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Occurrence and Distribution of *Legionella* Species in Composted Plant Materials

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Legionellae were found in many samples of composted plant matter obtained from home gardeners and from facilities which undertook bulk composting. The predominant species isolated from these composts was *Legionella pneumophila*, the strains of which belonged to serogroups other than serogroup 1. Other *Legionella* species were present in many samples. *Legionella longbeachae* serogroup 1, which is implicated in human infections in South Australia, was present in samples obtained from two of six facilities composting large volumes of material and from 3 of 30 gardeners. Many of the species or strains isolated from composts have not been implicated as causative agents of legionellosis in South Australia, but some cause infection in healthy and immunosuppressed persons.

Legionella longbeachae serogroup 1 infections are prevalent in South Australia and in recent years have outnumbered those due to *Legionella pneumophila* (1, 5). This species is common in composted waste wood products used in potting mixes in Australia (6) but has not been found in any water samples (7, 10). Although the most commonly identified species in potting mixes were *L. longbeachae* serogroup 1 and *Legionella bozemanii*, other species were also present in some samples (6). Legionellae were not consistently found in source materials used to make potting mixes, but they multiplied quickly in the early stages of composting and reached high numbers within 4 weeks. Composting of waste wood products requires adequate moisture and nitrogen to be effective, and the process generates considerable heat. Even in winter, temperatures in the outer 300 to 400 mm of composting heaps are maintained for days between 25 and 35°C and are in the optimal range for the multiplication of soil legionellae and free-living amoebae. Composting is therefore an important step in amplifying the numbers of *L. longbeachae* serogroup 1 and other legionellae in pine bark and sawdust. Many gardeners compost plant matter from their gardens, and recycling of larger volumes of waste plant materials from homes, parks, and gardens by composting has been adopted by many municipalities. The aim of this study was to determine if legionellae were present in municipal and home composted materials used by gardeners in Australia.

MATERIALS AND METHODS

Samples. Compost samples (n = 33), each approximately 500 g, were obtained from six facilities where large volumes of material (>10 m^3) were composted each time. Two of these were municipal (city) councils, three were botanic or zoological gardens, and one was a commercial manufacturer of organic composts. All facilities used plant waste including grass cuttings, shredded leaves and branches, and other vegetable materials from parks and from public and private gardens. This material was frequently mixed with soil and composted for 4 to 12 weeks. The commercial manufacturer added pine sawdust, straw, and animal and poultry manure to the composting plant material, which was mainly grass cuttings. Several samples from each facility were collected from the outer 30 cm of the heaps. Samples were collected from five facilities in two or three different seasons. Eighty samples of home composts were obtained from 30 gardeners, 26 in South Australia and 4 in Queensland. Most samples were collected in spring and summer, but 12 gardeners also submitted samples in winter.

Isolation and identification. The methods used to isolate and characterize legionellae from samples have been described previously (6, 7, 10). Briefly, suspensions of samples prepared as described below were decontaminated by treatment with 0.2 M HCl–KCl buffer followed by plating of 50 μl and 5 μl on selective *Legionella* agar medium (vancomycin-polymyxin-pimafucin) containing 1% bovine serum albumin (7). Methods were modified, as most composts were not sufficiently free draining for leachates to be prepared and culture plates from many samples were rapidly overgrown by numerous or spreading soil bacteria. To overcome these problems, a 1 in 3 (wt/vol) suspension of each sample was prepared in sterile tap water, mixed by vortexing, allowed to settle 30 min, and then treated with acid for 15 min after being diluted 1 in 100 in HCl-KCl buffer. If cultures were negative for legionellae or were overgrown by soil bacteria, the composts held at room temperature were retested at 2- to 3-week intervals on one or two further occasions before they were discarded as being unassessable. The limit of detection for most tests was 10^3 CFU of legionellae per g. Identification methods included tests for cysteine dependence, direct fluorescent antibody tests, and slide agglutination with latex-agglutinating sera. Ribotyping, with or without ubiquinone and fatty acid analyses, was used to identify *L. longbeachae* and other legionellae in selected cases. The monoclonal antibody MAb2, used to examine *L. pneumophila* serogroup 1 strains, was prepared from a hybridoma obtained from the American Type Culture Collection, Rockville, Md., and was used as recommended to identify epidemic strains (4, 9).

