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Characterization of Facultative Thermophilic Microbial Community of Composts by ARDRA

Shin-Tong Hong[1], Gan-Nan Chang[2] and Kuo-Chieh Ho[1,3]

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ABSTRACT: Bacterial activity is one of the factors to determine the maturity and quality of the composts. In order to have a better understanding of the composition of bacteria involving in the composting processes, the facultative thermophilic bacrerial community of local composts was analyzed. A total of 412 colonies were isolated and analyzed by amplified rDNA restriction analysis (ARDRA) and traditional colony observation, staining and biochemical tests. The isolates were divided into 24 groups mainly by ARDRA. Among these groups, the largest one contained 137 isolates. Mostly, the ARDRA analysis was in good conformity with the analysis of traditional methods: both results indicated that group 17 was a distinct one. The phylogenetic tree constructed based on the 16S rDNA sequence analysis demonstrated that the bacterial community of composts analyzed in this study was dominated by *Bacillus* spp., closely related to *Bacillus licheniformis* and *Bacillus sonorensis*. Only 16 isolates in group 17 were *Pseudomonas* spp., closely related to *Pseudomonas stutzer*.

KEY WORDS: Amplified rDNA restriction analysis (ARDRA), Compost, 16S rDNA, Thermophilic bacteria.

INTRODUCTION

Composting is a self-heating, aerobic, bio-decomposition of organic waste materials. Conversion of organic wastes from agricultural industries and animal husbandry through microbiological processes can decrease the amount of wastes disposed by landfilling and fuel required for waste incineration. Moreover, the composts produced can be used to fertilize the soil. In Taiwan, agricultural wastes, such as fruit-vegetable refuses, and flower wastes, are often directly treated by landfilling (Wu, 2000). With the very limited land resources in Taiwan, building of new land fields or incinerators is getting more and more difficult these days. To ameliorate the situation, it is of pressing urgency to popularize the utility of agricultural wastes via composting.

During composting process, the temperature rapidly increases to high level, sustains for a period of time, and then gradually cools down. Different microbial communities dominate in each composting phase and are responsible for a certain compost maturity and quality (Herrmann and Shann, 1997; Ryckeboer et al., 2003). Traditionally, the microorganisms in composts are analyzed by using selective plating techniques and physiological tests (de Brito Alvarez et al., 1995; Craft and Nelson, 1996). Molecular tools including fatty acid profiling, PCR-based single strand conformation polymorphism (PCR-SSCP) and denaturing gradient gel electrophoresis (PCR-DGGE), and 16S rRNA gene analysis are now in common use (Klamer and Baath, 1998; Peters et al., 2000; Dees et al., 2001; Marshall et al., 2003; Green et al., 2004; Halet et al., 2006). Among the method of 16S rRNA gene analysis, amplified rDNA restriction analysis (ARDRA) has been successfully used for the bacterial community analysis of various environments (Sessitsch et al., 2001; Lagace et al., 2004; Sasaki et al., 2004; Pourshafie et al., 2005; Sasaki et al., 2006; Yan et al., 2006).

The purpose of this work was to determine the thermogenic bacterial community of different composts from Taiwan Sugar Company at Huwei branch in Taiwan. Special attention was taken to develop a basic model of quick identification of microbial communities in composts via ARDRA analysis or traditional morphological and physiological classification. The result of ARDRA

1. Department of Life Science and Graduate Institute of Plant Biology, National Taiwan University, 1, Sec. 4, Roosevelt Rd., Taipei 106, Taiwan.
2. Department and Institute of Biotechnology, Tajen University, 20, Wei-Shin Rd., Shin-II Tsun, Yan-Puu Hsiang, Pingtung 907, Taiwan.
3. Corresponding author. Tel: 886-2-33662508; Fax: 886-2-23637274; Email: kch@ntu.edu.tw

analysis was well consistent with that of the traditional methods. Four hundred and twelve isolates were obtained. The dominant species in thermophilic composts were *Bacillus* spp., closely related to *Bacillus licheniformis* and *Bacillus sonorensis*.

MATERIALS AND METHODS

Sampling and composition of compost

The thermogenic samples were taken at 30 to 50 cm from the surface of huge composts at Taiwan Sugar Company at Huwei branch. The temperature lay between 45℃ - 60℃ and the pH ranged from 6.58 to 7.66. The composts were composed of wastes of fruit and vegetable, bagasse, and excrement of pigs at varied ratio as shown in Table 1.

The conventional microbiological methods

One gram of compost sample was mixed with 9 ml of sterile distilled water. Three hundred μl of solution from dilutions of 10^{-3}, 10^{-4} and 10^{-5} was spread on nutrient agar (NA) plates with a pH 7.0. The plates were incubated at 55℃ until colonies appeared. The colony was further purified by streak plate method, and then the single colony was picked up. The bacteria were preserved in 60% glycerol at -20℃ and -80℃ freezers. The pH of the compost sample was determined in a mixture of 20g fresh compost and 180 ml autoclaved distilled water.

The Gram straining and biochemical analyses were performed following the methods described by Cappuccino and Sherman (2002) with the necessary modifications including 55 ℃ for bacterial cultivation.

