1 **Unique hyper-thermal composting process in Kagoshima City forms distinct bacterial**
2 **community structures**

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16 Running title: Unique bacterial community in hyper-thermal compost

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Abstract

A unique compost, Satsuma soil, is produced from three types of wastewater sludge using hyper-thermal processes at temperatures much higher than that of general thermophilic processes in Kagoshima City, Japan. We analyzed the bacterial community structures of this hyper-thermal compost sample and other sludges and composts by a high-throughput barcoded pyrosequencing method targeting the 16S rRNA gene. In total, 621,076 reads were derived from 17 samples and filtered. Artificial sequences were deleted and the reads were clustered based on the operational taxonomic units (OTUs) at 97% similarity. Phylum-level analysis of the hyper-thermal compost revealed drastic changes of the sludge structures (each relative abundance) from Firmicutes (av. 47.8%), Proteobacteria (av. 22.3%), and Bacteroidetes (av. 10.1%) to two main phyla including Firmicutes (73.6%) and Actinobacteria (25.0%) with less Proteobacteria (~0.3%) and Bacteroidetes (~0.1%). Furthermore, we determined the predominant species (each relative abundance) of the hyper-thermal compost including Firmicutes related to *Staphylococcus cohnii* (13.8%), *Jeotgalicoccus coquinae* (8.01%), and *Staphylococcus lentus* (5.96%), and Actinobacteria related to *Corynebacterium stationis* (6.41%), and found that these species were not predominant in wastewater sludge. In contrast, we did not observe any common structures among eight other composts produced, using the hyper-thermal composts as the inoculums, under thermophilic conditions from different materials. Principle coordinate analysis of the hyper-thermal compost indicated a large difference in bacterial community structures from material sludge and other composts. These results suggested that a distinct bacterial community structure was formed by hyper-thermal composting.

Keywords hyper-thermal composting process; bacterial community structures; high-throughput 16S r RNA gene analysis; artificial sequences

1 **Introduction**

2 Composts are used as soil conditioners and organic fertilizers with the several functions
3 including enriching the soil, supplying nutrients such as nitrogen and minerals, and
4 controlling plant disease, which contributes to increased crop yield (1, 2). Composts have
5 been produced from different types of organic materials, mainly unutilized and renewable
6 biomasses including food waste (3), sewage sludge (4), lignocellulosic biomasses (5),
7 livestock manures (6), and inedible marine animal resources (7), which have contributed to
8 recycling organic wastes. Compositing is an aerobic process in batch or fed-batch
9 fermentation by which successive groups of microorganisms degrade organic materials (3, 5).
10 Based on the temperature profiles, the process is generally divided into two groups;
11 autothermal types (7, 8) and thermostatic control types (3, 9). Furthermore, composting
12 processes mainly exhibit broad ranges of temperatures, particularly mesophilic ($\leq$ around
13 50°C) and thermophilic (~50–70°C) conditions (3, 10, 11). Therefore, the temperatures
14 would affect the growth of microorganisms in individual composting processes.

15 Many studies have reported the analysis of microbial community structures in the
16 composting process using culture-independent and molecular biology approaches. In general,
17 bacteria belonging to phyla of Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, etc.
18 have been considered as predominant in most compositing processes for both, mesophilic and
19 thermophilic fermentation methods, by using clone library (4, 12), denaturing gradient gel
20 electrophoresis (DGGE) (3, 10), and terminal restriction fragment length polymorphism (T-
21 RFLP) (13) techniques. Recently, high-throughput 16S rRNA gene analysis using next-
22 generation sequencers are powerful methods for analysis of the bacterial community structure
23 in a compositing sample (14). Previous reports reveal that the varieties of predominant
24 bacteria among the different composting processes depend on the fermentation conditions
25 such as types of organic materials used and temperature (14).

1 A unique hyper-thermal composting process using sewage sludge as the organic material
2 has been employed in Kagoshima City, Japan, and is grouped into autothermal types (11, 15).
3 This process has been performed at a temperature higher than that in reported composting
4 processes in literature (~80–90°C for 40–45 days). Although the mechanism of self-heating
5 increase in temperature has not been elucidated, several thermophilic bacteria, especially
6 extreme thermophiles, are considered to be involved in this hyper-thermal composting
7 process. Previously, we isolated several extreme thermophilic bacteria not only from compost
8 products but also from material sewage sludge (11, 16). Additionally, the total bacteria in this
9 process have been quantified using culture-dependent plate counting and direct observation
10 (11, 15). Notably, the relationship between bacteria and hyper-thermal composting process is
11 still unknown and there are no reports on bacterial community structure analysis of composts
12 by using culture-independent and molecular biology approaches.

