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Bacterial species in raw and cured compost from a large-scale urban composter

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SUMMARY

Raw and cured compost samples from a large-scale urban composter were studied over a period of eight months to gain information on bacterial species present. Total viable, aerobic heterotrophic bacteria, lactose-positive bacteria, antibiotic and metal-resistant bacteria and thermophilic bacteria were enumerated. Both raw and cured compost samples contained metal and antibiotic-tolerant bacteria (<Log 3.0 to Log 8.5 CFU g^{-1} compost) as well as high numbers (as high as Log 7.4 CFU g^{-1} dry weight compost) of thermophilic bacteria isolated by growth at 55 °C. Selected colonies were also identified using the Biolog 95 substrate identification system. *Escherichia coli* and *Salmonella* spp. were not detected in compost samples.

INTRODUCTION

Composting involves the breakdown of complex and simple organic materials by aerobic microorganisms by an ecological succession of microorganisms during the composting process [1]. Hardy and Sivasithamparam [7] reported, in their study of the microbial, chemical and physical changes during composting of eucalyptus bark mix, that initially bacteria and filamentous fungi were the primary decomposers. However, over time yeasts and actinomycetes increased in numbers. In addition to these organisms, viruses, protozoan cysts and helminth ova may also be present. The abundance of these various organisms depends to some extent on the material being composted. A major goal of composting is to produce an end product that is free of animal and plant pathogens. Several studies have shown that aerobic composting can destroy pathogenic microorganisms [4,10].

Three main factors involved in eradication of pathogenic microorganisms from compost are: heat generated during the initial decomposition phase; toxicity of conversion products and other products such as antibiotics and volatile compounds formed primarily during the heat phase; and microbial antagonism during the entire composting process. Of these three, thermal death is thought to be the most important factor in eliminating both plant [8,12] and human pathogens [6]. Pathogenic organisms are destroyed provided the composting temperature reaches 60–70 °C for a minimum of three consecutive days.

According to Bollen [3], *Olpidium* species and wilt-causing formae species of *Fusarium oxysporum* are among the most heat-resistant fungal plant pathogens. In a study on the inactivation of soil-borne plant pathogens during small-scale composting, Bollen et al. [4] studied 17 plant pathogens, including *F. oxysporum* and *Olpidium* species. Only *F. oxysporum* f. sp. *melongenae*, *F. oxysporum* f. sp. *lilii* and *O. brassicae* survived composting, at very low numbers. In one instance, survival of *F. oxysporum* f. sp. *lilii* was attributed to poor composting. Where composting proceeded properly, the pathogen was totally eradicated and *O. brassicae* survived at extremely low levels. They also reported eradication of pathogens during the shorter heat phase of composting. Since microbial antagonism and formation of toxic conversion products are dependent on a number of factors (source material being composted, aeration, temperature, additives), these researchers considered heat killing the major contributing factor to destruction of pathogens. These findings are in agreement with an earlier study on survival of human pathogens in composted sewage [11], which used the yeast *Candida albicans* as the test organism because it was reported that *C. albicans* could withstand 60 °C temperatures for more than 90 min. *C. albicans* was killed by exposure to 70 °C for 60 min or 80 °C for 30 min.

In this report, we describe studies on enumeration and identification of bacterial species present in raw and cured compost from a large-scale urban compost facility, with an emphasis on viable heterotrophs, numbers of metal and antibiotic-resistant bacteria, and lactose-positive organisms. Aerobic, thermophilic bacterial numbers were also determined. Information from this study may be useful in

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determining the bacterial composition of compost as this waste management practice will be increasingly used in both urban and rural areas.

