Ecological studies on extremely thermophilic bacteria in various environments

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Thesis Summary

Recycling organic wastes as an alternative to chemical fertilizers has been considered a great option to promote environmentally sound waste disposal. Sewage sludge, or biosolid, is a by-product of wastewater treatment and the waste that remains after the process. Sewage sludge contains organic matters making it a good candidate for composting. Composting is a microbial process that converts organic waste into a nutrient-rich end product used in horticultural and agricultural applications. In Kagoshima City, Japan, a hyperthermal composting process wherein the internal temperature reaches 95˚C–100˚C was developed. In this process, sewage sludge is being employed as the organic material to produce a unique compost called Satsuma soil. This composting process is conducted for 40–45 days. Several thermophilic bacteria, including extreme thermophiles, are believed to be involved in the process of hyperthermal composting. To date, most thermophilic bacteria isolated from sludge compost are moderate thermophiles and these thermophiles are constantly being isolated from sludge compost in recent years. Over the last few years, we have been investigating the existence of extreme thermophiles in sewage sludge and compost using both qualitative and quantitative methods. Extreme thermophiles have been isolated from various environments exposed at both thermal and non-thermal temperatures. In the past, extreme thermophiles such as *Calditerricola* sp. and *Thermaerobacter* sp. found in sewage sludge and compost produced through hyperthermal composting were studied, and the occurrence of extreme thermophiles in sewage sludge was reported. We were also able to isolate these extreme thermophiles from Mt. Sakurajima volcanic ash samples. However, so far, their origin and ecology remain unknown, and a quantitative method for their detection has not been established yet. With this, we became interested with the bacterial composition of digested sludge, compost, and volcanic ash, and would like to determine the correlation of extreme thermophiles among these samples. Currently, no study has reported the existence of extreme thermophiles in volcanic ash, which has given us much interest in pursuing. This study aims to (1) establish a novel molecular biological detection method to qualitatively and quantitatively survey the ecological distribution of extreme thermophiles from various environments and to (2) determine the bacterial composition of various environments that could define the ecological features of extreme thermophiles such as *Calditerricola*.

Firstly, a survey on the ecological distribution of the extremely thermophilic bacteria *Calditerricola* spp. using a newly established enumeration method called enrichment most probable number (eMPN)-PCR was done. The extremely thermophilic bacterial genus *Calditerricola* was initially isolated from the high-temperature compost. Likewise, the bacteria were previously isolated from material sludge. It is believed that bacteria in this genus might be involved in the hyperthermal composting process. *Calditerricola* bacteria are distributed not only in compost, but also in all of its material sludge, and are more abundant in material sludge than in compost. Moreover, based on investigations of samples near geothermal areas in high temperature conditions, such as volcanoes, *Calditerricola* was presumed to originate in the volcanic ash of Mt. Sakurajima in Kagoshima City, Japan. However, its precise origin and ecology are unclear. Thus, in this study, a new method called eMPN-PCR was established and used to quantitatively investigate the population and distribution of *Calditerricola* spp. in environmental samples using genus-specific PCR primers. The eMPN-PCR method was an effective quantitative detection method with high sensitivity, yielding MPN estimates that were highly correlated with

CFU estimates but a low detection threshold value.

Next, the bacterial community structures of environmental samples such as digested sludge, compost, and volcanic ash from Mt. Sakurajima were studied using denaturing gradient gel electrophoresis (DGGE) and MiSeq analyses. For the DGGE analysis, VA1, VB1, VC1, and D2 were isolated from volcanic ash A, B, and C, and digested sludge, respectively. The identity of the isolates were revealed to be *T. marianensis* for VA1 and VB1, *T. composti* for VC1, and *C. satsumensis* for digested sludge. Species closely related to *T. marianensis* exists in volcanic ash B and D, digested sludge, and compost. Species closely related to *C. satsumensis* exists in volcanic ash B and C, digested sludge, and compost samples. Species closely related to *T. composti* exists in volcanic ash C and E, digested sludge, and compost samples. Isolation and identification of extreme thermophiles using 16S rRNA gene analysis were also done in this study. Isolates from digested sludge, compost, and volcanic ash samples were roughly divided into three clusters. The largest cluster is cluster 1, consisting of isolates from digested sludge, compost, and volcanic ash, which were found to be most closely related to *T. composti*. Cluster 2 was formed by isolates from volcanic ash B only and were closely related to *T. marianensis*. Cluster 3 was formed only by isolates from volcanic ash B (VB5) and digested sludge which were closely related to *C. satsumensis*. For the MiSeq analysis, results show a very little population shared by all four enriched samples (digested sludge, compost, volcanic ash A and B). Only five genera (1.2%) were found to be common in all the enriched samples that might suggest a common role in the process of hyperthermal composting. We expect that these bacteria present in all of the enriched samples were thermophiles since the samples were enriched at a very high temperature (75˚C). An OTU table representing the abundance of each OTU in each microbial sample was constructed and the generated OTU table was utilized to make a heatmap. The appearance of each OTU in a sample were quality filtered to 83, representing the dominant 1% of the total OTU count. Among the selected OTUs, only 11 were found to be thermophilic bacteria. Out of the 11 thermophilic bacteria, two are extremely thermophilic corresponding to *Caldicoprobacter faecalis* and *Calditerricola yamamurae*, respectively. An increase in population of *C. faecalis* and *C. yamamurae* after enrichment at 75˚C supports our hypothesis that the extreme thermophiles found in samples from Kagoshima City could have originated from Mt. Sakurajima through the (1) spread of bacterial cells or spores being carried in the volcanic ash, or through the (2) outflow of ground water contaminated with bacterial cells or spores.

Lastly, the thermophilic bacterial composition of the volcanic ash of Mt. Mayon in the Philippines was analyzed using the Illumina MiSeq sequencing platform. Previously, we studied the diversity and bacterial composition of volcanic ash from Kagoshima City, Japan. We were able to isolate and identify a few extreme thermophiles from these samples. In relation to this, both Japan and the Philippines are archipelagos in the Ring of Fire of the Pacific Ocean wherein a large number of earthquakes and volcanic eruptions occur. Therefore, a comparison between the thermophilic bacterial composition of volcanic ash samples from two different volcanoes, Mt. Sakurajima in Japan and Mt. Mayon in the Philippines, from the two countries seemed interesting. Experiments on determining the microbial diversity of Mt. Mayon volcanic ash, led to the discovery that volcanic ash samples from Mt. Mayon and Mt. Sakurajima share a common bacterial genus that is *Calditerricola*, which has also been observed in sludge and compost samples in the past.