Paper

Diagnostics of Susabi-nori (*Porphyra Yezoensis*) by Laser-Induced Fluorescence Method

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ABSTRACT

Susabi-nori (*Porphyra yezoensis*) was diagnosed by means of laser-induced fluorescence (LIF) method. Fluorescence peaks located at approximately 580, 660, 685 and 720 nm were observed in the LIF spectra of Susabi-nori. In the spectrum of the sample infected with the red rot disease, the intensity of 580 nm peak was relatively high as compared with that of the control sample. On the other hand, the intensities of 580 nm and 660 nm peaks drastically decreased by the influence of the chytrid disease. Furthermore, the intensity of the 580 nm peak increased by dipping into fresh water. These results indicate that LIF spectra of Susabi-nori are affected by the diseases and the stress of fresh water and that the diseases and the stress of Susabi-nori can be diagnosed by the LIF method.

KEYWORDS: Susabi-nori, *Porphyra yezoensis*, laser-induced fluorescence, red rot disease, chytrid disease

1. Introduction

The nori (laver; *Porphyra*) cultivation is greatly affected by weather and natural phenomena in ocean. In particular, the diseases cause damage to the nori cultivation. Furthermore, the nori may be influenced by fresh water, such as inflow of river water, and rain when it is exposed into the air during the ebb tide. Conventionally, fishermen diagnose the influences of the diseases and the stress by means of the visual inspection or the optical-microscope observation. However, a quantitative evaluation technique is required for more stable production in the nori cultivation. In addition, the technique for diagnostics in the early stage when we can not diagnose nori by the visual inspection or the optical-microscope observation is also required.

Therefore, we proposed laser-induced fluorescence (LIF) method for diagnostics of Susabi-nori. The LIF is a spectroscopic method, and used for remote sensing[1)-4)].

In this work, we investigated the influences of the diseases such as the red rot disease and the chytrid disease, and the stress by dipping into fresh water by means of LIF method.

2. Materials and methods

The samples of Susabi-nori (*Porphyra yezoensis*) were cultivated at the temperature of 18°C in Chiba Prefectural Fisheries Research Center. The used sea water was filtrated with 1-μm filter, and heat-treated at 90°C for 3 h for disinfection.

Figure 1 shows a schematic diagram of the LIF measurement system used in this work. The LIF spectra were measured at 20°C. The 488.0 nm emission line of a continuous-wave (cw) Ar^+ laser (GLG3020, NEC, Japan) was used as an excitation light. The laser power was kept at approximately 160 mW/cm^2. The laser line interference filter was used for removing the spurious background emissions from the laser. The LIF spectra were analyzed using a multichannel monochromator (PMA-11, Hamamatsu Photonics, Japan). The fluorescence was guided to the monochromator using the optical fiber. The short-

