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Chemical and Microbiological Evaluation of Vermicompost Made from School Food Waste in Japan

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Abstract

Vermicomposting technology is an environmentally friendly, sustainable, and low-cost tool used to convert agronomical and food waste into manure, facilitated by the decomposition and digestion by earthworms. In this study, we evaluated the chemical properties and microbial diversity of vermicompost (VC) derived from school lunch waste, which has long been a serious problem in many countries including Japan. The results revealed that 18-week-old VC promoted the root elongation of plants, and also showed a higher germination index (GI) and higher cation exchange capacity (CEC), indicating that vermicompost made from school food waste is an acceptable manure. As a result of the microbiome analysis by the sequencing of 16S ribosomal DNA (rDNA) using next generation sequencing (NGS), higher ratios of *Bacillus*, *Pseudomonas*, and *Paenibacillus* species, which may include beneficial bacteria for plant growth, were detected in VC than in the control compost (CC). These results demonstrate the significance of vermicompost in utilizing waste from school lunch, suggesting the possibility of implementing a waste recycling system that leads to reducing and recycling food waste in schools.

Discipline: Agricultural Environment
Additional key words: bacterial diversity, manure, microbial activity, school lunch

Introduction

It is important for children to live a healthy childhood with due consideration given to their maturation and physical growth, for which adequate nutrition during this period is essential. School lunch programs were first initiated in Japan in 1889, when a private elementary school in Yamagata Prefecture provided poor children with meals for free (Tanaka & Miyoshi 2012). In 2018, 95.2% of schools provided school lunches for about 6.3 and 2.7 million children in public elementary school and junior high school, respectively (MEXT, 2018). However, the proliferation of the school lunch program has resulted in a serious problem regarding school food waste. The Ministry of Environment (2013) reported that about 17.2 kg of food waste was discharged per student in Japanese elementary and junior high schools in 2013. As is the case with Japan, the problem has been serious in other countries, such as China and the United States (Byker et al. 2014, Liu et al. 2016, Cohen et al. 2013).

Vermicomposting is a biological recycling process of organic waste. In the process, earthworms are used to digest waste and convert it into manure. Noted for his work on the theory of evolution, Charles Darwin was one of the scientists who had a prominent interest in the role of earthworms, and published his findings in 1881 in a book titled "The Formation of Vegetable Mould through the Action of Worms, with Observations of their Habits" (Darwin 1881). More than 100 years since, vermicomposting technology is now being recognized as a sustainable, inexpensive and environmentally friendly approach to recycling organic waste. The final product of this technology offers more available nutrients and diversity microbial populations than those of normal composting (Frederickson et al. 1997, Edwards et al. 1988), and is considered an environmentally friendly organic fertilizer for agricultural application (Tajbakhsh

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et al. 2010). Previous studies have demonstrated that this technology can convert agricultural waste such as vegetables or fruits into useful compost (Mainoo et al. 2009, Fernández-Gómez et al. 2010a, Fernández-Gómez et al. 2010b, Suthar et al. 2009). However, fewer reports on converting agricultural and food waste into vermicompost in Japan have been available.

In recent years, sequencing technologies and bioinformatic tools have become widely available. Analysis of 16S ribosomal DNA (rDNA) has been used to determine the microbial communities in various samples including anaerobic sludge (Riviere et al. 2009), dairy farms, and the natural environment (Mulder et al. 2005, Øvreås et al. 2000, Pandey et al. 2018), and in vermicompost (You et al. 2019, Cai et al. 2018). However, bacterial communities in vermicompost derived from school lunch waste have yet to be analyzed or evaluated.

The purpose of this study is to estimate and compare the chemical properties and bacterial communities present in vermicompost and control compost produced using waste from school lunches in Japan. We suggest a cyclical waste recycling system comprised of farmers, schools and vermicomposting technology, whereby earthworms convert school lunch waste into vermicompost, with the resulting manure being used in turn to produce agricultural materials to make school lunches. Our suggestion is considered useful for reducing and recycling school lunch waste in the world.