Polymerase chain reaction (PCR)

The 16S rDNA fragment was amplified by colony PCR using the primers of Eu11F (5'-GTTTGATCCTGGCTCAG-3') and Eu1512R (5'-GGCTACCTTGTTACGACTT-3') based on the conserved regions of 16S rDNA gene of eubacteria (Amann et al., 1995; Cheng, 2000). The number in the names of the primers indicated the corresponding positions of *E. coli* 16S rDNA. The PCR reaction condition was as following: 95℃, 5 min, then 95℃, 45 sec; 52℃, 45 sec; 72℃, 2 min for 30 cycles. Finally, the reaction was continuated at 72℃ for 5 min.

Amplified rDNA restriction analysis (ARDRA)

The PCR products were digested with restriction enzyme *Hinf*I, *Hpa*II, *Mse*I, and *Sau*3AI, respectively. After complete digestion, the fragments were separated on an 8% polyacrylamide gel and stained with ethidium bromide. The bands between 1000 bp to 100bp were subjected to the restriction fragment length polymorphism (RFLP) analysis and then bacteria were grouped accordingly.

DNA sequencing and phylogenetic tree construction

After RFLP analysis, a representative colony was selected from each group. The 16S rDNA was amplified on each representative colony and purified by QIAquick PCR Purification Kit (QIAGEN, Germany). Both strands of the 16S rDNA were sequenced using primer of 123F (5'-ACGGGTGAGTAAC/TGC/TGT-3') or 1220R (5'-TTGTAGCACGTGTGTAGCCC-3') separately. The number in the names of the primers indicated the corresponding positions of *E. coli* 16S rDNA (Amann et al., 1995). DNA sequence was determined by a DNA automated sequencer (ABI Prism model 377, v 30; Applied Biosystems) as previously described (Ho et al., 2001). The sequence homology was searched using the BLAST of NCBI GeneBank database (http://www.ncbi.nlm.nih.gov/BLAST/). The nucleotide sequences of the 600-bp region from 24 group representatives were aligned using Clustal method of software DNASTAR/MegAlign, together with 17 well-known species that were selected by sequence homology, and a phylogenetic tree was constructed. The 600-bp region, from nucleotide 123 to 723, corresponds to the variable region of V2 to V4 of *E. coli* 16S rDNA (Neefs et al., 1993).

RESULTS

Bacterial isolation

The samples taken from inside of hot composts were dissolved in the sterile distilled water and then diluted properly. Aliquot from each dilution was spread on the NA plate and incubated at 55℃. The number of bacteria per gram of compost was various from 2.03×10^6 to 3.89×10^7 (Table 1). Four hundred and twelve colonies were isolated.

Morphological feature and biochemical properties

After single colony purification, bacteria from each colony were firstly characterized by gram staining and biochemical tests. All bacteria were rod-shaped. Most of them were gram positive (86%), others were gram negative (4%) and gram varied (10%). In the sugar utilization, 96% of isolates was sucrose-fermentative, 91% could use glucose; but none of them could use lactose. Sixty-five percent of

Table 1 Composition, temperature (T), pH, and bacterial number ($x10^4$/g compost) of various composts while sampling.

Sample No	Composition and the ratio	T	pH	Bacterial number
1	bagasse：excrement of pig (10:1)	54℃	7.32±0.03	2530.3±433.8
2	bagasse：excrement of pig (10:1)	56℃	7.14±0.02	862.7±253.4
3	waste of fruit and vegetable：bagasse：excrement of pig (5:5:1)	46℃	6.86±0.04	1773.5±248.2
4	waste of fruit and vegetable：bagasse：excrement of pig (5:5:1)	47℃	6.90±0.02	357.3±60.8
5	waste of flowers and plants：bagasse：excrement of pig (5:5:1)	46℃	7.22±0.05	1887.43±544.2
201	waste of flowers and plants：bagasse：excrement of pig (5:5:1)	48℃	7.16±0.03	203.2±30.6
203	waste of flowers and plants：bagasse：excrement of pig (2:5:1)	45℃	7.64±0.02	1779.87±640.3
205	waste of flowers and plants：bagasse：excrement of pig (2:5:1)	50℃	7.36±0.04	3886.43±838.2
207	waste of fruit and vegetable：bagasse：excrement of pig (5:2:1)	60℃	6.64±0.06	1223.8±133.5
209	waste of fruit and vegetable：bagasse：excrement of pig (5:2:1)	58℃	6.83±0.03	856.3±96.35
211	waste of fruit and vegetable：bagasse：excrement of pig (5:5:1)	60℃	7.12±0.08	2433.9±156.33
213	waste of fruit and vegetable：bagasse：excrement of pig (5:5:1)	60℃	7.04±0.02	1432.66±130.4

isolates could hydrolyze starch, 92% could hydrolyze gelatin, 91% could reduce nitrate. Ninety-two percent of isolates could use citrate as the carbon source, but none of them produced alkali in Simmon's citrate agar plate. All of the isolates were negative in indole production and MR tests, but positive in VP test and could hydrolyze casein (Table 2).

Amplified rDNA restriction analysis (ARDRA)

A 1.5-kbp 16S rDNA fragment was amplified on each bacterial genomic DNA by colony PCR. For the performance of ARDRA analysis, the amplified DNA fragment was restricted with different enzyme and then subjected to RFLP analysis. The total 412 isolates were divided into 24 groups mainly by ARDRA with the aid of conventional microbiological methods. The largest group contained 137 isolates. Figure 1 showed the restriction patterns for the representative isolates of each group.

