Microbial Ecology

Species Diversity and Substrate Utilization Patterns of Thermophilic Bacterial Communities in Hot Aerobic Poultry and Cattle Manure Composts

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Abstract

This study investigated the species diversity and substrate utilization patterns of culturable thermophilic bacterial communities in hot aerobic poultry and cattle manure composts by coupling 16S rDNA analysis with Biolog data. Based on the phylogenetic relationships of 16S rDNA sequences, 34 thermophilic (grown at 60°C) bacteria isolated during aerobic composting of poultry manure and cattle manure were classified as *Bacillus licheniformis*, *B. atrophaeus*, *Geobacillus stearothermophilus*, *G. thermodenitrificans*, *Brevibacillus thermoruber*, *Ureibacillus terrenus*, *U. thermosphaericus*, and *Paenibacillus cookii*. In this study, *B. atrophaeus*, *Br. thermoruber*, and *P. cookii* were recorded for the first time in hot compost. Physiological profiles of these bacteria, obtained from the Biolog Gram-positive (GP) microplate system, were subjected to principal component analysis (PCA). All isolates were categorized into eight different PCA groups based on their substrate utilization patterns. The bacterial community from poultry manure compost comprised more divergent species (21 isolates, seven species) and utilized more diverse substrates (eight PCA groups) than that from cattle manure compost (13 isolates, five species, and four PCA groups). Many thermophilic bacteria isolated in this study could use a variety of carboxylic acids. Isolate B110 (from poultry manure compost), which is 97.6% similar to *U. terrenus* in its 16S rDNA sequence, possesses particularly high activity in utilizing a broad spectrum of substrates. This isolate may have potential applications in industry.

Introduction

Aerobic composting is a self-heating, aerobic, and microbial degrading process that can convert organic waste materials into plant nutrients. A typical aerobic composting process comprises three stages: a short initial mesophilic phase, followed by a thermophilic phase, and a cooling or maturation phase [34]. Initially, organic wastes are decomposed by mesophilic fungi, actinomycetes, and bacteria. Later on, as the temperature increases, thermophilic bacteria become dominant in the compost, and the mesophilic microbes gradually decline. Finally, in the maturation phase, mesophilic microbes reappear in compost, whereas most thermophilic bacteria remain viable [17, 36].

Several methods have been used to investigate the microbial flora during aerobic composting. By using a culture-dependent approach, highly diversified bacteria, fungi, and actinomycetes were isolated from the mesophilic phase and the maturation phase, whereas limited species of bacteria, predominantly Gram-positive (GP) and spore-forming *Bacillus*, were isolated in the thermophilic phase [34]. In addition, a culture-independent approach that analyzed phospholipids of cell membrane also revealed few varieties of microbes in the thermophilic phase [5, 33]. Alternatively, by using a molecular–genetic approach of 16S rDNA sequence analysis, Blanc *et al.* [3] found *Thermus* and *Bacillus* species in hot compost. Moreover, recent studies by Dees and Ghiorse [9] and Juteau *et al.* [18] unanimously concluded that microbial communities in hot composts were dominated by *Bacillus* species. However, it is worth noting that these studies primarily focused on identification of compost bacteria, with little discussions about their substrate utilization preferences.

The aims of this study were to investigate culturable dominant thermophilic bacteria in hot aerobic poultry

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and cattle manure composts in Taiwan and to characterize their substrate utilization patterns. At a growth temperature of 60°C, we isolated dominant thermophilic bacteria from two hot aerobic composts. Based on genetic characteristics, these bacteria were classified by phylogenetic analysis of the 16S rRNA gene (rDNA). By employing the Biolog GP microplate system, we obtained substrate utilization patterns of individual bacteria. These Biolog physiological profiles were further subjected to principal component analysis (PCA) to characterize and compare the preference of substrate utilization between two bacterial communities.

Methods

Composting Facility and Sample Collection. Source B and C composts were processed from poultry manure and cattle manure, respectively. Poultry manure and cattle manure were mixed with sawdust and water to adjust the C/N ratio (20 for source B, 20—25 for source C) and moisture content (65%). Each composting pile was processed, with automated turning and aeration, in a semiclosed facility [$6 \times 1 \times 100$ m ($W \times H \times L$)]. Composting samples representing week 1 to week 8 composting piles were collected under a depth of 50 cm (composting temperature varied from 55°C to 74°C). Sample collections were conducted three times (compost triplicates) for each source of compost. The samples were maintained at 4°C before bacterial isolation, and bacteria were isolated within 24 h.

Bacterial Isolation and Culture Condition. The collected samples were serially diluted via the plate counting method. Aliquots (0.1 mL) of serial diluted samples were spread onto tryptic soy agar (TSA; Difco, Detroit, MI, USA) and incubated at 60°C for 24 h. After incubation, bacteria that could grow over 10^6 fold dilutions were isolated from plates and cultured in 1 mL tryptic soy broth (TSB; Difco) at 60°C.