Materials and methods

1. Composting

To make vermicompost (VC) and control compost (CC), we prepared two 300 L cylindrical plastic containers (φ 81.5 cm, height 85 cm) containing 18 L humus (Iris Ohyama Inc., Sendai, Japan). Humus was added as it provides suitable living conditions for earthworms (You et al. 2019). The composts were set up at the Fujiidera Support School (Fujiidera, Japan) on March 6, 2019. Average air temperature data on site was obtained from the nearest meteorological station in Yao City, Osaka, Japan. In the VC, 300 g of earthworms (*Eisenia foetida* Savigny, commercial name: "Kumatarofutomushi," Fishing Azumino Company, Azumino, Japan) was added to the humus. Approximately 5 kg of mainly vegetable waste and rice paste was collected on weekdays from the kitchen at the Fujiidera Support School. Thereafter, half the amount (i.e., approx. 2.5 kg) was placed in each compost. Through the composting process, both composts were watered once a week to maintain moisture at approximately 80% (w/w) according to You et al. (2019). The composts were not stirred to match the condition of CC with that of VC according to You et al. (2019), although originally composts should be stirred periodically. Water content was adjusted by adding about 5 kg of shredder paper waste from the school once a month. Samples were collected from each compost at 7, 12, and 18 weeks after the start of composting. For microbiome and physicochemical analysis, approximately 100 g of the compost samples was collected from three different locations for each compost and then mixed well to make one pooled sample. Three independent pooled samples were used for the germination test. Earthworms were removed from the VC sample to exclude the effect of chemical components derived from the earthworms themselves.

2. Germination test

The germination test was conducted as per the following procedure (Hase & Kawamura 2012). To obtain water extracts, 10 mL of boiled distilled water was added to 1 g of pooled sample in a conical beaker, and each mixture was placed for one hour at room temperature. The water extracts were poured onto a filter paper in a plastic Petri dish (φ 9 cm). Fifty Komatsuna (*Brassica rapa* var. *perviridis*) seeds were distributed on the filter paper, and incubated at 26°C in the dark for 48 h. Distilled water was used as a control. Water extracts were prepared from VC and CC, and the numbers of germinated seeds and the root length of each germinated seed were obtained. The germination rate (%) was calculated based on the numbers of germinated seeds and total seeds. The Germination Index (GI) was calculated according to the following formula by Zucconi et al. (1981).

$$GI\ (\%) = \frac{\text{Seed germination rate of treatment} \times \text{root length of treatment}}{\text{Seed germination rate of control} \times \text{root length of control}} \times 100$$

3. Microbial populations

The bacterial communities of VC and CC were further examined using next-generation sequencing (NGS). We analyzed the samples of VC and CC at 7, 12 and 18 weeks after starting composting. For DNA extraction by MORAEXTRACT (Kyokutoseiyaku Co., Ltd., Tokyo, Japan), 4 g of the subsample was used. The bacterial V3-V4 region of the 16S rDNA of each sample was amplified by a polymerase chain reaction (PCR) as described by Okano et al. (2016). The PCR products were sequenced using the Illumina MiSeq platform. The bacterial communities of each sample were identified by MacQIIME platform (Caporaso et al. 2010). In order to analyze the microbial diversity, operational taxonomic units were defined by clustering at 3% divergence (97% similarity).

4. Physicochemical analysis

Physicochemical analysis was performed by Kawata Research Co., Ltd. (Tsukuba, Japan). Samples of VC and CC were applied for analysis only once due to limited amount. Chemical properties were measured by following the methods described by the Editorial Committee of Analysis Methods of Soil Environment of Japan (1997) with some modification. Electrical conductivity (EC) was measured in a 1:5 (sample:water) slurry using a conductivity meter (model CM-14P, Toa Co., Tokyo, Japan). Soil pH was measured in a 1:5 (sample:water) slurry using a pH meter (model D-71, Horiba, Ltd, Kyoto, Japan). Cation exchange capacity (CEC) was measured according to the semimicro-Schollenberger method. CaO and MgO concentration was measured by the atomic absorption method. K_2O, P_2O_5, NH_4-N, and NO_3-H were measured by flame photometry, the Truog method, Nessler's method, and UV absorption photometry, respectively.

5. Statistics

Data are represented in terms of the mean ± SE for the germination test. The germination rate, root length, and GI were compared by Tukey's HSD test ($P < 0.05$) using SPSS (version 22; IBM, Co., Armonk, USA).