Sequencing analysis and phylogenetic tree construction

A 1078-bp PCR fragment corresponding to nucleotide 123 to 1220 of *E. coli* 16S rDNA from the representative isolates of each group was amplified and sequenced. The diversity of sequences was less than 5%. The result of nucleotide sequence homology search using BLAST of NCBI GenBank database indicated that most isolates belonged to *Bacillus* genus. Only one group (#17) was related to genus *Pseudomonas* (data not shown).

A phylogenetic tree was constructed based on the variable region corresponding to V2 to V4 region of *E. coli* and diagrammed in figure 2. The result also indicated that most of the groups were *Bacillus*-related, closed to *Bacillus licheniformis* and *Bacillus sonorensis*. They were the dominant species in thermophilic composts, consisting about 96% of total isolates. The bacteria of group 17 were closely related to *Pseudomonas stutzer*.

DISCUSSION

The management of various solid wastes is troublesome and costly, especially in the small island with very limited land and dense population like Taiwan. Composting becomes a valuable approach to solve this increasing problem. Moreover, composts from agricultural wastes can be used as fertilizer to improve the fertility and permeability of soil, and suppress some plant pathogens (Green et al., 2004). In order to produce high quality compost, the research on the microbial community of compost is intensively investigated (Strom, 1985a, 1985b; Halet et al., 2006 [and references therein]).

In this study, bacterial community composition of local composts was characterized. Among the 412 bacterial isolates, 24 groups were formed mainly according to the banding patterns obtained by ARDRA. The fragment of 16S rRNA gene for a representative isolate from each group was sequenced. The data of sequence comparison showed that most organisms were *Bacillus* spp., except those in group 17 that were related to *Pseudomonas* spp. The results were in agreement with those of conventional microbiological and biochemical methods, which also indicated the group 17 was a distinct group (Table 2). The phylogenetic tree demonstrated that the bacterial community of composts analyzed was dominated by *Bacillus* spp. closely related to *Bacillus licheniformis* and *Bacillus sonorensis*. The results well agreed with the work reported by Strom (1985a, b): The microbial diversity of hot compost is greatly limited by high temperature and dominated by low G+C content gram-positive spore-formed bacteria such as *Bacillus* spp.

Although few groups with the same restriction patterns were further separated by traditional methods, ARDAR was able to provide a quick method to identify the bacteria for composts. Our result will contribute to the research of microbial community in compost in Taiwan.

Table 2. Morphological and biochemical properties, and restriction patterns of the representative bacterial isolate in each group.

Group 1, 6[a]												
1-16[b]	Gram	Surcose	Glucose	Lactose	Starch	Citrate	Casein	Gelatin	Indole	MR	VP	Nitrate
Properties	V	A	A	(-)	(+)	(+)	(+)	(+)	(-)	(-)	(+)	(+)
Hinf I	620[c]	390	345	170								
Hpa II	750	550	380	220	150	130						
Mse I	715	615	430	280	130							
*Sau*3A I	680	445	320	190	150	120						
Group 2, 7												
1-20	Gram	Surcose	Glucose	Lactose	Starch	Citrate	Casein	Gelatin	Indole	MR	VP	Nitrate
Properties	V	A	(-)	(-)	(-)	(+)	(+)	(+)	(-)	(-)	(+)	(+)
Hinf I	620	390	345	315	170	130						
Hpa II	750	550	380	330	220	150	130	110				
Mse I	715	615	470	430	360	280	130					
*Sau*3A I	650	445	320	190	150	120						
Group 3, 13												
2-11	Gram	Surcose	Glucose	Lactose	Starch	Citrate	Casein	Gelatin	Indole	MR	VP	Nitrate
Properties	+	A	A	(-)	(-)	(+)	(+)	(+)	(-)	(-)	(+)	(+)
Hinf I	915	375	170									
Hpa II	850	750	380	220	150							
Mse I	900	715	615	530	430	360	280	165	130			
*Sau*3A I	320	250	150	120								
Group 4, 11												
4-12B	Gram	Surcose	Glucose	Lactose	Starch	Citrate	Casein	Gelatin	Indole	MR	VP	Nitrate
Properties	+	A	A	(-)	(-)	(+)	(+)	(+)	(-)	(-)	(+)	(+)
Hinf I	700	620	390	345	315	170	130					
Hpa II	750	550	520	455	380	330	280	150				
Mse I	615	470	430	360	280	130						
*Sau*3A I	680	445	320	280	190	150	120					
Group 5, 7												
201-17	Gram	Surcose	Glucose	Lactose	Starch	Citrate	Casein	Gelatin	Indole	MR	VP	Nitrate
Properties	V	A	(-)	(-)	(-)	(+)	(+)	(+)	(-)	(-)	(+)	(+)
Hinf I	620	390	345	170								
Hpa II	750	550	380	220	150	130						
Mse I	715	615	430	280	130							
*Sau*3A I	320	190	150	120								
Group 6, 6												
207-11	Gram	Surcose	Glucose	Lactose	Starch	Citrate	Casein	Gelatin	Indole	MR	VP	Nitrate
Properties	V	A	A	(-)	(+)	(+)	(+)	(+)	(-)	(-)	(+)	(+)
Hinf I	765	700	620	390	345	315	260	170	130			
Hpa II	550	520	380	330	220	180	150	130	110			
Mse I	715	615	470	430	360	280	165	130	110			
*Sau*3A I	680	445	320	280	190	150	120					
Group 7, 6												
211-11	Gram	Surcose	Glucose	Lactose	Starch	Citrate	Casein	Gelatin	Indole	MR	VP	Nitrate
Properties	V	A	A	(-)	(+)	(+)	(+)	(+)	(-)	(-)	(+)	(+)
Hinf I	520	500	390	220	130							
Hpa II	550	380	220	150	130							
Mse I	615	430	280	130								
*Sau*3A I	320	190	150	120								
Group 8, 12												
1-10	Gram	Surcose	Glucose	Lactose	Starch	Citrate	Casein	Gelatin	Indole	MR	VP	Nitrate
Properties	+	A	A	(-)	(-)	(-)	(+)	(+)	(-)	(-)	(+)	(+)
Hinf I	620	390	345	170								
Hpa II	850	750	550	455	380	330	220	180	150	130		
Mse I	715	615	430	280	165							
*Sau*3A I	320	190	150	120								
Group 9, 2												
1-19	Gram	Surcose	Glucose	Lactose	Starch	Citrate	Casein	Gelatin	Indole	MR	VP	Nitrate
Properties	V	A	(-)	(-)	(+)	(+)	(+)	(+)	(-)	(-)	(+)	(+)

