

Effect of high compost temperature on enzymatic activity and species diversity of culturable bacteria in cattle manure compost

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Abstract

To clarify the characteristics of thermophilic bacteria in cattle manure compost, enzymatic activity and species diversity of cultivated bacteria were investigated at 54, 60, 63, 66 and 70 °C, which were dependent on composting temperature. The highest level of thermophilic bacterial activity was observed at 54 °C. Following an increase in temperature to 63 °C, a reduction in bacterial diversity was observed. At 66 °C, bacterial diversity increased again, and diverse bacteria including *Thermus* spp. and thermophilic *Bacillus* spp. appeared to adapt to the higher temperature. At 70 °C, bacterial activity measured as superoxide dismutase and catalase activity was significantly higher than at 66 °C. However, the decomposition rate of protein in the compost was lower than the rate at 66 °C due to the higher compost temperature.

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1. Introduction

Composting is a biological process by which temperature rises to above 70 °C due to metabolic heat production from biodegradation (De Bertoldi et al., 1983). When compost temperature is greater than 60 °C, activity and diversity of compost bacteria are greatly suppressed, and strains related to *Bacillus stearothermophilus* are dominant (Jeris and Regan, 1973; Strom, 1985a; Suler and Finstein, 1977). However, several researchers have

suggested the presence of diverse bacteria in hot compost (Blanc et al., 1999; Dees and Ghiorse, 2001; Peters et al., 2000) and an increase in respiratory activity at temperatures above 65 °C (Beffa et al., 1996). Such findings are contrary to previous reports that bacterial activity and diversity are reduced dramatically at temperatures above 60 °C.

To clarify the characteristics of thermophilic bacteria in compost, it is necessary to determine the activity and diversity of the bacteria in more detail. In this paper, superoxide dismutase (SOD) and catalase activities were investigated as indicators of bacterial activity. SOD and catalase are antioxidative enzymes that reduce harmful reactive oxygen species produced during aerobic metabolism. Ma et al. (2003) has also used the antioxidative enzyme activity as a marker of microbial activity and respiration during composting process. Extracellular lactate dehydrogenase (LDH) activity was also measured to evaluate the amount of extinct bacteria. It is

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commonly used as an index for cell death (Bunthof et al., 2001). In addition to the enzymatic activities, we present a dendrogram based on random amplified polymorphic DNA (RAPD) profiles of culturable bacteria for analyzing species diversity.

2. Methods

2.1. Compost

Compost samples were prepared as follows. Dairy cattle manure from the University farm was dehydrated to about 60% wb at ambient temperature using an electrical fan. The manure was self-heated and then kept at 60 °C for several days in a laboratory-scale adiabatic compact reactor of one liter with a ventilation of 60 ml/min. Average temperature of the manure was 60.7 °C during the process. The purpose of preliminary composting was to eliminate all microorganisms irrelevant to composting (i.e. pathogenic bacteria) in order to create a more accurate microbiological analysis.

Seventy grams of the precomposted manure, which was adjusted to a moisture content of 60% wb using sterile water, was composted isothermally in a 250-ml glass reactor for three days with forced aeration of 15 ml/min through a humidifier. The composting of each treatment temperature was carried out each once. The compost samples were subjected to microbiological analysis following isothermal composting at temperatures of 54, 60, 63, 66 and 70 °C. Moisture contents in final composts decreased about 8% at most, and values of the pH ranged from 8.3 to 8.4.

2.2. Enzymatic activity analysis

For enzymatic activity analysis of compost bacteria, samples were prepared as follows. Compost samples (10 g wet weight) obtained at each temperature were homogenized at 9,500 rpm for 5 min with 90 ml of sterile water. One milliliter of suspension was seeded in 15 ml of Trypto-soya broth (Nissui, Japan) in dish. Five dishes were prepared for SOD activity assay, and three dishes for LDH per treatment. The dishes were incubated statically for one day at the same temperature as that from which the compost sample was taken. To obtain an enzymatic solution containing bacterial SOD and catalase, the culture was mixed with the equal volume of phosphate-buffer saline (PBS) including 0.2% (v/v) triton X-100, and sonicated for 10 min. After centrifugation at 10,000g for 30 min, the supernatant was used to determine the activity levels of SOD and catalase. To determine extracellular LDH activity, the supernatant of the incubated culture, which was centrifuged at 10,000g for 30 min, was subjected to measurement.