MATERIALS AND METHODS

Compost facility

A large-scale compost facility located in Toronto, Ontario, Canada was used as the source of compost samples for this study. The digester facility was in operation for several months prior to commencing this study. The assumption was made that compost samples removed from the digester and curing cells were representative. The digester was about 490 m^3 in capacity and housed in an enclosed structure. The air flow rate into the digester ranged from 43 to 115 L min^{-1}. The O_2 concentration in the digester varied between 20% in aerobic areas to less than 0.4% in anaerobic areas. The digester temperature ranged from 29 to 79 °C during operation. This depended on other operating parameters such as the loading rate which varied from 0 to 190 metric tons of source material per week. The entire digester tank was mixed once about every 45 min with large rotating augers. Compost resided in the digester for 2–5 weeks. The primary waste entering the digester was leaves, grass and food waste from urban residents. Digested compost was removed to an outdoor curing cell 6.7 m in width and 17 m in length. The final cured compost was also passed through a 6.4-mm mesh screen. The pH of the digester compost was 6.7–7.4 in the aerobic zones.

Agricultural sandy loam soil

For comparison purposes, agricultural sandy loam soil samples were also subjected to the same bacterial enumeration protocols as compost samples. The sandy loam soil has a pH of 7.8, a water holding capacity of 0.41 ml g^{-1}, 3.3% organic matter content, 78% sand, 10% silt and 12% clay [12]. The soil samples used have not been treated with agrochemicals or subjected to agricultural practices.

Bacterial enumeration and identification

Compost samples were removed from the digester and cured compost piles for microbiological studies. Two-liter samples were taken from the cured compost piles while 100-g compost samples were removed from larger samples taken from the digester.

Two 10-g subsamples of moist compost were oven-dried for 24 h at 105 °C to determine their dry weight. An initial compost suspension was made by adding 10 g (wet weight) of compost to 90 ml of 0.1% (w/v) sterile sodium pyrophosphate (pH 7.0). The suspension was shaken at 240 r.p.m. for 20 min at 22 °C. Serial dilutions of the initial compost suspension in sterile sodium pyrophosphate were then made. Aliquots (0.1 ml) of each dilution were spread onto Petri plates, in triplicate, containing the following agar media: Nutrient Agar (Difco, Detroit, MI, USA), MacConkey Agar (Difco), Nutrient Agar containing 100 μg ml^{-1} of cyloheximide to inhibit fungal growth plus 100 μg ml^{-1} of one of the following antibiotics: ampicillin, chloramphenicol, kanamycin, penicillin, streptomycin or tetracycline; Nutrient Agar containing 100 μg ml^{-1} of cycloheximide and one of the following metals: copper, lead or mercury each at a concentration of 100 μg ml^{-1} or cadmium at 50, 75 or 100 μg ml^{-1}. All plates were incubated at 30 °C for 24 h before determining the first colony counts. It was sometimes necessary to incubate plates for several days to allow colonies to develop.

Dilutions were also held at 80 °C for 15 min. Aliquots of 0.1 ml were then plated in triplicate onto Nutrient Agar plates and incubated at 55 °C for 24–48 h. This allowed enumeration of aerobic, thermophilic bacteria.

The number of colony-forming units was expressed as Log CFUs g^{-1} dry weight compost. Counts taken from the Nutrient Agar plates provided the total aerobic bacterial heterotrophs per g dry weight compost. Lac^+ colonies from MacConkey Agar plates yielded lactose positive CFUs g^{-1} dry weight compost. Counts obtained from metal- or antibiotic-amended plates yielded counts specific to those plates per g dry weight compost. Counts taken from the Nutrient Agar plates incubated at 55 °C yielded the viable, thermophilic, bacteria per g dry weight compost.

Organisms isolated from each compost sample were identified using the Biolog (Biolog Inc., Hayward, CA, USA) identification system. Different colonies were picked for Gram-staining and Biolog identification. Predominant colonies were selected from plates based on colony size, shape, texture and color. All bacterial isolates were first grown on Tryptic Soy Agar as recommended by the supplier of Biolog for growth of Gram-negative bacteria and their Gram reactions were determined. The Gram stain procedure also served as a check of the purity of the isolate, since the Biolog system is not designed for mixed cultures, and to reveal cell morphology. Isolates determined to be Gram-positive were subcultured onto Biolog Universal Growth Medium (BUGM) and isolates at 55 °C were grown on Biolog Universal Growth Medium plus glucose (BUGM+G) or yeast extract with glucose (YEG). BUGM+G and YEG are recommended by Biolog for growth of spore-forming rods. The identifications were performed according to the manufacturer's instructions and the results recorded after 4 and 24 h incubation at 30 °C for all isolates, except for tests for thermophiles which were conducted at 55 °C.