Table 2. Continued.

Hinf Ⅰ	700	620	390	345	315	170	130					
Hpa Ⅱ	850	750	550	520	455	380	280	240	220	180	130	110
Mse Ⅰ	615	470	430	360	280	165						
Sau3A Ⅰ	680	640	445	320	280	190	150	120				
Group 10, 43												
207-18	Gram	Surcose	Glucose	Lactose	Starch	Citrate	Casein	Gelatin	Indole	MR	VP	Nitrate
Properties	+	A	A	(-)	(-)	(+)	(+)	(+)	(-)	(-)	(+)	(+)
Hinf Ⅰ	700	620	390	345	315	170	130					
Hpa Ⅱ	850	550	520	455	380	330	280	240	220	180	150	110
Mse Ⅰ	715	615	470	430	360	280	130					
*Sau*3A Ⅰ	680	445	320	280	190	150	120					
Group 11, 10												
207-39	Gram	Surcose	Glucose	Lactose	Starch	Citrate	Casein	Gelatin	Indole	MR	VP	Nitrate
Properties	+	(-)	A	(-)	(+)	(+)	(+)	(-)	(-)	(-)	(+)	(-)
Hinf Ⅰ	620	390	345	170								
Hpa Ⅱ	520	380	220	150	130							
Mse Ⅰ	615	430	280	130								
*Sau*3A Ⅰ	320	190	150	120								
Group 12, 11												
213-31	Gram	Surcose	Glucose	Lactose	Starch	Citrate	Casein	Gelatin	Indole	MR	VP	Nitrate
Properties	+	A	A	(-)	(-)	(+)	(+)	(+)	(-)	(-)	(+)	(-)
Hinf Ⅰ	700	620	390	345	315	260	170	130				
Hpa Ⅱ	550	455	380	330	220	150	110					
Mse Ⅰ	715	615	470	430	360	280	130					
*Sau*3A Ⅰ	680	445	320	280	190	150	120					
Group 13, 19												
211-30	Gram	Surcose	Glucose	Lactose	Starch	Citrate	Casein	Gelatin	Indole	MR	VP	Nitrate
Properties	+	A	A	(-)	(-)	(+)	(+)	(+)	(-)	(-)	(+)	(+)
Hinf Ⅰ	700	500	390	220	130							
Hpa Ⅱ	850	750	550	520	455	380	330	280	240	220	180	150
Mse Ⅰ	615	430	280	165	130							
*Sau*3A Ⅰ	680	320	190	150	120							
Group 14, 8												
211-37	Gram	Surcose	Glucose	Lactose	Starch	Citrate	Casein	Gelatin	Indole	MR	VP	Nitrate
Properties	+	A	A	(-)	(+)	(+)	(+)	(-)	(-)	(-)	(+)	(+)
Hinf Ⅰ	700	620	390	345	315	170	130					
Hpa Ⅱ	550	455	380	330	220	180	150	110				
Mse Ⅰ	615	530	470	430	360	280	130					
*Sau*3A Ⅰ	680	445	320	280	190	150	120					
Group 15, 9												
211-2	Gram	Surcose	Glucose	Lactose	Starch	Citrate	Casein	Gelatin	Indole	MR	VP	Nitrate
Properties	+	A	A	(-)	(+)	(+)	(+)	(-)	(-)	(-)	(+)	(+)
Hinf Ⅰ	620	390	345	170								
Hpa Ⅱ	520	380	220	150	130							
Mse Ⅰ	615	430	280	165	130							
*Sau*3A Ⅰ	680	320	190	150	120							
Group 16, 4												
201-3	Gram	Surcose	Glucose	Lactose	Starch	Citrate	Casein	Gelatin	Indole	MR	VP	Nitrate
Properties	V	A	(-)	(-)	(+)	(+)	(+)	(+)	(-)	(-)	(+)	(+)
Hinf Ⅰ	915	765	620	390	345	260	170					
Hpa Ⅱ	850	750	550	455	380	330	220	180	150	130		
Mse Ⅰ	950	900	615	590	530	470	430	360	280	220	130	
*Sau*3A Ⅰ	720	650	320	280	190	150						
Group 17, 16												
205-4	Gram	Surcose	Glucose	Lactose	Starch	Citrate	Casein	Gelatin	Indole	MR	VP	Nitrate
Properties	-	(-)	A	(-)	(-)	(-)	(+)	(+)	(+)	(-)	(+)	(-)
Hinf Ⅰ	915	620	345	260	220	170						
Hpa Ⅱ	850	750	520	380	330	240	220	200	190	180	150	130
Mse Ⅰ	950	530	470	430	360	165	130					
*Sau*3A Ⅰ	820	400	190									