13 Although the barcoded pyrosequencing method has been increasingly applied for
14 bacterial community structure analysis of various environments such as fermented food (17),
15 intestinal tract (18), and soil (19), few studies have analyzed composts using this method (14,
16 20, 21). Here, we investigated the bacterial community structure of the compost produced by
17 the hyper-thermal composting process of waste sludge by barcoded pyrosequencing using the
18 next generation sequencer by Roche, 454 GS FLX Titanium XL+ platform, and compared the
19 results with those of other composts produced by different processes. We also analyzed the
20 bacterial community structures at a species level to elucidate detailed characteristics.

21

22 **Materials and Methods**

23

1 **Samples**

2 In total, 17 samples used in this study are listed in Table 1. Several samples produced by
3 hyper-thermal composting were obtained from Kagoshima City; namely, the compost
4 products (Satsuma soil; SSC.1 and SSC.2 provided from the same lot of composting process)
5 and their materials (various dehydrated sewage sludges: lime-dehydrated sludge, SS.1,
6 cation-dehydrated sludge; SS.2, digestive sludge through methane fermentation; SS.3). These
7 sewage samples were treated at atmospheric temperature from the excess sludge discharged
8 from the activated sludge method. SS.1 and SS.2 were flocculated with lime and cation
9 macromolecule, respectively, and dehydrated. SS.2 was further digested anaerobically,
10 yielding SS.3. SSC.1 and SSC.2 were produced by mixing SS.1, SS.2, and SS.3 sludge
11 samples by hyper-thermal composting. This process involves simple aerobic batch
12 fermentation with totally 4-times blending for a period of 40–45 d (15). In brief, the
13 temperature rapidly increases to reach 80–90°C, was maintained for several days, and then
14 gradually decreased. During the process, blending was performed only once every ~7–10 d
15 except for first blending (ca. 14 d), which led temporal decrease in the temperatures to ca.
16 60°C. Additionally, eight compost samples (KKC.1–KKC.8) derived from different materials
17 obtained from industrial wastes (sludge, wastewater, livestock manure, food waste, etc.) were
18 produced under thermal conditions at ~60–70°C with Satsuma soil as the inoculums. We also
19 analyzed the composts (JEC, TKC) produced at ~55–70°C and the sludge of mesophilic
20 methane fermentations (YMT, OSC) from different origins. All the samples were final
21 products in full-scale treatment processes, whose temperatures would be lower than those at
22 maximum in each process.

23

24 **DNA extraction from samples, PCR amplification of partial 16S rRNA gene, and**
25 **barcoded pyrosequencing**

1 Total DNA was extracted from each 0.25-g compost sample using PowerSoil™ DNA
2 Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA) according to the manufacturer's
3 instructions. For other sludge samples (YMT, and OSC), 0.25-g precipitates obtained by
4 centrifugation ($15,000 \times g$ for 10 min) were used. Before DNA extraction, all the samples
5 were homogenized and filtered with a mesh (pore size; 2 mm). The partial 16S rRNA gene
6 (V3–V5 region) was amplified using the extracted DNA samples as templates with universal
7 primer pairs for eubacteria, 357f (5'-CCTACGGGAGGCAGCAG-3') and 926r (5'-
8 CCGTCAATTCCTTTRAGTTT -3'), with 10-bp barcoded alignments in a total volume of 25
9 μ l containing 0.675 U of Ex Taq DNA polymerase (Takara Bio, Shiga, Japan), 0.2 mM
10 dNTPs, 0.5 μ M of each primer, and 1 μ l DNA template. The PCR cycling conditions were as
11 follows: 94°C for 40 s, 50°C for 40 s, and 72°C for 1 min, for 30 cycles, and final extension
12 at 72°C for 5 min. PCR products were purified using the QIAquick PCR purification kit
13 (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The purified PCR
14 products were sequenced by the 454 GS FLX Titanium XL+ platform (Roche, Basel,
15 Schwarz) according to the manufacturer's instruction. For pyrosequencing, ~5-fold amounts
16 of the DNA amplicon in SSC.1 was used to investigate the bacterial community structure in
17 detail (Fig. S1), which resulted in the highest raw reads (2,04,988), as compared with other
18 samples.