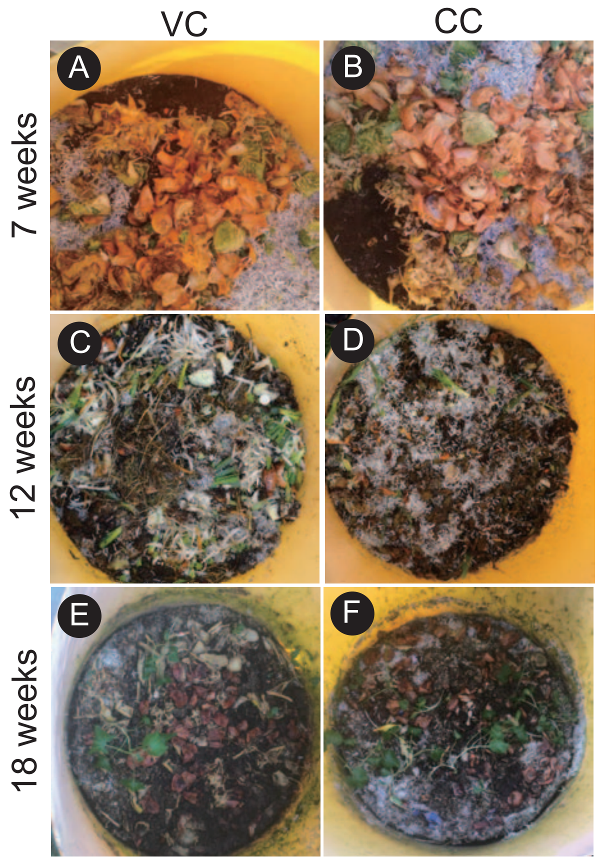


Fig. 1. Product appearance of vermicompost (VC) and control compost (CC)
Each compost was observed at 7 (A and B), 12 (C and D), and 18 weeks (E and F) after the start of composting.

Results and discussion

The composting process of vermicompost (VC) and control compost (CC) was observed at 7, 12 and 18 weeks after the start of the composting process (Fig. 1), and both composts were studied comparatively over time (Fig. 1). Changes in the appearance of the composts indicated that the composting of food waste was in progress (Fig. 1). VC at 18 weeks was almost odorless, whereas CC at the same time point was slightly foul smelling. Average outside air temperature during composting was measured in a range of approximately 10°C to 25°C (Fig. 2).

In order to evaluate the quantitative effect of VC for plant germination and root elongation, we conducted a seed germination test. As a result, the root length in the 18-week-old VC was observed to be significantly longer than those in the 18-week-old CC and water control (Fig. 3 A and B). No significant difference was observed among the treatments for the germination rate, except for that between 7 and 18-week-old VC (Fig. 3 C). It is interesting that the germination rate gradually increased in the 12 and 18-week-old VC samples, although in CC, it showed rather a constant value at all time points (Fig. 3 C). The Germination index (GI) value in VC increased approximately linearly as time passed, whereas GI gradually decreased during the composting process in CC (Fig. 3 D). The GI value in the 18-week-old VC was 193%, significantly higher than the 7-week-old VC, as well as the 18-week-old control compost (Fig. 3 D). The result indicates that the 18-week-old VC might be phytotoxin-free, and may be associated with some water-extractable mineral nutrients and biologically active metabolites. The positive effects of VC on plant growth have previously been reported. Arancon et al. (2012) showed that indole acetic acid (IAA), cytokinin, gibberellin acid (GA), and humic acids present in VC could enhance the germination of tomato seeds and initial root development. The treatment with 50% VC (i.e., 50% vermicompost and 50% soil) reportedly promoted the growth of pepper plants (*Capsicum annuum*) significantly as compared to GA and IAA treated plants (Rekha et al. 2018). Our results clearly indicate that 18-week-old VC offers great potential as an effective bio-fertilizer. Further studies may better clarify the exact reasons as to why VC from school lunch waste was so effective for komatsuna growth.

