Journal of Applied Phycology (2006) 18: 295–299
DOI: 10.1007/s10811-006-9031-0

Forecasting infections of the red rot disease on *Porphyra yezoensis* Ueda (Rhodophyta) cultivation farms

Chan Sun Park[1,*], Makoto Kakinuma[2] & Hideomi Amano[2]
[1]*Department of Marine Resources, Mokpo National University, 61 Torim-ri, Chounggye-myon, Muan-gun, Jeonnam 534–729, Korea;* [2]*Laboratory of Marine Biochemistry, Faculty of Bioresources, Mie University, 1515 Kamihama, Tsu, Mie 514–8507, Japan*

[*]Author for correspondence: e-mail: cspark85@mokpo.ac.kr; fax: 82-61-452-8875

Key words: *Porphyra yezoensis*, cultivation, red rot disease, *Pythium porphyrae*, forecast, zoospores

Abstract

Pythium porphyrae is a fungal pathogen responsible for red rot disease of the seaweed *Porphyra* (Rhodophyta). Infection forecasts of *Porphyra* by *P. porphyrae* were estimated from the epidemiological observations of *Porphyra* thalli and numbers of zoospore of *P. porphyrae* in laboratory and cultivation areas. Four features of forecasting infections were determined by relating zoospore concentrations to the incidence of thallus infection; infection (in more than 1000 zoospores L^{-1}), microscopic infection [less than 2 mm in diameter of lesion (in from 2000 to 3000 zoospores L^{-1})], macroscopic infection [more than 2 mm in diameter of lesion (in from 3000 to 4000 zoospores L^{-1}), and thallus disintegration (in more than 4000 zoospores L^{-1}). High zoospore concentrations led to more infection. The tendency that zoospore concentration of *P. porphyrae* increased with the rate of infection of *Porphyra* thalli was generally observed in forecasting infections in both the laboratory and in cultivation areas. Based on the *Porphyra* cultivation areas, the accuracy and consistency of forecasting infections suggest that this method could be employed to manage and control red rot disease.

Introduction

Red rot disease (Akagusare), caused by *Pythium porphyrae* (Oomycetes), is one of the most destructive fungal diseases of *Porphyra* and can seriously reduce both yield and quality in *Porphyra* farms every year (Amano et al., 1995). The causative organism of this disease is spread by zoospores released into seawater. After the zoospores attach to *Porphyra* thalli, they form hyphae to penetrate the cell of *Porphyra* thalli and to kill the alga within a few days (Sasaki & Sato, 1969; Fujita, 1990). There is then a massive release of new zoospores. Thus, the pathogen can survive as an endophyte, an epiphyte, or latent infections. The movement of infected asymptomatic *Porphyra* or *Porphyra* parts could serve as a means of introducing this serious disease into other geographic regions (Fujita & Zenitani, 1977; Kerwin et al., 1992). Therefore, it is important to be able to quickly assess the amount of zoospores in seawater prior to an outbreak of the disease at *Porphyra* farms.

To provide efficient protection of *Porphyra* farms from red rot disease, the development of an epidemiologically-based forecasting system for timely preventive application has been proposed by Park et al. (2001a). For example, a forecasting system can detect the disease more quickly than a routine such as visual observations (Sakaguchi et al., 2001; Uppalapati et al., 2001), and would allow farmers to use less fungicides (an organic acid-seawater mixture; pH about 2). This would give the opportunity to reduce the frequency of their treatments, thus lowering risks for the environment, yet still providing adequate or improved protection to *Porphyra* farms.

In the previous study, we designed the species-specific polymerase chain reaction (PCR) primers PP-1 (5′-TGTGTTCTGTGCTCCTCTCG-3′) and PP-2 (5′-CCCAAATTGGTGTTGCCTCC-3′) based on

internal transcribed spacers (ITS) rDNA sequences of *P. porphyrae* (Park et al., 2001b). We showed that it was possible to detect a single zoospore of *P. porphyrae* using these primers and to analyze quantitatively zoospores of *P. porphyrae* in the *Porphyra* cultivation farms by competitive PCR (Park et al., 2001a). However, we have yet to determine how many zoospores must be present in the seawater column to initiate an outbreak of the red rot disease.

The objective of this study was to describe the forecasting of infections of red rot disease from zoospore concentrations in the seawater of *Porphyra* cultivation farms by applying the results of susceptibility of *Porphyra* thalli infested artificially with *P. porphyrae* zoospores in the laboratory, and the relationships between the epidemiology of *Porphyra* thalli and the amount of *P. porphyrae* zoospores in seawater during the growing seasons at *Porphyra* farms.

