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**Repeated selective enrichment process of sediment microbiota occurred
in sea cucumber guts**

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Running title: Microbial selective enrichment in sea cucumber gut

Summary

Deposit-feeding sea cucumbers repeat ingestion of sediments and excretion of feces daily and consequently increase bacterial abundance in sediments and promote organic matter mineralization. Such ecological roles are expected to be collaborative activities of sea cucumbers and the gut microbiota. Here we performed a spatiotemporally-broad 16S rRNA gene analysis using 109 samples from sea cucumber feces and habitat sediments to explore potential contribution of their gut microbiota to the ecological roles. Most operational taxonomic units (OTUs) observed in the fecal samples were shared with the sediment samples, nevertheless fecal and sediment microbiota differed from each other in UniFrac analysis. Lower bacterial diversity and increased relative abundance of specific OTUs in the fecal microbiota strongly suggest selective enrichment of ingested sediment microbiota in their guts. Interestingly, representative fecal OTUs were more abundant in sea cucumber-populated sediments than in un-inhabited sediments, indicating bacteria selectively enriched in the guts were spread on ambient sediments via feces. Moreover, the predicted microbial community metabolic potential showed a higher abundance of genes related to carbohydrate and xenobiotics metabolisms in feces than in sediments. Our study suggests the

repeated selective enrichment transforms ambient sediment microbial communities and maintains the host's ecological roles by promoting organic matter mineralization.

Introduction

Holothuroidea (i.e. sea cucumber), member of the phylum Echinodermata, is one of the most abundant animals in marine benthic biomes. Currently more than 1,500 species have been described (Horton *et al.*, 2018). They are ubiquitous in marine environments, e.g. deep sea, coastal area, and coral reefs (Purcell *et al.*, 2012). Unlike other echinoderms (e.g. sea urchin, sea star, brittle star), a large number of sea cucumber species evolved as deposit-feeders consuming organic compounds derived from animal and plant detritus and microbial biomass (Yingst, 1976; Moriarty, 1982). Sea cucumbers rework huge amounts of sediments via ingestion and excretion (9-82 kg ind⁻¹ y⁻¹) (Uthicke, 1999) and can extensively blend and reform sea floor substrata. Considering their abundance, sea cucumber's biological behavior has greatly affected physico-chemical processes of both soft-bottom and reef ecosystems (Uthicke, 2001; Schneider *et al.*, 2011; MacTavish *et al.*, 2012; Purcell *et al.*, 2016).

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71 There are five major ecological functions of sea cucumbers: contributions to
72 sediment condition, recycling of nutrients, influencing seawater chemistry (i.e. pH,
73 alkalinity), forming pathways of energy transfer in food chains, and bolstering
74 biodiversity via symbiotic relationships (Purcell *et al.*, 2016). The first three are
75 likely to be more important in maintaining environmental conditions in marine
76 ecosystems. Bioturbation and sediment cleaning are the two main activities of the
77 animals in maintaining and improving sediment condition. Bioturbation, which is
78 defined as biological reworking and mixing of sediments and soils, impacts on
79 benthic primary producers, animals and microorganisms (Widdicombe and
80 Austen, 1999; Meysman *et al.*, 2006; Laverock *et al.*, 2010; MacTavish *et al.*,
81 2012). Burying and non-burying sea cucumbers distribute sediments vertically
82 and horizontally via their active ingestion and excretion of feces (Mercier *et al.*,
83 1999; Purcell, 2010; MacTavish *et al.*, 2012). Sediment cleaning is performed by
84 deposit-feeding sea cucumbers, which defecate less organic rich sand compared
85 to those of the ingested sediments (Yingst, 1976; Moriarty, 1982; Mercier *et al.*,
86 1999; Purcell *et al.*, 2016). Thus, sea cucumbers are used for integrated
87 multitrophic aquaculture with other aquatic animals (e.g. bivalves, finfish) to

reduce the accumulation of excess organic matter on the bottom of the farms (Slater and Carton, 2007, 2009; MacTavish *et al.*, 2012; Yokoyama *et al.*, 2015). This activity is analogous to the role of earthworms in soils (Drake and Horn, 2007). Moreover, sea cucumbers affect nutrient cycling by the conversion of organic nutrients into inorganic ones within their guts, and consequently influence the surrounding seawater chemistry (Uthicke, 2001; Schneider *et al.*, 2011; MacTavish *et al.*, 2012; Purcell *et al.*, 2016).