Chemical Analysis. Total nitrogen and carbon contents were determined by semimicro-Kjeldahl method and Walkley–Black method, respectively [11, 28]. Each sample was heated at 105°C for 24 h to measure its dry mass. The pH value was determined with a pH meter by suspending 10 g of the dry sample in 50 mL distilled water. Moisture content was calculated after deducting dry mass of the sample.

16S rDNA Amplification and Sequencing. Chromosomal DNA of bacterial isolates was extracted and used as the template for 16S rDNA amplification [29, 32]. DNA fragments, corresponding to positions 9 to 1510 of 16S rDNA of *Escherichia coli*, were amplified by polymerase chain reaction (PCR) using 27f and 1492r as primers [22]. Each PCR reaction mixture contained 10 mM of Tris–HCl (pH 9.0), 50 mM of KCl, 1.5 mM of $MgCl_2$, 0.2 mM of dNTP, 100 ng of chromosomal DNA template, 20 pM each of primers 27f and 1492r, and 2 U of *Taq* DNA polymerase (Promega, Madison, WI, USA) in a final volume of 50 μL. After a 30-s denaturation at 94°C, the reaction mixture was subjected to 25 cycles (50°C, 1 min; 72°C, 1.5 min; 94°C, 1.5 min) and a final extension at 72°C for 5 min in PerkinElmer GeneAmp 9600 PCR system (Applied Biosystems, Foster City, CA, USA). PCR products were purified with a Nucleo Trap PCR purification kit (Clontech, Palo Alto, CA, USA) and sequencing reactions were performed by using a SequiTherm Excel II DNA sequencing kit (Epicentre Technologies, Madison, WI, USA). Sequences were determined by LICOR automated DNA sequencer (model 4200SD; Licor, Lincoln, NE, USA). Both complementary strands of the amplified 16S rDNA fragments were sequenced using the primers 530f and 907r, respectively [22]. The GenBank accession numbers for the 16S rDNA sequence of 34 thermophilic isolates (C74, B39, B60, B64, B65, B66, B68, B72, B80, B88, B89, B99, B101, B103 to B110, C32, C34, C43, C54, C57, C59, C62, C66, C70 to C73, and B77) are from DQ153949 to DQ153982.

Phylogenetic Analysis of 16S rDNA Sequence. By using the Phylip 3.62 software [12], 16S rDNA sequences of bacterial isolates in this study were aligned and clustered against those of the family *Bacillaceae*, which were available from GenBank (*Geobacillus stearothermophilus*, X60640; *G. kaustophilus*, X60618; *G. thermocatenulatus*, Z26926; *G. uzenensis*, AF276304; *G. thermoleovorans*, Z26923; *G. thermodenitrificans*, Z26928; *G. caldoxylosilyticus*, AF067651; *G. thermoglucosidasius*, X60641; *G. toebii*, AB116117; *G. stearothermophilus*, X60640; *Bacillus firmus*, X60616; *B. azotoformans*, X60609; *B. circulans*, X60613; *B. licheniformis*, X60623; *B. pumilus*, X60637; *B. subtilis*, AB018486; B. amyloliquefaciens, X60605; *B. atrophaeus*, X60607; *B.* thuringiensis, X55062; *Ureibacillus terrenus*, AJ276403; *U.* thermosphaericus, X90640; *Brevibacillus thermoruber*, Z26921, *Br. levickii*, AJ715378; *Br. borstelensis*, D78456; *Br.* agri, D78454; *Paenibacillus granivorans*, AF237682; *P. agar*idevorans, AJ345023; *P. chibensis*, D85395; *P. azoreducens*, AJ272249; *P. popilliae*, AF071859; *P. borealis*, AJ011322; *P.* chinjuensis, AF164345; *P. cookii*, AJ250317). Genetic distance was computed based on Kimura's [20] two-parameter model, and phylogenetic trees were generated by the maximum parsimony method using Phylip 3.62 software.

