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Quantitative Assessment of Factors Affecting the Recovery of Indigenous and Released Thermophilic Bacteria from Compost

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Thermophilic actinomycetes and bacilli were recovered from mushroom compost by conventional dilution plating and sedimentation chamber-Andersen sampler methods. Excessive growth of thermophilic bacilli on dilution plates accounted for the poor recovery and limited diversity of actinomycete colonies, and this result was largely unaffected by the use of modified extraction procedures and diluents. Assessment of the actinomycete population was more successfully achieved by applying the sedimentation chamber method, by using selective media, or both. Background resistance of the compost microflora to selective agents (kanamycin, novobiocin, tetracycline, thiostrepton, and NaCl) was extremely varied, but both actinomycetes and bacilli were particularly sensitive to tetracycline. The selective isolation of *Thermoactinomyces* spp. and *Thermomonospora chromogena* by novobiocin and kanamycin, respectively, was shown to be reproducible, and the use of high concentrations of kanamycin resulted in the isolation of a novel group of unidentified thermophilic actinomycetes. Comparison of nonselective nutrient media demonstrated that the nutrient-rich protoplast regeneration medium R5 was surprisingly efficient for actinomycete recovery. This medium was found to be particularly appropriate for the recovery of *Saccharomonospora viridis* BD125, introduced as spores into both sterile and fresh samples of mushroom compost. This stable pigmented variant of the *S. viridis* strains indigenous to compost was released at concentrations of up to 10^7 spores g of compost^{-1} in order to provide information for future experiments on the release and recovery of genetically manipulated strains. The detection limits for this strain were in the region of 10^2 g^{-1} from sterilized compost but only 10^5 g^{-1} from nonsterile compost. These figures correspond to mean recovery efficiencies of approximately 70% (sterilized compost) and 53% (fresh compost) of viable spores released. Further improvements in the detection and recovery of *S. viridis* strains released into compost should be achieved by the introduction of selectable markers developed from this information on the antibiotic resistance profile of the indigenous compost microflora.

One of the concerns regarding the release of genetically manipulated microorganisms into the environment is that they may transfer novel DNA sequences to the indigenous microbial population, displace existing species from that population, or both. A prerequisite for any release, therefore, is the development of methodologies for monitoring the fate of particular microbes in the environment. Much attention has recently been directed toward improving direct detection methods, such as fluorescent-antibody staining (2, 23) and gene probes (8, 9), for this purpose. Such techniques allow specific organisms or genes to be monitored directly without the need for culturing. However, a return to conventional culture methods will be necessary for the isolation and identification of species receiving novel DNA sequences and for detecting population changes.

The aim of this study was to use and improve standard culture methods for characterizing the microbiology of a compost ecosystem before release experiments were conducted. Mushroom compost was selected as the model substrate because it contains a diverse and highly competitive microflora of which thermophilic actinomycetes and *Bacillus* spp. form an important component. Compost therefore provides a useful substrate for studying the population dynamics of these industrially important groups of bacteria. Emphasis was placed on the population of thermophilic actinomycetes, which becomes increasingly dominant during the second phase (the peak heat, or pasteurization, stage) of commercial compost preparation (6, 13, 25).

Specific recovery experiments were also conducted by using the thermophilic actinomycete *Saccharomonospora viridis*. This species is indigenous to compost and was selected because it can be readily identified on isolation media by the presence of a green pigment and characteristic arrangement of single spores on unbranched sporophores of the aerial hyphae. A variant of *S. viridis*, which is biochemically identical to wild-type strains but produces a deep lilac pigment (21), was used because of its potential use in future studies requiring differentiation of released strains from indigenous strains.

MATERIALS AND METHODS

Compost samples. The compost used in this study was a commercial preparation produced from a horse-bedding straw–deep-litter chicken manure mixture supplemented with gypsum (mean pH 7.3; moisture content, 67% [wt/wt]). Samples were collected at various stages of compost preparation, including the end of phase 2 (i.e., immediately before spawning). Samples were stored in plastic bags at 4°C and analyzed during a 4-week period. For certain *S. viridis* release and recovery experiments, compost samples were sterilized by autoclaving at 121°C for 20 min on 3 successive days.