Currently, many studies posit that animal biology and ecology could not be evaluated properly without their associated microbiomes (McFall-Ngai *et al.*, 2013). In this context, ecological roles, such as sediment cleaning and nutrient cycling promotion, are expected to be collaborative activities of sea cucumbers and their gut microbiota. Although previous studies showed community structures of sea cucumber guts differed from those of sediment microbiota (Plotieau *et al.*, 2013; Gao *et al.*, 2014), whether gut-unique microbes reside in the guts, and whether the gut microbes contribute to conversion of organic nutrients into inorganic ones, have not been evaluated. Furthermore, while it has been speculated that the deposition of sea cucumber's feces as a part of bioturbation

could increase benthic bacterial abundance (MacTavish *et al.*, 2012), effects of excreted gut microbiota via feces on benthic microbial community structures remain largely unexplored. To fill the gap between sea cucumber's ecological roles and the gut microbiota, in-depth comparison of gut and sediment microbiota is needed.

Sea cucumber wild stocks have been decreasing dramatically due to over-fishing, and some of them, e.g. *Apostichopus japonicus* and *Thelenota ananas*, are even listed in the IUCN Red List as “endangered species” (Conand *et al.*, 2013; Hamel and Mercier, 2013; Purcell *et al.*, 2013). These species are also representative sea cucumbers distributed in the North (e.g. Menagawa) and South (e.g. Ishigaki) of Japan (Fig. S1). For their biological conservation, establishment of a seed production system for sea cucumbers is urgently needed. In addition to optimization of biotic and abiotic factors for sea cucumber farming (Dong *et al.*, 2006, 2008; Xia *et al.*, 2012; Shi *et al.*, 2013), many studies have tried to develop probiotics to improve growth and immunity in the farmed individuals (Sun *et al.*, 2012; Zhao *et al.*, 2012; Chi *et al.*, 2014). Characterizing sea cucumber gut microbiota through comparison with sediment microbial communities could lead

to the development of more effective probiotics for these endangered species. To characterize sea cucumber gut microbiota and to explore potential contribution of their gut microbiota to the host's ecological roles, we performed a spatiotemporally-broad assessment of sea cucumber fecal microbiota and sediment microbiota by 16S rRNA gene sequencing analyses without dissection of specimens (Yamazaki *et al.*, 2016). Our results show sea cucumber gut microbiota is shaped by selective enrichment process of ingested microbes from sediments, and the repeated process might transform ambient sediment microbial community structures and promote organic matter mineralization.

Results and Discussion

The most abundant phylum observed in all samples was Proteobacteria; the relative abundance in feces (M-Feces), sediment (M-Sed), and seawater (M-SW) in Menagawa was $60.1 \pm 5.6\%$, $55.3 \pm 4.4\%$, 74.1% (mean $\pm$ SD), respectively (Fig. S2). Those from Ishigaki feces (I-Feces) and sediment (I-Sed) samples were $68.1 \pm 6.5\%$ and $55.3 \pm 1.3\%$, respectively (Fig. S2). The second most abundant phylum was Bacteroidetes in the Menagawa samples (M-Feces, $25.8 \pm 8.6\%$; M-Sed, $24.4 \pm 5.0\%$; M-SW, 20.5%) and Ishigaki sediment (I-Sed, $9.8 \pm 1.1\%$), and

Cyanobacteria in the feces of the Ishigaki sea cucumbers (I-Feces, $8.6 \pm 4.9\%$) (Fig. S2).

Sea cucumber fecal microbiota is shaped by selective enrichment of ingested microbes from sediments

We compared the microbiota of sea cucumber *A. japonicus* feces with sediments from Menagawa site through one whole year (from September 2016 to July 2017). Unweighted UniFrac analysis revealed that fecal microbiota differed from the sediment microbiota over the whole year tested [permutational multivariate analysis of variance (PERMANOVA)], and the fecal microbiota fluctuated in parallel with sediment microbiota along with seasonal changes based on UniFrac analysis (Fig. 1A). Such differences between gut and sediment microbiota have also been reported in *A. japonicus* maricultured in China (Gao *et al.*, 2014). Interestingly, most major fecal OTUs, which were defined as OTUs observed in all fecal samples studied in each month, were detected in sediment microbiota (Table S1). Few bacterial OTUs were unique to fecal samples, compared to sediment samples. Additionally, these unique OTUs varied among sampling months and accounted for less than 0.1% of fecal bacterial communities in each

month. Thus, we propose here that the sea cucumber fecal microbiota is occupied by transient fraction ingested with food (i.e. sediment).

