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Title	Repeated selective enrichment process of sediment microbiota occurred in sea cucumber guts
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Citation	Environmental microbiology reports, 11(6), 797-807 https://doi.org/10.1111/1758-2229.12791
Issue Date	2019-12
Doc URL	http://hdl.handle.net/2115/79857
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Type	article (author version)
File Information	YYamazaki_re-submitted_version.pdf

1 **Repeated selective enrichment process of sediment microbiota occurred**

2 **in sea cucumber guts**

3

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24 Running title: Microbial selective enrichment in sea cucumber gut

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34   **Summary**

35   Deposit-feeding sea cucumbers repeat ingestion of sediments and excretion of

36   feces daily and consequently increase bacterial abundance in sediments and

37   promote organic matter mineralization. Such ecological roles are expected to be

38   collaborative activities of sea cucumbers and the gut microbiota. Here we

39   performed a spatiotemporally-broad 16S rRNA gene analysis using 109 samples

40   from sea cucumber feces and habitat sediments to explore potential contribution

41   of their gut microbiota to the ecological roles. Most operational taxonomic units

42   (OTUs) observed in the fecal samples were shared with the sediment samples,

43   nevertheless fecal and sediment microbiota differed from each other in UniFrac

44   analysis. Lower bacterial diversity and increased relative abundance of specific

45   OTUs in the fecal microbiota strongly suggest selective enrichment of ingested

46   sediment microbiota in their guts. Interestingly, representative fecal OTUs were

47   more abundant in sea cucumber-populated sediments than in un-inhabited

48   sediments, indicating bacteria selectively enriched in the guts were spread on

49   ambient sediments via feces. Moreover, the predicted microbial community

50   metabolic potential showed a higher abundance of genes related to carbohydrate

51   and xenobiotics metabolisms in feces than in sediments. Our study suggests the

52 repeated selective enrichment transforms ambient sediment microbial

53 communities and maintains the host's ecological roles by promoting organic

54 matter mineralization.

55

56 **Introduction**

57 Holothuroidea (i.e. sea cucumber), member of the phylum Echinodermata, is one

58 of the most abundant animals in marine benthic biomes. Currently more than

59 1,500 species have been described (Horton *et al.*, 2018). They are ubiquitous in

60 marine environments, e.g. deep sea, coastal area, and coral reefs (Purcell *et al.*,

61 2012). Unlike other echinoderms (e.g. sea urchin, sea star, brittle star), a large

62 number of sea cucumber species evolved as deposit-feeders consuming organic

63 compounds derived from animal and plant detritus and microbial biomass (Yingst,

64 1976; Moriarty, 1982). Sea cucumbers rework huge amounts of sediments via

65 ingestion and excretion (9-82 kg ind^{-1} y^{-1}) (Uthicke, 1999) and can extensively

66 blend and reform sea floor substrata. Considering their abundance, sea

67 cucumber's biological behavior has greatly affected physico-chemical processes

68 of both soft-bottom and reef ecosystems (Uthicke, 2001; Schneider *et al.*, 2011;

69 MacTavish *et al.*, 2012; Purcell *et al.*, 2016).

70

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

88   reduce the accumulation of excess organic matter on the bottom of the farms

89   (Slater and Carton, 2007, 2009; MacTavish *et al.*, 2012; Yokoyama *et al.*, 2015).

90   This activity is analogous to the role of earthworms in soils (Drake and Horn,

91   2007). Moreover, sea cucumbers affect nutrient cycling by the conversion of

92   organic nutrients into inorganic ones within their guts, and consequently influence

93   the surrounding seawater chemistry (Uthicke, 2001; Schneider *et al.*, 2011;

94   MacTavish *et al.*, 2012; Purcell *et al.*, 2016).

95

96   Currently, many studies posit that animal biology and ecology could not be

97   evaluated properly without their associated microbiomes (McFall-Ngai *et al.*,

98   2013). In this context, ecological roles, such as sediment cleaning and nutrient

99   cycling promotion, are expected to be collaborative activities of sea cucumbers

100 and their gut microbiota. Although previous studies showed community structures

101 of sea cucumber guts differed from those of sediment microbiota (Plotieau *et al.*,

102 2013; Gao *et al.*, 2014), whether gut-unique microbes reside in the guts, and

103 whether the gut microbes contribute to conversion of organic nutrients into

104 inorganic ones, have not been evaluated. Furthermore, while it has been

105 speculated that the deposition of sea cucumber's feces as a part of bioturbation

106   could increase benthic bacterial abundance (MacTavish *et al.*, 2012), effects of

107   excreted gut microbiota via feces on benthic microbial community structures

108   remain largely unexplored. To fill the gap between sea cucumber's ecological

109   roles and the gut microbiota, in-depth comparison of gut and sediment microbiota

110   is needed.

