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ACTINOMYCETES IN COMPOSTS*

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Abstract: Actinomycetes, especially thermophilic species, are well known components of the microflora of composts. Composts for mushroom cultivation, prepared from animal manures and straw, have been most studied but actinomycetes may also colonise household and green waste composts. Actinomycetes are Gram-positive bacteria that mostly possess a mycelium. *Thermoactinomyces* spp. also produce a mycelium and have been generally considered with the actinomycetes but they produce endospores and are closely related to *Bacillus* spp. Many actinomycete species produce spores which easily become airborne in large numbers when the substrate is disturbed and some cause different forms of extrinsic allergic alveolitis. Composting for mushroom cultivation takes place in two phases, the first in windrows with large water contents and the second in humidified tunnels heated to 55-60°C. Actinomycetes, particularly white *Thermomonospora* spp., *Thermomonospora chromogena* and *Microtetraspora* spp., develop abundantly during the second phase and many spores are released during spawning. However, no one species has been implicated in mushroom worker's lung. A similar microflora occurs in composts made from household waste but those from green waste often have microfloras dominated by *Streptomyces* spp., especially during the cooler winter months when windrow temperatures may be lower than in summer. Sewage composts are also rich sources of actinomycetes which may include *Nocardia* and *Promicromonospora* spp. Actinomycete development is dependent on aerobic conditions, temperature and water content although the interrelationships of these factors and the occurrence of different taxa have not been closely studied in composts.

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INTRODUCTION

Actinomycetes are Gram-positive bacteria but are distinguished from other bacteria by their morphology, by DNA rich in guanine plus cytosine and on the basis of nucleic acid sequencing and pairing studies. Although some show pleomorphic and even coccoid elements, they characteristically have a filamentous mycelium and many produce spores that are easily detached and may become airborne when disturbed. They may thus be considered as the prokaryotic equivalent of fungi. However, the genus *Thermoactinomyces*, although producing a mycelium and often considered an actinomycete, is more closely related to the *Bacillaceae* than to other actinomycetes and produces endospores in an analogous way to *Bacillus* and *Clostridium* species [46]. Actinomycetes are well known for their ability to produce antibiotics and enzymes and for their ability to degrade complex and recalcitrant molecules, especially cellulose, lignocellulose and lignin, which makes them particularly important in composting [7]. Some species (*Actinomyces, Actinomadura, Nocardia*) can infect man and animals. Others (*Saccharopolyspora*,

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Thermoactinomyces) are important causes of occupational respiratory disease (extrinsic allergic alveolitis), e.g., farmer's lung (*Saccharopolyspora* (*Sap*.) *rectivirgula*), bagassosis (*Thermoactinomyces* (*Tha*.) *sacchari*) and, probably, mushroom compost worker's lung [9, 39, 71].

Composts have long been known as important sources of actinomycetes [55, 58, 70] and it was also soon discovered that many of the species in these materials are thermophilic and able to grow at temperatures up to 65-70°C. Such high temperatures result from the strong microbial activity, possible when there is adequate water, nutrient and oxygen, releasing energy through their respiration faster than it can be dissipated. Mushroom compost is generally prepared by a specialised two phase process [68], involving artificial heating during the second phase. However, the microbiology of this process has been studied more thoroughly than composting of other materials and it will be used as the basis for a discussion of the occurrence and role of actinomycetes in composts in this paper.

ISOLATION AND ENUMERATION OF ACTINOMYCETES

The traditional method of isolating actinomycetes from composts and other substrates is to suspend samples in water or a suspension fluid containing osmoprotectants, such as quarter-strength Ringer's solution or inositol. A logarithmic dilution series is then prepared and samples of suitable dilutions are spread on the surface of agar media in Petri dishes (dilution plating). However, excessive growth of bacilli often restricts isolation of actinomycetes and better recoveries have been obtained using air sampling methods. Lacey [38] used a wind tunnel/ Andersen sampler method [43] while Amner *et al*. [1] used a sedimentation chamber-Andersen sampler method [44] together with selective media containing different antibiotics. Half-strength nutrient and tryptone soya + casein hydrolysate agars have proved useful for the isolation of thermophilic actinomycetes [43]. Addition of novobiocin (25-50 $\mu g\ ml^{-1}$ agar medium) can be used for the selective isolation of *Thermoactinomyces* spp. and kanamycin ($25\mu g\ ml^{-1}$) or rifampicin ($5\mu g\ ml^{-1}$) for *Thermomonospora* (*Thm.*) spp., including *Thm. chromogena* [1, 2].

