Algae
Volume 20(2): 121-125, 2005

Effects of Six Antibiotics on the Activity of the Photosynthetic Apparatus and Ammonium Uptake of Thallus of *Porphyra yezoensis*

Min-Hyuk Oh, Yun Hee Kang[1], Choon-Hwan Lee and Ik Kyo Chung[1]*

Department of Molecular Biology and
[1]Department of Marine Science, Pusan National University, Busan 609-735, Korea

The modern integrated fish-seaweed mariculture has been tested to reduce the environmental impacts of an intensive fed culture. To obtain the best seaweed bioremediation performance, the effects of therapeutants used for fish disease control on the selected seaweed species should be considered. As a selected seaweed, *Porphyra yezoensis* was tested with six commercial antibiotics including erythromycin thiocyanate_A, erythromycin thiocyanate_B, oxytetracycline, doxycycline, pefloxacin, and amoxicillin trihydrate under the batch incubation at a photon flux density of 10 μmol $\cdot$ m^{-2} $\cdot$ s^{-1} at 15°C. Among the tested commercial antibiotics, erythromycin thiocyanate_A, erythromycin thiocyanate_B, oxytetracycline, and doxycycline showed decreases in Fv/Fm, the photochemical efficiency of photosystem II, with a dose-dependant and time-dependant manner. From the quenching analysis of chlorophyll fluorescence, three differential patterns were observed in the antibiotics-treated *Porphyra*: (1) high non-photochemical quenching (NPQ) and low photochemical quenching (qP) in the cases of Erythromycin thiocyanate_B and amoxicillin trihydrate, (2) high NPQ and high qP in the case of pefloxacin and (3) low NPQ and low qP in the case of oxytetracycline. These results indicated that antibiotics affected in various ways on the photosynthetic apparatus, reflecting differential lesion sites of antibiotics. In addition, the rates of ammonium uptake also decreased with a decrease of Fv/Fm in *P. yezoensis* thalli treated with erythromycin thiocyanate_B and oxytetracycline. Therefore, the four antibiotics mentioned could affect the bioremediation capacity of the selected seaweed species in the integrated fish-seaweed mariculture system due to the decrease of photosynthetic activity and the simultaneous decrease of ammonium uptake.

Key Words: ammonium uptake, antibiotics, chlorophyll fluorescence, *Porphyra*, seaweed

INTRODUCTION

The fed mariculture including the intensive finfish aquaculture has caused many environmental problems (Wu 1995). Modern intensive monoculture requires high inputs of water, feeds, fertilizers and chemicals and inevitably produces considerable wastes. Some types of the fed aquaculture fish farm operations in the coastal area can produce large amounts of waste. Therefore, many aquaculture operations put enormous pressure on coastal habitats (Black 2001). To reduce the nutrient burden of the fish farm effluents, an integration of seaweed cultivation with fish aquaculture has been proposed (e.g., Chopin *et al.* 2001). However, in waste effluents, some chemicals and drugs, such as pesticides, disinfectants and antibiotics have been included and which may negatively impact adjacent environment. Although antibiotics are no longer used routinely, they are still often used when a disease is diagnosed in the fish under cultivation. Nevertheless, antibiotics have been widely used in some countries for the protection of diseases caused by bacteria such as *Streptococcus*, *Edwardsiella*, *Vivrio*, and *Aeromonas*.

There are many different types of drugs used in finfish aquaculture. Erythromycin thiocyanate is most effective against gram-positive bacteria, such as *Streptococcus* species and is not very effective in a bath treatment and should only be administered by injection or mixed in to the feed. Tetracycline and related antibiotics are considered broad-spectrum antibiotics (effective against a wide variety of bacteria) and they work well when mixed with food. Terramycin is a brand of oxytetracycline manufactured by Pfizer that is FDA approved for use in the production of salmonids, channel catfish and lobsters. The quinolones, including nalidixic acid and oxolinic acid, like the tetracyclines, are considered broad-spectrum antibiotics, and they work

*Corresponding author (ikchung@pusan.ac.kr)

against a wide variety of bacteria. The sulfa drugs, including Romet®, are also considered to be broad-spectrum antibiotics.