111

112   Sea cucumber wild stocks have been decreasing dramatically due to over-fishing,

113   and some of them, e.g. *Apostichopus japonicus* and *Thelenota ananas*, are even

114   listed in the IUCN Red List as "endangered species" (Conand *et al.*, 2013; Hamel

115   and Mercier, 2013; Purcell *et al*., 2013). These species are also representative

116   sea cucumbers distributed in the North (e.g. Menagawa) and South (e.g. Ishigaki)

117   of Japan (Fig. S1). For their biological conservation, establishment of a seed

118   production system for sea cucumbers is urgently needed. In addition to

119   optimization of biotic and abiotic factors for sea cucumber farming (Dong *et al.*,

120   2006, 2008; Xia *et al.*, 2012; Shi *et al.*, 2013), many studies have tried to develop

121   probiotics to improve growth and immunity in the farmed individuals (Sun *et al*.,

122   2012; Zhao *et al.*, 2012; Chi *et al*., 2014). Characterizing sea cucumber gut

123   microbiota through comparison with sediment microbial communities could lead

124   to the development of more effective probiotics for these endangered species. To

125   characterize sea cucumber gut microbiota and to explore potential contribution of

126   their gut microbiota to the host's ecological roles, we performed a

127   spatiotemporally-broad assessment of sea cucumber fecal microbiota and

128   sediment microbiota by 16S rRNA gene sequencing analyses without dissection

129   of specimens (Yamazaki *et al.*, 2016). Our results show sea cucumber gut

130   microbiota is shaped by selective enrichment process of ingested microbes from

131   sediments, and the repeated process might transform ambient sediment

132   microbial community structures and promote organic matter mineralization.

133

134   **Results and Discussion**

135   The most abundant phylum observed in all samples was Proteobacteria; the

136   relative abundance in feces (M-Feces), sediment (M-Sed), and seawater (M-SW)

137   in Menagawa was 60.1±5.6%, 55.3 ± 4.4%, 74.1% (mean ± SD), respectively (Fig.

138   S2). Those from Ishigaki feces (I-Feces) and sediment (I-Sed) samples were

139   68.1±6.5% and 55.3±1.3%, respectively (Fig. S2). The second most abundant

140   phylum was Bacteroidetes in the Menagawa samples (M-Feces, 25.8 ± 8.6%; M-

141   Sed, 24.4 ± 5.0%; M-SW, 20.5%) and Ishigaki sediment (I-Sed, 9.8 ± 1.1%), and

142   Cyanobacteria in the feces of the Ishigaki sea cucumbers (I-Feces, 8.6 ± 4.9%)

143   (Fig. S2).

144

145   **Sea cucumber fecal microbiota is shaped by selective enrichment of**

146   **ingested microbes from sediments**

147   We compared the microbiota of sea cucumber *A. japonicus* feces with sediments

148   from Menagawa site through one whole year (from September 2016 to July 2017).

149   Unweighted UniFrac analysis revealed that fecal microbiota differed from the

150   sediment microbiota over the whole year tested [permutational multivariate

151   analysis of variance (PERMANOVA)], and the fecal microbiota fluctuated in

152   parallel with sediment microbiota along with seasonal changes based on UniFrac

153   analysis (Fig. 1A). Such differences between gut and sediment microbiota have

154   also been reported in *A. japonicus* maricultured in China (Gao *et al.*, 2014).

155   Interestingly, most major fecal OTUs, which were defined as OTUs observed in

156   all fecal samples studied in each month, were detected in sediment microbiota

157   (Table S1). Few bacterial OTUs were unique to fecal samples, compared to

158   sediment samples. Additionally, these unique OTUs varied among sampling

159   months and accounted for less than 0.1% of fecal bacterial communities in each

160 month. Thus, we propose here that the sea cucumber fecal microbiota is

161 occupied by transient fraction ingested with food (i.e. sediment).