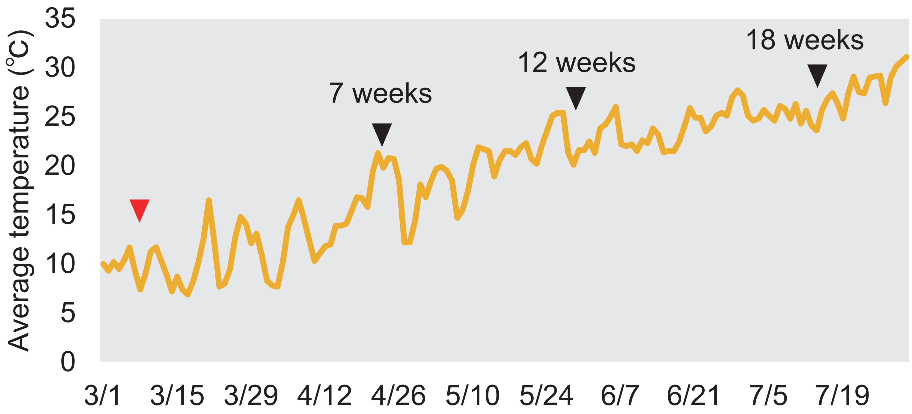


Fig. 2. Average air temperature data in 2019 during composting

Data was obtained from the nearest meteorological station in Yao City, Osaka, Japan. The red arrowhead in the graph denotes the starting time point of composting; black arrowheads denote the sampling time points.