Materials and methods

The incidence and expansion rate of red rot disease to *P. yezoensis* thalli were determined by d artificially infection with zoospores of *P. porphyrae*. Blade discs (4 mm in diameter) of *P. yezoensis* were cut with a cork-borer from uninfected healthy blades cultured in the laboratory. Twenty discs were placed in each beaker containing 1 L of sterile seawater (15 °C) with appropriate salinity (32‰) and pH (7.5). To obtain zoospores, five corn meal agar discs (6 mm in diameter) containing the edge of the *P. porphyrae* growth circle were transferred to Arasaki B liquid medium of 100 mL (Arasaki et al., 1968) and maintained for a further 4 days at 20 °C. Hyphae were gently shaken at 100 rpm on an orbital shaker (Iuchi Co. Ltd., Osaka, Japan) to release zoospores. After about 15 h, zoospores were discharged. The mycelia left were filtered out with a 20 μm nylon mesh. After zoospore concentration was determined by haemocytometer, about 10, 100, 500, 1000, 2000, and 4000 zoospores L^{-1} were inoculated.

The incidences of blade discs infected by *P. porphyrae* were determined by naked eye and using an inverted light microscope every day during the seven days after inoculation of the zoospores into the beaker containing *P. yezoensis* thalli discs. The disease incidence was categorized using an index as follows. No infection, -; infection, + and microscopic infection, ++ (less than 2 mm in diameter of lesion). The expansion of area per lesion on infected discs was determined after staining with 1% Erythrosin B solution using the microscope's digital camera computer system (DP50-A Olympus, Tokyo, Japan). Rates of disease expansion in terms of lesion area in infected discs were calculated from the mean area of lesions per disc measured in a sampling unit of three beakers, at the time of assessment. Three replicate sets of the treatments were completed.

The estimation of zoospores of *P. porphyrae* in the *Porphyra* cultivation areas was conducted at farms which use the floating net cultivation system in Wando, Korea (34°122′11″N; 127°21′35″E) during successive *Porphyra* cultivation seasons (field trial 1 in December 2002, field trial 2 in December 2003) using the methods described in the previous study (Park et al., 2001a). At the same time, to examine the relationship between the number of zoospores and incidence levels of red rot disease, the *Porphyra* thalli from the *Porphyra* nets were sampled and observations were carried out as described above. The experiment was conducted using a completely randomized design and each treatment was replicated in the three stations.

Results and discussion

The outbreak and the rates of disease incidence of red rot disease from zoospores infested artificially in the laboratory experiments were investigated (Figure 1). The time taken before disease outbreak differed with zoospore concentrations. In the case of 2000 and 4000 zoospores L^{-1}, infection occurred approximately 12 h

Number of zoospores L^{-1}	0	1	2	3	4	5	6	7
4000	+	+	++	++	++	++	++	++
2000	+	+	+	++	++	++	++	++
1000	-	+	+	+	++	++	++	++
500	-	-	+	+	+	++	++	++
100	-	-	-	+	+	+	++	++
10	-	-	-	-	+	+	+	++

Days from incubation

Figure 1. Features of infection of *Porphyra yezoensis* thalli artificially infested by zoospores of *Pythium porphyrae* [- : no infection; + : infection; ++ : microscopic infection (< 2 mm in diameter of lesion).

after zoospore inoculation. In the other hand, in the case of 10 and 100 zoospores, it took three to four days. The time taken to microscopic infection (less than 2 mm in diameter of lesion) after disease infection did not differ with zoospore concentrations. In 10 to 4000 zoospores L^{-1}, microscopic infection was observed in two and three days after disease infection. When the concentrations of fungal zoospores were high, the prevalence of disease was greater at higher concentrations of fungal zoospores than at lower zoospore concentrations. This result indicates that the outbreak of red rot disease was affected by the concentration of fungal zoospores. In the present study, on *in vivo* infection assays, the *Porphyra* thalli infested with high concentration of zoospores showed faster mycelial proliferation than either the *Porphyra* thalli infested with low concentration of zoospores and non-zoospore controls. Similar results were reported by Sakaguchi et al. (2001). These results suggest a possible critical threshold level that poses no disease threat to *Porphyra* farms during the growing season, if the number of *P. porphyrae* zoospores have been exactly estimated in the seawater at these farms.

Figure 2 shows the rates of expansion of lesion area per day in the laboratory experiments. The regression equation between rate of expansion of lesion area per lesion and time is given as: $y = 2689.4X^2 + 1006.8X$ with an r^2 value of 0.96. The expansion of lesion area per lesion was more than 40 000 μm^2 at 4 days after infection. The rate of expansion of lesion area per day was approximately 10 000 μm^2. Lesion area per lesion expanded slowly in the early stage of infection, but rapidly in the late stage of infection. As shown in Figure 1, the time taken to microscopic infection (less than 2 mm in diameter of lesion) was between three and four days after disease infection. Differences in