162

163 Shannon indices of sediment microbiota were 1.21 ± 0.05 times higher than those

164 of feces over the whole year (Monte Carlo permutation test) (Fig. 1B). We applied

165 Linear Discriminant Analysis Effect Size (LEfSe), to compare which OTUs may

166 be indicator of fecal or sediment samples (Segata *et al.*, 2011). LEfSe identified

167 110 OTUs (41 OTUs in September, 38 OTUs in December, 27 OTUs in April, 38

168 OTUs in May, 34 OTUs in June and 34 OTUs in July) whose relative abundance

169 was higher in fecal microbiota than in sediment microbiota. Heatmap analysis

170 based on difference of relative abundance of the 110 OTUs showed that 1) in

171 September, 14 OTUs belonging to Vibrionales were more abundant in fecal

172 microbiota, 2) in November and December, 10, 8, and 6 OTUs belonging to

173 Alteromonadales, Rhodobacterales, and Flavobacteriales, respectively, were

174 more abundant in fecal microbiota, and 3) from April to July, Rhodobacterales (25

175 OTUs), Flavobacteriales (18 OTUs) and Alteromonadales (9 OTUs) were more

176 abundant in feces than in sediments (Fig. 2A). These enriched bacterial groups,

177 Vibrionales, Alteromonadales and Rhodobacterales, have already been

9

178   proposed as probiotics candidates positively affecting physiology of *A. japonicus*

179   in previous study (Chi *et al.*, 2014; Yamazaki *et al.*, 2016). Additionally,

180   Flavobacteriales could also be a new target for probiotics in sea cucumbers.

181   LEfSe also identified 46 OTUs (13 OTUs in September, 12 OTUs in December,

182   11 OTUs in April, 26 OTUs in May, 27 OTUs in June and 17 OTUs in July) which

183   were more abundant in sediment microbiota than in fecal microbiota. The 46

184   OTUs were mainly affiliated to Flavobacteriales (16 OTUs) and Thiotrichales (9

185   OTUs), and notably Thiotrichales were more abundant in sediments than in feces

186   over the whole year (Fig. 2B). These results indicate sediment bacteria were

187   selectively enriched in the guts of *A. japonicus*.

188

189   To explore whether the above-mentioned process is common to other sea

190   cucumber species living in natural environments, we analyzed bacterial

191   communities of Ishigaki July samples including four species of sea cucumbers

192   (N=5) and Menagawa July samples as a reference. Although Ishigaki fecal

193   samples were taken from four different species of sea cucumbers, they were

194   clustered together, and fecal and sediment microbiota differed from each other

195   (PERMANOVA) (Fig. 3A). Most major fecal OTUs in Ishigaki samples were

196   present in sediment samples (Table S1). Results of Shannon index comparison

197   and LEfSe analysis were similar to those in *A. japonicus* (Fig. 3B and 4). The 38

198   OTUs including Rhodobacterales (15 OTUs), Desulfobacterales (7 OTUs),

199   Chroococcales (4 OTUs) were more abundant in feces than in sediments at the

200   Ishigaki site (Fig. 4A). On the other hand, the 9 OTUs including Thiotrichales (3

201   OTUs) and Flavobacteriales (2 OTUs) were more abundant in sediments than in

202   feces at the site (Fig. 4B). Overall, our results suggest that selective enrichment

203   of ingested microbes is the common process of shaping in fecal microbiota of

204   coastal sea cucumbers.

205

206   The feeding habits and the ecological roles of sea cucumbers are similar to that

207   of earthworms; they consume organic matter derived from bacteria, animals and

208   plants with inorganic components (i.e. sediments and soils), and they promote

209   organic matter mineralization (Drake and Horn, 2007; Purcell *et al.*, 2016).

210   Interestingly, our results suggest these animals share common process in

211   shaping of fecal microbiota. In both animals, 1) community structures of fecal

212   microbiota are different from that of ingested ones (e.g. sediment or soil), 2) high

213   number of common microbes are detected in both fecal and ingested microbiota,

214 and 3) bacterial diversity of fecal microbiota is lower than that of ingested

215 microbiota (Thakuria *et al.*, 2010; Gao *et al.*, 2014; Aira *et al.*, 2015). Although

216 processes shaping gut microbiota of both animals share similar properties,

217 taxonomic affiliation of gut microbiota differed from each other; In particular,

218 Firmicutes are the dominant phylum in earthworms' gut microbiota (Wüst *et al.*,

219 2011; Aira *et al.*, 2015), but relative abundance of this phylum was rare in sea

220 cucumbers' feces in all seasons tested. Our results suggest a convergence of

221 process of shaping in fecal microbiota between marine and terrestrial

222 invertebrates (Roberts, 2000; Drake and Horn, 2007).