Antibiotics may affect critical metabolic processes such as mitochondrial and/or photosynthetic activities of seaweeds growing in the biofiltration tank. If it is true, then it is a critical problem for the seaweed integrated aquaculture system, because the metabolic activities of seaweeds should be maintained in a proper level for the biofiltration. Therefore, we investigated whether antibiotics frequently used in the fish aquaculture system affect the activity of the photosynthetic apparatus and the nutrient removal rates in seaweeds or not for the establishment of a sustainable seaweed integrated system.

MATERIALS AND METHODS

Materials and Treatments of Antibiotics

Porphyra yezoensis samples used in this study were collected in Yeosu, Korea in February 2002. The thalli were rinsed with seawater to remove sediment and epiphytes. Before the experiment, the macroalgae were pre-equilibrated in the laboratory 4 days in filtered seawater at 10°C in darkness and used for experiments.

For treatments of antibiotics, we used six commercial antibiotics (Samyang Anipharm Co., Ltd) containing different chemicals: erythromycin thiocyanate_A, erythromycin thiocyanate_B, oxytetracycline hydrochloride, doxyclclin hyclate, pefloxacin, amoxicillin trihydrate. Erythromycin thiocyanate_A and erythromycin thiocyanate_B are two different commercial products containing erythromycin thiocyanate. The antibiotics were dissolved in filtered seawater and the macroalgae were grown in the seawater under the cool-white fluorescence lamps with an intensity of 10 μmol photons $\cdot m^{-2} \cdot s^{-1}$ at 15°C. The lowest concentration of the antibiotics treated was similar to that recommended by the manufacturers, and several higher concentrations were treated to test its effect when it was treated excessively compared with the recommended concentration or to test its effect when the antibiotics was treated for longer period. The period of the treatment was chosen from several hours up to 2 days depending on its effect. During experiments, the growth media were aerated by bubbling with air pump.

Chlorophyll *a* Fluorescence and Quenching Analysis

Chlorophyll *a* fluorescence was measured using a Plant Efficiency Analyzer (PEA, Hansatech Instruments Ltd., England) as described in Eu *et al*. (1996). The intensity of the saturating light beam was 1,200 μmol photons $\cdot m^{-2} \cdot s^{-1}$. The Photochemical efficiency of PSII (Fv/Fm) expressed as the ratio of variable fluorescence (Fv) to maximum yield of fluorescence (Fm) was measured. In addition, chlorophyll *a* fluorescence for quenching analysis was analyzed using Diving-PAM (Walz, Germany). Thallus samples were dark-acclimated for 10 min at room temperature. Photochemical quenching (qP) and non-photochemical quenching (NPQ) were calculated according to Schreiber *et al*. (1994).

For recovery experiment, the antibiotics-treated thalli were rinsed with seawater to remove antibiotics, incubated to nutrient (NH_4^+)-containing seawater for 2h, and than measured Fv/Fm using a PEA.

Ammonium Uptake

Ammonium contents and uptake of seawater were measured by detection at 640 nm using spectrophotometer (Shimadzu, Japan) using colorimetric methods (Parsons *et al*. 1984).