Air sampling has also utilised Andersen samplers, at 25 $l\ min^{-1}$, for general air samples but filtration, using disposable aerosol monitors loaded with polycarbonate (Nuclepore) filters and personal sampler pumps operating at 2 $l\ min^{-1}$, has been used to estimate worker exposure. Andersen samplers deposit airborne spores directly onto the agar surface in prepoured Petri dishes. Spores on aerosol monitor filters are resuspended in a suspension fluid (bacteriological peptone, 1 $g\ l^{-1}$; Tween 80, 0.5 $g\ l^{-1}$; inositol, 20 $g\ l^{-1}$). A logarithmic dilution series is then prepared, using quarter-strength Ringer's solution, for plating [47, 48]. The same media may be used as for direct isolation from composts.

ACTINOMYCETE TAXONOMY

Much confusion has surrounded the taxonomy of some thermophilic actinomycetes. This largely arose from the initial emphasis on morphological characteristics for classifying isolates, inadequate descriptions, lack of agreement over which characteristics were of taxonomic importance, changing concepts as new criteria were introduced and the failure of some authors to fully explore some of the earlier literature.

Many of these problems are illustrated by the history of *Thermoactinomyces* spp. *Tha. vulgaris* was first described by Tsiklinsky [70] as an actinomycete that was unable to degrade starch. Later, isolates were included in this species that had this ability and Küster and Locci [35] concluded that the taxon comprised a single, variable species. Kurup *et al*. [32] then concluded that *Tha. vulgaris* could be divided into two species on the basis of starch, aesculin and arbutin degradation, tyrosinase activity and melanin production. Unfortunately, they decided that isolates that hydrolysed aesculin and split arbutin but which were unable to degrade starch, hypoxanthine and tyrosine should be called *Tha. candidus* and the other isolates *Tha. vulgaris*. However, the original reference culture of *Tha. vulgaris*, as in Tsiklinsky's [70] description, lacked activity against starch and tyrosine. *Tha. vulgaris* is thus the legitimate name for *Tha. candidus* and *Tha. thalpophilus* for isolates degrading starch and tyrosine [42]. Further white *Thermoactinomyces* spp. have since been described having other combinations of characters. *Thermoactinomyces dichotomicus* ('*Actinobifida dichotomica*' [28]) differs in having yellow aerial mycelium and endospores borne on dichotomously branched sporophores.

Other single spored actinomycetes are included in the genera *Saccharomonospora* (*Sam.*), *Thermomonospora (Thm.*), *Promicromonospora* (*Prm*.) and *Micromonospora* (*Mim.*). Isolates of *Saccharomonospora* have variously been referred to as *Actinomyces monosporus* [66], *Thermoactinomyces glaucus* [18], *Tha. viridis* [67], *Thermopolyspora glauca* [5] and *Thermomonospora viridis* [34]. They were then renamed *Saccharomonospora viridis* [59] but this species has been shown to be heterogeneous and has been divided into at least three species [16]. Other species have been described from China [62, 63].

Cross and Lacey [10] described the almost continuous range of morphological types in the genus *Thermomonospora* between extremes represented by *Saccharomonospora viridis* and *Thermoactinomyces dichotomicus*. Henssen [18, 21] identified five species within this range, although not all were fully described and some cultures could not be purified or were later lost. These included *Thm. curvata, Thm. lineata, Thm. fusca, Thm. spiralis* and *Thm. falcata*. Another white *Thm.* species, *Thm. alba*, was added by Locci *et al*. [50] for isolates that were less thermophilic than the preceding species and mesophilic species have also been described from soil. *Thm. chromogena* was transferred to *Thermomonospora* from

Table 1. Morphological characteristics of actinomycetes found in composts and of some related taxa [after 27].