Principal Component Analysis of Biolog Data. Using the Biolog GP microplate system (Biolog, Hayward, CA, USA), we obtained 95 sole-carbon utilization patterns from bacterial isolates. Biolog data were converted into

Table 1. Chemical analysis of raw material and mature product of poultry (B) and cattle (C) manure composts

Source	*Material*	*pH*	*Moisture (%)*	*Nitrogen (%)*	*Carbon (%)*	*C/N Ratio*
B	Poultry manure	7.9–8.5	71–74	2.0–2.2	20.7–33.1	10–15
	Sawdust	4.8	38	1.5	58.0	39
	Mature compost	7.5–8.0	16	2.3–2.5	27.6–30.9	12–13
C	Cattle manure	8.3–8.7	82–83	1.5–2.0	38.5–39.1	19–26
	Sawdust	6.0	36	1.3	41.3	32
	Mature compost	7.9–8.3	16	2.3–2.4	26.5–31.4	12–13

five grades or values, including −1 (Biolog data < 0), 0 (0–29), 1 (30–39), 2 (40–99), and 4 (>100); these values were subjected to PCA using SPSS 12.0 (SPSS, Inc., Chicago, IL, USA) software. PCA simultaneously calculates many correlated variables and identifies the lowest number needed to accurately represent the structure of the data set. The first principal component (PC1) axis is formed from those original variables with the highest variance. The second principal component (PC2) is based on original variables that are uncorrelated with PC1.

Results

Chemical Characteristics of Poultry and Cattle Manure Composts. The feedstock or raw material of compost source B comprised poultry manure and sawdust, whereas that of compost source C comprised cattle manure and sawdust. Chemical characteristics of the raw material and mature product of both composts are listed in Table 1. Poultry manure contained higher nitrogen (2.0–2.2%) and lower carbon contents (20.7–33.1%) than cattle manure (1.5–2.0% for nitrogen, 38.5–39.1% for carbon) (Table 1). Consequently, the C/N ratio of poultry manure (10–15) was much lower than that of cattle manure (19–26).

Isolation of Dominant Thermophilic Bacteria. Serial dilution method was carried out to measure culturable bacterial numbers in hot aerobic poultry and cattle manure composts. At a growth temperature of 60°C, viable bacteria exceeded 10^6 CFU g compost^{-1}. In contrast, more than 10^8 CFU g compost^{-1} of viable bacteria were estimated when the growth temperature was set at 50°C. In this study, we recovered a total of 34 thermophilic (grew at 60°C) bacteria dominating (over 10^6 CFU g^{-1}) in hot aerobic composts, including 21 isolates (B39, B60, B64, B65, B66, B68, B72, B77, B80, B88, B89, B99, B101, and B103–B110) from compost source B (poultry manure) and 13 isolates (C32, C34, C43, C54, C57, C59, C62, C66, and C70–C74) from source C (cattle manure) (Table 2). These bacteria showed variable Gram stain results, and were identified as Gram-positive by KOH method [16]. They were endospore-forming bacilli that grew well at 60°C.

Species Diversity of Bacterial Communities. Based on phylogenetic analysis of the 16S rDNA sequence, 34

Table 2. Bacterial species determined by 16S rDNA sequence analysis and their PCA groups categorized according to substrate utilization patterns

Species	*PCA group*	*Isolates (Similarity %)*[a]
Bacillus licheniformis	4	C32 (100%), C57 (100%)
	5	C54 (99.8%)
	8	B68 (99.3%), B77 (98.8%)
Bacillus atrophaeus	4	C62 (100%)
	5	C34 (100%)
Brevibacillus thermoruber	3	B107 (99.5%), B109 (99.5%)
Geobacillus stearothermophilus	4	B66 (99.0%)
	5	B72 (97.6%)
	6	B88 (99.3%), B89 (99.0%)
	8	B60 (97.6%), B65 (99.5%)
Geobacillus thermodenitrificans	7	C43 (98.3%)
	8	B64 (99.0%), C66 (98.8%), C70 (99.0%)
Ureibacillus terrenus	1	B110 (97.6%)
	7	C72 (98.8%)
	8	C71 (98.8%)
Ureibacillus thermosphaericus	2	B105 (97.3%), B106 (97.6%)
	3	B103 (98.3%), B99 (98.8%), B108 (98.1%)
	7	B101 (98.1%), B104 (98.3%)
	8	B80 (97.1%), C59 (99.0%), C73 (98.8%), C74 (99.0%)
Paenibacillus cookii	8	B39 (99.0%)

[a]Similarity (in 16S rDNA sequence) to respective reference strain is shown in parentheses.