20 **Analysis of pyrosequencing data using bioinformatics**

21 The raw data was first filtered by using a Perl program to exclude reads with a low
22 quality such as, short reads less than 300-bp in length, undetermined bases, and alignment of
23 errant primers. The remaining sequences were sorted with specific barcoded sequences for
24 each sample. Subsequently, the dataset was depleted of chimeras by using the Black Box
25 Chimera Check (B2C2) software (22). A chimeric read is one where the starting and ending

1 100-bp regions of the sequence show different taxonomic phylum levels. After the chimera
2 check, the reads were grouped into operational taxonomic units (OTUs) at 97% similarity by
3 using the QIIME software package (23), and the most abundant read was selected as the
4 representative sequence for each OTU. The QIIME algorithm could not identify the bacteria
5 in the databases at a species-level; therefore, the Seqmatch program of RDP Classifier
6 (http://rdp.cme.msu.edu/seqmatch/seqmatch_intro.jsp) was used to assign the representative
7 sequences to the type of bacterial species. Furthermore, the closest relatives with the highest
8 RDP scores were extracted from the Seqmatch results by using the originally developed Perl
9 programs (named as Kugiri, mamax, and matome) in our laboratory. Moreover, alpha-
10 diversity (observed OTU, chao 1, and Shannon index) and beta-diversity (principle
11 coordinate analysis (PCoA)) were evaluated at a 3% OTU distance using the QIIME software
12 package.

13

14 **Results**

15

16 **Number of reads after filtration and chimera check from compost and sludge samples**

17 In total, 621,076 reads were obtained for various samples of materials and compost
18 products by barcoded pyrosequencing. The filtration process yielded 449,001 reads with high
19 quality scores and deleted ~28% of the reads with low quality scores (Table 2). Furthermore,
20 chimera check by the B2C2 software (22) detected chimeric reads from all the samples. The
21 frequency of chimeric occurrence was observed to have a wide range (1.81%–59.9%) among
22 all the samples (Table 2). Samples of the hyper-thermal compost (SSC.1 and SSC.2) had low
23 frequencies of chimeric occurrence (6.72% and 5.28%, respectively), while much higher
24 values of 45.8% (SS.3), 59.9% (YMT), and 50.8% (OSC) were obtained from sludge samples
25 of methane fermentation. KKC samples, produced from different materials by using the

hyper-thermal compost as the inoculums, exhibited a wide range of chimera frequencies (7.83%–44.4%). These results indicated that the frequency of chimeric occurrence in the pyrosequencing reads might depend on the origin of the samples.

Bacterial community structures in compost and sludge samples at a phylum-level

Figure 1 shows the overall bacterial community structures of samples analyzed in this study at a phylum-level after chimera check. Two major phyla were present in the hyper-thermal compost (SSC.1 and SSC.2) (Firmicutes; 74.3% and 72.9%, Actinobacteria; 24.1% and 25.9%, respectively). However, more than three phyla were highly abundant in sludge materials (SS.1, SS.2, SS.3). These results suggested that thermophilic bacteria in these two phyla grew selectively during the hyper-thermal composting process. KKC.1–8 compost samples showed higher diversity at a phylum-level than SSC.1 and SSC.2, although the hyper-thermal compost was used as the common inoculum. Additionally, there were fluctuations in the abundance of specific dominants among the samples, for example, Firmicutes; 2.99–75.1%, Bacteroidetes; 1.70–51.9%, Proteobacteria; 0.400–26.78%, Actinobacteria; 7.07–60.0%, were observed among KKC.1–8 compost samples. These results indicated that although the same inoculum (hyper-thermal compost) was used, the bacterial community structure in composts varied and were influenced by materials and fermentation processes. In contrast, the TKC compost had only one predominant phylum (Firmicutes; 91.1%) and the JEC compost had two predominant phyla (Firmicutes; 47.6%, Actinobacteria; 48.7%).