223

224 **The repeated selective enrichment process of sediment microbiota in sea**

225 **cucumber guts transform sediment microbial communities**

226 A previous study reported deposition of sea cucumber's feces might increase

227 benthic bacterial abundance (MacTavish *et al.*, 2012), but it remains largely

228 unexplored a possible linkage between sea cucumbers grazing and excretion

229 activities with sediment microbial community structure shifts. To address the

230 possible links, we selected six representative OTUs based on LEfSe:

231 denovo70836 (Flavobacteriales), denovo100805 (Rhodobacterales),

232 denovo17525 (Flavobacteriales), 669813 (Flavobacteriales), denovo147395

233 (Rhodobacterales), and denovo98634 (Flavobacteriales) (Fig. 2A). Relative

234 abundance of these OTUs was compared between those in feces, in those

235 sediments where sea cucumbers were densely populated (host-populated

236 sediments), and those in sediments with no record of habitation (control

237 sediments). Relative abundance of these six representative OTUs was highest in

238 feces, followed by the host-populated sediments (Fig. 5). Welch's *t* test showed

239 significantly higher abundance of these six OTUs in host-populated sediments

240 than in control ones (Fig. 5).

241

242 The above results suggest bacteria selectively enriched in sea cucumber guts

243 were spread on ambient sediments of the animals together with feces and drove

244 the transformation of the sediment microbial community structures. Sea

245 cucumber guts may also serve as reservoirs of several types of microbes

246 maintaining their abundance. Besides, after settlement, juvenile sea cucumbers

247 might be able to construct gut microbiota easily by ingesting the sediment

248 microbiota transformed by adult individuals in their habitat. Similarly, the

249 transformed sediment microbiota could also help gut-regenerated individuals re-

250 construct gut microbiota after the animals expel internal organs triggered by biotic

251 and abiotic stress (Mashanov and García-Arrarás, 2011). Further studies are

252 needed to examine whether heterogeneity in sediment microbial communities

253 formed by sea cucumbers have positive impacts on recruitment of their juveniles

254 and survival of gut-regenerated individuals.

255

256 **Bacterial metabolism in sea cucumber's gut and possible contributions to**

257 **the host's ecological roles**

258 To explore which bacterial functions increased in the feces through the selective

259 enrichment process of sediment microbiota in the guts, we used PICRUSt

260 software (Phylogenetic Investigation of Communities by Reconstruction of

261 Unobserved States) which predicts functional metabolic potential of microbial

262 communities from 16S rRNA gene libraries according to KEGG Orthology (KOs)

263 (Langille *et al.*, 2013). Although the PICRUSt prediction is limited to known

264 microbial species described in the reference (i.e. Greengenes) and metagenome

265 sequencing is more accurate to analyze functional profile in microbial community,

266 PICRUSt is still useful to discuss potential metabolic features in microbial

267 communities. The frequency of the bacterial functions was likely to be stable in

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

269

270 We found that carbohydrates degradation metabolism potential (i.e.

271 "Carbohydrate Metabolism" and "Xenobiotics Biodegradation and Metabolism"

272 KEGG categories) was significantly more abundant in sea cucumbers feces than

273 in sediment microbial communities (Fig. 6 and Table S2 for Welch's *t* test results).

274 To confirm if the enriched microbial taxa in fecal microbiota were responsible for

275 the increased carbohydrates degradation metabolisms, we removed all OTUs

276 enriched in the feces identified by LEfSe from dataset, such as Vibrionales,

277 Alteromonadales, Rhodobacterales, Flavobacteriales and Desulfobacterales.

278 Interestingly, after removing the OTUs, "Carbohydrate Metabolism" had no

279 significant difference, and the gene frequency of "Xenobiotics Biodegradation and

280 Metabolism" was significantly more abundant in feces than in sediments in only

281 one sampling point (Menagawa July) (Table S3). Therefore, these bacterial taxa

282 enriched in sea cucumber guts might actively decompose carbohydrates and

283 xenobiotics derived from animal and plant detritus thus contributing to organic

284 matter mineralization of marine sediments.