Shannon indices of sediment microbiota were 1.21 ± 0.05 times higher than those of feces over the whole year (Monte Carlo permutation test) (Fig. 1B). We applied Linear Discriminant Analysis Effect Size (LEfSe), to compare which OTUs may be indicator of fecal or sediment samples (Segata *et al.*, 2011). LEfSe identified 110 OTUs (41 OTUs in September, 38 OTUs in December, 27 OTUs in April, 38 OTUs in May, 34 OTUs in June and 34 OTUs in July) whose relative abundance was higher in fecal microbiota than in sediment microbiota. Heatmap analysis based on difference of relative abundance of the 110 OTUs showed that 1) in September, 14 OTUs belonging to Vibrionales were more abundant in fecal microbiota, 2) in November and December, 10, 8, and 6 OTUs belonging to Alteromonadales, Rhodobacterales, and Flavobacteriales, respectively, were more abundant in fecal microbiota, and 3) from April to July, Rhodobacterales (25 OTUs), Flavobacteriales (18 OTUs) and Alteromonadales (9 OTUs) were more abundant in feces than in sediments (Fig. 2A). These enriched bacterial groups, Vibrionales, Alteromonadales and Rhodobacterales, have already been

proposed as probiotics candidates positively affecting physiology of *A. japonicus* in previous study (Chi *et al.*, 2014; Yamazaki *et al.*, 2016). Additionally, Flavobacteriales could also be a new target for probiotics in sea cucumbers. LEfSe also identified 46 OTUs (13 OTUs in September, 12 OTUs in December, 11 OTUs in April, 26 OTUs in May, 27 OTUs in June and 17 OTUs in July) which were more abundant in sediment microbiota than in fecal microbiota. The 46 OTUs were mainly affiliated to Flavobacteriales (16 OTUs) and Thiotrichales (9 OTUs), and notably Thiotrichales were more abundant in sediments than in feces over the whole year (Fig. 2B). These results indicate sediment bacteria were selectively enriched in the guts of *A. japonicus*.

To explore whether the above-mentioned process is common to other sea cucumber species living in natural environments, we analyzed bacterial communities of Ishigaki July samples including four species of sea cucumbers (N=5) and Menagawa July samples as a reference. Although Ishigaki fecal samples were taken from four different species of sea cucumbers, they were clustered together, and fecal and sediment microbiota differed from each other (PERMANOVA) (Fig. 3A). Most major fecal OTUs in Ishigaki samples were

present in sediment samples (Table S1). Results of Shannon index comparison and LEfSe analysis were similar to those in *A. japonicus* (Fig. 3B and 4). The 38 OTUs including Rhodobacterales (15 OTUs), Desulfobacterales (7 OTUs), Chroococcales (4 OTUs) were more abundant in feces than in sediments at the Ishigaki site (Fig. 4A). On the other hand, the 9 OTUs including Thiotrichales (3 OTUs) and Flavobacteriales (2 OTUs) were more abundant in sediments than in feces at the site (Fig. 4B). Overall, our results suggest that selective enrichment of ingested microbes is the common process of shaping in fecal microbiota of coastal sea cucumbers.

The feeding habits and the ecological roles of sea cucumbers are similar to that of earthworms; they consume organic matter derived from bacteria, animals and plants with inorganic components (i.e. sediments and soils), and they promote organic matter mineralization (Drake and Horn, 2007; Purcell *et al.*, 2016). Interestingly, our results suggest these animals share common process in shaping of fecal microbiota. In both animals, 1) community structures of fecal microbiota are different from that of ingested ones (e.g. sediment or soil), 2) high number of common microbes are detected in both fecal and ingested microbiota,

and 3) bacterial diversity of fecal microbiota is lower than that of ingested microbiota (Thakuria *et al.*, 2010; Gao *et al.*, 2014; Aira *et al.*, 2015). Although processes shaping gut microbiota of both animals share similar properties, taxonomic affiliation of gut microbiota differed from each other; In particular, Firmicutes are the dominant phylum in earthworms' gut microbiota (Wüst *et al.*, 2011; Aira *et al.*, 2015), but relative abundance of this phylum was rare in sea cucumbers' feces in all seasons tested. Our results suggest a convergence of process of shaping in fecal microbiota between marine and terrestrial invertebrates (Roberts, 2000; Drake and Horn, 2007).