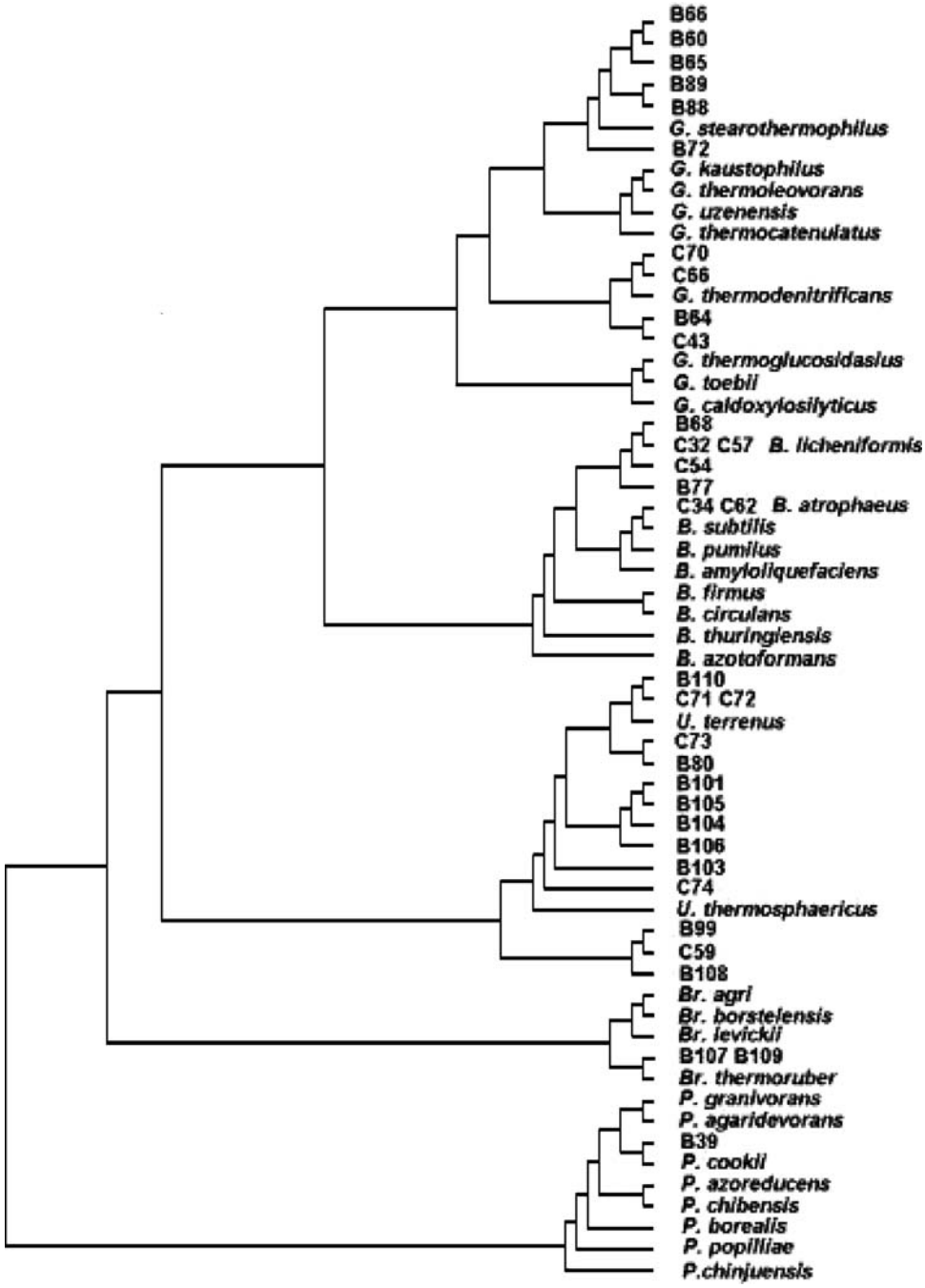

Figure 1. The phylogenetic relationship of thermophilic isolates determined by maximum parsimony analysis. *B.*: *Bacillus*; *G.*: *Geobacillus*; *Br.*: *Brevibacillus*; *P.*: *Paenibacillus*; *U.*: *Ureibacillus*.

thermophilic bacteria were classified into five genera: *Ureibacillus* spp., *Bacillus* spp., *Geobacillus* spp., *Brevibacillus* spp., and *Paenibacillus* spp. (Fig. 1, Table 2). *U. thermosphaericus* (eight isolates) and *G. stearothermophilus* (six isolates) were the dominant species in compost B. *U. thermosphaericus* (three isolates), *B. licheniformis* (three isolates), and *G. thermodenitrificans* (three isolates) were abundant in compost C. Although *G. stearothermophilus*, *Br. thermoruber* (two isolates), and *P. cookii* (one isolates) were only present in compost B, *B. atrophaeus* (two isolates) was found exclusively in compost C (Fig. 1). Overall, 21 isolates from compost B were classified into seven species whereas 13 from compost C were classified into five species (Fig. 1, Table 2).