RESULTS AND DISCUSSION

The bacterial enumeration data summarized in Tables 1 and 2 were derived from cured and raw compost as well as a sandy loam agricultural soil obtained from the University of Guelph field station at Cambridge, Ontario, Canada. The agricultural soil sample was processed in the same way as the compost and included for comparative purposes, especially with respect to numbers of metal and antibiotic-resistant bacteria. Cured compost exhibited counts of aerobic heterotrophs ranging from Log 7.4 to 8.6 CFU g^{-1} dry weight compost. Raw compost samples from the digester

TABLE 1

Total counts of heterotrophic, lactose positive and thermophilic bacteria in cured and raw (digester) compost and agricultural sandy loam soil

Sample	Log CFU g^{-1} dry weight compost (n = 3)		
	Heterotrophs	Lactose positive	Thermophilic
Cured compost			
Nov.16, 1992	7.4	<3.0	6.4
Feb. 1, 1993	7.6	<4.0	6.9
Feb. 8, 1993	8.6	<3.0	7.3*
Mar. 1, 1993	7.6	<3.0	7.1*
Mar. 2, 1993	7.6*	<3.0	7.4
Apr. 13, 1993	8.2	<3.0	6.0
Apr. 23, 1993	6.3	<3.0	6.3
Digester			
Dec. 9, 1992	8.7	<4.0	6.5
Dec. 18, 1992	7.8	5.1	6.0
Agricultural sandy loam soil			
Jan. 13, 1993	6.2*	<3.0	<3.0

*Average of two plates only.

TABLE 2

Selected counts of metal- and antibiotic-resistant bacteria in cured and raw (digester) compost and a sandy loam agricultural soil. Metals and antibiotics were individually added to nutrient agar at a concentration of 100 $\mu g\ ml^{-1}$ with the exception of cadmium which was used at three concentrations as specified

Sample	Log CFU g^{-1} dry weight compost (n = 3) in nutrient agar supplemented with									
	Cadmium	Copper	Lead	Mercury	Ampicillin	Chloramphenicol	Kanamycin	Penicillin	Streptomycin	Tetracycline
Cured compost										
Nov. 16, 1992	<3.0	5.7	7.2	4.6*	4.6	<3.0	<4.0	6.4	6.2	<3.0
Feb. 1, 1993	5.0*[b]	6.3	7.5	6.1	6.4	<4.0	6.3	7.5*	6.3	<4.0
Feb. 8, 1993	5.7[b]	7.5	>6.0	6.8	7.6	<3.0	8.3	8.5	7.3	<3.0
Mar. 1, 1993	5.1[b]	5.4	7.9*	<4.0	6.6	<3.0	6.8	7.7*	7.5	<3.0
Mar. 2, 1993	4.2*[b]	5.5	7.5*	<4.0	6.6	<3.0	6.1	7.5*	6.7	<3.0
Apr. 13, 1993	4.3[b]	5.5	8.1	5.7	6.0	<3.0	5.6	8.1	6.4	<3.0
Apr. 23, 1993	3.8[b]	<4.0	<5.0	<3.0	5.3	<3.0	<5.0	<5.0	5.8	<3.0
Digester										
Dec. 9, 1992	>6.0[a]	8.3	8.6	5.9*	8.1	6.4	6.5	8.3	6.7	6.5
Dec. 18, 1992	5.3[b]	7.3	7.7	4.9	5.5	5.0	5.0	7.5	6.3	5.1
Agricultural sandy loam soil										
Jan. 13, 1993	<3.0[b]	3.4	5.7*	<3.0	4.8	<3.0	4.1	6.0	4.2	<3.0