Table 2. Continued.

Group 18, 2												
201-7	Gram	Surcose	Glucose	Lactose	Starch	Citrate	Casein	Gelatin	Indole	MR	VP	Nitrate
Properties	V	A	(-)	(-)	(+)	(+)	(+)	(+)	(-)	(-)	(+)	(+)
Hinf I	915	765	620	390	345	260	170					
Hpa II	850	750	550	455	380	330	280	220	180	150	130	
Mse I	950	900	615	590	530	470	430	360	280	220	130	
*Sau*3A I	720	650	320	280	190	150						
Group 19, 5												
201-13A	Gram	Surcose	Glucose	Lactose	Starch	Citrate	Casein	Gelatin	Indole	MR	VP	Nitrate
Properties	V	A	(-)	(-)	(-)	(-)	(+)	(+)	(-)	(-)	(+)	(+)
Hinf I	915	765	620	390	345	260	170					
Hpa II	550	520	380	330	220	150	130	110				
Mse I	615	470	430	360	280	130	110					
*Sau*3A I	445	320	250	190	150	120						
Group 20, 137												
201-13B	Gram	Surcose	Glucose	Lactose	Starch	Citrate	Casein	Gelatin	Indole	MR	VP	Nitrate
Properties	+	A	A	(-)	(+)	(+)	(+)	(+)	(-)	(-)	(+)	(+)
Hinf I	915	765	620	390	345	170						
Hpa II	850	750	550	455	380	330	220	180	150	130		
Mse I	950	900	615	590	530	470	430	360	280	220	130	
*Sau*3A I	720	650	320	280	190	150						
Group 21, 4												
205-36	Gram	Surcose	Glucose	Lactose	Starch	Citrate	Casein	Gelatin	Indole	MR	VP	Nitrate
Properties	V	A	(-)	(-)	(+)	(+)	(+)	(+)	(-)	(-)	(+)	(+)
Hinf I	915	765	620	390	345	170						
Hpa II	850	750	550	455	380	330	220	180	150	130		
Mse I	950	900	615	590	530	470	430	360	280	220	130	
*Sau*3A I	720	680	650	320	280	190	150					
Group 22, 68												
4-3	Gram	Surcose	Glucose	Lactose	Starch	Citrate	Casein	Gelatin	Indole	MR	VP	Nitrate
Properties	+	A	A	(-)	(+)	(+)	(+)	(+)	(-)	(-)	(+)	(+)
Hinf I	620	390	345	170								
Hpa II	520	380	220	150	130							
Mse I	615	430	280	180	165	130	110					
*Sau*3A I	680	320	190	150	120							
Group 23, 2												
211-40	Gram	Surcose	Glucose	Lactose	Starch	Citrate	Casein	Gelatin	Indole	MR	VP	Nitrate
Properties	V	A	(-)	(-)	(+)	(+)	(+)	(+)	(-)	(-)	(+)	(+)
Hinf I	915	765	700	620	390	345	170					
Hpa II	850	750	550	455	380	330	220	180	150	130		
Mse I	950	900	615	590	530	470	430	360	280	220	130	
*Sau*3A I	720	680	650	320	280	190	150					
Group 24, 4												
213-30A	Gram	Surcose	Glucose	Lactose	Starch	Citrate	Casein	Gelatin	Indole	MR	VP	Nitrate
Properties	V	A	(-)	(-)	(+)	(+)	(+)	(-)	(-)	(-)	(+)	(+)
Hinf I	915	765	620	390	345	260	170					
Hpa II	850	750	550	455	380	330	220	180	150	130		
Mse I	950	900	615	590	530	470	430	360	280	220	130	
*Sau*3A I	720	680	650	320	280	190	150					

[a] number of isolates in the group, [b] bacterium number of the representative isolate in the group

[c] Length of restriction fragment (bp)