Isolation techniques. Thermophilic bacteria were isolated from compost samples by dilution plating and by the sedimentation chamber-Andersen sampler method (15). Three extraction techniques and four diluents were compared by the dilution plating method. Compost samples (10 g [fresh weight]) were suspended in 90 ml of sterile quarter-strength Ringer solution and either shaken for 30 min in 250-ml Erlenmeyer flasks (100 rpm at 50°C) with or without glass

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balls (3.4 to 4.5 mm in diameter) or homogenized in a Philips blender (model HL6803). Further samples were shaken as described above in 90 ml of the following sterile diluents: quarter-strength Ringer solution plus 0.01% sodium dodecyl sulfate (SDS), 0.01% peptone (Difco Laboratories, Detroit, Mich.), and 50 mM potassium phosphate buffer (pH 8). Logarithmic dilutions were prepared in triplicate for each of the compost-diluent slurries, using the corresponding diluent. Portions (0.1 ml) were spread onto the surfaces of petri dishes containing half-strength tryptone soy agar supplemented with 0.2% casein hydrolysate (1/2TSA+C) (14). The medium was adjusted to pH 8.0 and, after autoclaving, was amended with a filter-sterilized solution of cycloheximide (to a final concentration of 50 μg ml^{-1}) to suppress fungal growth. Isolation plates were incubated at 50°C for 96 h, and actinomycete colonies were identified by using a microscope (Leitz Wetzlar, E. Leitz [Instruments] Ltd., Luton, United Kingdom) fitted with an ×40 long-working-distance objective.

Further compost samples were dried at 37°C to constant weight and subjected to sedimentation chamber-Andersen sampler analysis as described previously (19). Dried samples (10 g) were agitated in a sedimentation chamber to produce a spore cloud which, after sedimentation for 90 min, was passed through an Andersen sampler loaded with plates of isolation media (1/2TSA+C) for 10 s at a rate of 25 liters min^{-1}. Isolations were performed in triplicate, and plates were incubated as described above.

Isolation media. Thermophilic bacteria were isolated from compost onto a range of selective and nonselective media. Either compost samples were dried and subjected to sedimentation chamber-Andersen sampler analysis as described above or fresh samples (3 g) were suspended in 30 ml of sterile quarter-strength Ringer solution in boiling tubes, vortexed for 60 s, and serially diluted. Bacteria were isolated on 1/2TSA+C, half-strength nutrient agar (1/2NA), R5 medium (10), and compost infusion agar (CIA). The latter medium was prepared by suspending 300 g of fresh compost in 1 liter of warm tap water in a muslin bag for 1 h and centrifuging the resulting suspension at 24,000 × *g* for 20 min to yield a supernatant to which agar at 18 g $liter^{-1}$ was added (5). Bacteria were also isolated on 1/2TSA+C amended with sodium chloride (5 and 10% [wt/vol]) and on 1/2TSA+C amended after being autoclaved with filter-sterilized solutions of kanamycin (25, 50, 100, and 200 μg ml^{-1}), novobiocin (25 and 50 μg ml^{-1}), tetracycline (25, 50, and 100 μg ml^{-1}), or thiostrepton (50 μg ml^{-1}). Isolations were performed in triplicate; plates were incubated and actinomycetes were identified as described above.

Release and recovery of *S. viridis* BD125 from compost. *S. viridis* BD125 (lilac variant) was routinely subcultured on R5 medium with incubation at 50°C. Stocks were maintained as suspensions of spores and hyphae in 20% (vol/vol) glycerol stored at −70°C. For release experiments, spore suspensions were prepared by agitating 5-day-old agar plate cultures in sterile distilled water and passing the resulting suspension through nonabsorbent cotton wool. Microscopic examination of the filtrate confirmed that hyphal fragments had been removed.