*Average of two plates only.
[a]Cadmium concentration = 50 $\mu g\ ml^{-1}$.
[b]Cadmium concentration = 75 $\mu g\ ml^{-1}$.

facility contained Log 8.7 CFU g^{-1} and Log 7.8 CFU g^{-1} in samples taken on 9 December and 18 December 1992, respectively, as determined by plating on Nutrient Agar. Lactose positive CFUs were usually less than Log 4.0 g^{-1} with one exception. Thermophilic counts ranged from Log 6.0 to Log 7.4 CFU g^{-1}.

Counts of thermophiles were not higher in digester samples compared to cured compost. The agricultural sandy loam soil contained less than Log 3.0 CFU thermophiles g^{-1} dry weight soil. In general, there were negligible differences in bacterial counts on the different media with respect to time of sampling and whether the sample was from the digester or the curing cells. This suggests that bacterial numbers in raw and cured compost were relatively stable.

All digester and cured compost samples contained bacterial species resistant to cadmium, copper, lead and mercury (Table 2). All metals were used at a concentration of 100 $\mu g\ ml^{-1}$ with the exception of cadmium which was used at 50, 75 and 100 $\mu g\ ml^{-1}$ concentrations depending on the sampling and plating time. A metal concentration of 100 $\mu g\ ml^{-1}$ is relatively high and should provide good selection for metal-tolerant species in compost samples. Whereas the agricultural soil contained from <Log 3 to Log 5.7 CFU g^{-1} dry weight compost, both cured and raw compost contained as high as Log 8.0 CFU g^{-1} dry weight compost tolerant to lead. The numbers of bacteria tolerant to the metals in compost did not exhibit any noticeable trends, nor did numbers of bacteria resistant to ampicillin, chloramphenicol, kanamycin, penicillin, streptomycin or tetracycline (Table 2). However, the numbers of antibiotic-resistant CFU g^{-1} compost ranged from <Log 3 to Log 8.5 g^{-1} dry weight compost. Antibiotic-tolerant bacterial numbers in the agricultural sandy loam soil ranged from <Log 3.0 to Log 6.0 CFU g^{-1} dry weight soil. Compost