In the methane-fermentation sludge samples, SS.3, YMT, and OSC, 2.67%, 4.71%, and 2.75% of archaea were detected, respectively, in spite of using universal eubacteria primers for PCR amplification; however, other samples (SS.1 and SS.2) contained $\leq 0.1\%$ archaea.

1

2 **Analysis of alpha- and beta-diversities in bacterial community structures in compost**
3 **and sludge samples at 3% OTU distance**

4 Table 3 shows the three parameters of alfa-diversity, Observed OTU, Chao 1, and Shannon.
5 Values of all the parameters of the hyper-thermal compost, SSC.1 and SSC.2, were much
6 lower than those (av. 208, 537, and 6.05, respectively) of material sludges, SS.1, SS.2, and
7 SS.3. These data suggested that the hyper-thermal composting process caused a drastic
8 decrease in the bacterial diversity. Respective values of the KKC composts (av. 121, 228, and
9 5.21) were comparable to those of SSC.1 and SSC.2, except for KKC.5 (186, 345, and 6.41,
10 respectively) and KKC.6 (204, 369, and 6.83, respectively). Overall, sludge samples (SS.1,
11 SS.2, SS.3, YMT, and OSC) exhibited higher bacterial diversity than compost samples
12 (SSC.1–2, KKC.1–4, 7–8, JEC, and TKC).

13 Figure 2 shows the beta-diversities obtained at 3% OTU distance by principal
14 coordinates analysis of all compost and sludge samples. The cluster of the hyper-thermal
15 compost (SSC.1 and SSC.2) was quite far from that of sludge material samples (SS.1, SS.2,
16 and SS.3). These results also indicated that the bacterial community structures of materials
17 changed drastically at a species-level by the hyper-thermal composting process. In contrast,
18 JEC and TKC samples did not belong to any cluster. Although percentages of several phyla
19 were not similar, as shown in Fig. 1, the KKC samples could be classified into another cluster.
20 Sludge samples, not only SS.1, SS.2, and SS.3 but also YMT and OSC, were located in one
21 cluster, which was separable from all the compost samples. Notably, similar bacterial
22 community structures were abundant in the methane-fermentation sludge samples (SS.3,
23 YMT, and OSC) although they were obtained from materials very different form one another.
24 These results indicated that the bacterial community structures of analyzed samples were

classified as the following clusters: hyper-thermal compost samples (SSC.1 and SSC.2), KKC samples (KKC.1–8), sludge samples (SS.1, SS.2, SS.3, YMT, and OSC), and two independent JEC and TKC samples.

Determination of predominant bacteria at a species-level in compost and sludge samples

To investigate the predominant bacteria in more detail, we assigned the representative OTU sequences in terms of the closely related strains of bacteria using the Seqmatch program of RDP Classifier. Figure 3 shows the heat map obtained from OTU assignments at a species-level that were higher than 3% OTU reads for any sample. Reproducible results were obtained for SSC.1 and SSC.2, which had two major related species, *Staphylococcus cohnii* (13.7% and 13.8%) and *Jeotgalicoccus coquinae* (8.11% and 7.91%), and other relatively minor species, *Corynebacterium stationis* (6.99% and 5.83%), *Bacillus foraminis* (5.10% and 3.72%), *Staphylococcus lentus* (6.10% and 5.82%), and *Virgibacillus kekensis* (5.26% and 3.44%). These results showed that the hyper-thermal composting process for Satsuma soil production from three types of sludge materials caused drastic changes in the bacterial community structures.

Different predominant species of representative OTU sequences were observed in SS.1 (*Bacillus subtilis*; 30.0%, *Clostridium bartlettii*; 23.3%), SS.2 (*Sporanaerobacter acetigenes*; 10.4%), and SS.3 (*Levilinea saccharolytica*; 10.0%), which might have resulted from different treatments of excess sludge by lime dehydration, cation dehydration, or methane fermentation, respectively. Although OSC had the closest bacterial community structures to beta-diversity of YMT, *L. saccharolytica* related species (43.2%) in OSC was the same predominant species in SS.3. The predominant species in YMT, *Prevotella heparinolytica* (14.3%), was different.