Samples of sterilized compost (10 g) were inoculated with 1-ml samples of a spore suspension (2.8×10^7 viable spores ml^{-1}) of *S. viridis* BD125. The different isolation techniques described above for thermophilic bacteria were applied to the recovery of *S. viridis* BD125. Portions (1 ml) of spore suspensions, containing up to 5.5×10^7 viable spores ml^{-1}, were also inoculated into boiling tubes containing 3-g amounts of either fresh or sterilized compost. All compost samples were extracted immediately by being vortexed in 30 ml of quarter-strength Ringer solution, and serially diluted portions were spread on petri dishes containing R5 medium. Isolation plates were incubated as described above, and the experiments were performed in triplicate.

TABLE 1. Recovery of thermophilic actinomycetes and of *S. viridis* BD125 by using variations of the dilution plate count technique[a]

Sample	Recovery	
	Thermophilic actinomycetes[b] (10^7 CFU g of fresh $compost^{-1}$)	*S. viridis* BD125[c] (10^5 CFU g of sterilized $compost^{-1}$)
Extracted in quarter-strength Ringer solution with:		
Shaking	13.0 (7.5)	24.3 (10.0)
Shaking with glass balls	5.7 (2.1)	27.3 (2.8)
Homogenizing	4.7 (4.2)	23.3 (0.8)
Shaken in:		
Quarter-strength Ringer solution	13.0 (7.5)	24.3 (10.0)
Quarter-strength Ringer solution + SDS (0.01%)	9.7 (6.5)	7.3 (1.5)[d]
Peptone (0.01%)	25.3 (7.4)	21.0 (7.6)
Phosphate buffer (pH 8.0)	24.3 (11.6)	20.6 (7.0)

[a] Isolation plates were incubated at 50°C for 96 h. Counts are the means of triplicates, with standard deviations given in parentheses.
[b] Recovered on 1/2TSA+C medium.
[c] Recovered on R5 medium.
[d] Significant difference ($P = 0.05$) from results for samples shaken in quarter-strength Ringer solution.

RESULTS

The microfloras of mushroom compost samples taken at different stages in the composting process were compared. The numbers of thermophilic bacilli were relatively constant throughout the process (10^9 to 10^{10} CFU g^{-1}), but the numbers and particularly the diversity of thermophilic actinomycetes increased as composting proceeded. Samples taken at the end of phase 2 yielded approximately 10^8 thermophilic actinomycete CFU g^{-1}, comprising representatives of the genera *Thermomonospora*, *Thermoactinomyces*, *Saccharomonospora*, and *Streptomyces* together with low numbers of unidentified strains. Mushroom compost from the end of phase 2 was therefore selected for use in this study and had the additional advantage of relative biological and chemical stability, since phase 2 is designed to pasteurize the compost and drive off excess ammonia.

Comparison of isolation techniques. Dilution plating of mushroom compost samples from the end of phase 2 on a nonselective agar medium (1/2TSA+C) yielded very high colony counts (up to 10^{10} colonies g of fresh $compost^{-1}$) of thermophilic bacteria, which were tentatively identified as thermophilic *Bacillus* spp. Growth of these bacteria tended to be confluent over the isolation plates, preventing enumeration of defined colonies. Recovery of thermophilic actinomycetes was reduced by these faster-growing *Bacillus* spp., and the viable counts obtained were highly variable (Table 1). The different extraction methods and diluents used had no significant effects on the recovery efficiency of thermophilic actinomycetes by dilution plating (Table 1). Furthermore, in these experiments the actinomycete species recovered by dilution plating were largely limited to white thermomonosporas and a few thermoactinomycetes.