V, Gram variable; (+), positive result; (-), negative result; A, acid produced

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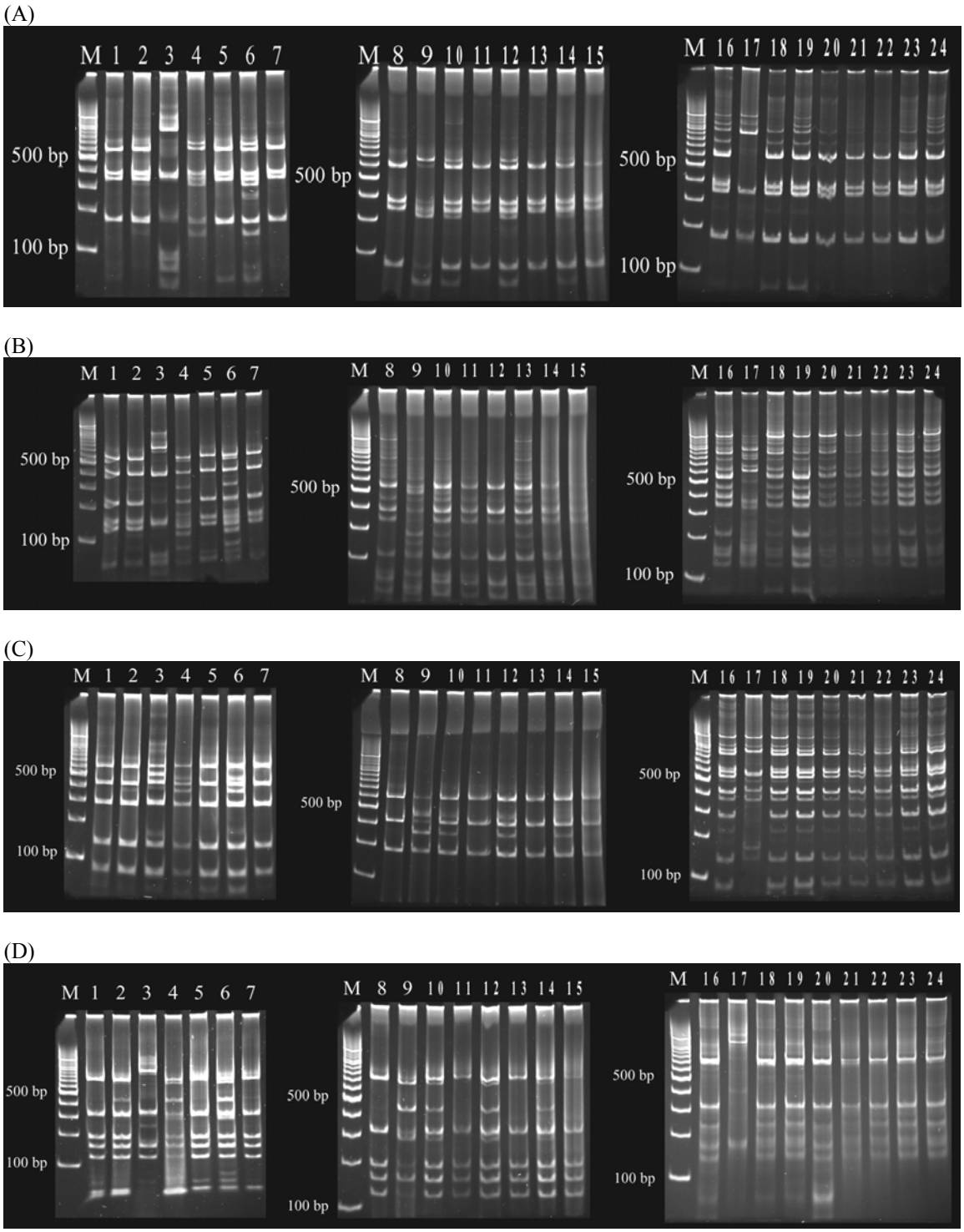

Fig. 1. ARDRA analysis of the representative in each group. The PCR-amplified16S rDNA fragment was digested completely with *Hinf* I (A), *Hpa*II (B), *Mse*I (C), and *Sau*3AI (D), respectively, and then subjected to an 8% polyacrylamide gel electrophoresis. Lane M: DNA marker (MBI Fermantas O'RangeRuler^{TM} 100bp DNA Ladder); Lane 1: 1-16; Lane 2: 1-20; Lane 3: 2-11; Lane 4: 4-12B; Lane 5: 201-17; Lane 6: 207-11; Lane 7: 211-11; Lane 8: 1-10; Lane 9: 1-19; Lane 10: 207-18; Lane 11: 207-39; Lane 12: 213-31; Lane 13: 211-30; Lane 14: 211-37; Lane 15: 211-2; Lane 16: 201-3; Lane17: 205-4; Lane 18: 201-7; Lane 19: 201-13; Lane 20: 201-13B; Lane 21: 205-36; Lane 22: 4-3; Lane 23: 211-40; Lane 24: 213-30A.