285

286 The relative abundance of Thiotrichales, which consist of diverse sulfur oxidizing

287 bacteria, was lower in sea cucumber guts than in sediments throughout the year

288 (Fig. 2 and 4), suggesting anaerobic environments in their guts. In addition,

289 Vibrionales known as facultative anaerobes and Desulfobacterales known as

290 sulfate reducers were enriched in northern and southern sea cucumber guts,

291 respectively (Fig. 2 and 4). Previous studies showed that Vibrionales is often

292 dominant in sea cucumber guts (Enomoto *et al.*, 2012; Plotieau *et al.*, 2013; Gao

293 *et al.*, 2017). These bacterial taxa cause fermentation within sea cucumber guts

294 and may contribute to the decomposition of organic matter and further

295 dissimilation processes in cooperation with other bacteria and host metabolism.

296 Similar patterns were observed in organic matter mineralization by collaborative

297 activity of earthworms and their gut microbiota (Horn *et al.*, 2003; Drake and Horn,

298 2007; Wüst *et al.*, 2011).

299

300 One previous study found sea cucumbers facilitates organic matter mineralization

301 in sediments through increasing bacterial abundance and decreasing

302 microphytobenthos abundance in sediments (i.e. shifting the microbial balance

303 from producers to decomposers) (MacTavish et al., 2012). Our results showed

304 sea cucumber feces contain more abundant Alteromonadales and

305 Flavobacteriales, representative heterotrophic bacteria in marine environment,

306 than in sediments (Fig. 2 and 4). The excreted feces could increase the

307 abundance of such microbes in sediments and thus have a great impact on

308 mineralization of sedimentary organic matters. This present study suggests a

309 mechanism of increased bacterial abundance in sediments (MacTavish et al.,

310 2012) and also a potential contribution of sea cucumber gut microbiota to

311 sedimentary organic matter mineralization outside of their guts.

312

313 **Conclusion**

314 Our results indicate sea cucumber fecal bacterial communities are shaped by the

315 selective enrichment of heterotrophic microbes acquired from ingested sediments.

316 The repeated process transforms sediment microbial community structures

317 around the host territory and can also promote organic matter mineralization

318 inside and outside of the guts. Our study suggests, similar to earthworms, that

319 sea cucumber gut microbiota can maintain the host's ecological roles including

320 sediment cleaning and nutrient cycling promotion.

321

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463

464 **Figure Legends**

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

466 UniFrac analysis was performed based on phylogenic tree using 8000 of

467 subsampled reads. The PCoA 2D plot shows fecal, sediment and seawater

468 microbiota in the Menagawa. Different shapes of samples indicate different

469 sample types (i.e. feces, sediment, seawater), and samples were colored by

470 months. Significant difference of UniFrac distance was confirmed by

471 permutational multivariate analysis of variance (PERMANOVA) (FDR-corrected

472 $p<0.05$). (B) Using the same number of subsampled reads, Shannon index of

473 bacterial community diversity from sea cucumber fecal and sediment samples

474 considering their sampling months was calculated. Asterisks show significant

475 differences between mean Shannon indices within fecal and sediment samples

476 in each month. Significance was evaluated based on 999 Monte Carlo

477 permutation test with false discovery rate [false discovery rate (FDR)-corrected

478 $p<0.05$].

479

480 **Figure 2. Dendrogram-connected heatmaps of LEfSe-identified OTUs of the**

481 **Menagawa site.** Difference of mean relative abundance of each OTU between

482 feces and sediments were calculated [= effect size (%)]. More vivid magenta and

483 cyan corresponds to more abundant OTUs in feces and sediments, respectively.

484   X axis of the heatmaps were aligned by time series of sampling. Y axis were

485   aligned by maximum-likelihood tree of representative reads of each OTU. Color

486   bars show order level affiliation of each OTU. Unassigned and minor taxa (more

487   abundant OTUs in feces or sediments than the other at one time only) were

488   combined into others. The fecal sample in November was excluded from LEfSe

489   analysis due to limited available samples (N=1). (A) The heatmap shows OTUs

490   which were more abundant in fecal microbiota than in sediment microbiota. Red

491   circles indicate six representative OTUs which were more abundant in feces than

492   in sediments in the months of September and December in 2016, and May, June

493   and July in 2017 based on LEfSe. (B) The heatmap shows OTUs which were

494   more abundant in sediment microbiota than in fecal microbiota.

495

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

497   **samples.** (A) Unweighted UniFrac analysis was performed based on phylogenic

498   tree using 8000 of subsampled reads. The 2D PCoA plot shows fecal and

499   sediment microbiota in the Ishigaki site in July and in the Menagawa site in July.