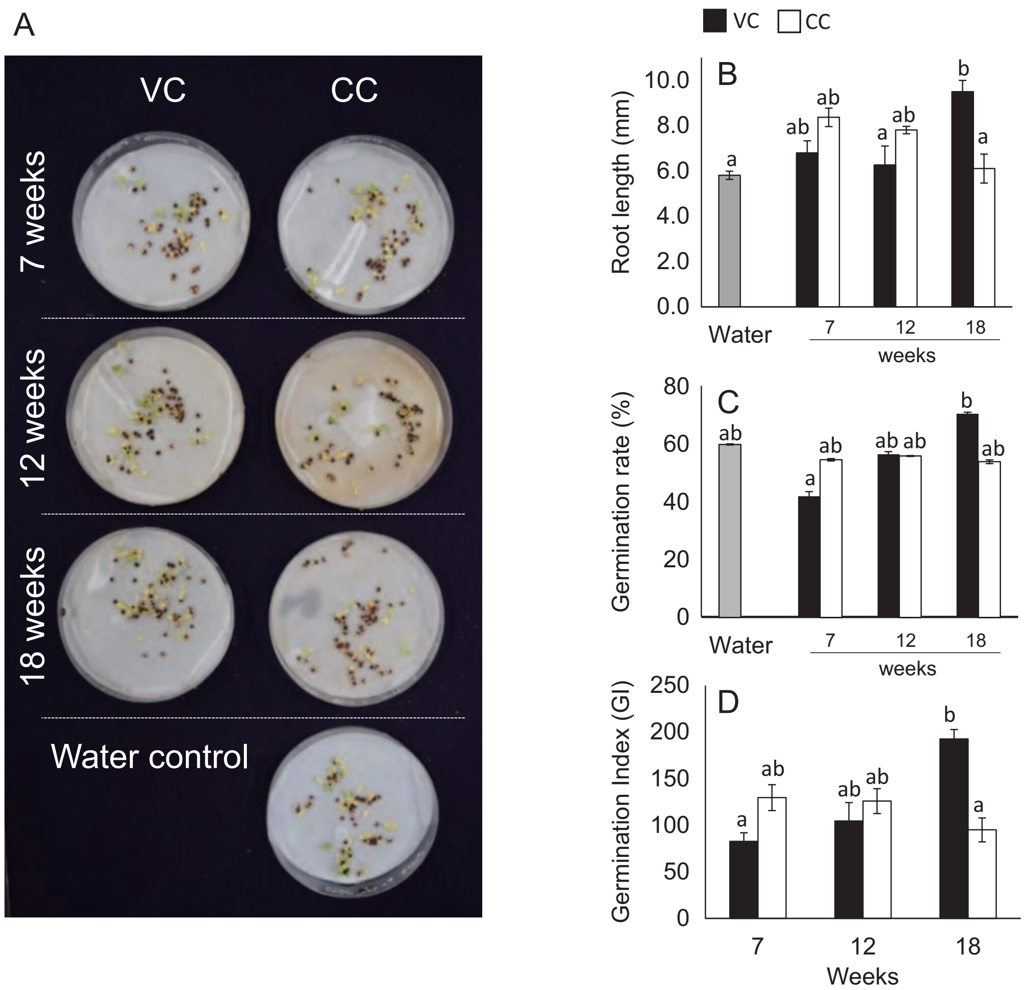


Fig. 3. Komatsuna seed germination test

Water extract from compost was poured onto a filter paper in a plastic Petri dish with Komatsuna seeds, which were incubated at 26°C in the dark for 48 hours (A). The numbers of germinating seeds were counted, and the root length of each seed was measured (B and C). The Germination Index (GI) was calculated by the formula according to Zucconi et al. (1981) (see Materials and methods). Different lowercase letters in each plot (B-D) indicate significant differences (Tukey HSD test, $P < 0.05$, n = 3).

Table 1 lists the chemical properties of VC. In the present study, the C/N ratio of VC and CC was found to be 11.08 and 11.04, respectively, and thus below the indicative value of 20 for acceptable maturity (Morais & Queda 2003). Furthermore, both composts have relatively higher pH (9.9 in VC and 9.5 in CC) than in earlier reports that analyzed vermicomposted vegetable waste or bamboo powder, which showed pH of around 7.5 (Huang et al. 2017 & You et al. 2019). However, it corresponds to compost prepared from kitchen waste whose pH had been previously observed to be around 9 (Garg et al. 2006). Thus, high pH observed in this study may be attributed to complicated food composition as well as kitchen waste. Although high pH is generally inappropriate for composts, it can be effective for improving the acidic soil widely found in Japan. Moreover, the CEC value was approximately two times higher in VC compared with those in CC. CEC is mainly related to clay minerals and organic matter such as humic substances, and vermicompost is enriched in humic substances including humic acid that can promote plant development, especially for root systems (Canellas et al. 2002, Arancon et al. 2003, Arancon et al. 2004). Therefore, in the present study, higher CEC in VC is most likely caused by the increase in humic substances.

Interestingly, NO_3-N content was higher in VC, whereas NH_4-N content was lower compared with CC, suggesting that earthworms enriched the nitrate content and promoted nitrification in VC, as previously reported (Huang et al. 2017). The fact that VC at 18 weeks was almost odorless may be due to the relatively lower NH_4-N content in VC than in CC. There are some reports on vermicompost with NH_4-N and NO_3-N values similar to the values in this study (Mupondi et al. 2010, Cai et al. 2020). Considering that these reports prepared vermicompost from high-carbon wastes such as paper waste and sugarcane bagasse (Mupondi et al. 2010, Cai et al. 2020), the NH_4-N and NO_3-N values obtained in the present study may be influenced by the paper shredder waste added to the compost (see Materials and methods).

In addition, K_2O content in VC was almost two times higher than in CC (Table 1). Higher K content in the VC was observed in Kaviraj & Sharma (2003), reporting that *Eisenia foetida* increased the K content by 10%. The enhanced population of microflora present in the gut of earthworms might have played an important role in increased K_2O over the control (Kaviraj & Sharma (2003). These results indicate VC from school food waste, which showed a low C/N ratio, high CEC, and high content of minerals, has substantial agronomic potential as a useful soil fertilizer, although the results listed in the Table 1 have no replication.

