(第3号様式) (Form No. 3)

学 位 論 文 要 旨
Dissertation Summary

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論　文　名: Collaborative processing of proteins by bacteria and protists in marine microbial loop
(Dissertation Title)

The microbial loop consists of various microbes, which is an essential part of food webs in the ocean. Organic matters in the ocean comprised ca. 700 Pg of dissolved organic matters (DOM) and ca. 3 Pg of particulate organic matters (POM). It has been thought that only oligotrophic bacteria are transformers of DOM to POM. When bacteria incorporate high molecular substances, they should decompose the high molecular matters to oligo- or monomeric substances. Decomposition and utilization process of DOM by bacteria should be based on enzymatic reactions; however, the processing of high molecular weight matters has not been clarified.

Protein is an important organic matter, which should be hydrolyzed by enzymes into oligopeptides and amino acids, then utilized by diverse bacteria. In several reports, bacteria are the major contributor to the enormous bulk enzyme pool. The research group of Ehime University has first reported that various proteases were found in seawater and trypsin-type enzymes were mainly present in dissolved fraction, whereas aminopeptidases were attributed to bacterial-cell-size fraction. From this new finding, the question is raised that who is the source of these diverse enzymes. The aim of this thesis work is to clear the origin of proteolytic enzymes. This evidence gives an epoch-making knowledge in biological oceanography. I have hypothesized from the above finding that not only bacteria but also other living things would produce various enzymes, which act in the processing of DOM within microbial loop.

This thesis study firstly aimed to reveal black box of the protein transformation process in seawater in terms of biochemical and experimental ecosystem studies. *Pseudomonas aeruginosa* distributes wide area of the ocean, and its porin protein OprP could be ubiquitously detected in seawater. To chase protein destiny, the inactivated-*P. aeruginosa* cells (Pa) were used as a proteinaceous substrate in enzyme reaction. In the Chapter 2, microcosms including fractionated seawater and Pa cells were prepared. Eight treatments

of microcosms were set in duplicate: natural seawater with Pa (SW+Pa), autoclaved seawater with Pa (Auto-SW+Pa), GF/F-filtered seawater with Pa (SW-0.7+Pa), 0.2-μm-filtered seawater with Pa (SW-0.2+Pa), natural seawater (SW), autoclaved seawater (Auto-SW), GF/F-filtered seawater (SW-0.7) and 0.2-μm-filtered seawater (SW-0.2). Bacterial abundance, protist abundance, protease activities and protein degradation profiles were monitored during 30 days. Result showed that protease activity increased substantially in SW-0.7+Pa, but Pa proteins were retained. This microcosm did not contain protists, suggesting that natural bacterial and Pa-derived enzymes did not completely decompose Pa proteins. Complete degradation of Pa proteins was observed in only SW+Pa, in which protists coexisted. This indicated that natural bacteria produced high protease activities in the absence of predators. However, bacterial proteases could not completely decompose bacterial proteins by alone, suggesting the necessity of proteases originated from larger microbes. The result reminds us the protists' roles as enzyme sources and protein utilizers. This concept is unique and further studied in the next chapter.

It was found that the bulk enzyme pool can be contributed by different microbes in the community. I investigated in Chapter 3 the protease production dynamics in the presence of both marine protists and bacteria along 10-day incubation to know the contribution of them to the enzyme pool. Three microcosms were established in two experiments at two different protein concentrations. The microcosms were: only Pa (Pa), Pa and ciliates (Pa+CB), Pa and separated ciliate-associated bacteria (Pa+B). Results showed that significant activity hydrolyzing Boc-Val-Leu-Lys-MCA was observed in the Pa+CB microcosms, whereas Pa+B showed negligible activity in both cell-associated and cell-free fractions throughout incubation time. This indicated that the activity was high when the ciliates present. Moreover, protease profiles in the cell associated- and cell-free fractions were different, suggesting that the proteases detected were actively released by the ciliates, not via cell lysis. Although the protein concentrations in both Pa+CB and Pa+B declined, the decreasing amount was considerably higher in the Pa+CB microcosms, suggesting that most dissolved proteins were transferred to ciliate biomass. Until now, the oligotrophic bacteria have been believed to produce proteases, which is needed for their growth. The present study evidenced that ciliates produced trypsin-type proteases, which could transform the proteins to oligopeptides, then bacterial aminopeptidases could hydrolyze oligopeptides to amino acids. The co-work of ciliates and bacteria makes the hydrolysis of proteins effective. This might be a new concept "enzyme (molecular) symbiosis", proposing additional possibility that protists are potential players in the organic matter degradation in water columns with their specific enzymes to support the cascade hydrolysis of proteins. Through the study in Chapter 3, the role of protists as protease producers hypothesized in Chapter 2 was successfully proved.

In the last Chapter 4, I examined pH dependency of protease activity of ciliate, which is indirect evidence to show the enzymes are released, not leaked. The pH dependent activity showed that bulk enzymes are active in neutral pH range, suggesting these enzymes are adapted to outside cell, because the cytosolic digestive enzymes are generally active at acidic pH also. Increase of protease activity was found in the CB+L(live)Pa+Pa and CB+Pa, suggesting that the protists released the enzymes into environment.

As a conclusion, the present thesis focusing on protease activity and protein degradation showed protein degradation is completed by presence of protists in the process of protein matter cycling in marine microbial loop. This is a new evidence on the proteolytic process in the microbial loop.