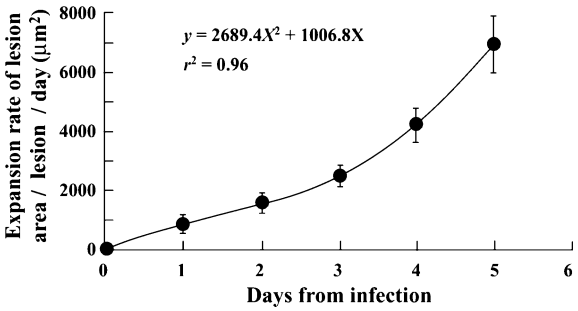

Figure 2. Expansion rates of lesion area per lesion on infected *Porphyra yezoensis* thalli with respect to time after artificial infection by zoospores of *Pythium porphyrae*.

the number of infection sites and the expansion of lesions on different thalli accounted for the difference of susceptibility of the initial steps of host-pathogen reactions of *Porphyra* thalli. Involvement of nutritional and/ or metabolic status of the host plant in the growth of *P. porphyrae* or degree on colonization of *Porphyra* thalli have been described (Arasaki, 1947; Kato et al., 1973; Uppalapati & Fujita, 2001).

In the Wando area, *Porphyra* cultivation starts between early October and late September every year when the temperature of seawater drops to below approximately 20 °C. Zoospores of *P. porphyrae* were detected on 5 December for the first time in both field trials 1 and 2, and the concentration of zoospore was approximately 50 zoospores L^{-1} (Figure 3). Thereafter, the number of zoospores in the seawater gradually increased depending on growing periods of *Porphyra*, and the concentration of zoospores increased rapidly until late December, when it peaked at approximately 4500 zoospores L^{-1}. Even though seawater sampling in the present study was done at one-day intervals, data analyses were based on a convenient, discrete daily period, which was necessary to represent continuous epidemic processes, such as zoospore production and dispersal, and subsequent infection. The results of this study clearly showed that the degree of infection of red rot disease in the *Porphyra* cultivation areas was related to the number of *P. porphyrae* zoospores.

As in the results of epidemiological observation, the infection of *Porphyra* thalli was found on 11 to 13 December for the first time in field trial 1 and 2. The number of zoospores of *P. porphyrae* at that time was approximately 1000 zoospores L^{-1}, and the prevalence of infected thalli (microscopic infection) was visible by microscopy on 14 to 17 December when the number of zoospores was more than 2000 zoospores L^{-1}. Macroscopic infection was clearly visible to the naked eye between 17 and 20 December when the number of zoospores was more than 3000 zoospores L^{-1}. Thus, the time taken from microscopic infection to macroscopic infection (more than 2 mm in diameter of lesion) was three to four days, and the disintegration of *Porphyra* thalli by disease infection occurred six to seven days after macroscopic infection. These results are similar to those of Amano et al. (1996), observed during *Porphyra* cultivation periods, using a monoclonal antibody.

Some methods, including acid treatment, freezing, and exposure of nets, are used to control the spread of red rot disease on *Porphyra* farms. These treatments,

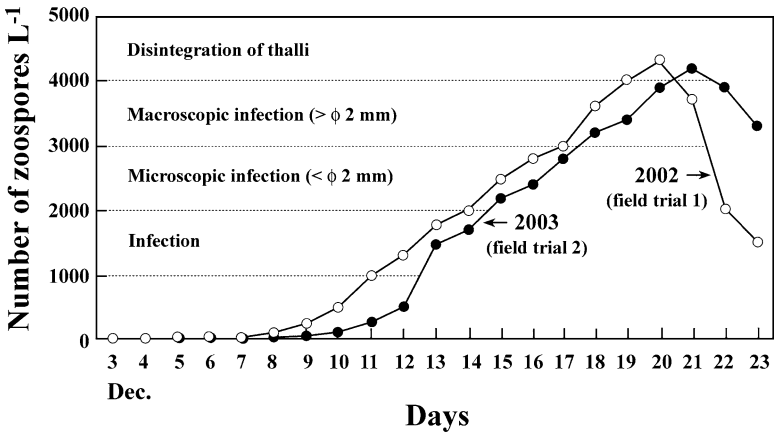

Figure 3. The number of zoospores of *Pythium porphyrae* per litre of seawater and the incidences of observed infection of *Porphyra yezoensis* thalli in *Porphyra* cultivation areas in Wando, Korea during successive *Porphyra* cultivation seasons (field trial 1; December 2002, field trial 2; December 2003) using the methods described in the previous study by Park et al. (2001a).

however, are only effective if they are implemented at the early stages of infection (Arashima et al., 1994; Uppalapati & Fujita, 2000). The results described in this paper are probably more easily used in the field, in *Porphyra* cultivations, because they are based both on data obtained from the laboratory and from cultivation areas. The present study shows that, based on the occurrence of zoospores of *P. porphyrae,* infections of red rot disease on *Porphyra* farms can be detected and forecast earlier than was possible with previous detection methods. It could predict the disease nine to ten days before the microscopic infections could be observed by conventional light microscopy and twelve to thirteen days before the macroscopic infections (more than 2 mm in diameter of lesion) could be observed by the naked eye. Thus, on the basis of the number of zoospores in the seawater, the forecasting system of recognizable thresholds can be applied on a cultivation-by-cultivation basis so that farmers can take appropriate measures where necessary. This means that infections of red rot disease on *Porphyra* farms can be more effectively forecast than using previous methods such as observation by the naked eye.