Isolates B80, B99, B103, B104, B106, B108, C59, C73, and C74 had more than 97.1% sequence similarity to *U. thermosphaericus*. Isolates B110, C71, and C72 were classified as *U. terrenus*, with 97.6%, 98.8%, and 98.8% similarity to reference strain, respectively. Among isolates that were grouped into *Bacillus* spp., isolates C34 and C62 were 100% identical to *B. atrophaeus*, and isolates C32, C54, C57, B68, and B77 were clustered with *B.*

Table 3. Carbon substrates that contribute most to the first and the second principal components (PC1 and PC2) in analysis of Biolog GP microplate data

Substrates	*PC1*	*PC2*
Carbohydrates		
3-Methyl glucose	0.855	
Sedoheptulosan	0.813	
α-Methyl D-galactoside	0.788	
β-Nethyl D-galactoside	0.745	
L-Rhammose	0.742	
D-Psicose	0.731	
Stachyose	0.722	
D-Raffinose	0.710	
Xylose	0.709	
D-Mannitol	0.705	
Turanose	0.689	
D-Trehalose	0.675	
D-Sorbitol	0.656	
α-D-Glucose	0.655	
D-Melezitose	0.643	
Maltotriose	0.641	
α-Methyl D-mannoside	0.623	
Palatinose	0.614	
Cellobiose		−0.628
D-Mannose		−0.604
Polymers		
Mannan	0.634	
Tween80	0.605	
Dextrin	0.601	
Carboxylic acids		
ρ-Hydroxy butyric acid	0.790	
γ-Hydroxy butyric acid	0.771	
L-Lactic acid	0.684	
α-Keto glutaric acid	0.658	
α-Keto valenic acid		0.640
Phosphorylated chemicals		
Glucose-1-phosphate		0.690
Amino acids		
L-Asparagine		0.803
L-Glutamic acid		0.732
L-Alanine		0.695
L-Serine		0.611

Only those variables larger than |0.6| are shown. These variables are computed and applied to the principal component analysis presented in Fig 2.

licheniformis with over 98.8% similarity. Six isolates (B60, B65, B66, B72, B88, and B89) were classified as *G. stearothermophilus* (with over 97.6% similarity), and isolates C66, C70, C43, and B64 were related to *G. thermodenitrificans* with more than 98.3% sequence similarity. In addition, isolate B39 had more than 99% sequence similarity to *P. cookii*, and isolates B107 and B109 were 99.5% similar to *Br. thermoruber* (Table 2).

Substrate Utilization Patterns of Bacterial Communities. Biolog GP microplate profiles of 34 thermophilic bacteria were subjected to PCA. In this study, 95 sole-carbon source substrates on Biolog GP microplate were categorized into polymers, carbohydrates, carboxylic acids, amides, amines, esters, amino acids, alcohols, and aromatic and phosphorylated chemicals. The first and second principal components (PC1 and PC2) accounted for 26.8% and 16.5% of total variations among GP plate data, respectively. Substrates that contributed most to these two PCs are listed in Table 3. The PCA result of 34 bacteria is illustrated in a two-dimensional plot (Fig. 2). These thermophilic bacteria were categorized into eight PCA groups according to their physiological profiles (Fig. 2; Table 4). PC1 mainly consists of polymers, carbohydrates, and carboxylic acids. PC2 primarily contains amino acids, phosphorylated chemicals, and carbohydrates (Table 3).

B110, located alone in the first quadrant of PCA plot with high PC1 and PC2 scores, utilized almost all substrates of the Biolog GP microplate. It was listed as the only member of group 1. Isolates B105 and B106, located in the second quadrant, were ascribed to group 2. This group utilized all the phosphorylated chemicals of the Biolog GP microplate and some carbohydrates, but was incapable of using any of the polymers tested. Isolates B99, B103, B107, B108 and B109 of group 3, located in the third quadrant with low PCA scores, could only use few carbohydrates (Table 4). Groups 4 (B66, C32, C57, C62) and 5 (C54, B72) in the fourth quadrant possessed different utilization patterns for amino acids, alcohols, and aromatic chemicals. Groups 6, 7, and 8 were distributed in the central part of PCA plot. None of the carbohydrates, alcohols, and aromatic and phosphorylated chemicals could serve as substrates for groups 6 and 7. In contrast, few of the esters, carbohydrates, and carboxylic acids could be utilized as carbon source by group 8 (Fig. 2, Table 4).

The frequently utilized substrates are listed in Table 5. Dextrin and mannan (polymers) were common substrates for bacteria isolated from source C (cattle manure) compost. Except for D-raffinose and salicin that were used more frequently by bacteria from source C than those from source B, ribose, sucrose and xylose were well utilized by both sources of bacteria. Several kinds of carboxylic acids were used to a higher degree than other substrates by these thermophilic bacteria. The tricarboxylic acid (TCA) cycle intermediates such as malate and pyruvate that are involved in many metabolic pathways were suitable carbon source for thermophilic bacteria isolated in this study. Bacteria from source C used hydroxybutyric acid and succinic acid, and bacteria from source B used keto-valeric acid among all carboxylic acids. Fructose-6-phosphate, instead of other phosphorylated chemicals, was a common substrate shared by bacteria of source C.

