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

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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).