus genomes and effects of viral latency on the physiological performance and fitness of the hosts are necessary before a comprehensive understanding of the impressive virus pandemy in *Ectocarpus* and related brown algae is possible.

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References

Asensi, A. O. 1974. Observaciones sobre la citologia y morfogenesis de los esporocistos anormales de *Ectocarpus dimorphus* Silva (Phaeophyta, Ectocarpales). *Physis* Sección A *86*: 139–147.

Bräutigam, M., M. Klein, R. Knippers and D. G. Müller. 1995. Inheritance and meiotic elimination of a virus genome in the host *Ectocarpus siliculosus* (Phaeophyceae). *J. Phycol. 31*: 823–827.

Cardinal, A. 1964. Étude sur les Éctocarpacées de la Manche. *Beih. Nova Hedwigia 15*: 1–86.

Clitheroe, S. B. and Evans, L. V. 1974. Viruslike particles in the brown alga *Ectocarpus*. *J. Ultrastruct. Res. 49*: 211–217.

Delaroque, N., I. Maier, R. Knippers and D. G. Müller. 1999. Persistent virus integration into the genome of its algal host *Ectocarpus siliculosus* (Phaeophyceae). *J. gen. Virology 80*: 1367–1370.

Müller, D. G. 1991. Mendelian segregation of a virus genome during host meiosis in the marine brown alga *Ectocarpus siliculosus*. *J. Plant Physiol. 137*: 739–743.

Müller, D. G., H. Kawai, B. Stache and S. Lanka. 1990. A virus infection in the marine brown alga *Ectocarpus siliculosus* (Phaeophyceae). *Botanica Acta 103*: 72–82.

Müller, D. G., M. Sengco, S. Wolf, M. Bräutigam, C. E. Schmid, M. Kapp and R. Knippers. 1996. Comparison of two DNA viruses infecting the marine brown algae *Ectocarpus siliculosus* and *E. fasciculatus*. *J. Gen. Virology 77*: 2329–2333.

Russell, G. 1966. The genus *Ectocarpus* in Britain. I. The attached forms. *J. Mar. Biol. Ass. U. K. 46*: 267–294.

Sauvageau, C. 1896. Sur la nature des sporanges en chapelet de l'*Ectocarpus confervoides*. *J. Botanique 10*: 140–144.

Sengco, M., M. Bräutigam, M. Kapp and D. G. Müller. 1996. Detection of virus DNA in *Ectocarpus siliculosus* and *E. fasciculatus* (Phaeophyceae) from various geographic areas. *Eur. J. Phycol. 31*: 73–78.

Silva, P. C. 1957. Notes on Pacific marine algae. *Madroño 14*: 41–51.

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