TABLE 2. Effect of medium composition on the recovery of thermophilic actinomycetes and bacilli from compost by the sedimentation chamber method

Isolation medium	No. of colonies recovered/stack of Andersen sampler plates[a]						
	White thermomonosporas	Thermoactinomycetes	*T. chromogena*	Streptomycetes	*S. viridis*	Other actinomycetes	Bacilli
Nonselective							
1/2TSA+C	745 (49)	9 (3)	655 (115)	29 (13)	0.3 (0.6)	0	NC
1/2NA	705 (316)	11 (2)	623 (197)	54 (42)	0.3 (0.6)	0	NC
R5	360 (225)	0	269 (33)	242 (116)	2.0 (1.7)	0	2 (1)
CIA	NC	NC	NC	NC	0	0	NC
Selective, 1/2TSA+C containing:							
Kanamycin (μg ml^{-1})							
25	0	0	412 (9)	0	0	0	9 (4)
50	0	0	0	0	0	22 (4)	0.3 (1)
100, 200	0	0	0	0	0	0	0
Novobiocin (μg ml^{-1})							
25	0	68 (6)	0	0	0	0	23 (6)
50	0	56 (15)	0	0	0	0	14 (3)
Tetracycline (25, 50, 100 μg ml^{-1})	0	0	0	0	0	0	0
Thiostrepton (50 μg ml^{-1})	0	0	0	25 (4)	0	0	0.3 (1)
NaCl (% [wt/vol])							
5	34 (28)	0	0	12 (1)	0	0	16 (4)
10	0	0	0	0	0	0	1 (1)

[a] Colony counts are the means of triplicates, with standard deviations given in parentheses. NC, Colonies not countable because of confluent growth.

The same diluents and extraction methods were applied to the recovery of *S. viridis* BD125 released into sterilized compost to determine whether they had any effect on recovery in a simple one-membered population. The extraction methods again showed no significant differences from each other. However, use of quarter-strength Ringer solution plus 0.01% SDS as the diluent resulted in a significant reduction in the number of *S. viridis* colonies isolated compared with quarter-strength Ringer solution alone (Table 1).

Thermophilic actinomycetes were more readily isolated from compost onto the nonselective 1/2TSA+C medium when the sedimentation chamber-Andersen sampler method was used (Table 2). Colonies of thermophilic bacilli were still recovered but in significantly lower numbers, which permitted the growth of a greater diversity of actinomycete species. Thermomonosporas were the dominant actinomycete group recovered, with an average of 745 white thermomonosporas (*Thermomonospora curvata* and *Thermomonospora fusca*) and 655 *Thermomonospora chromogena* colonies isolated per stack of six Andersen sampler plates. Colonies were concentrated on plates from stages 5 and 6 of the sampler, where the critical air velocity for impaction of actinomycete spores is attained. It is therefore necessary to apply positive-hole statistics (1) to correct for single colonies arising from multiple impaction of viable particles. This resulted in estimates for the numbers of white thermomonospora and *T. chromogena* viable spores impacted of 1,689 and 978, respectively. In addition to this large population of thermomonosporas, discrete colonies of streptomycetes (with grey aerial mycelia), thermoactinomycetes, and *S. viridis* were recovered (Table 2).

Comparison of isolation media. A range of media was used in an attempt to improve the recovery of thermophilic actinomycetes from compost samples from the end of phase 2 (Tables 2 and 3). The use of nonselective media (particularly 1/2TSA+C and 1/2NA) in conjunction with the dilution plate count technique resulted in isolation plates covered with confluent bacteria as before (Fig. 1a). Thermophilic actinomycetes were nevertheless enumerated at concentrations of up to 10^7 colonies g of fresh compost^{-1}, although in many cases only a few colonies were recovered, with poor reproducibility (Table 3). As before, the problem of confluent bacterial growth was overcome to a large extent by the sedimentation chamber method (Fig. 1b). Spreading bacterial colonies were still recovered on Andersen sampler plates but were more discrete, which enabled actinomycetes to colonize a greater area of the isolation plate. There were no significant differences in the numbers or diversity of actinomycetes isolated on the two nonselective media, 1/2TSA+C and 1/2NA (Table 2).