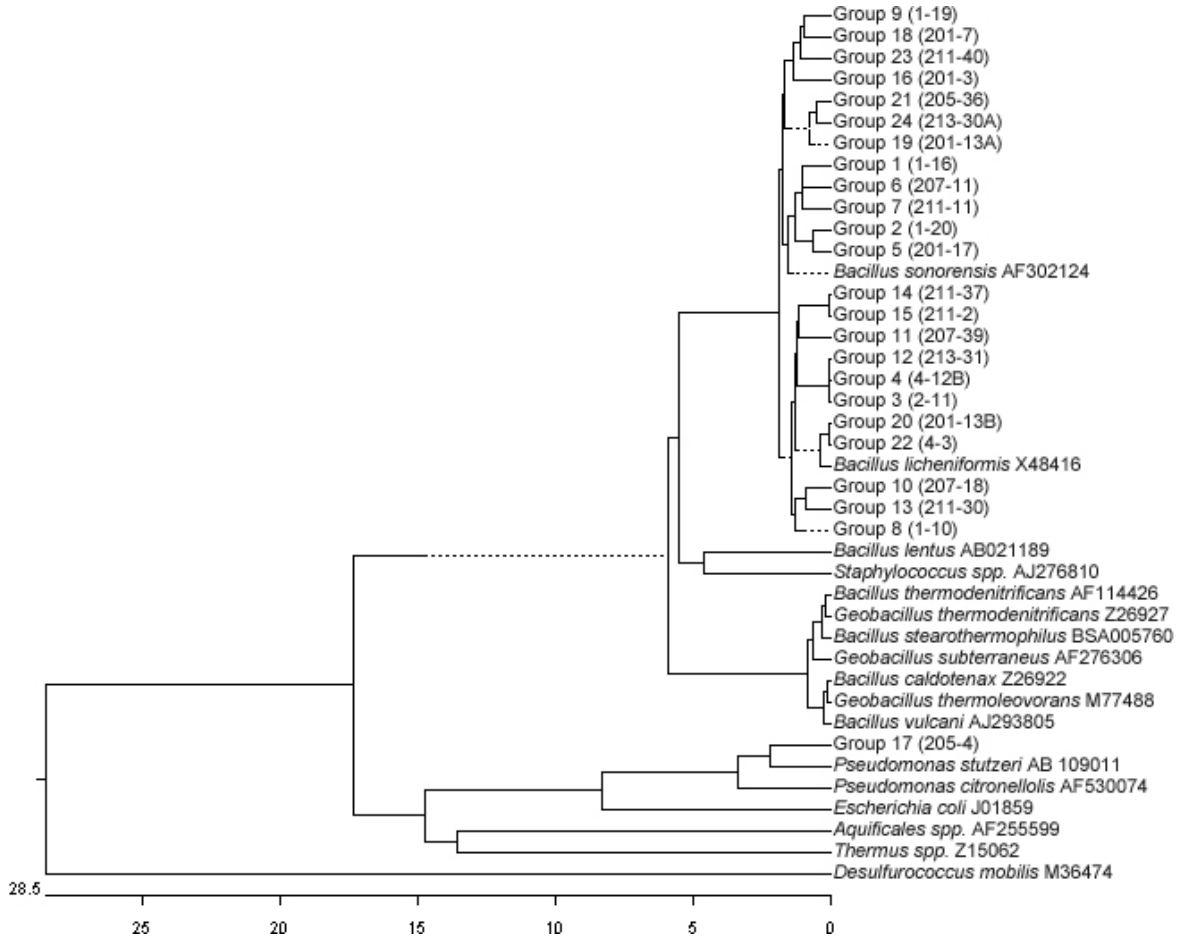

Fig. 2. Phylogenetic tree of 24 representative bacteria from each group based on the partial sequence of 16S rDNA. The region corresponding to the variable region (V2 toV4 region, 123 to 723 bp) of *E. coli*16S rDNA were used. The rDNA sequences of 17 known bacterial species deposited in public databases were included as the references. *Desulfrococcus mobilis* (accession number M36474) was used as an outgroup. Alignment was performed using the clustal method of DNASTAR/MegAlign program. The solid line represents the evolution distance.

LITERATURE CITED

Amann, R. I., W. Ludwig and K. H. Schlieifer. 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol. Rev. **59**: 143-169.

de Brito Alvarez, M. A., S. Gagne and H. Antoun. 1995. Effect of compost on rhizosphere microflora of the yomato and on the incidence of plant growth-promoting rhizobacteria. Appl. Environ. Microbiol. **61**: 194-199.

Cappuccino, J. G. and N. Sherman. 2002. Microbiology: A laboratory manual, 6th ed. Benjamin Cummings. San Francisco, Boston, New York, USA. 491pp.

Cheng, M.-Q. 2000. Study on cultivable bacterial communities of the reactors capable of phosphorous removal biologically. Master thesis, National Central University, TaoYun, Taiwan. 53pp. (in Chinese with an English abstract)

Craft, C. M. and E. B. Nelson. 1996. Microbial properties of composts that suppress damping-off and root rot of creeping bentgrass caused by *Pythuim graminicola*. Appl. Environ. Microbiol. **62**: 1550-1557.

Dees, P. M. and W. C. Ghiorse. 2001. Microbial diversity in hot synthetic compost as revealed by PCR-amplified rRNA sequence from cultivated isolates and extracted DNA. FEMS Microbiol. Ecol. **35**: 207-216.

Green, S. J., F. C. Michel, Y. Hadar and D. Minz. 2004. Similarity of bacterial communities in sawdust- and straw-amended cow manure composts. FEMS Microbiol. Lett. **233**: 115-123.

Halet, D., N. Boon. and W. Verstraete. 2006. Community dynamics of Methanotrophic

bacteria during composting of organic matter. J. Bios. Bioeng. **101**: 297-302.

Herrmann, R. F. and J. F. Shann. 1997. Microbial community changes during the composting of municipal solid waste. Microbiol. Ecol. 33:78-85.