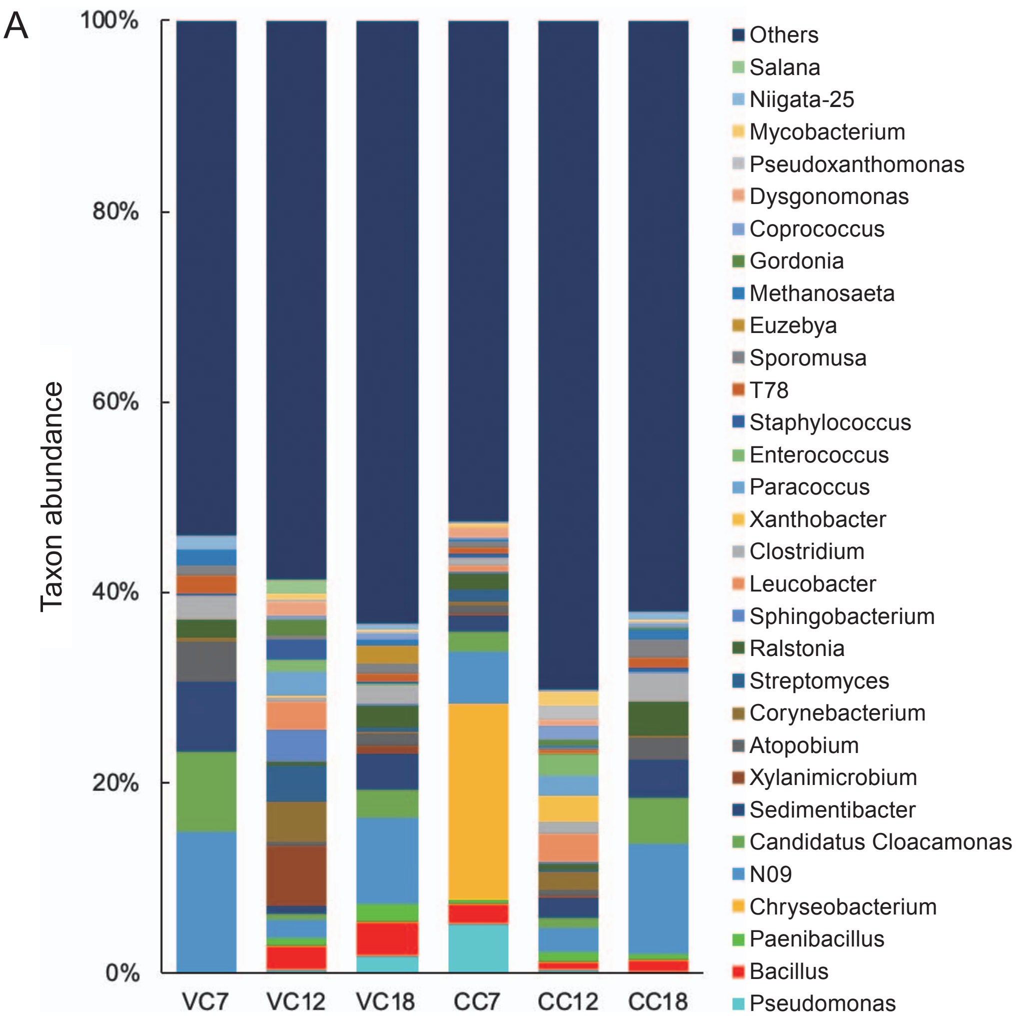
Next-generation sequencing (NGS) analysis revealed that the predominant bacterial genera in both the CC and VC changed at different sample times (Fig. 4 A). In the VC at 7 and 18 weeks, the most dominant genus was N09 (14.7% and 9.0% of the total taxon abundance, respectively). At 12 weeks in the VC, *Xylanimicrobium* was the most dominant (6.3%). In the CC, *Chryseobacterium* (20.6%), *Leucobacter* (3.0%), and N09 (11.6%) were the most dominant genus at 7, 12 and 18 weeks, respectively. In the 18-week-old VC, besides N09, the dominant genera (relative abundance > 1%) include *Sedimentibacter* (3.7%), *Bacillus* (3.5%), *Candidatus Cloacamonas* (3.0%), *Ralstonia* (2.3%), *Paenibacillus* (2.0%), *Euzebya* (1.9%), *Clostridium* (1.9%), *Pseudomonas* (1.8%), and *Atopobium* (1.4%). In the 18-week-old CC, besides N09, the other dominant genera (relative abundance > 1%) were *Candidatus Cloacamonas* (4.7%), *Sedimentibacter* (4.2%), *Ralstonia*

Table 1. Chemical properties of vermicompost (VC) and control compost (CC) at 18 weeks after the start of composting

Parameters	VC	CC
C/N ratio*	11.08	11.04
EC (mS/cm)	5.6	1.8
pH	9.9	9.5
CEC (me/100g)	42	21
CaO (mg/100g)	1,000	1,200
MgO (mg/100g)	310	260
K_2O (mg/100g)	2,500	1,400
P_2O_5 (mg/100g)	460	490
NH_4-N (mg/100g)	0.6	1.3
NO_3-N (mg/100g)	1.1	0.4

* The samples at 22 weeks after the start of composting were used.