Viable counts of the two predominant groups, white thermomonosporas and *T. chromogena*, were clearly reduced when recovered on R5 agar, a rich medium designed for the regeneration of streptomycete protoplasts (Table 2). On this medium, however, streptomycetes were isolated in higher numbers but were nonsporulating and could be identified only when subcultured on nutrient agar, where they developed grey aerial mycelia. *S. viridis* was also recovered in higher numbers on R5, the only isolation medium on which these actinomycetes were observed when dilution plating was used as the isolation method (Table 3). The reduced numbers of thermomonosporas, the bald nature of the streptomycetes, and the less confluent growth and often raised shape of the *Bacillus* colonies ensured that *S. viridis* was readily identifiable on R5 isolation plates. CIA supported confluent growth of actinomycete mycelia, which were thin and poorly developed because of the low-nutrient status of this medium. Microscopic examination of CIA isolation plates revealed the presence of characteristic streptomycete, thermoactinomycete, and thermomonospora sporulating hyphae, but these were sparse.

Recovery of thermophilic bacilli and actinomycetes on 1/2TSA+C amended with selective agents was extremely variable (Tables 2 and 3). The compost microflora exhibited particularly low resistance to the antibiotic tetracycline.

TABLE 3. Effect of medium composition on the recovery of thermophilic actinomycetes and bacilli from compost by dilution plating

Isolation medium	Highest dilution at which colonies were recorded (relative abundance)[a]						
	White thermomonosporas	Thermoactinomycetes	*T. chromogena*	Streptomycetes	*S. viridis*	Other actinomycetes	Bacilli
Nonselective							
1/2TSA+C	10^{-7} (++)	10^{-5} (±)	10^{-6} (±)	10^{-5} (±)	ND	ND	10^{-10} (++)
1/2 NA	10^{-7} (++)	10^{-4} (±)	10^{-7} (+)	10^{-7} (++)	ND	ND	10^{-8} (++)
R5	10^{-6} (++)	10^{-6} (++)	ND	10^{-7} (++)	10^{-4} (±)	ND	10^{-7} (++)
CIA	10^{-6} (++)	10^{-10} (++)	ND	10^{-7} (++)	ND	ND	10^{-8} (++)
Selective, 1/2TSA+C containing:							
Kanamycin (μg ml^{-1})							
25	10^{-6} (+)	ND	10^{-6} (++)	ND	ND	ND	10^{-8} (++)
50, 100	ND	ND	ND	ND	ND	10^{-6} (++)	10^{-7} (++)
200	ND	ND	ND	ND	ND	ND	10^{-7} (++)
Novobiocin (μg ml^{-1})							
25	10^{-6} (±)	10^{-8} (++)	ND	ND	ND	ND	10^{-8} (++)
50	ND	10^{-7} (++)	ND	ND	ND	ND	10^{-8} (++)
Tetracycline (μg ml^{-1})							
25	ND	ND	ND	ND	ND	ND	10^{-5} (++)
50	ND	ND	ND	ND	ND	ND	10^{-3} (++)
100	ND	ND	ND	ND	ND	ND	ND
Thiostrepton (50 μg ml^{-1})	10^{-5} (±)	ND	ND	10^{-5} (+)	ND	ND	10^{-6} (++)
NaCl (% [wt/vol])							
5	10^{-6} (±)	ND	ND	10^{-5} (+)	ND	ND	10^{-7} (++)
10	ND	ND	ND	ND	ND	ND	10^{-5} (++)

[a] Colonies recovered: ND, none detected; (±) infrequently; (+) consistently at 1 dilution; (++) consistently at >1 dilution.