Discussion

Based on phylogenetic relationships of 16S rDNA sequences, 34 thermophilic bacteria reported here were

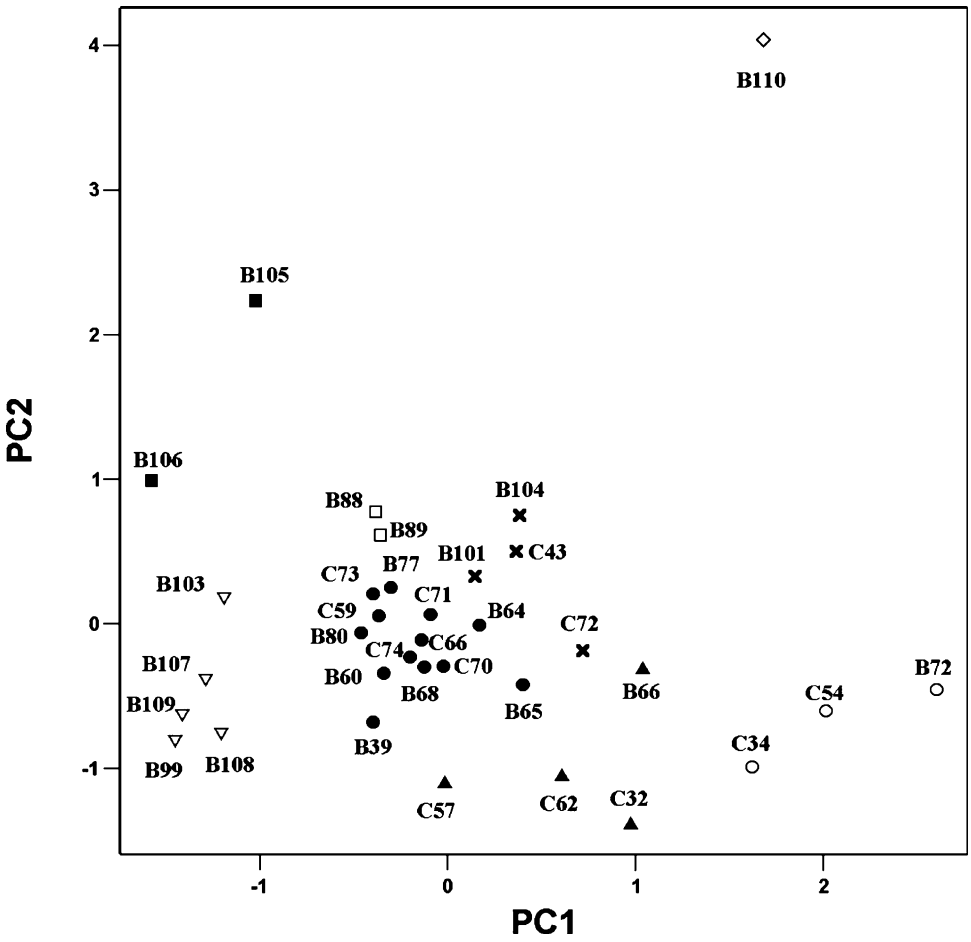

Figure 2. Principal component analysis (PCA) showing differences in utilizing carbon substrates of Biolog GP microplate among thermophilic bacteria. Principal component 1 (PC1) mainly consists of polymers, carbohydrates, and carboxylic acids. Principal component 2 (PC2) primarily contains amino acids, phosphorylated chemicals, and carbohydrates. Bacteria isolated in this study are categorized into eight PCA groups. Group 1 (♦): B110; group 2 (■): B105, B106; group 3 (▽): B99, B103, B107, B108, B109; group 4 (▲): B66, C32, C57, C62; group 5 (○): C34, C54, B72; group 6 (□): B88, B89; group 7 (×): C43, C72, B101, B104; group 8 (●): B39, B60, B64, B65, B68, B77, B80, C59, C66, C70, C71, C73, C74.

classified into five genera of GP endospore-forming bacilli: *Bacillus*, *Geobacillus*, *Ureibacillus*, *Brevibacillus*, and *Paenibacillus* (Fig. 1, Table 2). In this study, isolates that were related to *B. atrophaeus*, *Br. thermoruber*, and *P. cookii* were recovered for the first time from hot compost. Conversely, some isolates were closely related to *U. thermosphaericus*, *G. stearothermophilus*, and *G. thermodenitrificans*, which had been previously isolated from compost [27, 34, 38].