SOD activity was determined using the xanthine-xanthine oxidase-nitroblue tetrazolium (NBT) method (Beauchamp and Fridovich, 1971), catalase activity was determined using the spectrophotometric method (Aebi, 1963) and extracellular LDH activity was determined following the method of Wróblewski and LaDue (1955).

2.3. RAPD and diversity analysis

To obtain pure strains from the compost samples at 54, 60, 63, 66 and 70 °C, samples were prepared as follows. The homogenate compost described above was serially diluted in sterile water spread onto Trypticase soy agar (TSA; Difco, USA) plates. After incubation for one day at the same temperature that each compost sample was taken, 20 colonies on each plate were randomly selected, and subcultured again using a TSA agar plate.

Genomic DNA of each of the pure strains was extracted as described by Tanaka et al. (2000) with slight modifications. Fragments of the DNA were amplified utilizing the RAPD-polymerase chain reaction (PCR) method (Williams et al., 1990). PCR reaction mixtures were prepared according to the manufacturer's instructions for DNA polymerase (Amplitaq Gold; Applied Biosystems, USA) using five random Primers (Operon 10-mer kit A, OPA-2, 3, 7, 10, 13; Operon Technologies, Inc., USA). The PCR protocol was as follows: initial denaturation at 95 °C for 9 min; 45 cycles of 94 °C for 1 min, 36 °C for 1 min, 72 °C for 2 min; and a final elongation for 10 min at 72 °C. PCR products were then electrophoretically separated and visualized in 1.5% agarose gels stained with ethidium bromide. The visualized PCR products had RAPD profiles that consisted of various sizes of DNA fragments. A dendrogram based on these RAPD profiles was constructed using the unweighted pair group method with arithmetic average (UPGMA) (Sneath and Sokal, 1973) and the similarity coefficients were calculated as described by Nei and Li (1979).

Bacterial diversity analysis was based on the results of the dendrogram. The species diversity index was calculated by using Shannon index (Shannon and Weaver, 1949).

2.4. Decomposition rate of protein

The decomposition rate of protein was calculated by comparing the protein content of initial and final compost samples. For protein assay, each 1.0 g of wet compost was extracted with 9 ml of 0.1 N NaOH (McKinley and Vestal, 1985). After vortexing and sonication for 10 min, the protein in supernatant, which was centrifuged at 10,000g for 30 min, was measured using the method of Lowry et al. (1951).

2.5. Statistical analysis

Data are expressed as means \pm standard errors. Statistical comparison was performed using the Bonferroni–Dunn test. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Enzymatic activities of compost bacteria

Enzymatic activities of bacteria related to composting temperature are shown in Figs. 1 and 2.

Fig. 1 shows that SOD and catalase activities at 54 °C were significantly higher than those at the other temperatures. At 70 °C, an increase in SOD activity was again observed but a corresponding rise in catalase activity was not detected.

Fig. 2 shows extracellular LDH activity increased at temperatures above 63 °C, suggesting that a certain type of compost microorganism began to die at this temperature.

3.2. Dendrogram and species diversity

Fig. 3 shows the UPGMA dendrogram based on the RAPD profiles of compost bacteria. These profiles were classified into 27 distinct bacteria, labeled 1 to 27, and 14 separate groups, labeled A to N, according to a level of 75% similarity. Strains in groups A and B were isolated