RESULTS

Large-scale composts. The results for 33 composts made in six facilities are shown in Table 1. Populations of legionellae in compost generally ranged from 1×10^3 to 5×10^5 CFU/g. In one facility the population of *L. pneumophila* in a sample

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TABLE 1. Occurrence of various *Legionella* species in composted material obtained from large-scale composting facilities

Organism(s)	Legionellae in composts from facility:					
	1	2	3	4	5	6
L. pneumophila serogroup 1	–[a]	–	–	+[b]	–	–
L. pneumophila serogroups 2 to 14	+	+	+	+	+	+
L. bozemanii serogroup 1	+	–	+	+	+	+
L. longbeachae serogroup 1	+	+	–	–	–	–
L. micdadei	–	+	–	–	–	–
L. quinlivanii	+	–	–	–	–	–
L. santicrucis	–	–	–	+	–	–
L. anisa group[c]	–	–	+	–	–	–
Unidentified *Legionella* species	+	–	–	+	+	–

[a] Not detected.
[b] Present.
[c] Includes *L. bozemanii* serogroup 2.

collected in summer was 1.7×10^6 CFU/g. The *L. pneumophila* serogroup 1 strain isolated from one facility did not react with the monoclonal antibody MAb2. *L. pneumophila* strains from four facilities reacted strongly with direct fluorescent antibodies to *L. pneumophila* serogroups 3, 4, and 6. Strains that reacted with latex-agglutinating antisera to *L. pneumophila* serogroups 2 to 14 but did not give a strong reaction with direct fluorescent-antibody reagents to serogroups 1 to 6 were also present in many samples. The lack of suitable reagents prevented serotyping of these strains of *L. pneumophila*. *L. longbeachae* serogroup 1 was present in a population of $\geq 2 \times 10^4$ CFU/g in two facilities. Composts from all facilities frequently contained more than one *Legionella* species or more than one serogroup of *L. pneumophila*. Species other than *L. pneumophila* and *L. longbeachae* were found in some facilities, as detailed in Table 1. The populations of legionellae were generally higher in samples of composted material collected in summer than in those collected in cooler months, but there was a wide variation in populations within each season. Overgrowth of culture plates with indigenous soil bacteria did occur but was not a major problem. A high percentage of samples (85%) from large-scale composting facilities yielded legionellae (Table 2).

Home composts. Legionellae were isolated from 45 of the 80 samples tested. These positive samples were obtained from 20 gardeners. Legionellae were not detected in 24 samples obtained from seven gardeners (Table 2). With repeat testing, 11 samples from three gardeners remained unassessable because of overgrowth of plates by other soil bacteria. Results for the *Legionella* species and strains isolated from home composts are shown in Table 3. Some samples contained more than one species or more than one serogroup of *L. pneumophila*. One *L. pneumophila* serogroup 1 strain was isolated from home composts. This did not react with the monoclonal antibody MAb2. The majority of *L. pneumophila* strains belonged to serogroups other than serogroups 1 to 6. We did not identify to the species level blue-white autofluorescent strains that reacted with *Legionella anisa* antiserum. This serum also reacted strongly with *L. bozemanii* serogroup 2. Unidentified legionellae were very common in these composts and were present in samples obtained from 14 gardeners. Unidentified strains were the only legionellae present in samples from six gardeners. Preliminary investigations of these strains using ribotyping, ubiquinone, and/or fatty acid analyses suggested that they comprised a number of distinct species, some of which may be novel. There was no obvious seasonal variation in the numbers of legionellae present in samples. Samples from four gardeners had a tenfold-higher population in spring than in summer, while those from five had the reverse. In four cases, populations were within 1 $\log_{10}$ unit in both seasons. Populations of legionellae were usually in the range of 10^3 to 10^5 CFU/g.

As legionellae were prevalent in home composts, we obtained garden soil samples from 14 gardeners shown during the survey to have legionellae present in their composts. These samples were collected about 1 month after the winter samples of compost were examined. Legionellae were present in garden soils obtained from six gardeners (43%) and were identified as *L. pneumophila* in samples from five of these six. This species had been present in at least one sample of composted material obtained from each of these five gardeners. The populations of legionellae in garden soils were similar to those found in many home composts and ranged from 1×10^3 to 5×10^4 CFU/g.