When tetracycline was included in the isolation medium, no colonies were recovered on Andersen sampler plates and only low numbers of thermophilic bacilli were recovered on dilution plates. High concentrations of kanamycin (100 and 200 μg ml^{-1}) also inhibited growth on Andersen sampler plates, but up to 10^7 CFU of bacilli g of compost^{-1} was recovered on dilution plates containing these concentrations of kanamycin. At a lower concentration (25 μg ml^{-1}), use of kanamycin selected *T. chromogena* colonies in addition to bacilli (Fig. 1c; Tables 2 and 3). Other thermophilic actinomycetes were also isolated on both dilution and Andersen sampler plates in the presence of kanamycin; these were all of one morphological type, characterized by apparent production of spore vesicles (sporangia) on a white aerial mycelium. This finding illustrates how selective media can be used to reveal the presence of thermophilic actinomycete taxa other than those normally associated with composts.

Thermoactinomycetes were selectively isolated on media containing novobiocin, whereas nonsporulating streptomycete colonies were abundant on media containing thiostrepton (50 μg ml^{-1}) or NaCl (5% [wt/vol]). When the concentration of NaCl was increased to 10% (wt/vol), no actinomycetes were isolated and plates were dominated by thermophilic *Bacillus* colonies with a characteristic flat, crenulated appearance. White thermomonosporas were occasionally isolated on dilution plates containing low concentrations of kanamycin, novobiocin, and thiostrepton but not on Andersen sampler plates. This discrepancy may be attributable to the fact that these isolates appeared to develop over spreading *Bacillus* colonies, which may have protected them from the effects of the antibiotics.

Recovery of *S. viridis* BD125 released into compost. *S. viridis* BD125 was inoculated into fresh and sterilized composts at a range of spore concentrations in order to deter-

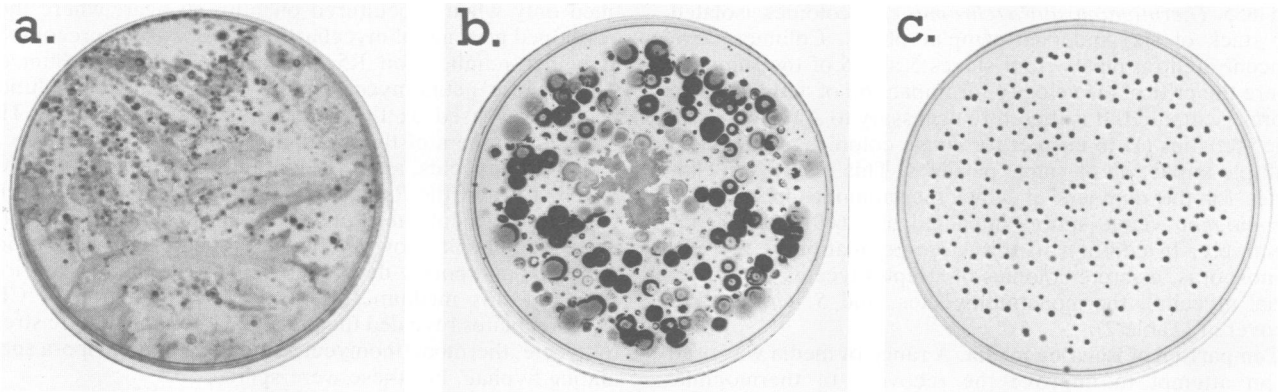

FIG. 1. Representative isolation plates illustrating the recovery of thermophilic actinomycetes from compost. (a) Nonselective (1/2TSA+C) dilution plate showing spreading growth of bacilli and limited development of actinomycete colonies; (b) nonselective (1/2TSA+C) Andersen sampler plate showing well-isolated actinomycete colonies and minimal growth of bacilli; (c) selective isolation of *T. chromogena* on 1/2TSA+C containing 25 μg of kanamycin ml^{-1} by the sedimentation chamber method.