Ho, K.-C., C.-C. Tsai and T.-L. Chung. 2001. Organization of ribosomal genes from a loofah witches' broom phytoplasma. DNA Cell Biol. **20**: 112-122.

Klamer, M. and E. Baath. 1998. Microbial community dynamics during composting of straw material using phospholipid fatty acid analysis. FEMS Microbiol. Ecol. **27**: 9-12.

Lagace, L., M. Pitre, M. Jacques and D. Roy. 2004. Identification of the bacterial community of maple sap by using amplified ribosomal DNA (rDNA) restriction analysis and rDNA sequencing. Appl. Environ. Microbiol. **70**: 2052-2060.

Marshall, M. N., L. Cocolin, D. A. Mills and J. S. Vander-Gheynst. 2003. Evaluation of PCR primers for denaturing gradient gel electriphoresis analysis of fungal communities in compost. J. Appl. Microbiol. **95**: 934-948.

Neefs, J.-M., Y. V. de Peer, P. D. Rijk, S. Chapelle and R. D. Wachter. 1993. Compilation of small ribosomal subunit RNA structures. Nucl. Acid Res. **21**: 3025-3049.

Peters, S., S. Koschinsky, F. Schwieger and C. C. Tebbe. 2000. Succession of microbial communities during hot composting as detected by PCR-single-strand-conformation-polymorphism -based genetic profiles of small-subunit rRNA genes. Appl. Environ. Microbiol. **66**: 930-936.

Pourshafie, M., P. Vahdani and M. Popoff. 2005. Genotyping *Clostridium botulinum* toxinotype A isolates from patients using amplified rDNA restriction analysis. J. Med. Microbiol. **54**: 933-936.

Ryckeboer, J., J. Mergaert, K. Vaes, S. Klammer, D. de Clereq, J. Coosemans, H. Insam and J. Swings. 2003. A survey of bacteria and fungi occurring during composting and self-heating processes. Ann. Microbiol. **53**: 349-410.

Sasaki, E., R. Osawa, Y. Nishitani and R. A. Whiley. 2004. ARDRA and RAPD analysis of human and animal isolates of *Streptococcus gallolyticus*. J. Vet. Med. Sci. **66**: 1467-1470.

Sasaki, H., E. Kawamoto, E. Okiyama, H. Ueshiba, K. Mikazuki, H. Amao and T. Sawada. 2006. Molecular typing of *Pasteurella pneumotropica* isolated from rodents by amplified 16S ribosomal DNA restriction analysis and pulsed-field gel electrophoresis. Microbiol. Immunol. **50**: 265-272.

Sessitsch, A., A. Weilharter, M. H. Gerzabek, H. Kirchmann and E. Kandeler. 2001. Microbial population structures in soil particle size fractions of a long-term fertilizer field experiment. Appl. Environ. Microbiol. **67**: 4215-4224.

Strom, P. F. 1985a. Effect of temperature on bacterial species diversity in thermophilic solid-waste composting. Appl. Environ. Microbiol. **50**: 899-905.

Strom, P. F. 1985b. Identification of thermophilic bacteria in solid-waste composting. Appl. Environ. Microbiol. **50**: 906-913.

Wu, C.-S. 2000. Establishing composting procedure for treatment of the agricultural waste from fruit-vegetable market. Annual report to Environmental Protection Administration, Executive Yuan, Taiwan. 42pp. (in Chinese)

Yan, B., K. Hong and Z.-N. Yu. 2006. Archaeal communities in mangrove soil characterized by 16S rRNA gene clones. J. Microbiol. **44**: 566-571.

堆肥中兼氧性嗜熱細菌相之研究

洪新棠[1]、張甘楠[2]、何國傑[1,3]

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摘　　要

細菌的活性是堆肥成熟度及品質的重要決定因素之一。為了瞭解本土堆肥在堆肥化過程中細菌的活性，我們分析了臺糖虎尾廠堆肥中兼氧性嗜熱細菌的族群。總共分離了 412 個菌落，依據擴增核糖體 DNA 限制片段分析（ARDAR）並輔以傳統的菌落觀察，格蘭氏染色，及生化測驗，可將他們分成 24 群。整體而言，ARDAR 與傳統方法的結果相當一致，均顯示 24 群中之第 17 群有別於其它的菌群。根據擴增的核糖體 DNA 序列及親緣樹的分析，證明第 17 群為親緣上接近 *Pseudomonas stutzer* 的綠膿桿菌屬，其餘的 23 菌群屬於枯草桿菌，在親緣上接近 *Bacillcus licheniformis* 及 *Bacillus sonorensis*。這些研究結果將有助於台灣區域性之堆肥研究。本研究同時證明 ARDAR 可直接用於鑑定堆肥中的菌種。

關鍵詞：擴增核糖體 DNA 限制片段分析、堆肥、16S 核糖體 DNA、嗜熱細菌。

1. 國立臺灣大學生命科學系及植物科學研究所，106 台北市羅斯福路 4 段 1 號，臺灣。
2. 私立大仁科技大學生物科技系暨研究所，907 屏東縣鹽埔鄉新二村維新路 20 號，臺灣。
3. 通信作者。Tel: 886-2 -33662508; Fax: 886-2-23637274; Email: kch@ntu.edu.tw