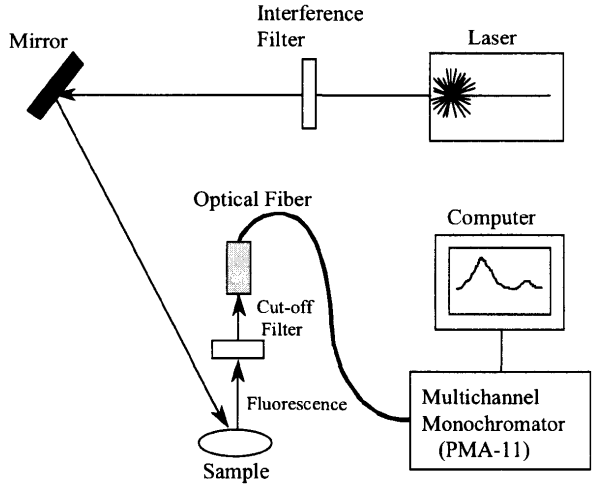

Figure 1 Schematic diagram of the LIF measurement system

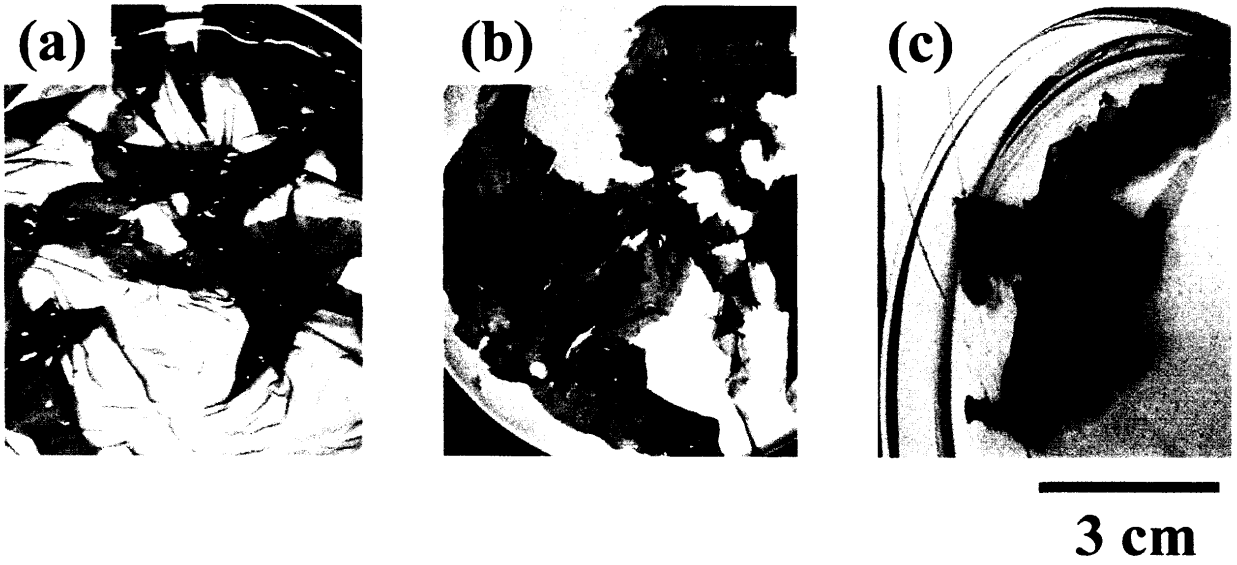

Figure 2 The pictures of (a) the control sample and the samples infected with (b) the red rot disease and (c) the chytrid disease

wavelength cut-off filter down to 530 nm was attached to the optical fiber in order to prevent the influence of the excitation light. The exposure time was 1 s.

3. Results and discussion

3.1 Influences of the diseases on the LIF spectra

First of all, we investigated the influences of the diseases such as the red rot disease and the chytrid disease. Figure 2 shows the pictures of (a) the control sample and the samples infected with (b) the red rot disease and (c) the chytrid disease. The red rot disease is caused by a parasitic fungus, *Pythium* species. The cells penetrated by the hyphae are shrunk and the color of the leaves changes to green. The chytrid disease is caused by a fungus, *Olpidiopsis* species, and the leaves are crinkled.

Figure 3 shows the typical LIF spectra of (a) the control sample and the samples infected with (b) the red rot disease and (c) the chytrid disease. In these spectra, the fluorescence peaks located at approximately 580 nm (peak A), 660 nm (peak B), 685 nm (peak C) and 720 nm (peak D) were observed. Susabi-nori belongs to red algae, and it contains chlorophyll-a and phycobilins such as phycoerythrin, phycocyanin and allophycocyanin, as photosynthetic pigments. The emissions at approximately 580 nm (peak A) and 660 nm (peak B) probably originate in phycoerythrin and allophycocyanin, respectively[5]. The emissions at approximately 685 nm (peak C) and 720 nm (peak D) are probably due to chlorophyll-a[1)-4)]. Furthermore, a shoulder peak located at approximately 640 nm was observed (marked by an arrow in the figure), and probably originates in phycocyanin[5]. In the spectrum of the control sample, the intensities of the peaks at 660 nm (peak B), 685 nm (peak C) and 720 nm (peak D) were almost the same, however the 580 nm peak (peak A) had