Each KKC sample had different predominant species from those in SSC.1 and SSC.2; KKC.1 (*Allobacillus halotolerans*; 28.9%, *Lentibacillus salicampi*; 15.5%), KKC.2 (*Actinomadura sputi*; 9.71%, *Amaricoccus macauensis*; 8.71%), KKC.3 (*Bacillus thermoamylovorans*; 35.8%, *Brevibacterium sanguinis*; 9.80%), KKC.4 (*Allobacillus halotolerans*; 14.5%), KKC.5 (*Mycobacterium thermoresistibile*; 13.1%), KKC.6 (*Aciditerrimonas ferrireducens*; 5.83%), KKC. 7 (*Georgenia ruanii*; 15.7%, *Paraliobacillus ryukyuensis*; 14.1%), and KKC.8 (*Pedobacter lentus*; 34.1%). Furthermore, different predominant species were present in JEC (*Saccharopolyspora rosea*; 32.9%) and TKC (*Pediococcus acidilactici*; 28.6%, *Bacillus subtilis*; 20.4%). However, beta-diversity of bacterial community structures of KKC samples grouped into the same cluster (Fig. 2). The most predominant bacteria would be distinct at species-level in each sample of compost and sludge.

Discussion

Bacterial community structure of composts is generally affected by several factors including materials and temperatures (mesophilic or thermophilic, type of temperature controls [autothermal type and thermostatic control type]). The hyper-thermal composting process for production of Satsuma soil in Kagoshima City showed several noteworthy characteristics. This process was carried out with three types of materials, lime-dehydrated, cation-dehydrated, and digested sludges, at temperatures as high as 80–90°C, with the autothermal type being reported the most in literature so far, and then the product was analyzed as the hyper-thermal compost sample, whose temperatures would be lower than those at maximum. In this study, we first investigated the bacterial community structures of the hyper-thermal compost, its sludge materials, composts using the hyper-thermal compost as the inoculums, other composts, and anaerobically digested sludges by barcoded

1 pyrosequencing using a next-generation sequencer, 454 GS FLX Titanium XL+ platform.
2 Although some reports have analyzed bacterial community structures of composts by next-
3 generation sequencing, which can give much more information than other previous methods
4 (14, 20, 21), we report the alfa- and beta-diversities at OTU level here for the first time.

5 PCR amplification targeting bacterial 16S rRNA gene is known to yield chimeric reads,
6 particularly using DNA templates derived from bacterial communities (24, 25). Regarding
7 the 454 sequencer used in this study, chimeric reads were observed in all 17 samples used
8 with different frequencies of chimeric occurrence (1.81–59.9%) (Table 2). The frequencies of
9 chimeric occurrence have been influenced by several factors including PCR conditions (25),
10 sequence similarities of 16S rRNA genes among the bacterial communities in the template
11 DNA extracted from environmental samples (26), and presence of damaged DNA (25).
12 Additionally, many chimeric sequences have been reportedly been deposited into the
13 databases (26), which would lead to misunderstanding of the homology searches. Although
14 the relationship between frequencies of chimeric occurrence and alfa-diversity has not been
15 reported, to our knowledge, these results suggested that the samples (SS.3, KKC.6, YMT, and
16 OSC) with relatively high alfa-diversity index values also exhibited high frequencies of
17 chimeric occurrence (44.4–59.9%) (Tables 2 and 3). Therefore, a chimera check would be a
18 useful and essential tool to determine the bacterial community structure more accurately from
19 mega data obtained from the next generation sequencer.

20 Previous studies on bacterial community structure analysis indicate that three phyla,
21 Firmicutes, Proteobacteria, and Actinobacteria, are generally major members in the
22 composting process under both, mesophilic and thermophilic conditions (14). Furthermore,
23 thermophilic composting processes have been shown to increase the distributions of
24 Firmicutes and decrease those of Proteobacteria, compared with mesophilic composting
25 processes (14). Our results revealed that hyper-thermal composting processes almost

1 completely diminished Proteobacteria to less than 0.5% from Proteobacteria-rich sludge
2 materials (~7–45% in SS.1–SS.3), and only two phyla, Firmicutes (~74%) and Actinobacteria
3 (~25%), became predominant in SSC samples (Fig. 1). Additionally, even by using the
4 hyper-thermal compost as the inoculums, the predominant phyla were diverse and specific in
5 each compost of KKC and SSC (Fig. 1). These results are consistent with the phenomena
6 suggested in literature, which state that various factors including temperature, moisture, pH,
7 C/N ratio, aeration, and materials, influence the bacterial community structures in composting
8 processes (10, 14, 21, 27).