500   Circles indicate fecal samples, and triangles do sediment samples. Ishigaki

501   samples were colored by orange, and Menagawa samples were colored by dark

502   purple. Significant difference of UniFrac distance was confirmed by

503   PERMANOVA (FDR-corrected *p*<0.05). (B) Using the same number of

504   subsampled reads, we compared bacterial community diversity (Shannon index)

505   from sea cucumber fecal and sediment samples considering their geographical

506   locations. Mean Shannon indices between fecal and sediment samples in each

507   habitat were compared and evaluated based on 999 Monte Carlo permutation

508   test with false discovery rate (FDR-corrected *p*<0.05).

509

510   **Figure 4. Dendrogram-connected heatmaps of LEfSe-identified OTUs of**

511   **Ishigaki July and Menagawa July samples.** Difference of mean proportion of

512   each OTU between feces and sediments was calculated [= effect size (%)] using

513   samples collected in the Ishigaki site in July and the Menagawa site in July. More

514   vivid magenta and cyan corresponds to more abundant OTUs in feces and in

515   sediments, respectively. Y axis were aligned by Maximum-Likelihood tree of

516   representative reads of each OTU. Color bars show order level affiliation of each

517   OTU. Unassigned and minor taxa (more abundant OTUs in feces or sediments

518   than the other at one time only) were combined into others. (A) The heatmap

519   shows OTUs which were more abundant in fecal microbiota than in sediment

520 microbiota. (B) The heatmap shows OTUs which were more abundant in

521 sediment microbiota than in fecal microbiota.

522

523 **Figure 5. Relative abundance of six representative OTUs by feces, host-**

524 **populated sediments and controls.** We selected the six representative OTUs

525 based on LEfSe analysis in the Menagawa site. The bar plot shows mean relative

526 abundance of the OTUs between those in feces, those in sediments where sea

527 cucumbers were densely populated (host-populated sediments, i.e. Popul.) and

528 those in sediments with no record of habitation (controls, i.e. Cont.). Error bars

529 indicate standard errors. Bars marked by asterisks indicate relative abundances

530 of host-populated sediments which were significantly higher than those of control

531 sediments in sampling months (Welch's *t* test, FDR-corrected $p < 0.05$).

532

533 **Figure 6. Frequency of predicted KEGG functions.** Row names indicate

534 KEGG functions, and column names indicate sampling points. Difference of gene

535 frequencies between feces and sediments was calculated [= effect size (%)]. Red

536 and blue colors indicate functions which were more abundant in feces than in

537 sediments and more abundant in sediments than in feces, respectively.

538

Supporting Information

540   All sequences were deposited in DDBJ/GenBank/EMBL database under the

541   accession no. PRJDB7862.

542

543   Appendix S1. Detailed methodology and related references.

544

545   Table S1. The number of major fecal OTUs shared by sediments. Major fecal

546   OTUs are defined as OTUs observed in all fecal samples studied in each month.

547

548   Table S2. Difference of frequencies of predicted KEGG functions between feces

549   and sediments. "M-" and "I-" stand for the Menagawa and the Ishigaki sites,

550   respectively. Proportion of predicted functions were compared between those in

551   fecal and those in sediment samples, and twelve features (the left column) were

552   significantly more abundant in feces than in sediments at least one sampling point.

553   Yellow colors indicate statistical significance determined by Welch's t-test (FDR-

554   corrected $p<0.05$). Numbers in cells show proportion of difference between those

555   in feces and those in sediments by feature [effect size (%)]. Positive values

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

557

558   Table S3. Difference of frequencies of predicted KEGG functions between feces

559   and sediments using dataset excluding OTUs which were more abundant in feces

560   than in sediments based on LEfSe analysis. "M-" and "I-" stand for the Menagawa

561   and the Ishigaki sites, respectively. After excluding OTUs which were more

562   abundant in feces than in sediments based on LEfSe analysis, proportion of

563   predicted functions was compared between those in fecal and those in sediment

564   samples. Five features were more abundant in feces than in sediments. Yellow

565   colors indicate statistical significance determined by Welch's *t* test. Numbers in

566   cells show proportion of difference between those in feces and those in sediments

567   by feature [effect size (%)]. Positive values indicate a feature is more abundant

568   in feces than in sediments.

569

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

571

572   Figure S1. Location of the Menagawa and the Ishigaki sites. The blue circle is the

573   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.