Taxon	Colony colour	Spore chain and mycelium morphology	Spore surface	Wall chemotype[1]
Thermoactinomyces	White, yellow	Endospores formed singly on aerial and substrate mycelium, sessile or on short sporophores, dichotomously branched in one species.	Polygonal or ridged	III
Micromonospora	Orange - black	Branched substrate mycelium carrying single spores	Smooth, warty, spiny	II
Promicromonospora	Yellow	Mycelium breaking up into fragments of variable size and shape, giving rise to single rod-shaped, coccoid or chlamydospore-like elements	Smooth	VI
Thermomonospora	White	Single spores, densely packed sessile or on dichotomously branched sporophores	Smooth, spiny	III
Saccharomonospora	Blue, green, violet	Single, densely packed spores	Smooth, warty	IV
Saccharopolyspora	White, pink, brownish grey	Short or long chains, straight, loops and spirals	Smooth, roughened, spiny, hairy	IV
Microtetraspora	Blue-grey, cream, grey, pink, violet, yellow, white	Straight, hooked or spiral spore chains, up to 30 spores long, formed on branched aerial mycelium	Smooth, irregular, warty	III
Actinomadura	White, yellow, pink, blue, green, grey	Short or long chains, straight, loops and spirals, pseudosporangia in some species	Smooth, warty, spiny, uneven	III
Nocardiopsis	White to yellowish grey	Long, straight, flexuous or zig-zag-shaped hyphae, completely fragmenting into spores	Smooth	III
Saccharothrix	Yellowish-white, yellowish-grey	Aerial and substrate hyphae fragment into coccoid elements	Smooth	III
Nocardia	White, pink	Mycelium fragmenting into rod-shaped and coccoid elements often in zig-zag arrangement	Smooth	IV
Pseudonocardia	White	Zig-zag-shaped, budding, long chains fragmenting into squarish or oval fragments	Smooth, spiny	IV
Thermocrispum	White	Long chains, pseudosporangia fragmenting into rod-like structures	Smooth	III
Amycolatopsis	White	Long chains, squarish to oval fragments, spore-like structures	Smooth	IV
Streptomyces	White, yellow, green, grey, blue, pink, red, purple	Spores on aerial mycelium in straight, flexuous, looped or spiral chains, in some species arranged in verticils	Smooth, spiny, hairy, warty	I

[1]Cell wall types: Type I, LL-2,6-diaminopimelic acid (DAP) and glycine; Type II, meso-DAP and glycine; Type III, meso-DAP (with, in *Actinomadura*, galactose and madurose (3-O-methyl-D-galactose) and, in *Microtetraspora*, trace amounts of madurose); Type IV, meso-DAP, arabinose and galactose (with, in *Nocardia*, nocardomycolic acids); Type VI, lysine.

the genus *Actinobifida* [30, 53]. *Thm. curvata*, *Thm. fusca*, *Thm. alba* and *Thm. chromogena* have been accepted as valid while *Thm. falcata* has been shown to be a synonym of *Thm. chromogena*. *Thm spiralis* was never fully described and has never again been isolated. *Micromonospora* has sometimes been confused with *Thermomonospora* but it is not thermophilic and produces orange to black colonies without aerial mycelium. The spores are produced singly on the substrate mycelium or on the agar surface. *Prm. citrea* also produces little or no aerial mycelium but has yellow colonies with a vegetative mycelium that fragments and produces single rod-shaped, coccoid or chlamydospore elements, sometimes up to 5 µm diameter that have been regarded as spores [23].

The original Russian description of '*Thermopolyspora rectivirgula*' [29] was misleading and actinomycetes implicated in farmer's lung were first named '*Thermopolyspora polyspora*'. It was later shown that identification of these isolates with '*T. polyspora*' was erroneous and that the genus *Thermopolyspora* was illegitimate. Farmer's lung actinomycetes were subsequently named *Micropolyspora faeni* but were later shown to be synonymous with '*T. rectivirgula*' and were given the name, *Faenia rectivirgula* [33], since the earlier specific

Table 2. Occurrence of actinomycetes in cereal straw [37].