9 In microbial ecology, the information pertaining to a genus and species of the present
10 microorganisms in targeted samples has greater significance and utility than the upper taxon
11 such as phylum, class, order, family, and tribe for understanding ‘which microbe are
12 present?’ and ‘what is their underlying action mechanism?’. Although some reports have
13 analyzed the bacterial community structures in composts by pyrosequencing, which would
14 provide higher resolutions with much more information than conventional methods (DGGE,
15 T-RFLP, clone library), they did not show the bacteria at the species level (14, 20, 21). Here,
16 we affiliated approximately half of OTUs at the species level with similarity scores greater
17 than 0.9 (Fig. 3), which indicated meaningful and helpful information. We determined the
18 predominant species belonging to Firmicutes (*St. cohnii* (av. 13.8%), *J. coquinae* (av. 8.01%),
19 and *St. lentus* (av. 5.96%)), and Actinobacteria (*Cory. stationis* (av. 6.41%)) in the hyper-
20 thermal compost (SSC.1, SSC.2) with 0.953–0.990 similarity scores (Fig. 3). By using a
21 clone library method targeting ~1500 bp-length of the 16S rRNA gene, *Staphylococcus* sp.,
22 *Jeotgalicoccus* sp., and *St. lentus* were also detected in the same compost samples with more
23 than 99% similarities (data not shown). To our knowledge, these species, except *St. lentus*,
24 have not been reported to be predominant in any other composts (28, 29), and little is known
25 of their roles and functions in the hyper-thermal composting process. Contrary to our

1 expectation, these closely-related bacteria have been shown not to be thermotolerant but
2 mesophilic (30–33). We postulated that these bacteria became predominant after the
3 temperature decreased to the suitable conditions to grow well or that unisolated bacteria
4 closely-related to the known species grew under the thermophilic condition. To validate these
5 postulations, therefore, their quantitative and functional analysis and their isolation and
6 identification still have to be investigated to gain a better understanding of the hyper-thermal
7 composting process.

8 It was assumed that extreme thermophiles growing at $> \sim 65^{\circ}\text{C}$ were dominant over
9 moderate thermophiles growing at $\leq \sim 65^{\circ}\text{C}$ in the hyper-thermal composting process because
10 we previously observed central viable cells by the high-temperature direct viable count
11 method and isolated extreme thermophiles such as *Calditerricola* sp. (11) and
12 *Thermaerobacter* sp. (data not shown) from the hyper-thermal compost. Notably, in this
13 study, these extreme thermophiles were not detectable in the compost by barcoded
14 pyrosequencing. Therefore, the reason that the hyper-thermal composting process can be
15 conducted at the highest temperature remains unknown and unisolated thermophiles in new
16 taxa might contribute to the process. Further studies are required to monitor dynamics of
17 bacterial community structures, particularly extreme thermophiles, during the hyper-thermal
18 composting process.

19 In conclusion, we analyzed alfa- and beta-diversities, at the OTU level, of the compost
20 (Satsuma soil) produced hyper-thermally from three types of sludge at temperatures higher
21 than $\sim 90^{\circ}\text{C}$ in Kagoshima City by using high-throughput pyrosequencing. A chimera check
22 was considered useful because it deleted artificial sequences to decrease unclassified bacteria;
23 however, the frequencies of chimeric occurrence depended on the analyzed samples. We
24 suggest that the bacterial community structure of hyper-thermal composts was drastically
25 changed from that of material sludge, and were different among the other composts produced

from distinct materials under thermophilic condition. Although the mechanism by which hyper-thermal composting process is achieved remains unknown, related strains to *St. cohnii*, *J. coquinae*, *St. lentus*, *Cory. stationis* were predominant species in hyper-thermally produced compost.

Acknowledgements

This work was supported partly by JST/JICA, SATREPS (Science and Technology Research Partnership for Sustainable Development), by JSPS KAKENHI Grant number 26740050, and by Kyushu University interdisciplinary programs in education and projects in research development. We acknowledge Dr. Tairo Oshima at Kyowakako Co., Ltd. Corp., for providing KKC compost samples.

