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学 位 論 文 要 旨
Dissertation Abstract

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学位論文題目： Persistence of multi-drug resistance plasmid in seawater environment
Title of （多剤耐性プラスミドの海水環境での残存）
Dissertation

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The emergence of antibiotic resistant bacteria (ARB) has become a public health problem worldwide. Transferable plasmids play a major role in the rapid dissemination of antibiotic resistance genes (ARGs). The ARGs coded on plasmid are developed in clinical setting and environments, flowing into river and sea. The fate of the plasmids in aquatic environment, especially in seawater, has not been known. Existent status and fate of ARGs in environment should be essential information to mitigate spread of ARB and reduce ARGs risks. Present study aims to elucidate the persistence status of a multi-drug resistance (MDR) plasmid under very low selective pressure and high grazing pressure in seawater.

Chapter 1 describes background of this research, followed by Chapter 2 where the persistence of MDR plasmids conveyed by original host and transconjugant was examined in seawater microcosms. The defined microcosms were prepared with a marine bacterium *Photobacterium damselae* subsp. *damselae* 04Ya311 harboring a MDR plasmid pAQU1 or transconjugant *E. coli* W3110 conjugated by pAQU1. The microcosm was incubated in the presence or absence of tetracycline. A transferable incompatibility FIB group plasmid (IncFIB) in *E. coli* 133 was used as a comparison. Results showed that pAQU1 was stable with minor fluctuations in original host strain 04Ya311, and nearly constant in transconjugant

W3110, although colony-forming unit gradually declined during 30-day starvation. The stable profile was also observed for plasmid IncFIB in the strain 133. This indicates that MDR plasmids stably remained not only in the original host, but also in a transconjugant, even after being in a non-culturable state. The results suggest a phenomenon “easy to get, hard to lose” of plasmid. This means the elimination of a plasmid from a natural environment is unlikely.

Chapter 3 shows the release of ARG/plasmid into environment by the protist grazing, and constant retaining in seawater and bacterial cells. The 04Ya311 strain with pAQU1 was cultured with marine ciliates or heterotrophic nanoflagellates (HNFs). The significant increase of tetracycline resistance gene *tet*(M) in seawater (ex-tetM) was found at day 3 in cultures with ciliates and day 20 in cultures with HNFs. The ex-tetM increased with decrease of bacterial number, and remained in seawater throughout incubation period. The ex-tetM did not increase in microcosms containing only bacteria. The results indicate the involvement of marine ciliates and HNFs in the release of ARGs into seawater, and extracellular ARGs are preserved in the environment. Additionally, pAQU1 plasmid stably retained in bacterial cells under the presence of grazing pressure. The presence of oxytetracycline did not affect to the release and constancy of ARGs, as well as stability of resistance plasmid in the population during experimental period.

In conclusion, seawater environment can serve as reservoir and source of ARGs even under absence of selective pressure and presence of grazing pressure. This should be a reason that the ARGs are not rapidly extinguished in marine environment. The evidences here are useful to give an answer to the question of ARGs being continuously present in environments.