Species	Frequency of isolation (%)	Samples containing $> 2 \times 10^5$ cfu g^{-1} dry wt. (%)
Saccharopolyspora rectivirgula	67	42
Saccharomonospora spp.	58	14
Streptomyces albus	67	19
Streptomyces griseus	61	36
Streptomyces spp. (grey)	83	28

epithet took priority [40]. Finally, *Faenia* was shown to be indistinguishable from the genus *Saccharopolyspora* [26, 41, 45]. Another species with short chains of spores has also been reported from mushroom and domestic waste composts, sometimes in large numbers. This has now been referred to *Microtetraspora* (*Mit.*) *flexuosa* with *Actinomadura flexuosa, Microtetraspora flexuosa* and the earlier '*Thermopolyspora flexuosa*' considered to be synonyms [15, 31]. *Actinomadura* (*Acd.*) spp. generally produce short chains of spores, sometimes arranged in pseudosporangia. Many species have been isolated from soils although not from compost if *Acd. flexuosa* is excluded.

A range of species having long chains of spores have been described from composts. Many of these are *Streptomyces* (*Stm.*) species, including previously undescribed species, but others have been referred to such genera as *Nocardia, Pseudonocardia, Amycolata, Amycolatopsis* and *Thermocrispum* [11, 18, 21, 27, 49]. These differ in their morphology, cell wall composition and in other biochemical characteristics although *Amycolata* is now considered a synonym of *Pseudonocardia. Nocardiopsis* and *Saccharothrix* produce chains of spores or ovoid-coccoid elements of indeterminate length. Neither has so far been reported from compost but they have been isolated from soil.

Characteristics of the principal actinomycete genera to be found in composts, including *Thermoactinomyces*, and other similar taxa are summarised in Table 1.

ACTINOMYCETES IN MUSHROOM COMPOSTS

Composting for production of mushrooms (*Agaricus bisporus*) is a two phase process with Phase 1 completed out of doors and Phase 2 indoors. In Phase 1, horse manure is mixed with straw, gypsum and, if necessary, a nitrogen supplement. It is then stacked in long windrows, 2 m square in cross section, and is left to heat. The piles are turned two to three times over seven to ten days to encourage even degradation. Temperatures may rise to 75-80°C during this process and the pH value increases to 8.5 as ammonia is released. After Phase 1, the compost is transferred in trays or in bulk to insulated rooms or tunnels in which it is heated rapidly to 60°C with heated air containing 15-20% oxygen and maintained at this temperature for three to ten days by thermogenesis. It is then cooled and mixed with mushroom spawn.

Studies of the development of actinomycetes in mushroom composts [3, 6, 12, 17, 25, 60] have shown a predominance of *Streptomyces* spp. with grey aerial mycelium, *Micromonospora* spp. and, occasionally, *Thermoactinomyces, Thermomonospora* and '*Actinobifida*' spp. Reports of *Micromonospora* and '*Actinobifida*' spp. are likely to refer to *Thermoactinomyces* and *Thermomonospora* spp. but these species are likely to be underestimated using dilution plating for their isolation. Actinomycetes actively decompose mannans and xylans in hemicellulose, cellulose and lignin and may also degrade many other organic materials. They thus have important roles in the changes that take place in composts.

Phase 1. The straw used for the preparation of composts may already contain actinomycetes before the start of Phase 1 composting (Tab. 2) [37]. However, subsequently during Phase 1, temperatures are probably too great for much actinomycete growth, except at the edges of piles and early in the process. Nevertheless, Waksman *et al.* [73] showed that thermophilic actinomycetes grew well on animal manures. They isolated three *Streptomyces* species and three, described as *Micromonospora,* which were probably *Thermoactinomyces vulgaris* and *Thermomonospora* species. Populations

Table 3. Occurrence of actinomycetes during preparation of mushroom composts [after 36].