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16

1 **Figure captions**

2 Fig. 1. Comparison of bacterial community structure at phylum level among hyper-thermal

3 composting process, materials, and other processes

4 Fig. 2 Beta- diversity of bacterial community structure in compost and sludge samples at 3%

5 OTU distance

6 Fig. 3 OTU Heat map at species level accounted for higher than 3% OTU reads in each

7 sample

Table 1. List of samples for analysis of bacterial community structure

Sample's abbreviations	Characteristics	Materials	Water content (%)	pH
SSC.1, SSC.2	Compost (Satsuma soil)	Digestive sludge, lime-dehydrated sludge, cation-dehydrated sludge	25.6	8.3
SS.1	Lime-dehydrated sludge	Excess sludge through activated sludge method	62.1	10.9
SS.2	Cation-dehydrated sludge	Excess sludge through activated sludge method	78.7	6.1
SS.3	Digestive (methane fermentation) sludge	Cation-dehydrated sludge	87.5	8.8
KKC.1	Compost using Satsuma soil as the seed	Excess sludge, fisheries industrial waste	28.9	8.3
KKC.2	Compost using Satsuma soil as the seed	Industrial effluent sludge, excess sludge	32.1	8.1
KKC.3	Compost using Satsuma soil as the seed	Excess sludge	23.7	8.0
KKC.4	Compost using Satsuma soil as the seed	Industrial effluent sludge, fisheries industrial waste, food waste	37.9	8.1

KKC.5	Compost using Satsuma soil as the seed	Industrial effluent sludge	23.6	8.3
KKC.6	Compost using Satsuma soil as the seed	Livestock waste, straw	ND	ND
KKC.7	Compost using Satsuma soil as the seed	Industrial waste	21.1	8.1
KKC.8	Compost using Satsuma soil as the seed	Peanut hull	32.7	8.3
JEC	Compost	Fisheries industrial waste, coffee extraction residue	10.9	7.8
TKC	Compost	Food waste	17.3	6.8
YMT	Sludge through mesophilic methane fermentation	Pig manure	ND	ND
OSC	Sludge through mesophilic methane fermentation	Food waste, human excreta, sludge from septic tank	ND	ND

ND: not determined.

Table 2. The numbers of raw, filtered, chimera and non-chimera reads, and ratio of chimera occurrence in each analyzed sample

Sample's abbreviations	Raw reads	Filtered reads	Chimera reads	Non-chimera reads	Frequency of chimeric occurrence (%)
SSC.1	204988	146902	9870	137032	6.72
SSC.2	49559	35841	1894	33947	5.28
SS.1	56266	41549	4655	36894	11.2
SS.2	46170	35095	6239	28856	17.8
SS.3	43018	33023	15108	17915	45.8
KKC.1	14771	11478	2466	9012	21.5
KKC.2	11568	8106	1456	6650	18.0
KKC.3	3491	2559	504	2055	19.7
KKC.4	24422	18552	4622	13930	24.9
KKC.5	22614	14891	2222	12669	14.9
KKC.6	35724	26206	11644	14562	44.4
KKC.7	21696	13894	1088	12806	7.83
KKC.8	15475	10327	2725	7602	26.4
JEC	32025	21184	3976	17208	18.8
TKC	9147	6856	124	6732	1.81
YMT	10635	8006	4799	3207	59.9
OSC	19507	14532	7381	7151	50.8

Table 3. Alfa-diversity of bacterial community structure in each analyzed compost and sludge sample at 3% OTU distance

	Observed OTU ^a	Chao 1 ^a	Shannon ^a
SSC.1	126	249	5.43
SSC.2	135	256	5.63
SS.1	157	464	4.59
SS.2	258	694	6.98
SS.3	208	452	6.59
KKC.1	104	188	4.69
KKC.2	135	249	5.76
KKC.3	99.7	186	4.44
KKC.4	122	226	5.76
KKC.5	186	345	6.41
KKC.6	204	369	6.83
KKC.7	130	252	5.32
KKC.8	135	265	5.31
JEC	138	364	4.93
TKC	80.9	140	4.14
YMT	181	335	6.24
OSC	140	348	5.13

^a All the indexes were calculated per 629 reads.

Fig. 1.

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Fig. 2.