TABLE 4. Effect of inoculum size on the recovery of *S. viridis* BD125 released into compost

Inoculum size (spores g of compost^{-1})	Dilution plate	Recovery[a]			
		Fresh compost		Sterilized compost	
		Viable count (g^{-1})	Mean recovery (%)	Viable count (g^{-1})	Mean recovery (%)
1.8×10^7	10^{-5}	$6.6\ (1.8) \times 10^6$	36	$1.1\ (0.2) \times 10^7$	59
	10^{-6}	$1.2\ (0.5) \times 10^7$	68	$1.5\ (0.8) \times 10^7$	82
1.8×10^6	10^{-4}	+		$1.04\ (0.1) \times 10^6$	58
	10^{-5}	$1.0\ (0.2) \times 10^7$	55	$1.2\ (0.4) \times 10^6$	65
1.8×10^5	10^{-3}	+		$1.3\ (0.4) \times 10^5$	70
	10^{-4}	+		$1.6\ (0.7) \times 10^5$	89
1.8×10^4	10^{-2}	−		$9.6\ (0.6) \times 10^3$	53
	10^{-3}	−		$1.6\ (0.6) \times 10^4$	87
1.8×10^3	10^{-2}	−		$1.4\ (0.2) \times 10^3$	78
	10^{-3}	−		$1.0\ (1.0) \times 10^3$	55
1.8×10^2	10^{-2}	−		$3.0\ (3.0) \times 10^2$	167
1.8×10^1	10^{-2}	−		+	

[a] Viable counts are the means of triplicates, with standard deviations given in parentheses. —, No colonies detected; +, colonies observed but not countable.

mine the detection limits for this organism by dilution plating on R5 isolation medium. The mean recovery of BD125 from sterilized compost was approximately 70% of viable spores released for inocula containing 1.8×10^3 to 1.8×10^7 spores g^{-1} (Table 4). When smaller inocula were released (<180 viable spores g of sterilized compost^{-1}), colony counts were extremely variable and unreproducible. *S. viridis* BD125 was isolated from nonsterile compost only when inoculated at high concentrations, i.e., $>1.8 \times 10^5$ viable spores g^{-1} (Table 4). At these levels, recovery rates were lower than in sterilized compost and averaged 53% of viable spores released. Detection and enumeration of *S. viridis* BD125 in the nonsterile compost samples was limited by the confluent growth of thermophilic bacilli on isolation plates, as described above.

DISCUSSION

The tendency for thermophilic actinomycetes to be growth inhibited by faster-growing *Bacillus* spp. on isolation plates has been reported previously and highlights the limitations of dilution plate techniques for isolation of actinomycetes from compost samples (17). Our attempts to increase the efficiency of actinomycete recovery with various diluents and extraction procedures were unsuccessful. Although any effects on thermophilic actinomycete recovery may have been masked by competition with *Bacillus* colonies, the experiments with *S. viridis* released into sterile compost should have revealed these differences. This result was disappointing, since peptone has been shown to promote greater viability of different bacterial strains than do other diluents, such as distilled water, Ringer solution, and phosphate buffer (24). Detergents have also been used to improve the recovery of bacteria from soils (22), and an investigation of the effect of SDS was therefore included in this study. However, SDS appeared to have an inhibitory effect on the isolation of *S. viridis* from sterilized compost, which resulted in a significant reduction in the number of colonies isolated.

The sedimentation chamber-Andersen sampler method recovered a greater diversity of actinomycetes, with white thermomonosporas predominant on nonselective media. Thermomonosporas have been found to be dominant in other compost samples (5, 7, 19), a fact which presumably reflects their improved growth and sporulation at high pH values and their ability to degrade lignocellulose (18). When CIA was used as the isolation medium, streptomycetes were isolated in greater abundance. Nutrient-deficient media such as CIA have traditionally been used for the isolation of actinomycetes (5, 12), but, as we have demonstrated, they tend to select for streptomycetes. In addition, the intense opacity of this medium and the sparse development of actinomycete colonies recovered on it limit the application of CIA for the enumeration and identification of species. Our observations indicate that 1/2TSA+C, which is convenient to prepare and has been used effectively by other workers (14, 19), is an efficient medium for the nonselective isolation of thermophilic actinomycetes from mushroom compost.