Organism	Cfu × 10^4 g^{-1} dry wt.				
	End of Phase 1	During Phase 2	End of Phase 2	After spawning	Before casing
Saccharomonospora spp.	0.8	0.4	4.3	-	-
Saccharopolyspora spp.	6.4	5.6	0.1	7.0	2.2
S. rectivirgula	-	-	0.2	-	-
Streptomyces spp.	19.1	24.3	24.7	7.9	1.4
Thermoactinomyces spp.	7.8	16.5	4.7	13.1	-
Thermomonospora spp. (white)	15.2	54.4	48.1	7.0	-
T. chromogena	14.4	60.5	72.4	72.3	4.3
Others	4.2	9.9	17.1	34.9	2.9

Table 4. Actinomycete content of mushroom composts during preparation [after 36].

Stage of composting	Actinomycete population (determined by)		
	Balla [3] (Dilution plating) cfu × 10^3 g^{-1} dry wt.	Craveri *et al*. [6] (Dilution plating) cfu × 10^6 g^{-1} dry wt.	Lacey [36] (Wind tunnel/Andersen sampler) cfu × 10^6 g^{-1} dry wt.
Before Phase 1	4	0.57	-
End Phase 1	20	6.59	0.68
During Phase 2	2-9	-	1.66
End Phase 2	3-20	3.68	1.72
After spawning	-	-	1.42
Before casing	-	1.54	1.07
Mushroom production	3	0.21	-

reached 12×10^9 g^{-1} moist compost after 10 days at 50°C. Populations were slightly smaller after incubation at 65°C but few were grown after incubation at 28°C and none after 75°C [72]. Henssen [18, 20] and Henssen and Schnepf [22] used anaerobic techniques to isolate thermophilic actinomycetes from manures. Of the 11 species isolated, eight were previously unknown and some grew equally well in anaerobic and aerobic conditions [19], although there has been no subsequent confirmation of this by other workers.

Populations of actinomycetes in some British composts at the end of Phase 1, measured using a wind tunnel/ Andersen Sampler method [43], are shown in Table 3 and in other composts in Table 4. Often populations exceeded 10^6 colony forming units (cfu) g^{-1} dry compost but were sometimes only 10^3 g^{-1} in improperly heated composts.

Table 5. Predominant actinomycetes in GCRI Revised Formula 2 and in rapidly-prepared non-manure compost [after 12].

Time (h)	Formula 2 compost	Rapidly-prepared compost
Start	*Thermoactinomyces vulgaris*	*Thermoactinomyces vulgaris*
20	*T. vulgaris*	
44	*T. vulgaris*	
48		*T. vulgaris*
68	*T. vulgaris*	
72	*T. vulgaris*	
96		*Saccharomonospora* spp. *Streptomyces thermovulgaris* *Streptomyces* spp. (grey) *T. vulgaris* *Thermomonospora* spp.
160		*Saccharomonospora* spp. *Streptomyces thermovulgaris* *T. vulgaris* *Thermomonospora* spp.
168	*Streptomyces thermovulgaris* *Streptomyces* spp. (grey) *T. vulgaris*	
264	*Saccharomonospora* spp. *Streptomyces thermovulgaris* *Streptomyces* spp. (grey) *T. vulgaris* *Thermomonospora* spp.	
Appearance of 'fire fang'	192–240 h	72–96 h

