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学位論文要旨 Dissertation Summary

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論文名: Occurrence and diversity of tetracycline resistance gene, *tet(M)*, in Korean
(Dissertation Title) coastal seawater

Antibiotics can be metabolized after administration; however, up to 90 % of antibiotics are excreted through urine or feces without complete decomposition, which are still active. Substantial amount of excreted antibiotics are released into the aquatic environment, and thus have been detected in aquatic environments. The antibiotic contamination increases number of antibiotic resistant bacteria (ARB). ARB and antibiotic resistance genes (ARGs) are becoming recognized as environmental pollutants, and action is being sought to preserve the efficacy of antibiotics also the expanding problem of emergence of ARGs and their resistance genes is becoming a major issue.

Oxytetracycline (OTC) is a broad-spectrum antibiotic and widely used around the world for treating and preventing the bacterial infectious diseases. OTC resistant bacteria and resistance genes have been reported in fish pathogens and environmental bacteria. In addition, more than 40 tetracycline resistance genes (*tet*) have been reported in various bacterial species.

Previous studies have focused on the isolated, however, in the aquatic environment, most bacteria are non-culturable on agar plate. Little is known regarding the diversity, distribution and origin of resistance genes among the non-culturable majority. To solve this problem, the quantitative real-time PCR (qPCR) assay has been frequently employed to study the resistance levels. A number of studies reported that *tet* gene concentrations were significantly correlated with the levels of tetracycline residues in the environments. Among them, the *tet(M)* gene is well studied in not only clinic but also environments, which is known to have the widest host range of any *tet* genes since it is often associated with mobile genetic elements which enhance its transferability from one bacteria to another. Also, several studies have reported a variety of genotypes of the *tet(M)* gene from natural environments or resistance isolates and various genotypes of the *tet(M)* are known to occur in human and animal pathogens.

In Korea, the amount of veterinary antibiotics consumes about 1.5 times greater than other countries including USA, UK and Europe thus they might be released into the aquatic environments. Although OTC is major antibiotics in Korea, the contamination status in Korean coastal sites has not been well understood. Therefore, the present situation of contamination by OTC and OTC resistance genes is needed to estimate the potential risk of antibiotics and the resistance genes in aquatic

environment. Therefore, the aims of this study were (1) to estimate the OTC concentration in coastal seawater in Korea; (2) to enumerate OTC resistant (OTC^r) bacteria and their profiling of multidrug resistance; (3) to confirm the persistence of *tet(M)* and its genetic diversity in natural assemblages, and (4) to describe the diversity of *tet(M)*-possessing bacteria.

Sampling of coastal seawater was collected in Yeosu (KYS-1, 2 and 3), Geoje (KGJ-1, 2 and 3) and Wando (KWD-1, 2 and 3) in 2 sampling trials in December, 2010 and May to June 2011. The OTC concentration was below detection limit (0.1 µg/mL) in all the sampling sites. Therefore, these areas are not contaminated with OTC.

The occurrence rate of OTC^r bacteria was for 0.0 to 0.35 % in 2010 and 0.0 to 10.6 % in 2011 of the total colony-forming unit (CFU) in collected seawater. The occurrence rate of OTC^r bacteria was increased in 2011; even the sampling sites were not contaminated with OTC indicating the OTC^r bacteria are existed in non-contaminated natural seawater in Korean coastal seawater and OTC^r bacteria are not related to OTC contamination in Korea.

The 35 isolates OTC^r bacteria in 2010 were showed a nine patterns multidrug resistance patterns in this study. 8 (23%) isolates showed the 4 drug resistance, 12 isolates showed the more than 2 drug resistance indicating that OTC^r bacteria might be linked to other antibiotics such as ampicillin and erythromycin suggesting OTC^r bacteria convey macrolide, sulfonamide and β-lactam resistance gene also.

Total 106 OTC^r isolates in 2010 (35 isolates) and 2011 (71 isolates) were randomly selected for screening of *tet(M)* gene. 30 isolates out of 35 isolates in 2010 and 40 isolates out of 71 isolates in 2011 were positive for *tet(M)*. Overall, 66 % were positive for *tet(M)* whereas 34 % were negative for *tet(M)*. Most of *tet(M)* negative strains (27/36) were isolated in Geoje (KGJ-3) site whereas all of the positive strains were isolated in Wando (KWD-1 and KWD-2) sites indicating that this gene is widely distributed in Wando sites not in Geoje (KGJ-3) site suggesting different distribution of *tet(M)* even in neighboring areas.

To quantify the prevalence of *tet(M)* gene in Korean coastal seawater, the qPCR was performed. The relative abundance of *tet(M)* was normalized by 16S rRNA gene. The relative abundance of *tet(M)* was under detection limit ($<1.5 \times 10^{-4}$ copies/mL) at the most of sampling sites in 2010, whereas that in 2011 ranged from 10^{-3} to 10^{-2} copies/mL, indicating that the *tet(M)* distribution is changeable in seawater. This result suggests that the *tet(M)* gene is persisted in natural seawater in Korean coastal sites and also suggesting the non-culturable bacteria have harbored the *tet(M)* gene in Korean coastal seawater.

The genetic diversity of *tet(M)* showed 99 to 100 % homogeneity among the isolates and natural assemblage in KWD, suggesting that the *tet(M)* is homogenous in KWD. All *tet(M)* in KWD were the same as aquaculture origin *tet(M)* in previous studies. On the other hand, KWD showed different result. The *tet(M)* showed 91.7 to 100 % similarity, indicating diverse in natural assemblages. Since the high homology was obtained with human origin *tet(M)*, KWD site should be received by human and/or animal effluent. This study suggests a possibility of different source of *tet(M)* in Korean coastal seawater. The result of *tet(M)*-possessing bacteria obtained from KWD revealed that only Gammaproteobacteria, *Vibrio* sp. and *Photobacterium* sp., possessed *tet(M)*. In addition, specific group is suggested to be *tet(M)* reservoir when monitored by culture-method. In Gram negative groups, Gamma-proteobacteria are frequently isolated as *tet(M)*-reserving bacteria in human and animal sources. This study found that this phylum is a major group possessing *tet(M)* in marine derived bacteria also, suggesting this phylum should be ubiquitous reservoir of *tet(M)* in natural environment.

Through this study, new findings were obtained that (1) the distribution of OTC^r bacteria and *tet(M)* was not related to OTC contamination; (2) different genotypes of *tet(M)* were found in Korean coastal sites; and (3) the major reservoir of *tet(M)* was *Vibrio* sp and *Photobacterium* sp. in *tet(M)*-possessing bacteria. These are the first evidences in Korean environment.