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Succession of Bacterial Communities during Early Plant Development: Transition from Seed to Root and Effect of Compost Amendment

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Compost amendments to soils and potting mixes are routinely applied to improve soil fertility and plant growth and health. These amendments, which contain high levels of organic matter and microbial cells, can influence microbial communities associated with plants grown in such soils. The purpose of this study was to follow the bacterial community compositions of seed and subsequent root surfaces in the presence and absence of compost in the potting mix. The bacterial community compositions of potting mixes, seed, and root surfaces sampled at three stages of plant growth were analyzed via general and newly developed *Bacteroidetes*-specific, PCR-denaturing gradient gel electrophoresis methodologies. These analyses revealed that seed surfaces were colonized primarily by populations detected in the initial potting mixes, many of which were not detected in subsequent root analyses. The most persistent bacterial populations detected in this study belonged to the genus *Chryseobacterium* (*Bacteroidetes*) and the family *Oxalobacteraceae* (*Betaproteobacteria*). The patterns of colonization by populations within these taxa differed significantly and may reflect differences in the physiology of these organisms. Overall, analyses of bacterial community composition revealed a surprising prevalence and diversity of *Bacteroidetes* in all treatments.

The chemical, physical, and biological properties of soil in conjunction with various plant characteristics have profound effects on seed- and root-associated microbial communities (10, 11, 20, 23, 33, 34, 46, 53). Distinct microbial communities have been shown to develop on plant surfaces during different plant developmental stages, suggesting that a succession of microbial communities accompanies plant development (3, 10, 11, 29, 30, 34, 53). In addition to plant-specific effects, microbial communities associated with plants during development also can be influenced by exogenous amendments, such as compost, to plant soils or potting media (4, 25, 49). Compost amendment introduces copious amounts of organic matter and high numbers of microbial cells into soils or potting mixes. These microorganisms are often metabolically diverse, and some can degrade polymeric substances such as cellulose, hemicellulose, and lignin (5, 18, 42, 50). Saison et al. (43) recently reported that the community composition of soil-compost environments was influenced primarily by the organic-rich compost matrix rather than by the native compost microbiota. However, the extent to which such amendments can influence microbial communities in the rhizosphere and can serve as sources for rhizosphere populations has not been well characterized. Since composts are routinely applied to agricultural soils and potting mixes to improve soil fertility and plant growth and health, there is a need to characterize compost-plant interactions (15, 19, 24, 31).

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In this study, we examined the bacterial community composition associated with cucumber seeds and seedling roots grown in compost-amended mixes by using PCR-denaturing gradient gel electrophoresis (DGGE) and subsequent sequence analyses. Our objective was to follow the effect of compost amendment to potting mixes on the bacterial community compositions of seed and subsequent root surface communities.

MATERIALS AND METHODS

Cucumber growth, sampling, and DNA extraction. Three peat-based potting mixes were formulated as previously described (21). Briefly, sphagnum peat moss and perlite were combined with sawdust-incorporated cow manure compost ("sawdust compost") or straw-incorporated cow manure compost ("straw compost") in a 5:4:1 ratio, all on a volume basis (13). A "peat-only" treatment consisted of peat and perlite in a 6:4 ratio, also on a volume basis. Potting mixes were irrigated in 500-ml Styrofoam pots and incubated for 2 days prior to sowing. Two cucumber seeds (*Cucumis sativus* L. 'Straight Eight') were then sown in 500-ml Styrofoam pots and incubated under greenhouse conditions (22 to 27°C). Potting mix and plant material were sampled from three separate pots at three stages of plant development: seed germination (24 h postsowing), seedlings with fully extended cotyledons (1 week postsowing), and seedlings with four true leaves (3 weeks postsowing). Seed and roots were removed from each pot, shaken to remove loosely adhering potting mix, and washed twice with distilled water. Roots were homogenized using sterile razors and comprised rhizoplane, endosphere, and tightly adhering rhizospheric potting mix. Total DNA was extracted from these samples in triplicate using the UltraClean soil DNA isolation kit (MoBio Laboratories, Inc., California).

DNA-based molecular analyses of bacterial community composition. Two strategies were used to analyze bacterial communities in this study. First, for each sample, fragments of 16S rRNA genes were PCR amplified from extracted DNA with the "general bacterial" primer set 11F (5′-GTT TGA TCM TGG CTC AG-3′) (21)/907R (5′-CCG TCA ATT CMT TTG AGT TT-3′) (38) and subsequently PCR amplified with the "general bacterial for DGGE" primer set 341FGC (5′-CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCC CCG CCC GCC TAC GGG AGG CAG CAG-3′) (38)/907R, as described previously (21). The general bacterial primer 11F, which fortuitously has a single mismatch with the cucumber plastid 16S rRNA gene sequence (and others) (matching

TABLE 1. Analysis of 16S rRNA gene sequences recovered from DGGE bands excised and cloned from general bacterial analyses of potting mix, seed, and root samples