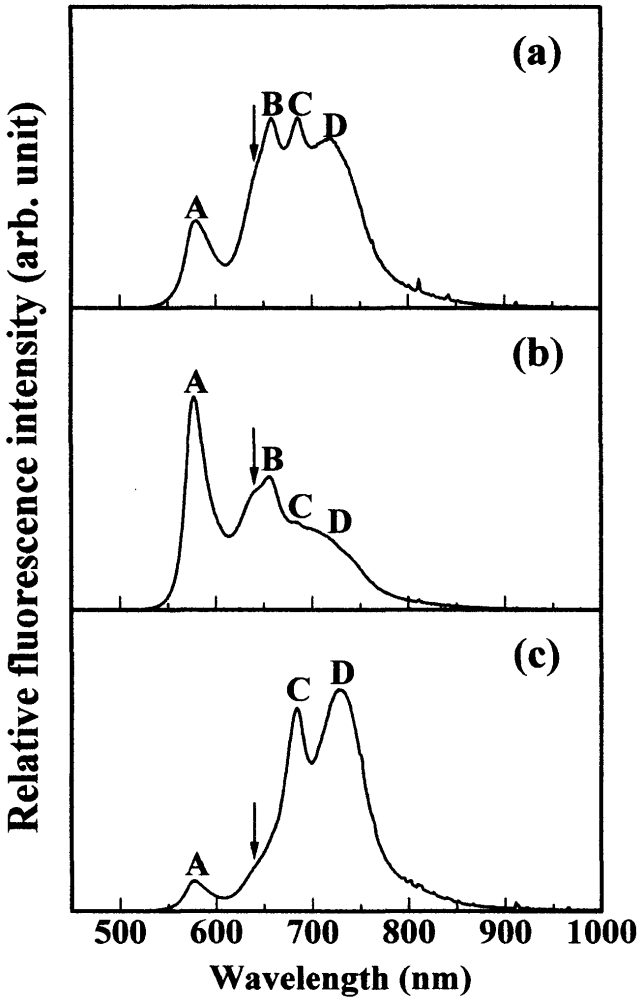

Figure 3 Typical LIF spectra of (a) the control sample and the samples infected with (b) the red rot disease and (c) the chytrid disease

about a half intensity as compared with the other peaks.

In the spectrum of the sample infected with the red rot disease, the intensity of 580 nm peak (peak A), which probably originated in phycoerythrin, was relatively high as compared with that of the control sample. The intensity ratios of the peak A to the peak B in the sample infected with the red rot disease and the control sample are 1.6 and 0.46, respectively, and the ratios of the peak A to the peak C are 2.4 and 0.46, respectively. (The intensities of the peak A, B and C include the intensities of the other peaks superimposed on each peak.) We speculate that the intensity of 580 nm peak (peak A), which probably originated in phycoerythrin, increases because the infection with the red rot disease disturbs the energy transfer from phycoerythrin to chlorophyll-a as a photosynthetic reaction center.

On the other hand, the intensities of the peaks at 580 nm (peak A) and 660 nm (peak B), which probably originated in phycobilins, drastically decreased by the influence of the chytrid disease. The intensity ratios of the peak A to the peak C in the sample infected with the red rot disease and the control sample are 0.15 and 0.46, respectively. This result is probably due to the destruction of phycoerythrin, phycocyanin and allophycocyanin.

These results indicate that LIF spectra of Susabi-nori are affected by the diseases such as red rot disease and chytrid disease and that the diseases of Susabi-nori can be diagnosed by the LIF method.