TABLE 3

Biolog identification of bacterial colony types selected from various media

Sample	Colony origin	Gram-reaction	Biolog identification
Cured compost			
Sept. 29, 1992	Cadmium Agar	–	*Klebsiella pneumoniae* A
		–	*Flavimonas oryzihabitans*
	Tetracyline Agar	–	*Serratia marcescens*
	Lead Agar	–	*Serratia marcescens*
	MacConkey Agar	–	*Serratia marcescens*
			Pseudomonas putida A
	Ampicillin Agar	–	*Pseudomonas fluorescens* B
	Penicillin Agar	–	*Pseudomonas putida* B
	Cadmium Agar	–	*Methylobacterium extorquens*
Nov. 16, 1992	Copper Agar	–	*Pseudomonas stutzeri*
	Lead Agar	–	*Serratia entomophilia*
	Nutrient Agar	–	*Moraxella bovis*
	Penicillin Agar	–	*Psychrobacter immobilis*
		–	*Pseudomonas fluorescens* E
	Ampicillin Agar	–	*Xanthomonas maltophilia*
		–	*Pseudomonas fluorescens* E
	MacConkey Agar	–	*Pseudomonas fluorescens* E
Digester			
Dec. 9, 1992	MacConkey Agar	–	*Pseudomonas fragi*
	Lead Agar	–	*Pseudomonas fragi*
	Tetracycline Agar	–	*Pseudomonas fragi*
	Ampicillin Agar	–	*Pseudomonas fragi*
	Copper Agar	–	*Pseudomonas fragi*
Dec. 18, 1992	Lead Agar	–	*Pseudomonas fragi*
	MacConkey Agar	–	*Pseudomonas fragi*
	Ampicillin Agar	–	*Pseudomonas fragi*
	Penicillin Agar	–	*Leuconostoc mesenteroides*
Agricultural sandy loam soil			
Jan. 13, 1993	Penicillin Agar	–	*Xanthomonas oryzae*
	Lead Agar	+	*Bacillus thuringiensis*
	Chloramphenicol Agar	–	*Acinetobacter baumannii*
	Copper Agar	–	*Alcaligenes paradoxus*
		–	*Comamonas acidovorons*
Feb. 1, 1993	Lead Agar	–	*Pseudomonas fragi*
	Nutrient Agar	–	*Pseudomonas fluorescens* B
		–	CDC Group DF-3
	Chloramphenicol Agar	–	*Pseudomonas fluorescens* B
	Streptomycin Agar	–	*Corynebacterium jeikeium*
	Copper Agar	–	*Acinetobacter radioresistens*
	Penicillin Agar	–	*Alteromonas haloplanktis*
Feb. 8, 1993	Chloramphenicol Agar	–	*Acinetobacter baumannii*
	Streptomycin Agar	–	CDC Group II–I
		+	*Corynebacterium jeikeium*
	Kanamycin Agar	–	*Ochrobactrum anthropi*
	MacConkey Agar	–	*Pseudomonas fluorescens* B
	Copper Agar	–	*Pseudomonas fluorescens* B
	Ampicillin Agar	–	*Pseudomonas fluorescens* B
		–	*Pseudomonas viridilivida* A
	Lead Agar	–	*Pseudomonas viridilivida* A
	Penicillin Agar	–	*Cytophaga johnsonae*
	Tetracycline Agar	–	*Acinetobacter calocetius*

(Continued over)

TABLE 3

Continued

Sample	Colony origin	Gram-reaction	Biolog identification
Mar. 1, 1993	MacConkey Agar	–	*Pseudomonas marginalis*
		–	*Pseudomonas fragi*
	Nutrient Agar	–	*Pseudomonas putida*
		–	*Pseudomonas fragi*
	Copper Agar	–	*Pseudomonas putida* B
	Cadmium Agar	–	*Cytophaga johnsonae*
	Ampicillin Agar	–	*Pseudomonas fluorescens* B
Mar. 2, 1993	MacConkey Agar	–	*Serratia marcescens*
	Nutrient Agar	–	*Xanthomonas oryzae*
		–	*Pseudomonas fluorescens* B
	Ampicillin Agar	–	*Pseudomonas fluorescens* B
	Penicillin Agar	–	*Pseudomonas fragi*
	Kanamycin Agar	–	CDC Group II–I
		–	*Sphingobacterium mizutaii*
	Lead Agar	–	*Pseudomonas marginalis*
	Cadmium Agar	–	*Pseudomonas fragi*
Apr. 13, 1993	MacConkey Agar	–	*Pseudomonas taetrolens*
		–	*Pseudomonas fluorescens* B
	Nutrient Agar	–	*Pseudomonas fluorescens* B
	Cadmium Agar	–	*Flavobacterium indologenes*
	Mercury Agar	–	*Pseudomonas diminuta*
	Copper Agar	–	*Pseudomonas fluorescens* C
		–	CDC Group II-E A
	Ampicillin Agar	–	*Pseudomonas fluorescens* B
	Streptomycin Agar	–	CDC Group II-I
Apr. 23, 1993	MacConkey Agar	–	*Pseudomonas putida* A
		+	*Bacillus brevis*
		+	*Staphylococcus capitis* ssp. *ureolyticus*
	Ampicillin Agar	–	*Pseudomonas putida* A
		–	*Flavobacterium gleum*
	Copper Agar	–	*Pseudomonas fluorescens* A

samples contained a higher number of bacterial numbers (Log 8.5 g^{-1} in the February 8, 1993 sample) resistant to penicillin. Antibiotic-resistant colonies are found in soil and aquatic environments, and as observed in this study, also in compost.