Phase 2. Thermophilic actinomycetes grow extensively during Phase 2 of composting and are often evident macroscopically from the white wefts of mycelium ('fire fang') in the compost. Fergus [11] reported 11 species of actinomycetes in Phase 2 composts and Kleyn and Wetzler [25] six. These included *Nocardia brasiliensis*, *Pseudonocardia thermophila*, *Saccharomonospora viridis*, *Saccharopolyspora rectivirgula*, *Stm. diastaticus*, *Stm. griseus*, *Stm. rectus*, *Stm. thermoviolaceus*, *Stm. thermovulgaris*, *Stm. violaceoruber*, *Tha. thalpophilus*, *Tha. vulgaris*, *Thm. chromogena*, *Thm. curvata* and *Thm. fusca*. Identification of *Nocardia brasiliensis*, a human pathogen, has not been confirmed however. *Stm. albus* was also reported from spent compost. Craveri *et al*. [6] reported 3.7×10^6 actinomycete cfu g^{-1} in composts at the end of Phase 2 and Lacey [36] 1.72×10^6 cfu g^{-1} (Tables 3 and 4). Actinomycete populations in composts are often dominated by *Thermomonospora* spp., both species with white aerial mycelium (including *Thm. curvata*, *Thm. alba* and *Thm. fusca*) and *Thm. chromogena* [52, 53]. However, *Tha. vulgaris* has been reported as predominant in Italian and American composts [6, 25] but this could be a consequence of the isolation method used. *Microtetraspora flexuosa* has also sometimes been isolated in large numbers [9, 71]. However, it can be difficult to differentiate the different species of white actinomycetes on crowded isolation plates and slow growing species, such as *M. flexuosa*, can easily be missed. *Thermoactinomyces* spp., including *Tha. dichotomicus*, and thermophilic and mesophilic *Streptomyces* spp., including *Stm. megasporus*, *Stm. rectus*, *Stm. thermovulgaris*, *Stm. albus* and *Stm. griseus*, have also been recorded in smaller numbers.

Table 6. Concentrations of airborne actinomycete spores on a mushroom farm [9].

Microorganism	Log_{10} colony forming units m^{-3} air		
	Spawning	Picking	Cookout
Total	7-8	2-6	3-4
Microtetraspora	6-7	nd[a]-2	2-4
Saccharomonospora	2-5	nd-2	4-5
Saccharopolyspora	nd	nd-2	4
Streptomyces	3-6	nd-2	5
Thermoactinomyces	3-5	nd-3	3-5
Thermomonospora (white)	6-7	2-4	4-5
T. chromogena	6-7	1-2	2-4

[a]not detected.

The development of actinomycetes in two mushroom composts based on wheat straw, one of which also contained deep litter chicken manure, was studied by Fermor *et al.* [12]. Initially, numbers of thermophilic bacteria differed considerably between the two composts but the patterns of development of thermophilic actinomycete were similar (Tab. 5). Initially, *Tha. vulgaris* and *Streptomyces* spp. were most frequent. However, after 7 days in compost with chicken litter and 4 days in the rapidly prepared compost, coincidentally with the development of 'fire fang', *Thermomonospora*, *Saccharomonospora* spp. and grey *Streptomyces* spp., including *Stm. thermovulgaris* were isolated. In the rapidly-prepared compost, actinomycete numbers increased from 10^3 cfu g^{-1} to 10^7 cfu g^{-1} during the first 50 h but then remained close to 10^7 g^{-1} up to 200 h.

The spores of actinomycetes are easily dispersed into the air during the spawning process, giving large concentrations in the air of spawning sheds (Tab. 6) [9]. Concentrations up to 2×10^7 actinomycete spores m^{-3} may be general in such environments with up to 7.4×10^8 spores m^{-3} occurring close to spawning lines [38]. Van den Bogart *et al.* [71] also report 10^9 cfu m^{-3} air in fermentation tunnels and during spawning. Concentrations of airborne actinomycete spores in other parts of mushroom farms are generally smaller. They are usually about 10^4 cfu m^{-3} in the cropping sheds or during cookout when the compost is steamed and tipped out of the trays and only rarely reach 10^6 cfu m^{-3} [9].