3.2 Influences of the stress by dipping into fresh water on the LIF spectra

Next, we investigated the influences of the stress by dipping into fresh water (distilled water) by the LIF method. Figure 4 shows the time dependence of LIF spectrum by moving the sample from sea water into fresh water. After moving into fresh water, it plotted to 200 s at intervals of 10 s. From this result, it was found that the intensity of the 580 nm peak, which probably originated in phycoerythrin, drastically increased as time passed and became almost constant at approximately 100 s. The increase in the 580 nm peak intensity is probably due to the disturbance of the energy transfer from phycoerythrin to chlorophyll-a by the stress of fresh water.

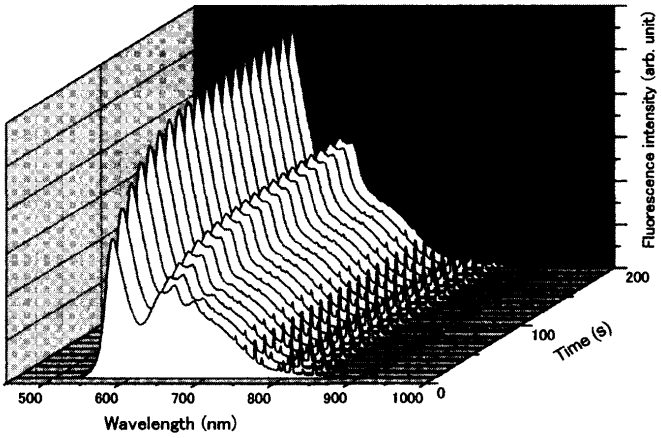

Figure 4 Time dependence of LIF spectrum by moving the sample from sea water into fresh water

Furthermore, we investigated the spectral change by returning the sample from fresh water into sea water. Figure 5 shows the time dependence of LIF spectrum by returning the sample from fresh water into sea water after dipping into fresh water for 3 h. After returning into sea water, it plotted to 200 s at intervals of 10 s. The intensity of the 580 nm peak decreased as time passed and recovered to that of the control sample at approximately 100 s.

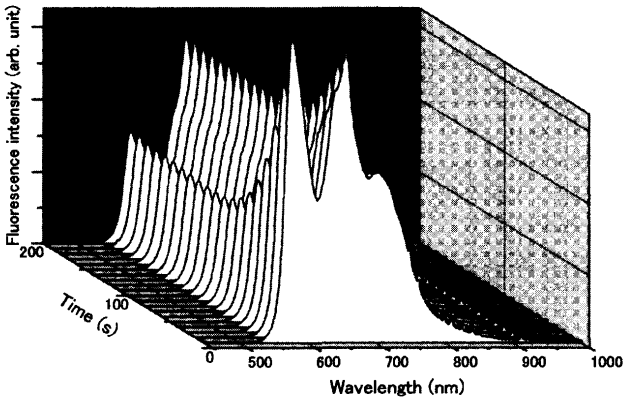

Figure 5 Time dependence of LIF spectrum by returning the sample from fresh water into sea water after dipping into fresh water for 3 h

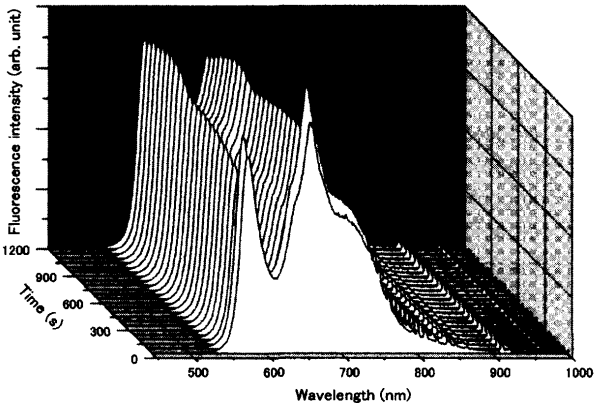

Figure 6 Time dependence of LIF spectrum by returning the sample from fresh water into sea water after dipping into fresh water for 36 h