The successful isolation of thermophilic actinomycetes and reduction in *Bacillus* growth on the nutrient-rich medium R5 was unexpected. This medium was originally designed for the regeneration of streptomycete protoplasts and contains excess amounts of nutrients in the form of sucrose, glucose, yeast extract, amino acids, and divalent cations (10). Nutrient-rich media are not typically used for the isolation of bacteria from soils since they are associated with reduced bacterial counts (16, 22). Our observations indicate that use of R5 results in reduced recovery of thermophilic bacilli, which may have been responsible for changes observed in the recovery profile of different thermophilic actinomycetes. In the case of *S. viridis*, however, improved recovery on R5 appears to be a direct result of the medium composition, as discussed below.

Addition of selective agents to 1/2TSA+C enhanced the recovery of particular taxa. The selective isolation of thermoactinomycetes and *T. chromogena* on media containing novobiocin (50 μg ml^{-1}) and kanamycin (25 μg ml^{-1}), respectively, has already been documented (3, 20). In our study, the efficacy of these selective agents was thoroughly tested and shown to be reproducible, a result which supported these earlier observations. Furthermore, increasing the concentration of kanamycin (to 50 to 100 μg ml^{-1}) in the isolation medium resulted in the isolation of a group of unidentified actinomycetes not encountered elsewhere in this study. Thiostrepton was found to have only a limited inhibitory effect on the recovery of thermophilic streptomycetes. This result was unexpected, since the gene for thiostrepton resistance has been isolated on a plasmid (11) whose derivatives are the most commonly used cloning vectors in streptomycetes, with expression of thiostrepton

resistance as the selection for transformants. However, it is likely that our results merely reflect the poor thermostability of thiostrepton rather than indicating the presence of a significant reservoir of thiostrepton resistance in natural streptomycete populations.

Release of an *S. viridis* strain (BD125) into compost demonstrated how the inoculum size influences the detection limits for any microorganism. This pigmented strain was selected for study because it is readily identifiable on isolation plates and because it will allow us to differentiate between introduced and resident strains of *S. viridis* in future release experiments. On the basis of the results described above, we selected R5 as the isolation medium for *S. viridis* BD125. Furthermore, sporulation and pigment production by this and other *S. viridis* strains were found to be enhanced on R5 medium, which offers considerable advantages for identification and enumeration. Consequently, *S. viridis* BD125 could be detected at densities as low as 3×10^2 g of sterilized compost^{-1}. However, recovery of this strain from fresh compost on R5 isolation plates where competing bacteria were present was reduced, with detection limits in the range of 10^5 g of compost^{-1}. An inoculum of $>10^5$ viable spores g^{-1} is therefore required for detectable recovery. This compares poorly with reported detection limits for a range of other bacteria of 1 to 20 cells g of soil^{-1} by the plate count technique (4), 10^2 cells ml of water^{-1} by the fluorescent-antibody–direct viable count method (2), and 10^4 cells g of soil^{-1} with DNA probes (9). However, such data on detection limits are not directly comparable because different criteria are used and results are seldom expressed in terms of the proportion of released CFU immediately recoverable. Detection limits for *S. viridis* BD125 could be further improved by the introduction of specific selectable markers together with improvements in the isolation medium. We have recently identified yeast extract, L-proline, and the high concentration of divalent cations as the active components in R5 responsible for the enhanced growth of *S. viridis* BD125. This medium is now being modified to increase the recovery of *S. viridis*; on the basis of our knowledge of the antibiotic resistance profile of the indigenous compost microflora, selectable markers will be developed.

There have been a number of publications characterizing the population of thermophilic bacteria present in a variety of composts (5, 7, 13, 25, 26). However, the study reported here is an attempt to assess the detection limits for a defined species in a heterogeneous compost population. Data of this nature will provide essential information on which to base future experiments on the release and recovery of genetically manipulated bacteria.

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