The repeated selective enrichment process of sediment microbiota in sea cucumber guts transform sediment microbial communities

A previous study reported deposition of sea cucumber's feces might increase benthic bacterial abundance (MacTavish *et al.*, 2012), but it remains largely unexplored a possible linkage between sea cucumbers grazing and excretion activities with sediment microbial community structure shifts. To address the possible links, we selected six representative OTUs based on LEfSe: denovo70836 (Flavobacteriales), denovo100805 (Rhodobacterales),

denovo17525 (Flavobacteriales), 669813 (Flavobacteriales), denovo147395 (Rhodobacterales), and denovo98634 (Flavobacteriales) (Fig. 2A). Relative abundance of these OTUs was compared between those in feces, in those sediments where sea cucumbers were densely populated (host-populated sediments), and those in sediments with no record of habitation (control sediments). Relative abundance of these six representative OTUs was highest in feces, followed by the host-populated sediments (Fig. 5). Welch's *t* test showed significantly higher abundance of these six OTUs in host-populated sediments than in control ones (Fig. 5).

The above results suggest bacteria selectively enriched in sea cucumber guts were spread on ambient sediments of the animals together with feces and drove the transformation of the sediment microbial community structures. Sea cucumber guts may also serve as reservoirs of several types of microbes maintaining their abundance. Besides, after settlement, juvenile sea cucumbers might be able to construct gut microbiota easily by ingesting the sediment microbiota transformed by adult individuals in their habitat. Similarly, the transformed sediment microbiota could also help gut-regenerated individuals re-

construct gut microbiota after the animals expel internal organs triggered by biotic and abiotic stress (Mashanov and García-Arrarás, 2011). Further studies are needed to examine whether heterogeneity in sediment microbial communities formed by sea cucumbers have positive impacts on recruitment of their juveniles and survival of gut-regenerated individuals.

Bacterial metabolism in sea cucumber's gut and possible contributions to the host's ecological roles

To explore which bacterial functions increased in the feces through the selective enrichment process of sediment microbiota in the guts, we used PICRUST software (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) which predicts functional metabolic potential of microbial communities from 16S rRNA gene libraries according to KEGG Orthology (KOs) (Langille *et al.*, 2013). Although the PICRUST prediction is limited to known microbial species described in the reference (i.e. Greengenes) and metagenome sequencing is more accurate to analyze functional profile in microbial community, PICRUST is still useful to discuss potential metabolic features in microbial communities. The frequency of the bacterial functions was likely to be stable in

all sample types (i.e. feces, sediment, seawater) collected in all seasons (Fig. S3).

We found that carbohydrates degradation metabolism potential (i.e. “Carbohydrate Metabolism” and “Xenobiotics Biodegradation and Metabolism” KEGG categories) was significantly more abundant in sea cucumbers feces than in sediment microbial communities (Fig. 6 and Table S2 for Welch’s *t* test results).

To confirm if the enriched microbial taxa in fecal microbiota were responsible for the increased carbohydrates degradation metabolisms, we removed all OTUs enriched in the feces identified by LEfSe from dataset, such as Vibrionales, Alteromonadales, Rhodobacterales, Flavobacteriales and Desulfobacterales. Interestingly, after removing the OTUs, “Carbohydrate Metabolism” had no significant difference, and the gene frequency of “Xenobiotics Biodegradation and Metabolism” was significantly more abundant in feces than in sediments in only one sampling point (Menagawa July) (Table S3). Therefore, these bacterial taxa enriched in sea cucumber guts might actively decompose carbohydrates and xenobiotics derived from animal and plant detritus thus contributing to organic matter mineralization of marine sediments.