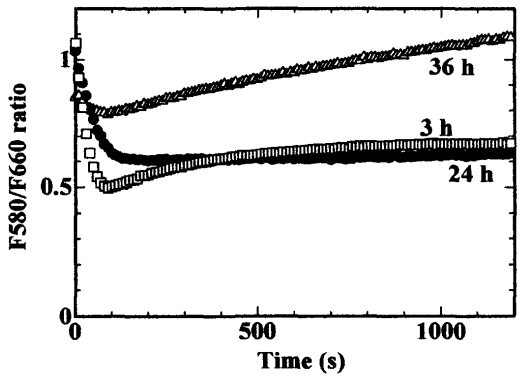

Figure 7 Time dependence of the intensity ratio of the 580-nm peak to the 660-nm peak (F580/F660 ratio) after returning into sea water with various dipping times into fresh water

Moreover, we investigated the influences of the dipping time into fresh water. Figure 6 shows the time dependence of LIF spectrum by returning the sample from fresh water into sea water after dipping into fresh water for 36 h. It plotted to 1200 s at intervals of 50 s, after returning into sea water. In this figure, the remarkable spectral change was not observed after returning the sample from fresh water into sea water, and the intensity of the 580 nm peak did not recover to that of the control sample. Figure 7 shows the time dependence of the intensity ratio of the 580 nm peak to the 660 nm peak (F580/F660 ratio) after returning into sea water with various dipping times into fresh water. In the dipping times of 3 to 24 h, the F580/F660 ratio decreased as time passed and recovered to that of the control sample (approximately 0.5) at approximately 100 s. However, it did not recover to that of the control sample in the dipping time of 36 h. These results suggest that the cells of the nori are destroyed by dipping into fresh water with the dipping times more than approximately 36 h.

4. Conclusions

We diagnosed Susabi-nori by means of laser-induced fluorescence (LIF) method. Fluorescence peaks located at approximately 580, 660, 685 and 720 nm were observed in the LIF spectra of Susabi-nori. The 580 nm and 660 nm peaks probably originate in phycoerythrin and allophycocyanin, respectively, and the 685 nm and 720 nm peaks are probably due to chlorophyll-a. In the spectrum of the sample infected with the red rot disease, the intensity of 580 nm peak was relatively high as compared with that of the control sample. On the other hand, the intensities of 580 nm and 660 nm peaks drastically decreased by the influence of the chytrid disease. Furthermore, the intensity of the 580 nm peak increased by dipping into fresh water. These results indicate that LIF spectra of Susabi-nori are affected by the diseases and the stress of fresh water and that the diseases and the stress of Susabi-nori can be diagnosed by the LIF method.

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References

(1) Takahashi, K., Mineuchi, K., Emori, Y., Kobayashi, S. and Ishii, H.: Study of a method of measuring the transverse distribution of illuminating laser light and laser-induced chlorophyll fluorescence in plant leaves using a micro-fluorescence imaging (MFI) system, J. Light and Visual Environment, Vol. 22 (1), pp. 53-62, (1998).

(2) Saito, Y., Kurihara, K., Takahashi, H., Kobayashi, F., Kawahara, T., Nomura, A. and Takeda, S.: Remote estimation of the chlorophyll concentration of living trees using laser-induced fluorescence image lidar, Optical Review, Vol. 9, pp. 37-39, (2002).

(3) Schmuck, G. and Moya, I.: Time-resolved chlorophyll fluorescence spectra of intact leaves, Remote Sens. Environ., Vol. 47, pp. 72-76, (1994).

(4) Cecchi, G., Mazzinghi, P., Pantani, L., Valentini, R., Tirelli, D. and De Angelis, P.: Remote sensing of chlorophyll a fluorescence of vegetation canopies; 1. Near and far field measurement techniques, Remote Sens. Environ., Vol. 47, pp. 18-28, (1994).

(5) Glazer, A. N.: Light harvesting by phycobilisomes, Ann. Rev. Biophys. Biophys. Chem., Vol. 14, pp. 47-77, (1985).