Previously, Choi and Dobbs [7] had applied PCA on Biolog microplate (GN and ECO) data to distinguish between aquatic microbial communities. In this study, we performed PCA on Biolog GP microplate data to separate compost bacteria. Interestingly, our PCA results showed differences in substrate utilization patterns between bacterial communities from poultry and cattle manure composts. Bacteria isolated from compost B (poultry manure) were classified into eight different PCA groups (1 to 8), and those from compost C (cattle manure) were classified into four groups (4, 5, 7, and 8). Although Biolog substrate utilization patterns of each bacterial community may not necessarily reflect the natural composition of its compost source, the fact that bacteria from compost B are categorized into more complex PCA groups (all eight

Table 4. The substrate utilization pattern of bacteria in different PCA groups

PCA group	*Polymers*	*Carbohydrates*	*Carboxylic Acids*	*Amides, Amines, Esters*	*Amino Acids*	*Alcohols, Aromatic Chemicals*	*Phosphorylated Chemicals*
1	+	++	++++	++++	++++	++++	+++
2	–	+	++	+	+	++	++++
3	–	+	–	–	–	–	–
4	++	+++	+++	+	–	–	+
5	+++	+++	+++	++	+	++	+
6	+	–	+++	++	++	–	–
7	+	–	++	++	++	–	–
8	–	++	+++	++	–	–	–

+: 0–25% utilization; ++: 25–50% utilization; +++: 50–75% utilization; ++++: 75–100% utilization.

Table 5. Carbon substrates that are frequently utilized by bacterial isolates from poultry (source B) and cattle (source C) manure composts

	Source B (n = 21)	*Source C (n = 13)*
Substrates	*No. of Isolates (%)*	*No. of Isolates (%)*
Polymers		
Dextrin	5 (23.8%)	8 (61.5%)
Mannan	3 (14.3%)	7 (53.8%)
Carbohydrates		
D-Raffinose	6 (28.6%)	8 (61.5%)
D-Ribose	14 (66.7%)	9 (69.2%)
Salicin	4 (19.0%)	8 (61.5%)
Sucrose	9 (42.9%)	9 (69.2%)
D-Xylose	13 (61.9%)	8 (61.5%)
Carboxylic acids		
Acetic acid	14 (66.7%)	9 (69.2%)
Hydroxybutyric acid	10 (47.6%)	10 (76.9%)
Keto-valeric acid	16 (76.2%)	6 (46.1%)
L-Malic acid	14 (66.7%)	8 (61.5%)
Pyruvic acid	16 (76.2%)	9 (69.2%)
Succinic acid	10 (47.6%)	10 (76.9%)
Methyl-pyruvate	16 (76.2%)	12 (92.3%)
Mono-methyl-succinate	12 (57.1%)	9 (69.2%)
Phosphorylated chemicals		
Fructose-6-phosphate	6 (28.6%)	8 (61.5%)

groups) suggests that poultry manure compost may provide more diversified organic nutrients during the thermophilic phase than cattle manure compost. Remarkably, isolate B110 (PCA group 1) from compost B had the highest utilization of nitrogen-containing substrates, including amides, amines, and amino acids (Table 4). Compost B might be rich in these nitrogen-containing substrates because our chemical analysis showed that the nitrogen content of poultry manure was higher than that of cattle manure (Table 1). Moreover, the result of PCA indicated that PCA groups 4 and 5 primarily included isolates (five out of seven) from compost C and PCA groups 1, 2, 3, and 6 exclusively consisted of isolates (10) from compost B (Fig. 2). With the exception of carboxylic acids, groups 4 and 5 presented with higher utilizations of other carbon-containing substrates (polymers and carbohydrates) than groups 1, 2, 3, and 6 (Table 4). Such observations could be explained by the chemical analysis that the carbon content of cattle manure was higher than that of poultry manure (Table 1). In fact, among carbon substrates that were frequently utilized by all isolates presented here, isolates from compost C usually utilized polymer, carbohydrates, carboxylic acids, and phosphorylated chemicals more frequently than those from compost B (Table 5). These isolates apparently adapted well to the carbon substrates-enriched cattle manure compost.

Many thermophilic bacteria isolated in this study could use a variety of carboxylic acids, primarily malate and succinate of TCA cycle intermediates, alpha-keto valeric acid, pyruvate, and acetate. All these aerobic endospore-forming bacilli are members of *Bacillus* and *Bacillus*-derived genera. It has been know that *B. subtilis*, the type species of *Bacillus*, possesses a complete set of TCA cycle enzymes and hence can grow on most TCA cycle intermediates [13, 21]. Like *B. subtilis*, many isolates presented in this study may encode C4-dicarboxylate-binding proteins and transporters, such as MaeN for malate transport, to be able to use these TCA cycle intermediates as sole carbon sources [2, 35]. Although *Bacillus* can express pyruvate dehydrogenase to utilize pyruvate [4, 6], little is known about how *Bacillus* or related species can grow on alpha-keto valeric acid, a metabolic product of aromatic hydrocarbons. Furthermore, according to the genomic sequence of *B. licheniformis* DSM13, five isolates that we identified as *B. licheniformis* may as well possess glyoxylate bypass genes to grow on acetate [37]. Acetate can be converted from pyruvate and secreted by a number of enteric bacteria, e.g., *Escherichia*, *Enterobacter*, *Klebsiella*, *Shigella*, and *Salmonella* [24], which may be present in the compost pile during a mesophilic phase.