RESULTS AND DISCUSSION

Effects of Antibioics Treatement on the Photochemical Efficiency of PSII (Fv/Fm)

We investigated the change of the chlorophyll *a* fluorescence to understand the effect of antibiotics on the photosynthetic activity of *Porphyra yezoensis*. When antibiotics were treated with various concentration to filtered seawater, the photochemical efficiency of PSII (Fv/Fm) showed the substantial differences in sensitivity to antibiotics as indicated by reduction of Fv/Fm (Fig. 1) and color change (data not shown). Thus, the effects of the antibiotics for the photosynthetic activity of *Porphyra* sp. is divided into three groups (Fig. 1). (1) Type I: pefloxacin and amoxicillin trihydrate; (2) Type II: erythromycin thiocyanate_A and Erythromycin thiocyanate_B; (3) Type III: oxytetracycline hydrochloride, and doxyclclin hyclate. The Type I antibiotics did not show the decrease of Fv/Fm value in all concentration points for 48 h treatment, but the Type II and Type III show the dose- and time-dependant decreases of Fv/Fm. In case of erythromycin thiocyanate_A treatment, Fv/Fm was small-ranged between 0.5 and 0.6 at the concentration of 7.5 g/L for 18h, and erythromycin thiocyanate_A could be observed

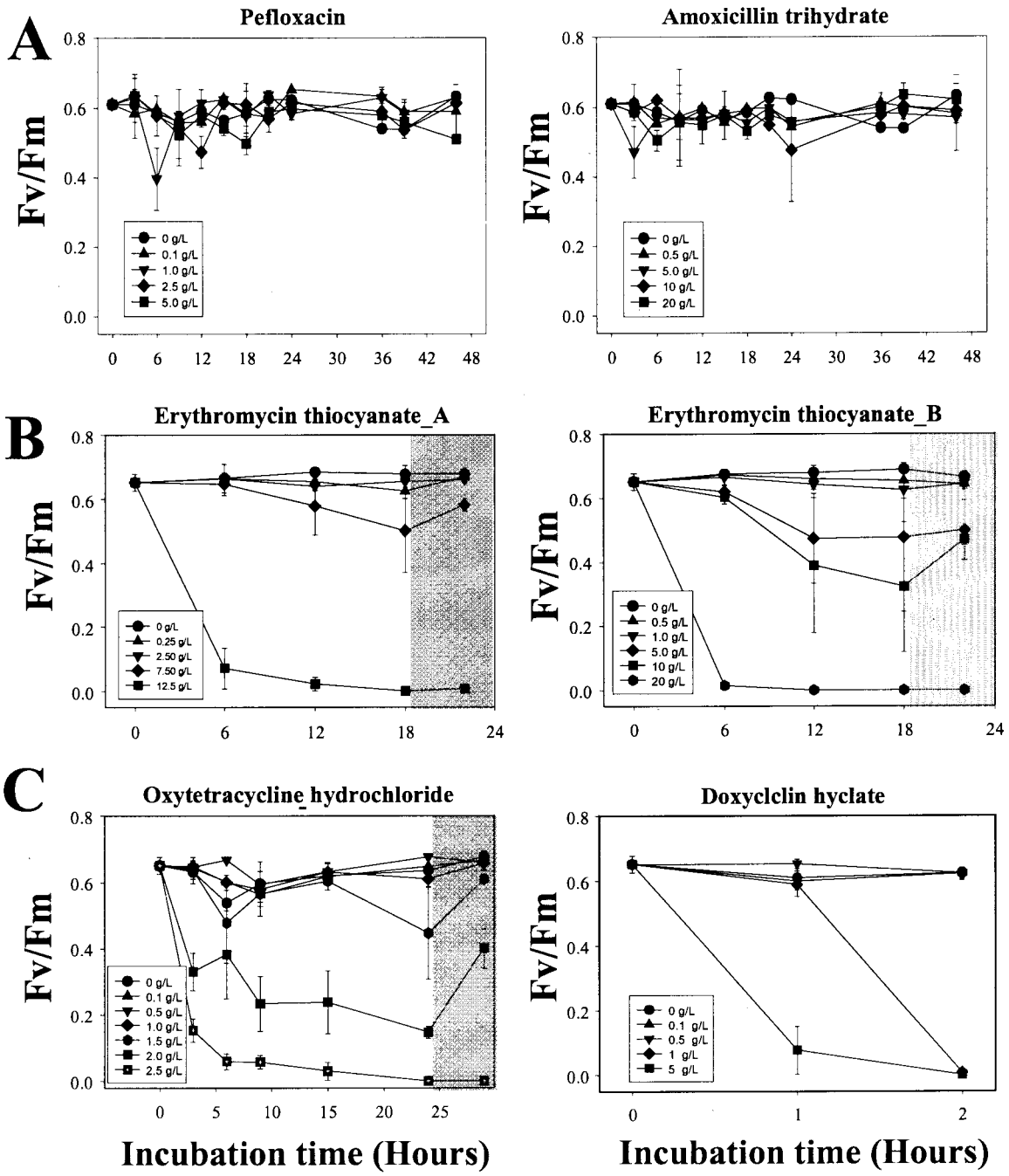

Fig. 1. Changes in PSII photochemical efficiency (Fv/Fm) from thalli of *Porphyra* treated with various concentrations of several antibiotics. (**A**) Type I antibiotics, (**B**) Type II antibiotics, (**C**) Type III antibiotics. The macroalgae were pre-equilibrated in the laboratory 4 days in filtered seawater at 10°C in darkness, and then tested in filtered