TABLE 2. Detection of legionellae in composted materials subjected to repeated testing, with cumulative results from first and final test shown

Sample type	Test	No. (%) of samples with *Legionella* species:		
		Present	Not detected	Overgrown
Large-scale compost (n = 33)	1st	21 (64)	5 (15)	7 (21)
	3rd	28 (85)	3 (9)	2 (6)
Home compost (n = 80)	1st	34 (42)	19 (24)	27 (34)
	3rd	45 (56)	24 (30)	11 (14)

TABLE 3. *Legionella* strains and species isolated from composts obtained from 20 gardeners with positive samples

Organism(s)	No. (%) of gardeners with positive samples
L. pneumophila serogroup 1	1 (5)
L. pneumophila serogroups 2 to 14	11 (55)
L. longbeachae serogroup 1	3 (15)
L. bozemanii serogroup 1	3 (15)
L. anisa group[a]	5 (25)
Unidentified strains	6 (30)

[a] Includes *L. bozemanii* serogroup 2.

DISCUSSION

This study demonstrated that legionellae were common in composted plant materials obtained from many sites, in numbers comparable to those found in bark-based potting mixes (6). However, the predominant species in these composts was *L. pneumophila*, rather than *L. longbeachae* serogroup 1. *L. longbeachae* serogroup 1 was present in a small number of home composts and in two large-scale composting facilities. It is possible that its presence in small numbers in some samples could have been obscured by other legionellae, particularly *L. pneumophila*. The methods used in this study did not permit the selective isolation of minority species from samples. The factors determining the predominance of particular species are unknown. The nature of the material being composted, the type and availability of nitrogen, and the complexity of the bacterial and protozoal populations could all influence the species of legionellae present.

The significance for human health of legionellae in garden composts is unknown. Epidemiological studies in South Aus-

tralia (1) found that gardening was a major risk factor for developing *L. longbeachae* infection. Infections with this species have been documented for individuals who have used only commercial potting mixes, for persons who have used only home-made composts, and once for a person exposed to dust created by the landscaping of an adjacent park, who denied having gardened in the 3 months before infection. The role of soil as a source of legionellosis is unclear but was suspected in an outbreak of Legionnaires' disease in 1965 (8). *L. pneumophila* serogroup 1, implicated in that outbreak, has rarely been found in soils but was recently isolated from sedimentary soils at depths up to 190 feet (57 m) in South Carolina (3). An investigation of 34 natural soil samples mainly from South Australia failed to detect *L. longbeachae* or other recognized species, but a species or strain antigenically related to *L. longbeachae* serogroup 1 and having a unique ribotype was found in one garden soil to which home compost had been added (6). The present study confirmed that legionellae were relatively common in garden soils mixed with composted materials.

Most of the *Legionella* species or strains found in these composted materials have not been implicated in infections in South Australia. We have documented occasional infections due to *L. pneumophila* serogroups 2, 3, and 5 by culture of respiratory secretions over the last 5 years. The sporadic nature of these cases and the occurrence of these serogroups in some water samples have led to the view that they were acquired from an unidentified water source. Home composts are normally applied directly to soil, a practice which minimizes the risk of aerosol formation. Aerosols are more likely to occur during the watering of free-draining materials such as potting mixes used for container plants. However, legionellae in all types of composted materials could be ingested accidentally while gardening. If aspiration is an important route of *Legionella* infection, as has been suggested (2), home composts and other materials containing these organisms could act as sources of infection for susceptible individuals. Many *Legionella* species, including *L. pneumophila*, have been implicated in infections of immunosuppressed individuals (2). *L. longbeachae* serogroup 1 organisms remained viable for periods up to 30 min on hands contaminated by handling potting mix but were readily removed by washing with soap and water (unpublished observations). Gardeners, especially those with impaired immunity, should be warned about the potential risks of handling composted materials containing legionellae and other respiratory pathogens. They should be advised to avoid creating aerosols while watering plants and on the importance of handwashing to prevent ingestion of contaminated material.

The source of legionellae in composts is unknown. The wide diversity in and disparate sources of materials used virtually preclude an association between particular materials and legionellae. Legionellae may be ubiquitous in soils in numbers too low to be detected by the culture methods employed in this study. Since legionellae flourish in composting materials, composts may represent an important ecological niche for the multiplication and dissemination of many species. The concept that legionellae are primarily aquatic bacteria should be reassessed.

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