Table 3 summarizes bacterial species identified on the basis of their Gram stain reaction and Biolog identification. A wide diversity of Gram-negative bacteria were identified. Numerous *Pseudomonas* species were present as well as *Serratia*, *Xanthomonas*, and *Klebsiella*. *Corynebacterium jeikeium* (Gram-positive) was isolated from cured compost and *Serratia marcescens* was identified from several samples. Several *Pseudomonas* spp. were tolerant to lead, cadmium, copper, ampicillin, chloramphenicol, tetracycline and penicillin (Table 3). Difficulty was experienced with identification of bacterial isolates from Nutrient Agar plates incubated at 55 °C for enumeration of thermophilic bacteria. It is noteworthy that *Escherichia coli* and *Salmonella* species were not found.

Hussong et al. [9] reported that in sewage sludge composts from 30 municipalities, 4 (12%) contained salmonellae. It was concluded that active indigenous microbial populations in compost established a barrier to colonization by salmonellae. Species such as *S. marcescens* can be considered an opportunistic animal pathogen. However, most of the isolates are relatively common soil organisms. The significance of the number of antibiotic and metal-tolerant bacteria in the compost samples is interesting, given that no metals were deliberately added to the compost facility. It appears that a large number of bacterial species both in compost and in agricultural soil are tolerant to antibiotics and metals. It is not known if the resistances are plasmid-encoded in the isolates studied. However, some percentage of the metal- and antibiotic-tolerant isolates is likely due to plasmid-encoded mechanisms.

In summary, there were no distinct trends or differences observed between raw and cured compost samples over the duration of this study. Thermophilic bacterial numbers in

compost samples were considerably higher than in the agricultural soil (Table 1). However, isolates were not easily identified using the GP Biolog system due to the necessity to incubate the Biolog GP microplates at 55 °C for growth of thermophiles. It may be necessary to use other identification methods to obtain accurate identification of these thermophilic bacteria. Several *Bacillus* spp. were isolated by growth at 55 °C. They were identified by their rod-shaped cell morphology and their Gram-positive staining reactions. *Bacillus* spp. are typically aerobic endospore-forming rods, that produce spores resistant to heat, with vegetative cells capable of degrading many organic substrates [2]. This was expected due to the high operating temperatures of the compost facility for efficient composting and killing of plant and animal pathogenic microorganisms. The bacteriological composition of compost varies depending on the facility and the composition and nature of wastes entering the digester. If the curing cells are exposed to the environment, then the compost will also contain microbial species that come into contact with the compost and survive or multiply in it.

Collins and Kennedy [5] recently published an excellent review on microbiological hazards of municipal and clinical wastes. Pathogenic organisms were listed that may be found in the wastes, as well as endotoxin-producing microbial strains found in the air of waste-handling facilities. Some *Pseudomonas* spp. are pathogens and *P. putida* was listed as an endotoxin-producing strain [4]. *P. putida* was detected in compost samples in this study.

The antibiotic- and metal-resistant isolates found in this study were not investigated further to ascertain the resistance mechanisms. However, many metal- and antibiotic-resistant mechanisms are encoded on self-transmissible, mobilizable and non-conjugative plasmids. There is a paucity of information on plasmid transfer at elevated temperatures during composting. The same information is also lacking in mature, cured compost. Since antibiotics and metals are not deliberately added to the incoming waste that will be transformed to compost, microbial strains in the compost should not require metal-resistance plasmid(s) for normal growth and activity unless the plasmids also encode for catabolic metabolism of organic compounds during the composting process. As more information becomes available on microbiological populations in compost, it may be possible to use selected strains to inoculate compost to achieve a more controlled composting process. For example, pesticide residues which may be found in grass clippings entering composters may be more rapidly degraded by selected microbial strains.

Although composting is an ancient art and science, studies on microbial populations in compost will likely be necessary as large-scale urban compost facilities become more common and occupational health and safety regulations evolve with expanding use of composting.

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