seawater under the light at 10 μmol photons $\cdot m^{-2} \cdot s^{-1}$ at 15°C during the subsequent experiment. Aeration was provided by bubbling with air pump. The subsequent recovery experiments were carried out in 75 uM nutrient (NH_4^+)-containing seawater for 2h (gray shading). Chlorophyll *a* fluorescence was measured after 10 min dark adaptation at room temperature.

serially the decrease of Fv/Fm with the dose-dependant manner within 12h. In the oxytetracycline hydrochloride and doxyclclin hyclate as a Type III, rapid reduction of Fv/Fm could be observed within 2h although low concentrated antibiotics were treated, and also showed a chlorosis, differ to Type II, resulting the significant decrease of dry weight (data not shown). From these results, we could observed some antibiotics down-regulates the photosynthetic activity in *Pophyra* sp. Interestingly, it was notable that some antibiotics-treated *Porphyra* leaves showed the recovery pattern in terms of increase of Fv/Fm when antibiotics-treated thalli were

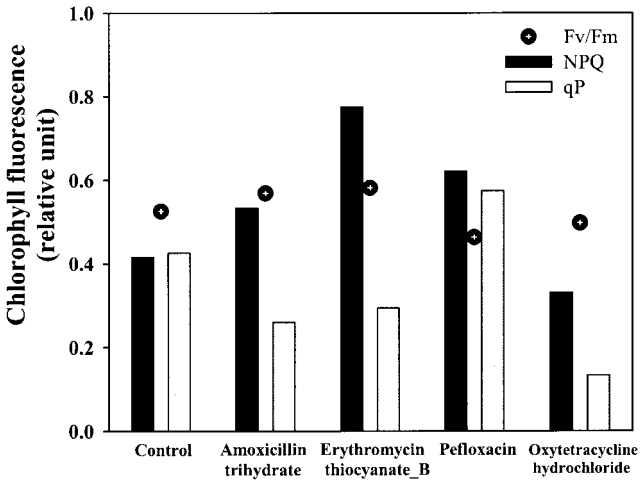

Fig. 2. Quencing analysis from thalli of *Porphyra* treated with several antibiotics. The experimental conditions are the same as in Fig. 1. The concentration of antibiotics were treated during 32 h as followed: 20 g/L Amoxicillin trihydrate, 7.5 g/L Erythromycin thiocyanate_B, 5 g/L Pefloxacin, and 1.0 g/L Oxytetracycline hydrochloride. Chlorophyll fluorescence was measured after 10 min dark adaptation at room temperature.

transferred to nutrient (NH_4^+)-containing seawater after rinse with filter seawater and than were incubated for 2h.