ACTINOMYCETES IN DOMESTIC WASTE COMPOSTS

There are few studies of the development of actinomycetes in domestic waste composts but their abundance in these composts is demonstrated by their abundance in the air spora when composts are handled. Freshly collected domestic waste contains few actinomycetes. The mean concentration of total thermophilic bacteria and actinomycetes growing at 55°C in the air at some British waste transfer stations was 5.3×10^3 cfu m^{-3} [8]. Species isolated included *Tha. vulgaris*, *Tha. thalpophilus* and, occasionally, *Sap. rectivirgula*. However, numbers increased rapidly in holding bunkers where emissions could reach 3.0×10^5 cfu m^{-3}, with *Thm. fusca* numbering up to 1.2×10^4 cfu m^{-3} and *Tha. vulgaris* up to 1.9×10^5 cfu m^{-3} when waste was moved by overhead cranes. Rész *et al.* [61] isolated *Thermoactinomyces vulgaris*, *Thermomonospora alba*, *Thm. curvata* and *Thm. lineata* from German domestic waste composts. These mostly grew best close to 50°C, with maxima at 60-65°C, and showed proteolytic enzyme activity against casein, gelatine, collagen and elastin and, to a lesser extent, against keratin. We have studied emissions on two sites although these cannot be considered representative of handling on commercial sites. The first [48] concerned emissions during the conveying and trommel screening of experimental composts indoors at Warren Spring Laboratory and the second [47] emissions while turning experimental batches of compost out of doors with a front-end loader at a site at Castle Bromwich, U.K. However, they do demonstrate the species of actinomycetes that occur and their numbers in the air during these processes (Tab. 7).

The range of actinomycetes isolated from domestic waste composts was similar to that found in mushroom spawning sheds but with maximum concentrations of the order of 10^6 cfu m^{-3} air, during both air classification indoors and turning out of doors. Personal samplers on workers yielded 10^7 cfu m^{-3} air. This compares with a reported concentration of 2×10^4 cfu m^{-3} in the pile composting area of a German composting site [13].

Table 7. Mean concentrations of airborne actinomycete spores during air classification indoors and turning out of doors of experimental composts made from domestic waste.

Actinomycete taxa	Colony forming units of actinomycetes × 10^5 m^{-3} air		
Site	Indoors, trommel screening	Out of doors, turning	
Sampling method	Andersen sampler	Andersen sampler	Personal samplers
Saccharomonospora	4.0	3.9	26
Saccharopolyspora rectivirgula	4.1	0.01	13
Thermoactinomyces spp.	3.3	1.8	14
Thermomonospora spp. (white)	0.63	2.2	62
T. chromogena	0.04	2.4	0.55
Streptomyces spp.	2.3	4.9	18
Unidentified white thermophiles	3.8	-	-

Table 8. Airborne actinomycete spores dispersed from green waste composts.

Taxon	cfu × 10^4 m^{-3} air	
	Fresh waste	Composted waste
Microtetraspora spp.	nd[a]	1.67
Streptomyces albus	0.78	22.4
Grey *Streptomyces* spp.	0.26	26.2
Other *Streptomyces* spp.	0.59	0.20
Saccharomonospora spp.	1.51	1.68
Saccharopolyspora rectivirgula	0.001	0.47
Thermoactinomyces spp.	0.87	5.29
Thermomonospora spp. (white)	0.17	8.09
T. chromogena	nd	1.17
Other actinomycetes	2.66	44.2

[a]not detected.

GREEN WASTE COMPOSTS

There have been few studies of the microbiology of vegetable composts although studies of fodder deterioration are relevant [36]. Actinomycete numbers have again been found to increase when spontaneous heating occurs. Earlier papers have shown that only *Tha. vulgaris* could be isolated from grass compost although *Streptomyces* spp. were reported to be predominant in composts made from lucerne, oat straw and maize stalks [35].

Microbial emissions from green waste composts have recently been the subject of a study in the United Kingdom and Denmark. Studies in Denmark suggest that there are few actinomycetes in freshly collected garden waste which gave rise to fewer than 18 cfu of thermophilic actinomycetes and 22 mesophilic actinomycetes m^{-3} air [4]. Results from the British study are incomplete but are sufficient to show that many actinomycetes are released from composts when they are turned (Tab. 8). During shredding of fresh green waste, concentrations of airborne actinomycetes were smaller than 5×10^4 cfu m^{-3} but they averaged about 10^6 cfu m^{-3} air close to compost piles during turning. *Saccharomonospora* spp. formed the most abundant taxon during shredding but *Stm. albus* and *Thermoactinomyces* were also frequently isolated. During composting, *Streptomyces* spp. with grey aerial mycelium, *Stm. albus* and a group of unidentified actinomycetes that represented several taxa became most abundant. Some of the unidentified colonies probably represent *Streptomyces* and *Saccharopolyspora* spp. but others possibly represent *Amycolata*, non-sporing *Microtetraspora* and *Thermomonospora chromogena* colonies and perhaps even *Thermocrispum*, a new species recently described from urban waste composts in Germany [27]. *Thermomonospora* spp., including *Thm. chromogena*, and *Microtetraspora* were less numerous than is sometimes found in mushroom composts. There was a suggestion that populations at shredding were less thermotolerant than those dispersed from compost piles.

