Molecular ecological analysis on microfauna in freshwater ponds and aquaculture ponds for clarification of pond water

Chapter 1 Introduction
This chapter provides backgrounds and objectives of the thesis. The morphological identification of protozoan and metazoan in aquatics environment demands identification expertise. To conduct the quick identification under a microscope, it is important to train the skill. However, this is a time-consuming work even after obtaining the enough technique. Thus, if a molecular taxonomical method for the identification of protozoan and metazoan was introduced, more accurate and systematic identification can be quickly performed. Therefore, I firstly studied on the one cell and one individual isolation, direct PCR and sequencing method to determine the 18S rRNA rapidly. Then, I surveyed typical microfauna in reservoirs and ponds in Nagasaki prefecture. Then, several rotifer species were focused because these rotifers potentially graze toxic cyanobacteria. Then, I carried out the isolation of rotifers from aquaculture ponds, then direct PCR and the sequencing were performed in Maejo university (Chiang Mai, Thailand). Finally, Phylodina sp., which graze toxic Microcystis sp. cells, was studied to apply for clarifying the polluted ponds water with microcystin.

Chapter 2 Literature review
I reviewed on previous achievements on the main theme of study including aquatic environments, toxic cyanobacteria blooms, protozoan and metazoan in freshwater environment. I found the several studies on toxic cyanobacteria degradation by zooplanktons and microorganisms and the molecular ecological methods for monitoring and identifying protozoan and metazoan were also summarized. Finally, the potential future works are described.

Chapter 3 Development of one cell or one individual direct PCR of Protozoan and Metazoan 18S rRNA gene for Molecular ecological method
The experiments were conducted to evaluate the conventional and development of one cell or one individual direct PCR of protozoan and metazoan 18S rRNA gene for molecular ecological method. The new method for one cell or one individual direct PCR is important to build a local DNA database of protozoans and metazoans for molecular ecological studies. At first, we applied a glass capillary method for isolated a protozoa cell and a metazoan individual. The other sources were from wastewater treatment systems in Nagasaki. The addition of BSA to a water droplet was very useful for the quick isolation of a protozoan cell due to reducing the protozoan motion. To decompose dissolved DNA of other organisms, DNase-I was added to the PCR tube and incubated for 30 min. Then, 70 percent EtOH of 100μl was added to the PCR tube. It was sequentially treated by sonication for 30 sec and heated for 2min in microwave oven. We applied a nested PCR for 18S rRNA gene of the isolated rotifer individual and a protozoan cell. Finally, we determined the sequence of each PCR amplicon for the rotifer individual or the protozoan. As a result, using the developed new method, we could correctly determine the partial sequence of 18S RNA genes of 17 samples of ciliates and rotifiers in 20 samples from natural ponds and activated sludge systems (Figure 3.1).
Chapter 4 Molecular ecological analysis in aquaculture ponds in Chiang Mai Thailand

Comparison the character of rotifers which can prey on toxic cyanobacteria in Thailand and Japan using the molecular cloning method. The sequencing of the 18S rRNA gene of the isolated rotifer species was achieved using molecular cloning method. We success to get the longer sequence of 18S rRNA gene of bdelloidea rotifers isolated from wastewater treatment plant J1 Bdelloidea sp. R14, J2 Philodina acuticornis, J3 Ahrickia morexian and J4 Philodina acuticornis Bdelloidea rotifer isolated from aquaculture ponds in Chiang Mai, Thailand T1 Rotaria rotatoria and T2 Adineta vaga.

Apply the one cell or one individual direct PCR method for investigation the microfauna in biofloc aquaculture ponds. We collected sample from Thailand Aquaculture Revolution TAR Bio Floc Technology, San Pa Tong, Chiang Mai, Thailand. Succeeded in developing an isolation method using micro capillary for uncultured protozoan and metazoan in Biofloc. Then the resulted 100% successes to get the true 18Sr RNA gene sequence.

Chapter 5 Monitoring of rotifer in bio-carrier for remove of toxic cyanobacteria from ponds water

The result of designed primer sets for real-time PCR to quantify the rotifers based on the the 18Sr RNA gene partial sequence of bdelloidea rotifer. we get primer sets of 55F-395R cheek by 2.5% agarose gel electrophoresis and apply this primer for real time PCR to obtain population dynamic of bdelloidea rotifer in bioreactor. Application of the column type charcoal bioreactor using the several kind of charcoal included the wood charcoal and corn crop charcoal, were filled with crashed charcoal granules (1-2mm) as carriers, were used for the experiment on removing cyanobacterial cell. The efficiency percentage removal of chlorophyll-a the wood charcoal is 71.64% better than the corn crop charcoal is 65.78% in the day 17. Compare efficiency of pretreatment and direct DNA extraction method of bdelloidea rotifers DNA from charcoal ball bio-carrier using real time PCR.

Figure 5.1 The result of real time PCR (a) Standard of artificial gene (b) graph show correlation between gene copy number of 18Sr DNA and number of rotifer population from direct method

Chapter 6 Conclusion

I summarized the major results and provide further discussions. To developed a new method for one cell or one individual direct PCR of protozoan and metazoan 18Sr RNA gene sequencing, evaluated the genetic diversity and analysis on the population dynamics of rotifers using real-time PCR to explore the effect of rotifer on removal of microcystis sp. in aquatics environments.

Keywords: Protozoan and metazoan, 18S rRNA gene, Specific primer, Molecular ecology.