Hokkaido University
Leading Graduate School
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Antibiotic resistance genes (ARGs) and its horizontal gene transfer (HGT) in aquaculture environment

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ACADEMIC DEGREES:

B.Sc. 1980, Hokkaido University (Dept. Food Science & Technology, Faculty of Fisheries)
M.Sc. 1982, Hokkaido University (Lab. Microbiology, Faculty of Fisheries)
Ph.D. 1985, Hokkaido University (Faculty of Pharmaceutical Sciences)

PROFESSIONAL APPOINTMENTS:

1985 – 1987, Research Fellow of Alberta Heritage Foundation for Medical Research, Dept. Biochemistry, University of Alberta, Edmonton, Canada
1987 – 1992, Assistant Professor, Faculty of Fisheries, Hokkaido University
1992 – 2000, Associate Professor, Faculty of Agriculture, Kochi University
2000 – present, Professor, CMES, Ehime University

RESEARCH INTERESTS:

1980-1987,1992-2000 Virology (Chemotherapy of Herpes and Hepatitis B viruses, and molecular ecology of Birnaviruses)
1987-1992, Food Hygienic Microbiology (*Campylobacter jejuni* toxins, and polyamine synthesis of marine bacteria)
2000- present, Environmental Microbiology (Antibiotic resistance in aquatic environment, and organic matter dynamics in marine environment)

We focus on marine microbes which play in integral role in the microbial food web, and act as a buffer to ecological stress and chemical pollutants. Studies include advanced research on the microbial loop and molecular ecology of marine microbes. Recent project covers antibiotic resistance and the horizontal gene transfer among various environmental microbes. Results from these studies can be used to develop technology to improve marine industries related to aquaculture and seawater utilization, and monitoring of human and ecosystem health.

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Since the 1960's, issues associated with antibiotic resistant bacteria (ARB) have been a long-pending problem in the aquaculture industry. This has predominantly occurred in fish pathogenic bacteria. Dosage of antibiotics to fish is performed by mixing with the feed, a method which does not adequately deliver the antibiotics to each fish and the residual antibiotics are dispersed into the environment. Some of the residual antibiotics are subsequently retained in the sediments under the net culture pen.

Various transmittable elements conveying antimicrobial resistance genes (ARGs) have been discovered from seawater and sediment. The structure of the plasmids is diverse, but core unit of the plasmid is known to be common from different aquaculture sites, suggesting a history of developing the ARGs and movement of the plasmid among various sites. This talk will show an example of the transferable plasmids and ARGs from aquaculture sites of Japan.

A recent study from Japan has shown a new plasmid possessing multi-ARGs. The plasmid, pAQU1[1)], was sequenced and found to have core structure of IncA/C series in terms of *tra* genes series; however the *rep* gene was unique. This suggests that transfer mechanism is common but host range and replication property are different. Different gene vehicles were isolated from the same sample. Furthermore, the same *tet*(M) gene was found on the various HGT vehicles. This suggests history of reconstruction of plasmids through horizontal gene transfer (HGT) in the same aquaculture site.

Our ecological studies in aquaculture sites revealed that tetracycline resistance genes (*tet*) are retained in sediments of aquaculture sites even after fish culture has ceased for several years[2)], suggesting that the sediments are an ARGs-reservoir in the natural environment. We also found the *tet*(M) in offshore sediments[3)]. It has not yet been evaluated whether marine environmental bacteria are a risk for humans. Exposure and transmission of ARGs to humans from aquaculture activities should be studied further. Although we recently issued a representation paper on reduction of release of antibiotics and ARGs[4)], the risk assessment of the environmental ARGs should be paid attention world widely.

Finally, the new findings of a reality of ARGs in seawater will be shown. A sulfonamide resistance gene, *sul3*, was found in non-culturable bacterial community in coastal environment in the Philippines[5)]. ARB studies have been performed in culturable bacteria, however our evidence suggests importance of non-culturable community of the environments.

References

1) Nonaka L et al. (2012) Non conjugative transferable multiple drug resistance plasmid pAQU1 from *Photobacterium damselae* subsp. *damselae* isolated from marine aquaculture environment. *Microbes Environ*., 27, 263-272.
2) Tamminen M et al. (2011) Tetracycline resistance genes persist at aquaculture farms in the absence of selection pressure. *Environ. Sci. Technol*., 45, 386-391.
3) Rahman HM et al. (2008) Occurrence of two genotypes of tetracycline (TC) resistance gene *tet*(M) in the TC-resistant bacteria in marine sediments of Japan. *Environ. Sci. Technol*., 42, 5055-5061.
4) Pruden A et al. (2013) Management options for reducing the release of antibiotics and antibiotic resistance genes to the environment. *Environ. Health Perspect*., 121, 878-885. http://dx.doi.org/10.1289/ehp.1206446.
5) Suzuki S et al. (2013) Who possesses drug resistance genes in the aquatic environment? : sulfamethoxazole (SMX) resistance genes among the bacterial community in water environment of Metro-Manila, Philippines. *Front. Microbiol*., 4, doi:10.3389/fmicb.2013.00102.

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