Chapter I

Introduction

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Sea cucumbers (class : Holothuroidea) are a group of Echinodermata. As ancient creatures, they appeared from 400 million years ago (Lambert, 1997). Sea cucumbers are exclusively marine animal, that habit from deep sea to shallow intertidal areas in both tropical and temperate oceans. Totally, ~ 1430 species have been known (Uthicke et al., 2009).

Sea cucumbers are common benthic organisms in both seacoast and deep-sea, and play an important role in marine ecosystem through bioturbation, bioirrigation and organic matter cycling (Shiell et al, 2010). By ingesting and assimilating sediments, sea cucumber can affect sendimentary nutrient recycling including carbon, nitrogen and calcium. (Uthicke et al., 1998; Paltzat et al, 2008; Yu et al, 2011; Schneider et al., 2011).

Sea cucumbers (holothurians) are divided into two feeding groups: suspension feeding and deposit-feeding (Sewell and Levitan, 1992; Graham and Thompson, 2009; Kerr and Kim., 2001). Members of the order Dendrochirotida the class Holothuroidea are believed to be suspension feeders which feed on suspended particles in the water column. In contrast to suspension-feeding sea cucumbers, members of other five orders e.g. Apodida, Aspidochirotida, Dactylochirotida, Elasipodida and Molpadiida of the class Holothuroidea are deposit feeders (Roberts et al., 2000). The deposit-feeding sea cucumbers are typical marine detritivores that ingest superficial sediments and feed on

detritus including microorganisms (Yingst, 1976; Massin, 1982; Moriarty, 1982).

Deposit-feeding holothurians, marine detritus and sedimentary bacteria are important compartments revealed in food web structure (Deming and Colwell., 1982; Iken et al., 2001; Wilson et al., 2003). Detritus is composed of the bodies or fragments of dead plants and animals as well as fecal material (Frankenburg et al, 1967; Johannes et al., 1967). Heterotrophic and autotrophic microorganisms are common composition of marine sediment (e.g. bacteria, algae, protozoans and fungi) (Meng et al., 2011; Mote et al., 2012; Kawasaki et al., 2011).

Deposit-feeding sea cucumbers feed on and ingest sediments that are rich source of nutrients. It was considered that sea cucumbers could utilize the organic matter (OM) in sediment. In marine epibenthic area, 22% of sendimentary OM was utilized by the deposit-feeding holothurian *Parastichopus parvimensis* (Clark) (Yingst, 1976). Moriarty reported that 60-80% of detrital OM might be utilizable for *Holothuria atra* and *Stichopus chloronotus* (Moriarty, 1982).

Study on deep-sea holothurians indicated that *Mesothuria verrilli*, *Psychropotes semperiana*, and *Benthodytes linqua* could utilize decomposed seagrass in abyssal detritus (Suchanek et al., 1985). By cultural experiment, Gao et al. found that *A. japonicus* feeding on mixed diet of algae and natural benthic matter (e.g. microalgae, bacterial and muddy material) showed better growth than feeding on single diet of algae (Gao et al., 2012).

By carbon isotope technique, Yingst indicated the shallow water holothurian

Parastichopus parvimensis could directly utilize benthic bacteria as food source (Yingst, 1976). While Deming and Colwell studied on an abyssal sea cucumebr and proposed that contribution of bacteria as food was very low, about 3% of total organic carbon. Study on prokaryotic biodiversity associated with gut contents of the deep-sea holothurians *Molpadia musculus* also indicated prokaryotes were not the major food source, because prokaryotes contributed only 0.5% of the utilized sedimentary organic carbon (Amaro et al., 2012). Other researches indicated that diatoms were nutrient source for sea cucumbers (Uthicke, 1999; Harder et al., 2002; Giraspy and Ivy, 2005). So, the value of bacteria as food for sea cucumbers is still unclear.

Though over three decades, there were numerous investigations about the feeding habits of sea cucumbers, there has never been a common understanding of how holothurians fulfill their dietary and energetic requirements. It is still unclear what the principal nutrient unit(s) is/are for sea cucumbers.

There have been only a few reports on the microbiota in the digestive tract of sea cucumbers (Amaro et al., 2009, 2012, Ward-Rainey et al., 1996). Ward-Rainey et al. reported aerobic bacterial microbiota of *Holothuria atra*. In their report, only 23 isolates were identified by 16S rRNA gene sequences analysis and they were affiliated to the genera *Vibrio* and *Bacillus*. Amaro et al. used non-culturing methods to analyze bacterial community of the abyssal holothurian *Molpadia musculus*. Their results suggested that the gut bacterial composition was similar to that of the organic matter-rich sediments. Members of the phylum Bacteroidetes dominated in the bacterial

community (Amaro et al., 2009). Recently Amaro et al. also reported the occurrence of wide and highly diversified interactions between prokaryotes and deep-sea holothurians (Amaro et al., 2012). Enomoto et al. reported that Gammaproteobacteria members were mainly isolated as culturable bacteria from the intestine of the Japanese spiky sea cucumber *Apostichopus japonicus* (Enomoto et al., 2012). Using the molecular techniques, they also found that Proteobacteria members were main metabolically active microbial populations in the intestine of *A. japonicus*.

Though the diversity of intestinal bacteria had been investigated for several holothurians species using culture-independent methods, studies on the function of the gut associated bacteria is limited. It is well known that gut microflora play an important role for a host, such as ingestion, contribution of extroenzymes, gene transfer (Harris, 1993; Flint et al., 2008; Kudo, 2009; Hehemann et al., 2010). The relationships between sea cucumbers and their gut bacteria and bacterial functions were not still clear. The diversity and function of aerobic culturable bacteria in the intestine of sea cucumbers are also till unclear. Much more work is needed to determine what substrates in detritus are bioavailable to and preferentially used by sea cucumbers.

The microbe diversity in gut of sea cucumbers (*Holothuria leucospilota*, deep-sea holothurian and *Apostichopus japonicas*) and the physiological characteristics of the aerobic culturable isolates were studied in this study. This study had three principal objectives:

1. To isolate the culturable bacteria from sea cucumbers and to investigate whether

there are differences in the bacterial community between different sea cucumber species or not, and what kinds of bacteria exist in the gut of sea cucumber.

2. To examine the function of these intestinal bacteria. It is important to determine decomposition ability of polysaccharide of intestinal bacteria from sea cucumbers and to understand symbiotic interaction between the two organisms.
3. To examine the physiological characteristics of these intestinal bacteria. It is necessary to investigate whether these bacteria are alive or active in the gut of sea cucumbers.