This study shows that by using competitive polymerase chain reaction techniques it is possible to predict the potential occurrence of fungal infection and the critical threshold levels that pose disease threats to *Porphyra* cultivation. This technique is now being extended as a more simple way to prevent or control red rot disease in seawater at *Porphyra* cultivation farms.

Acknowledgments

This work was supported by grant No. R08-2003-000-10074-0 from the Basic Research Program of the Korea Science & Engineering Foundation.

References

Amano H, Sakaguchi K, Maegawa M, Noda H (1996) The use of a monoclonal antibody for the detection of fungal parasite, *Pythium* sp., the causative organism of red rot disease, in seawater from *Porphyra* cultivation farms. Fish. Sci. 62: 556–560.

Amano H, Suginaga R, Arashima K, Noda H (1995) Immunological detection of the fungal parasite, *Pythium* sp.; the causative organism of red rot disease in *Porphyra yezoensis*. J. Appl. Phycol. 7: 53–58.

Arasaki S (1947) Studies on red rot of *Porphyra tenera*. Nippon 280 Suisan Gakkaishi 13: 74–90.

Arasaki S, Akino K, Tomiyama T (1968) A comparison of some physiological aspects in a marine *Pythium* on the host and on the artificial medium. Bulletin of Misaki Marine Biology Institute of Kyoto University 12: 203–206.

Arashima K, Amano H, Suginaga R, Noda H (1994) Preparation of monoclonal antibodies against the fungal parasite, *Pythium* sp., the causative organism of laver red rot. Fish. Sci. 60: 481–482.

Fujita Y (1990) Introduction to Applied Phycology. In Akatsuka I, Sheath R.G (eds.), Diseases of cultivation *Porphyra* in Japan. SPB Academic Publishing, The Netherlands: 177–190.

Fujita Y, Zenitani B (1977) Studies on pathogenic *Pythium* of laver red rot in Ariake Sea farm-II. Experimental conditions and nutritional requirements for growth. Nippon Suisan Gakkaishi 43: 89–95.

Kato S, Watanabe T, Sato Y (1973) Studies on the diseases of cultural *Porphyra*-VII. A comparison of physiological properties among

the different isolates of the causal fungus of the red wasting disease. Nippon Suisan Gakkaishi 39: 859–865.

Kerwin JL, Johnson LM, Whisler HC, Thininga AR (1992) Infection and morphologensis of *Pythium marinum* in species of *Porphyra* and other red algae. Can. J. Bot. 70: 1017–1024.

Park CS, Kakinuma M, Amano H (2001a) Detection and quantitative analysis of zoospores of *Pythium porphyrae*, causative organism of red rot disease in *Porphyra*, by competitive PCR. J. Appl. Phycol. 13: 433–441.

Park CS, Kakinuma M, Amano H (2001b) Detection of red rot disease fungi *Pythium* spp. by polymerase chain reaction. Fish. Sci. 67: 197–199.

Sakaguchi K, Park CS, Kakinuma M, Amano H (2001) Effects of varying temperature, salinity, and acidity in the treatment of *Porphyra* infected by red rot disease. Suisanzoshoku 49: 77–83.

Sasaki M, Sato S (1969) Composition of medium and cultural temperature of *Pythium* sp., a pathogenic fungus, of the 'Akagusare' disease of cultivated *Porphyra*. Bulletin of Tohoku Region National Fisheries Research Institute 29: 125–132.

Uppalapati SR, Fujita Y (2000) Carbohydrate regulation of attachment, encystment, and appressorium formation by *Pythium porphyrae* (Oomycota) zoospores on *Porphyra yezoensis* (Rhodophyta). J. Phycol. 36: 359–366.

Uppalapati SR, Fujita Y (2001) The relative resistance of *Porphyra* species (Banggiales, Rhodophyta) to infection by *Pythium porphyrae* (Peronosporales, Oomycota). Bot. Mar. 44: 1–7.

Uppalapati SR, Kerwin JL, Fujita Y (2001) Epifluorescence and scanning electron microscopy of host-pathogen interactions between *Pythium porphyrae* (Peronosporales, Oomycota) and *Porphyra yezoensis* (Bangiales, Rhodophyta). Bot. Mar. 44: 139–145.