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SSC.1	SSC.2	SS.1	SS.2	SS.3	KKC.1	KKC.2	KKC.3	KKC.4	KKC.5	KKC.6	KKC.7	KKC.8	JEC	TKC	YMT	OSC	Phylum	Closest species	Score
4.03	1.30	0.02	0.02			0.34	0.20	1.99	0.65				9.15				Firmicutes (Bacilli)	<i>Bacillus thermocloacae</i>	1.00
5.10	3.72		0.04		0.09	0.81	4.50	5.07	3.85		0.10	0.05	0.06		5.73			<i>Bacillus foraminis</i>	0.864
																		<i>Paenibacillus cookii</i>	0.943
					15.5						0.16							<i>Lentibacillus salicampi</i>	0.823
0.53	0.39				0.02				0.10		14.1	0.91						<i>Paraliobacillus ryukyuensis</i>	0.814
5.26	3.44		0.03		0.15	0.54		0.78	0.14				0.11					<i>Virgibacillus kekensis</i>	0.825
		30.0	0.27		28.9			14.5			0.18	0.16						<i>Allobacillus halotolerans</i>	0.901, 0.966
											0.02			20.4				<i>Bacillus subtilis</i>	0.986
8.11	7.91		0.08						0.06		0.22		0.03		0.12			<i>Jeotgalicoccus coquinae</i>	0.978
6.10	5.82		0.06			0.24	0.90	0.03	0.34		0.51		0.02					<i>Staphylococcus lentus</i>	0.990
13.7	13.8	0.03	0.35	0.08		0.24	1.60		0.54		0.57		0.02	4.00	0.12	0.05		<i>Staphylococcus cohnii</i>	0.966
1.09	5.53	0.04	0.08			0.20	35.8	0.11	2.48	0.42	0.65		0.05					<i>Bacillus thermoamylovorans</i>	0.860
0.02														28.6				<i>Pediococcus acidilactici</i>	0.991
					0.02				0.02		0.27		10.1		1.67	8.02	Firmicutes	<i>Acholeplasma morum</i>	0.723
															0.12			<i>Atopostipes suicloacalis</i>	0.975
				5.14													Firmicutes (Clostridia)	<i>Sedimentibacter hydroxybenzoicus</i>	0.803
		0.04	0.14	5.70												0.03		<i>Alkalibaculum bacchi</i>	0.558
			10.4															<i>Sporanaerobacter acetigenes</i>	0.888
		23.3	0.59	0.01														<i>Clostridium bartlettii</i>	1.00
		4.10	0.11								0.04				0.19			<i>Clostridium glycolicum</i>	0.905
	0.01	0.12	5.15	0.40	0.04	0.13				1.52					0.09		Proteobacteria	<i>Paracoccus aminophilus</i>	0.956
0.04						8.71		1.21	1.76	1.55	0.39	1.76	0.12					<i>Amaricoccus macauensis</i>	0.835
								0.02			0.51	5.31					Bacteroidetes	<i>Aquiflexum balticum</i>	0.648
							7.90			6.45	34.1				14.3			<i>Prevotella heparinolytica</i>	0.544
																		<i>Pedobacter lentus</i>	0.568, 0.592
0.02				10.0											0.37	43.2	Chloroflexi	<i>Levilinea saccharolytica</i>	0.458, 0.509, 0.542, 0.634
						3.66		4.05	0.59	5.83								<i>Aciditerrimonas ferrireducens</i>	0.604
3.48	3.15		0.03				9.80	0.06	0.89	0.08	0.24	1.97	0.94				Actinobacteria	<i>Brevibacterium sanguinis</i>	1.00
0.04	0.02				0.89	0.07		0.10	0.02		15.7	0.45	0.09					<i>Georgenia ruanii</i>	0.920
					0.02						6.47	0.11						<i>Isoptericola halotolerans</i>	0.848
0.02					0.39			0.08	0.04		5.12	0.83	0.03					<i>Haloactinobacterium album</i>	0.742
						9.71	0.30	1.85	3.53	3.32	0.37	1.87						<i>Actinomadura sputi</i>	0.975
						5.24	0.10		0.08	0.60								<i>Glycomyces arizonensis</i>	0.797
									0.02				32.9					<i>Saccharopolyspora rosea</i>	0.725, 0.720
6.99	5.83		0.05			0.10		0.12	0.06		0.04							<i>Corynebacterium stationis</i>	0.953
0.01						0.24		1.04	13.1			0.08						<i>Mycobacterium thermoresistibile</i>	0.957

Fig. 3.