The relative abundance of Thiotrichales, which consist of diverse sulfur oxidizing bacteria, was lower in sea cucumber guts than in sediments throughout the year (Fig. 2 and 4), suggesting anaerobic environments in their guts. In addition, Vibrionales known as facultative anaerobes and Desulfobacterales known as sulfate reducers were enriched in northern and southern sea cucumber guts, respectively (Fig. 2 and 4). Previous studies showed that Vibrionales is often dominant in sea cucumber guts (Enomoto *et al.*, 2012; Plotieau *et al.*, 2013; Gao *et al.*, 2017). These bacterial taxa cause fermentation within sea cucumber guts and may contribute to the decomposition of organic matter and further dissimilation processes in cooperation with other bacteria and host metabolism. Similar patterns were observed in organic matter mineralization by collaborative activity of earthworms and their gut microbiota (Horn *et al.*, 2003; Drake and Horn, 2007; Wüst *et al.*, 2011).

One previous study found sea cucumbers facilitates organic matter mineralization in sediments through increasing bacterial abundance and decreasing microphytobenthos abundance in sediments (i.e. shifting the microbial balance from producers to decomposers) (MacTavish *et al.*, 2012). Our results showed

sea cucumber feces contain more abundant Alteromonadales and Flavobacteriales, representative heterotrophic bacteria in marine environment, than in sediments (Fig. 2 and 4). The excreted feces could increase the abundance of such microbes in sediments and thus have a great impact on mineralization of sedimentary organic matters. This present study suggests a mechanism of increased bacterial abundance in sediments (MacTavish et al., 2012) and also a potential contribution of sea cucumber gut microbiota to sedimentary organic matter mineralization outside of their guts.

Conclusion

Our results indicate sea cucumber fecal bacterial communities are shaped by the selective enrichment of heterotrophic microbes acquired from ingested sediments. The repeated process transforms sediment microbial community structures around the host territory and can also promote organic matter mineralization inside and outside of the guts. Our study suggests, similar to earthworms, that sea cucumber gut microbiota can maintain the host's ecological roles including sediment cleaning and nutrient cycling promotion.

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Figure Legends

Figure 1. Bacterial diversity analyses in the Menagawa site. (A) Unweighted

UniFrac analysis was performed based on phylogenetic tree using 8000 of subsampled reads. The PCoA 2D plot shows fecal, sediment and seawater microbiota in the Menagawa. Different shapes of samples indicate different sample types (i.e. feces, sediment, seawater), and samples were colored by months. Significant difference of UniFrac distance was confirmed by permutational multivariate analysis of variance (PERMANOVA) (FDR-corrected $p < 0.05$). (B) Using the same number of subsampled reads, Shannon index of bacterial community diversity from sea cucumber fecal and sediment samples considering their sampling months was calculated. Asterisks show significant differences between mean Shannon indices within fecal and sediment samples in each month. Significance was evaluated based on 999 Monte Carlo permutation test with false discovery rate [false discovery rate (FDR)-corrected $p < 0.05$].

Figure 2. Dendrogram-connected heatmaps of LEfSe-identified OTUs of the Menagawa site. Difference of mean relative abundance of each OTU between feces and sediments were calculated [= effect size (%)]. More vivid magenta and cyan corresponds to more abundant OTUs in feces and sediments, respectively.

X axis of the heatmaps were aligned by time series of sampling. Y axis were aligned by maximum-likelihood tree of representative reads of each OTU. Color bars show order level affiliation of each OTU. Unassigned and minor taxa (more abundant OTUs in feces or sediments than the other at one time only) were combined into others. The fecal sample in November was excluded from LEfSe analysis due to limited available samples (N=1). (A) The heatmap shows OTUs which were more abundant in fecal microbiota than in sediment microbiota. Red circles indicate six representative OTUs which were more abundant in feces than in sediments in the months of September and December in 2016, and May, June and July in 2017 based on LEfSe. (B) The heatmap shows OTUs which were more abundant in sediment microbiota than in fecal microbiota.

Figure 3. Bacterial diversity analyses of Ishigaki July and Menagawa July

samples. (A) Unweighted UniFrac analysis was performed based on phylogenetic tree using 8000 of subsampled reads. The 2D PCoA plot shows fecal and sediment microbiota in the Ishigaki site in July and in the Menagawa site in July. Circles indicate fecal samples, and triangles do sediment samples. Ishigaki samples were colored by orange, and Menagawa samples were colored by dark

purple. Significant difference of UniFrac distance was confirmed by PERMANOVA (FDR-corrected $p < 0.05$). (B) Using the same number of subsampled reads, we compared bacterial community diversity (Shannon index) from sea cucumber fecal and sediment samples considering their geographical locations. Mean Shannon indices between fecal and sediment samples in each habitat were compared and evaluated based on 999 Monte Carlo permutation test with false discovery rate (FDR-corrected $p < 0.05$).