Quenching Analysis of Chlorophyll *a* Fluorescence of the Antibiotics-Treated Porphyra

As shown in Fig. 2, in order to find the difference in the effects among the antibiotics, the chlorophyll *a* fluorescence quenching analysis were conducted at concentration and time with near 0.5 value of Fv/Fm after treatment of the antibiotics. NPQ is induced by changes in the transthylakoid pH-gradient, as well as by activation xanthophyll cycle; whereas qP changes when photochemical energy conversation by the charge separation in reaction centers of PSII lowers due to changing linear electron transport rate to PSI through cytochrome b6/f complex (Rohacek 2002). The effect of the antibiotics in *Porphyra* is divided into three groups for pattern of NPQ and qP compared with control (Fig. 2). (1) High NPQ and low qP; These pattern may be results of the activated delta pH or xanthophyll cycle data in case of amoxicillin trihydrate and erythromycin thiocyanate_B, whereas suppress electron transport rate and the amount of actual fraction of PSII reaction centers that are in the open state. (2) High NPQ, high qP; Both reaction, NPQ and qP, due to re-oxidation of the QA in the case of pefloxacin. (3) Low NPQ, low qP; oxytetracycline hydrochloride is reduced both NPQ and qP shown that depressed electron transport and whole photosynthetic capacity. From chlorophyll *a* fluorescence quenching analysis, we could differentiate antibiotics into 3 groups with different action mechanism in photosynthetic apparatus, indicating the different target or action site of antibiotics in the photosynthetic apparatus.

The Effects of Antibiotics in Ammonium Uptake of Thalli

To understand interrelation between the degeneration of photosynthetic apparatus and the ammonium uptake in *Porphyra*, we tested the changes of the concentration of the soluble ammonium in seawater after transfer the antibiotic-treated samples to the 75 μM ammonium standard solution (Fig. 3). Ammonium uptake in the *Porphyra* were showed biphasic pattern: (1) Surge uptake, initially high uptake rate with time; (2) Constant uptake, comparatively constant uptake at a fixed rate; after surge uptake (Dy and Yap 2001). The decrease of ammonium uptake in the erythromycin thiocyanate_B and oxytetracycline hydrochloride-treated *Porphyra* also was similar with decrease in Fv/Fm. Generally, the ability of ammonium uptake in *Porphyra* was significantly decreased in antibiotics-treated leaves compared with control showing the dose-dependant manner. It has been demonstrated that concurrent oscillations in chlorophyll *a* fluorescence and changes in oxygen exchange after additions of N to N-stressed *Dunaliella tertiolecta* reflect tight coupling between photosynthesis and N metabolism (Young and Beardall 2003). The effect of the antibiotics on the decrease in the ammonium uptake causally linked to its effect on decrease of Fv/Fm, suggesting the quantity of the antibiotics treated often limits of aquaculture production.

Conclusions

In an attempt to avoid the creation of resistant bacteria in a facility, some farms will rotate the antibiotics they use every few months or every year. However, the best solution is to positively identify the bacteria by running culture and sensitivity tests, and thereby avoid unnecessary, costly and potentially harmful treatments in modern aquaculture. Our study revealed that misuse of any antibiotic can lead to loss of the ammonium uptake through degradation of the photosynthetic apparatus in *Pophyra*. Therefore, we assume that the proper management of the antibiotics is important to