Interestingly, 11 isolates that we found closely related to *U. thermosphaericus* in 16S rDNA sequence showed four different patterns of substrate utilization on Biolog microplates. Among them, isolates B99, B103, and B108 were categorized as PCA group 3 with few carbohydrates utilizing activities, whereas B105 and B106 were in group 2 with broader activities than group 3. On the other hand,

two isolates (B101 and B104) and four isolates (B80, C59, C73, and C74) were further divided to PCA group 7 and group 8, respectively. Both groups 7 and 8 showed higher preference in using polymers, carbohydrates, and carboxylic acids than groups 2 and 3 (Table 4). Similar to our study, Gagné *et al.* [15] reported that eight thermophilic isolates from aerobic swine waste compost, which were closely related to *U. thermosphaericus* in 16S rDNA analysis, presented with different morphological, biochemical, and physiological characteristics. Furthermore, three isolates (B110, C71, and C72) related to *U. terrenus* also diverged phenotypically to three different groups (groups 1, 8, and 7, respectively) based on the physiological plot of PCA (Table 2, Fig. 2). B110, in particular, possessed the highest activity in utilizing a broad spectrum of substrates. In contrast to C71 and C72, which share higher similarity (98.8%) in 16S rDNA sequence to *U. terrenus*, B110 is 97.6% similar to *U. terrenus*. Based on its genetic and phenotypic variations, B110 should be further characterized to determine if it can be classified as a new species.

Based on 16S rDNA analysis, we identified isolates C34 and C62 as *B. atrophaeus*. This species was reclassified in 2001 from *B. subtilis* [14]. Although *B. atrophaeus* had not been reported as a compost bacterium, we are not surprised to find it in hot compost because it is used as a bioindicator for sterilization procedures due to its heat resistance [19]. The physiological characteristics of isolates C34 and C62 were similar, except for having some difference in utilizing amino acids, alcohols, and aromatic chemicals.

Many isolates presented here could utilize mannans (Table 5). Among them, two isolates (B66 and B72) and three isolates (C32, C54, and C57) were related or identical to *G. stearothermophilus* and *B. licheniformis* in their 16S rDNA sequences. In fact, thermophilic *G. stearothermophilus* and mesophilic *B. licheniformis* have been exploited in industry for their ability to secrete amylases and mannases, respectively [1, 10, 29]. Additionally, isolates B88 and B89 that were identified as *G. stearothermophilus* by 16S rDNA analysis could not utilize most of the polymers and carbohydrates. Because substrate utilization patterns of B88 and B89 were different from that of *G. stearothermophilus*, both isolates may represent new species. Other taxonomic methods such as fatty acid analysis, DNA base composition, and DNA–DNA hybridization should be performed in the future to clarify this issue.

Interestingly, B39 isolated in this study shared a 99% similarity in 16S rDNA with *P. cookii*, a novel species newly discovered by Logan *et al.* [25]. *P. cookii* was first isolated from the geothermal soil of an active volcano on Candlemas Island. As a comparison, B39 in this study could grow at higher temperatures than the reference strain LMG419T of *P. cookii* [25]. In addition, B39 presented with other different phenotypes (data not shown) from that of strain LMG419T.

Overall, several isolates that were closely related to each other or to respective reference strains in their 16S rDNA sequence nonetheless showed quite diverse phenotypes in substrate utilization. As reported earlier by others, phylogenetic relationship of 16S rDNA may not always consist with Biolog classification [23, 26]. One explanation for this discrepancy is that the 16S rRNA gene has a slower evolutionary rate compared to many protein-coding genes [31]. However, many other factors, such as lateral gene transfer, genetic deficiency, or attenuation of enzyme, could also account for the phenotypic diversity. Lateral gene transfer, in particular, may contribute to genetic variability between phenotypically distinct strains of *Prochlorococcus* that are highly similar in 16S rDNA sequences [8]. In the future, we may perform whole-genome comparisons among the phenotypically diverse isolates to resolve this issue.

In conclusion, bacterial communities from poultry manure compost comprised more divergent species (21 isolates, seven species) and utilized more diverse substrates (eight PCA groups) than that from cattle manure compost (13 isolates, five species, and four PCA groups). Furthermore, by coupling Biolog data with 16S rDNA analysis, we investigated phenotypic variations among genetically similar thermophilic compost bacteria. Some potential novel species may be discovered by this approach. Most of all, among the 34 thermophilic isolates reported here, B110, closely related to *U. terrenus*, possesses particularly high activity in utilizing a broad spectrum of substrates. This isolate may be of use in the biotechnology field and should be investigated further.

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