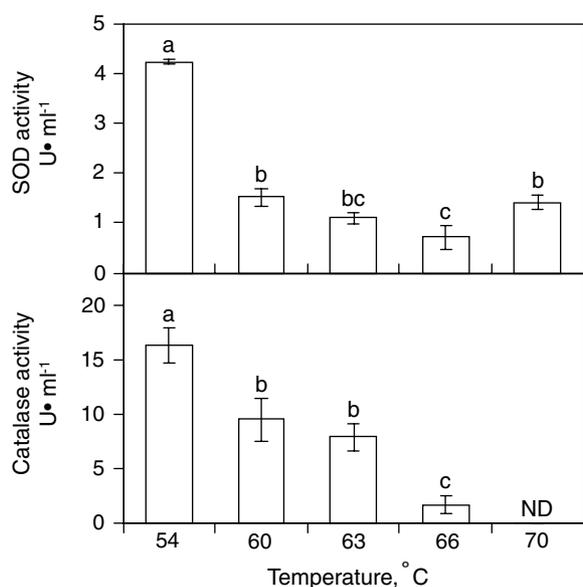


Fig. 1. Effect of temperature on SOD and catalase activities ($n = 5$) of bacteria. Data are expressed as means \pm standard errors. Values with the same letter are not significantly different at $P < 0.05$. ND: not detected.

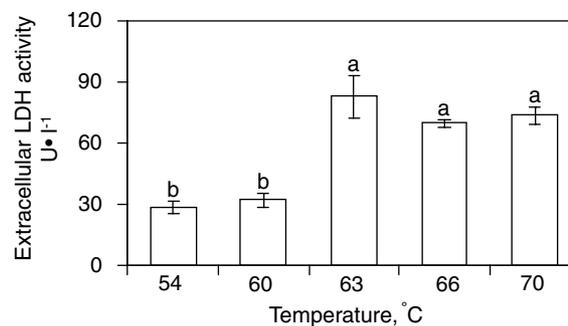


Fig. 2. Effect of temperature on extracellular LDH activity ($n = 3$) of bacteria. Data are expressed as means \pm standard errors. Values with the same letter are not significantly different at $P < 0.05$.

at a wide range of temperatures from 54 to 70 °C. Approximately 95% of strains in groups C, D, E, and F were isolated at 66 and 70 °C. Strains in groups I to N were isolated at 54 and 60 °C.

According to microscopic observation, the strains in groups C, E, and F were spore-forming bacteria, while the strains in group D were a mixture of spore-forming and nonspore-forming bacteria. In particular, the non-spore-forming bacteria had long rods or filaments, which were similar to *Thermus* strains isolated from hot compost by Beffa et al. (1996).

The species diversity index of compost bacteria at each temperature presented large values at both ends of the temperature range of 54 and 70 °C, and a minimum value at 63 °C (2.97, 2.90, 1.90, 2.74 and 3.21 for 54, 60, 63, 66 and 70 °C respectively).

3.3. Protein decomposition

The decomposition rate of protein in compost samples was found to decrease as temperature increased (Fig. 4). The maximum decomposition rate was 65.5% at 54 °C and the minimum rate was 22.3% at 70 °C.

4. Discussion

The aim of this study was to investigate the effects of high compost temperature on enzymatic activity and species diversity of culturable bacteria in dairy cattle manure. Based on the results, three important characteristics of thermophilic compost bacteria can be more fully understood.

First, the highest levels of SOD and catalase activity in thermophilic bacteria were observed at 54 °C and decreased sharply after 60 °C (Fig. 1). The decline in activity did not coincide with microbial extinction but with a decrease in metabolic activity. In fact, extracellular LDH activity and the species diversity index value at 60 °C were almost the same as those at 54 °C (Fig. 3 and see Section 3.2). Our observation of a decrease in