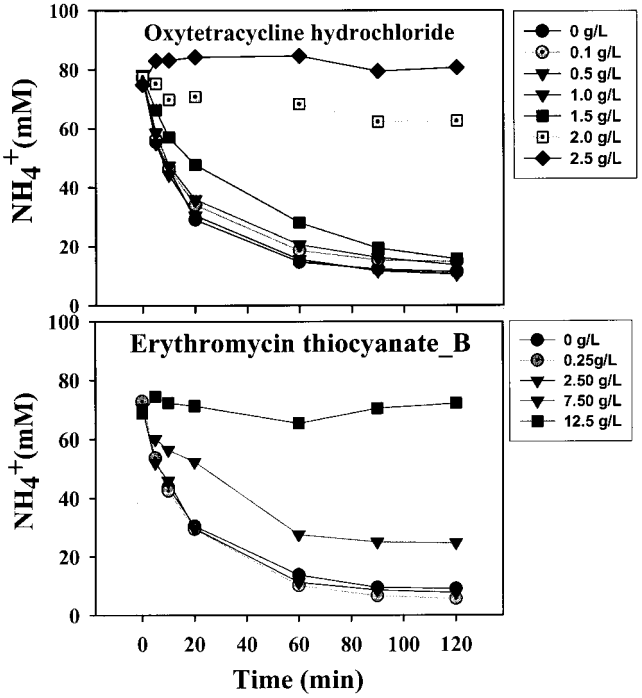

Fig. 3. Ammonium uptake expertiment. Time course in depletion of ammonium by *Porphyra yezoensis* in the initial 75 uM concentration of NH_4^+. The antibiotics-treated thalli were rinsed with seawater to remove antibiotics, incubated to nutrient (NH_4^+)-containing seawater for 2h, and than measured ammonium concentration by detection at 640 nm using spectrophotometer.

eliminate or reduce all contributing stresses for seaweed plant to remove fish-excreated inorganic nutrient, making the healthy fish.

ACKNOWLEDGEMENTS

This work has been financed by the Ministry of Science and Technology through the International Science & Technology Cooperation Program (KISTEP: M6-0203-00-0041) to I.K. Chung and by the Ministry of Marine Affairs and Fisheries through Korean Sea Grant Program to C.-H. Lee. A financial support from the Korea Science and Engineering Foundation (M02-2003-000-20089-0) was also given to M.-H. Oh. We thank the entire SSIAS group for a fruitful collaboration and Drs. J.A. Shin and T.H. Seo for their assistance in the field at Yeosu and finally to the Marine Research Institute (Contribution No. 32) in Pusan National University.

REFERENCES

Black K.D. 2001. *Environmental Impacts of aquaculture*. Scheffield Academic Press, Sheffield.

Chopin T., Buschmann A.H., Halling C., Troell M, Kautsky N., Neffus A., Kraemer G.P., Zertuche-Gonzalez J.A., Yarish C. and Neefus C. 2001. Integrated seaweeds into marine aquaculture systems: a key toward sustainability. *J. Phycol.* **37:** 975-986.

Dy D.T. and Yap H.T. 2001. Surge ammonium uptake of the culture seaweed, *Kappaphycus alvarezii* (Doty) Doty (Rhodophyra: Gigartinalse). *J. Exp. Mar. Biol. Ecol.* **265:** 89-100.

Eu Y.-J., Ha, S.-B. and Lee C.-H. 1996. Effects of chilling injury in the light on chlorophyll fluorescence and D1 protein turnover in cucumber and pea leaves. *J. Biochem. Mol. Biol.* **29:** 398-404.

Parsons T.M., Maita Y. and Lalli C.M. 1984. *A manual for chemical and biological methods for seawater analysis*. Pergamon Press, New York.

Rohacek K. 2002. Chlorophyll fluorescence parameters: the definitions, photosynthetic meaning, and mutual relationships. *Photosynthetica* **40:** 13-29.

Schreiber U., Bilger W. and Neubauer C. 1994. Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of in vivo photosynthesis. In: Schulze E.D. and Caldwell M.M. (eds), *Ecophysiology of Photosynthesis*. Springer-Verlag, Berlin, pp 49-70.

Wu R.S.S. 1995. The environmental impacts of marine fish culture: towards a sustainable future. *Mar. Pollut. Bull.* **31:** 159-166.

Young E.B. and Beardall J. 2003. Papid ammonium- and nitrate-induced pertubationsd to Chl *a* fluorescence in nitrogen-streessed *Dunaliella tertiolecta* (Chlorophyta). *J. Phycol.* **39:** 332-342.

Received 14 March 2005
Accepted 17 March 2005