Figure 4. Dendrogram-connected heatmaps of LEfSe-identified OTUs of Ishigaki July and Menagawa July samples. Difference of mean proportion of each OTU between feces and sediments was calculated [= effect size (%)] using samples collected in the Ishigaki site in July and the Menagawa site in July. More vivid magenta and cyan corresponds to more abundant OTUs in feces and in sediments, respectively. Y axis were aligned by Maximum-Likelihood tree of representative reads of each OTU. Color bars show order level affiliation of each OTU. Unassigned and minor taxa (more abundant OTUs in feces or sediments than the other at one time only) were combined into others. (A) The heatmap shows OTUs which were more abundant in fecal microbiota than in sediment

microbiota. (B) The heatmap shows OTUs which were more abundant in sediment microbiota than in fecal microbiota.

Figure 5. Relative abundance of six representative OTUs by feces, host-populated sediments and controls. We selected the six representative OTUs based on LEfSe analysis in the Menagawa site. The bar plot shows mean relative abundance of the OTUs between those in feces, those in sediments where sea cucumbers were densely populated (host-populated sediments, i.e. Popul.) and those in sediments with no record of habitation (controls, i.e. Cont.). Error bars indicate standard errors. Bars marked by asterisks indicate relative abundances of host-populated sediments which were significantly higher than those of control sediments in sampling months (Welch's t test, FDR-corrected $p < 0.05$).

Figure 6. Frequency of predicted KEGG functions. Row names indicate KEGG functions, and column names indicate sampling points. Difference of gene frequencies between feces and sediments was calculated [= effect size (%)]. Red and blue colors indicate functions which were more abundant in feces than in sediments and more abundant in sediments than in feces, respectively.

Supporting Information

All sequences were deposited in DDBJ/GenBank/EMBL database under the accession no. PRJDB7862.

Appendix S1. Detailed methodology and related references.

Table S1. The number of major fecal OTUs shared by sediments. Major fecal OTUs are defined as OTUs observed in all fecal samples studied in each month.

Table S2. Difference of frequencies of predicted KEGG functions between feces and sediments. “M-” and “I-” stand for the Menagawa and the Ishigaki sites, respectively. Proportion of predicted functions were compared between those in fecal and those in sediment samples, and twelve features (the left column) were significantly more abundant in feces than in sediments at least one sampling point.

Yellow colors indicate statistical significance determined by Welch’s *t*-test (FDR-corrected $p < 0.05$). Numbers in cells show proportion of difference between those in feces and those in sediments by feature [effect size (%)]. Positive values

indicate a feature is more abundant in feces than in sediments.

Table S3. Difference of frequencies of predicted KEGG functions between feces and sediments using dataset excluding OTUs which were more abundant in feces than in sediments based on LEfSe analysis. “M-” and “I-” stand for the Menagawa and the Ishigaki sites, respectively. After excluding OTUs which were more abundant in feces than in sediments based on LEfSe analysis, proportion of predicted functions was compared between those in fecal and those in sediment samples. Five features were more abundant in feces than in sediments. Yellow colors indicate statistical significance determined by Welch’s *t* test. Numbers in cells show proportion of difference between those in feces and those in sediments by feature [effect size (%)]. Positive values indicate a feature is more abundant in feces than in sediments.

Table S4. Sample information of 16S rRNA gene sequencing.

Figure S1. Location of the Menagawa and the Ishigaki sites. The blue circle is the Menagawa site, Hokkaido, Japan (41°45’N, 141°5’E), where feces of

574 *Apostichopus japonicus* and sediments were collected. The orange circle is the
575 ishigaki site, Okinawa, Japan (24°21'N, 124°00'E), where feces of four species
576 of sea cucumbers (*Holothuria edulis*, *H. atra*, *Stichopus chloronotus*, *Thelenota*
577 *ananas*) and sediments were collected.

578

579 Figure S2. Bacterial community structures (phylum level) of sea cucumber feces
580 and their habitat's sediment and seawater. The Bar plot shows relative
581 abundance of the top 10 phyla. Unassigned and under top 10 phyla were
582 combined into others.

583

584 Figure S3. Functional proportion of individual bacterial communities based on
585 KEGG pathway.

586

587 Figure S4. A satellite image of Menagawa aquarium.