(3.4%), *Clostridium* (3.0%), *Atopobium* (2.2%), *Sporomusa* (1.9%), *Bacillus* (1.1%), T78 (1.1%), *Methanosaeta* (1.0%). In both the 18-week-old VC and CC, the genus of N09, *Sedimentibacter*, *Bacillus*, *Candidatus Cloacamonas*, *Ralstonia*, *Clostridium* and *Atopobium* were dominant. *Paenibacillus, Euzebya*, and *Pseudomonas* were dominant in the 18-week-old VC, but not in the 18-week-old CC. *Sporomusa*, T78, and *Methanosaeta* were dominant in the 18-week-old CC, but not in the 18-week-old VC. In addition, the relative abundance of *Bacillus* in the 18-week-old VC was three times higher than in the 18-week-old CC (Fig. 4 A). Among them, *Bacillus*, *Paenibacillus*, and *Pseudomonas* contain many beneficial species that have antagonistic activities against a wide range of phytopathogens, and can promote plant growth. For example, *Bacillus amyloliquefaciens* subsp. *plantarum* FZB42 has nearly 10% of its genome devoted to synthesizing antimicrobial compounds (Chowdhury et al. 2015). Owing to its high ability to suppress phytopathogens, it has been used



B

Family genus	V7	V12	V18	C7	C12	C18
Nitrosomonadaceae	2.25×10^{-2}	2.21×10^{-2}	1.15×10^{-1}	3.78×10^{-2}	1.00×10^{-1}	1.80×10^{-1}
Nitrosomonadaceae Nitrosovibrio	0	0	0	0	5.02×10^{-3}	0
Nitrospiraceae Nitrospira	0	0	1.30×10^{-2}	4.59×10^{-3}	0	0

Fig. 4. Microbial diversity during control composting and vermicomposting of school food waste

The soil samples of VC and CC at 7, 12, and 18 weeks after we started composting were analyzed and taxon abundance (%) was calculated (A). B: The abundance (%) of bacteria involved in the nitrification process. *Nitrosomonadaceae* was indicated at the family level because the family could not be classified into a specific genus except *Nitrosovibrio*.

commercially as a biocontrol agent in agriculture. Interestingly, the relative abundances of all three genera increased as vermicomposting progressed. It can be inferred that their microbial activities may be stimulated by earthworms. This agrees with several previous studies demonstrating that earthworms strongly modify the microbial diversity and activity during vermicomposting (Aira et al. 2007, Huang et al. 2013).

Intriguingly, nitrite-oxidizing bacteria *Nitrospira*, which oxidizes nitrite to nitrate, were detected in VC product at 18 weeks (Fig. 4 B). Huang et al. (2017) investigated the mechanism of earthworms promoting nitrification and demonstrated that final vermicompost products were abundant in the members of *Nitrosomonas* and *Nitrosospira*. Thus, *Nitrospira* detected in this study probably contributed to nitrification in VC. This is supported by the result that demonstrated relatively higher NO_3- N content in VC than in the CC (Table 1).

It is important that final vermicomposting products do not contain pathogens for human and plants for application to farms. Regarding this point, vermicomposting has reportedly eliminated *Salmonella* and *Escherichia* sp., and earthworm gut analysis also proved that *Salmonella* sp. ranging 15-17 × 10^3 CFU/g and *Escherichia* sp. ranging 10-14 × 10^2 CFU/g were completely eliminated in the gut after 70 days of the vermicomposting period (Pathma & Sakthivel 2012, Ganesh Kumar & Sekaran 2005). Therefore, vermicompost in our study is expected to be acceptable manure, although a more detailed investigation is still necessary.

The Japanese school lunch system is attracting more worldwide attention these days as it can provide flavorful and nutritionally balanced food beneficial for schoolchildren. Consequently, school food waste will also pose a serious problem if other countries introduce this system in the future. Our present study is valuable for reducing and recycling food waste in schools, not only in Japan but also worldwide.

Conclusion

The final product of vermicomposting made from school food waste may be more suitable for application in agriculture, as evidenced by its positive effect on komatsuna seed germination, and the abundant populations of bacteria. These results demonstrate the significance of vermicompost in utilizing waste from school lunch, suggesting a viable waste recycling system that focuses on reducing and recycling food waste in schools through vermicomposting.

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