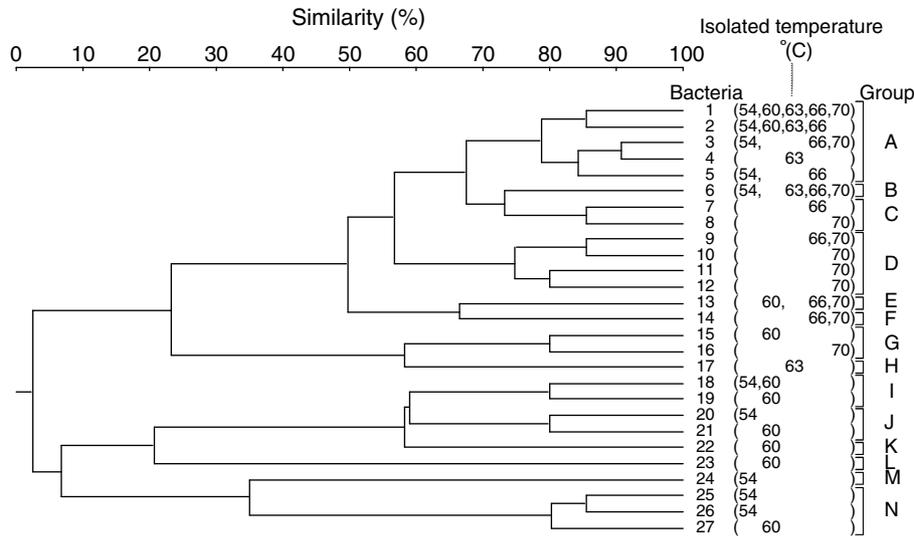


Fig. 3. UPGMA dendrogram based on RAPD profiles of cultivated bacteria.

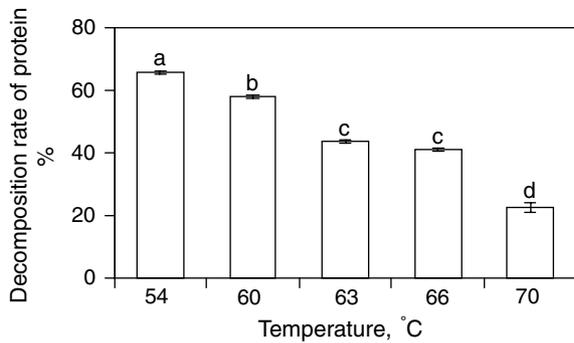


Fig. 4. Relationship between temperature and decomposition rate of protein ($n = 5$). Data are expressed as means \pm standard errors. Values with the same letter are not significantly different at $P < 0.05$.

bacterial activity at temperatures of 60 °C and higher is strikingly similar to previous studies that reported a decrease in CO₂ production and oxygen consumption at temperatures above 60 °C (Jeris and Regan, 1973; Suler and Finstein, 1977).

Second, at 63 °C, extracellular LDH activity reached the highest level (Fig. 2), and the species diversity index value was the lowest (see Section 3.2), indicating that bacterial diversity is reduced and certain bacteria die at 63 °C. According to the dendrogram, strains in groups I to N were not isolated at temperatures of 63 °C or higher (Fig. 3). Thus, the extinction of the strains in groups I to N induced the increase in extracellular LDH activity and the decrease in the species diversity index.

Third, an increase in SOD activity was observed at 70 °C (Fig. 1) without a corresponding increase in catalase activity. This suggests that the bacterial metabolism functioning at 60 °C differs from that functioning at other temperatures. This data indicates that activating

bacteria appear in extremely high-temperature conditions. Furthermore, the species diversity increased at temperatures above 66 °C (increase from 1.90 for 63 °C to 2.74 for 66 °C). According to the dendrogram, the strains in groups C, D, E, and F were isolated at 66 and 70 °C (Fig. 3). Beffa et al. (1996) reported the presence of large numbers of thermophilic bacteria related to the genus *Thermus* with rapid growth and high respiratory-activity rates from 65 to 75 °C. We also observed *Thermus* strains, as well as thermophilic *Bacillus* spp. that were frequently observed in hot compost at temperatures above 66 °C (Strom, 1985a,b). These diverse bacteria, including *Thermus* spp. and thermophilic *Bacillus* spp., may lead to the increase in SOD activity observed at 70 °C. However, the decomposition rate of protein decreased with the increasing compost temperature (Fig. 4), and therefore no increase in decomposition of organic matter in manure can be expected at 70 °C. These results show that diversity and activity of bacteria increase between 66 and 70 °C, although the organic decomposition rate declines.

In the present paper, we demonstrated that enzymatic activity and species diversity of thermophilic compost bacteria are affected by composting temperatures between 54 and 70 °C. The results obtained show that the highest activity of thermophilic bacteria was observed at 54 °C. When the temperature increased to 63 °C, a certain group of bacteria died out, resulting in an overall reduction in bacterial diversity. At 66 °C, bacterial diversity increased again, and diverse bacteria including *Thermus* spp. and thermophilic *Bacillus* spp. appeared to adapt to the higher temperature. At 70 °C, activity of these diverse bacteria increased, but the decomposition of manure organic matter was reduced due to the higher temperature.

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