SEWAGE COMPOSTS

Up to 15,000 thermophiles actinomycete cfu m^{-3} were found downwind during turning of compost windrows containing a mixture of woodchips (2.5 parts) and sewage sludge (1 part) [56]. Isolates included *Nocardia*, *Saccharopolyspora* and *Saccharomonospora* spp., *Streptomyces* spp., including the thermophilic *Stm. megasporeus*, *Stm. macrosporeus*, *Stm. thermolineatus* [14], *Promicromonospora citrea* and *Thermoactinomyces vulgaris* (Lacey, unpublished).

CONCLUSION

Actinomycetes are undoubtedly numerous in composts and have important roles in their decomposition. Many of the species are thermophilic, with temperature optima at about 55°C, and depend on thermogenesis within the compost piles to provide temperatures suitable for their growth. Their spores readily become airborne when compost piles are turned giving rise to large concentrations in the vicinity which may be carried downwind although numbers probably decrease as the piles mature and as water content increases. Especially large concentrations of airborne spores occur in the spawning sheds of mushroom farms where they have traditionally been associated with outbreaks of mushroom worker's lung, a form of extrinsic allergic alveolitis, in exposed workers.

Following the implication of thermophilic actinomycetes in farmer's lung, actinomycetes were suspected of causing mushroom worker's lung. Sakula [64] first suggested that mushroom worker's lung might be caused by the same actinomycetes as farmer's lung, after finding precipitins against *Tha. vulgaris* in one worker and again *Sap. rectivirgula* in another. However, these two species seem unlikely causes since they are rarely, if ever, numerous in bioaerosols from mushroom composts [38]. Sensitivity to these organisms appears more likely from exposure to mouldy straw before composting than to the compost itself. Subsequent attempts to identify a specific antigen causing mushroom worker's lung have been unsuccessful [51, 64, 68]. However, Kleyn *et al*. [24] found strong reactivity in some workers to *Tha. vulgaris* and *Sap. rectivirgula* while van den Bogart *et al*. [71] and Crook *et al*. [9] found sensitivity to a range of fungal and actinomycete antigens in exposed workers. Van den Bogart *et al*. [71] also reported that symptoms characteristic of mushroom worker's lung were induced in inhalation provocation tests with air containing large numbers of *Mit. flexuosa*, *Thm. curvata*, *Thm. fusca* and *Thm. alba* and concluded that these were the causes of the disease.

Concentrations associated with handling of composts made from domestic, green or sewage sludge, at least out of doors, have generally been smaller than those associated with mushroom compost. Up to 10^9 actinomycete spores have been reported in spawning sheds from mushroom composts [71] but those from other composts are generally of the order of 10^6-10^7 cfu m^{-3} although these figures probably underestimate the concentration of antigenic material which may include dead spores. Up to 10^8 spores of *Sap. rectivirgula* m^{-3} air are considered necessary for sensitization in farmer's lung [54]. Such exposure is only likely among workers in close contact with composts and is unlikely to affect the local population residing around compost sites. However, estimates of concentrations of *Aspergillus fumigatus* downwind from composting sites suggest that concentrations only approach ambient levels after about 500 m in unstable climatic conditions and after a greater distance in neutral or stable conditions [57]. Dust control measures or enclosure of composting facilities may therefore be necessary if houses occur within 500 m. Workers should be fully protected against inhalation of actinomycetes and fungi at all times.

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