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, Vibrio, 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...
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 · m⁻² · 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 · m⁻² · 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 (NH₄⁺)-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 Antibiotics Treatment 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
serially the decrease of $Fv/Fm$ with the dose-dependant manner within 12h. In the oxytetracycline hydrochloride and doxycyclin 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 Porphyra 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...
transferred to nutrient ($\text{NH}_4^+$)-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 $F_v/F_m$ 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 $\mu$M 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 $F_v/F_m$. 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 $F_v/F_m$, 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 Porphyra. Therefore, we assume that the proper management of the antibiotics is important to
eliminate or reduce all contributing stresses for seaweed plant to remove fish-excreted inorganic nutrient, making the healthy fish.

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