Treatment or organelle and band	Accession no.	Sample (time postsowing)	Most similar sequence (by BLAST analysis)			
			Organism	Accession no.	% Similarity	Phylum
Peat						
P1	AY332573	Mix (0)	*Chryseobacterium formosense*	AJ715377	99.82	*Bacteroidetes*
P1-9	AY341108	Root (1 wk)	Potato plant root bacterium RC-III-62	AJ252725	97.78	*Bacteroidetes*
P2	AY341109	Root (1 wk)	*Flavobacterium* sp. strain R-20822	AJ786788	94.24	*Bacteroidetes*
P3	AY332574	Mix (0)	Rhizosphere soil bacterium RSI-35	AJ252602	97.97	*Bacteroidetes*
P5	AY341110	Root (3 wk)	*Bacteriovorax stolpii*	AY094131	98.73	*Deltaproteobacteria*
P6a	AY332575	Mix (0)	Uncultured bacterium clone O-CF-31	AF443566	98.72	*Betaproteobacteria*
P6b	AY332576	Mix (0)	Uncultured *Acidobacteria* clone W1-1H	AY192198	97.20	*Acidobacterium*
P9	AY332577	Mix (0)	Uncultured bacterium clone D	AJ459874	99.43	*Alphaproteobacteria*
P10	AY341111	Seed (1 day)	Uncultured bacterium clone C-CF-23	AF443568	97.81	*Betaproteobacteria*
P12	AY341112	Root (1 wk)	Subseafloor sediment bacterial clone	AB177044	97.03	*Bacteroidetes*
P13	AY341113	Root (1 wk)	Uncultured *Bacteroidetes* strain PRD18H08	AY948070	93.63	*Bacteroidetes*
P15	AY341114	Root (1 wk)	Uncultured soil bacterium clone TIIA5	DQ297951	94.95	*Bacteroidetes*
P16a	AY341115	Seed (1 day)	*Bacillus* sp. strain HSCC 1649T	AB045097	95.99	*Firmicutes*
P16-14	AY341116	Root (3 wk)	*Sphingobacteriaceae* bacterium Tibet-IIK55	DQ177471	93.14	*Bacteroidetes*
P17	AY341117	Root (3 wk)	*Bacteroidetes* bacterium LC9	AY337604	96.29	*Bacteroidetes*
P19-2	AY332578	Mix (0)	Betaproteobacterium Ellin152	AF408994	96.20	*Betaproteobacteria*
P19-4	AY341118	Seed (1 day)	*Oxalobacter* sp. strain 62AP11	AB242751	99.64	*Betaproteobacteria*
P20	AY561506	Mix (0)	Uncultured soil bacterium clone 845-2	AY326591	97.64	*Gammaproteobacteria*
PS5	AY341119	Root (1 wk)	Uncultured *Bacteroidetes* clone PRD18H08	AY948070	93.15	*Bacteroidetes*
Sawdust						
S2	AY332586	Mix (0)	Uncultured *Bacteroidetes* clone CrystalBog5D8	AY792301	96.67	*Bacteroidetes*
S3	AY341120	Root (1 wk)	Uncultured bacterium BIjii2	AJ318153	91.01	*Bacteroidetes*
S5	AY332587	Mix (0)	*Chryseobacterium formosense*	AJ715377	98.52	*Bacteroidetes*
S6	AY332588	Mix (0)	*Fluviicola taffensis* strain RW262	AF493694	97.05	*Bacteroidetes*
S7	AY332589	Mix (0)	*Sphingobacterium composta*	AB244764	96.13	*Bacteroidetes*
S8	AY332590	Mix (0)	Uncultured bacterium clone PE37	AY838493	93.87	*Bacteroidetes*
ST-9	AY332591	Mix (0)	Uncultured bacterium clone C7-K9	AJ421162	88.95	*Bacteroidetes*
S9	AY561507	Seed (1 day)	Uncultured bacterium O-CF-31	AF443566	99.27	*Betaproteobacteria*
S10	AY332592	Mix (0)	*Exiguobacterium* sp. strain NIPHL090904/K2	AY748915	100.00	*Firmicutes*
S11	AY332593	Mix (0)	Uncultured gammaproteobacterium clone AKYG1610	AY921806	97.27	*Gammaproteobacteria*
S13	AY332594	Mix (0)	Uncultured bacterium clone CYCU-NirS-16S-NH-123	DQ010317	98.89	*Bacteroidetes*
S14	AY341121	Seed (1 day)	Uncultured *Bacteroidetes* strain BIsii5	AJ318181	95.84	*Bacteroidetes*
S24	AY341122	Root (3 wk)	Uncultured bacterium BIjii2	AJ318153	92.42	*Bacteroidetes*
S26	AY341123	Root (3 wk)	Uncultured bacterium HP1A92	AF502211	98.70	*Bacteroidetes*
S27	AY341124	Root (3 wk)	Uncultured *Bacteroidetes* strain BIsii5	AJ318181	94.61	*Bacteroidetes*
S28	AY341125	Root (1 wk)	Uncultured *Bacteroidetes* clone EB 19	AM168117	97.58	*Bacteroidetes*
S30	AY341126	Root (1 wk)	*Sporocytophaga myxococcoides*	AJ310654	98.86	*Bacteroidetes*
S32	AY341127	Root (3 wk)	Uncultured betaproteobacterium clone CW13	AY956663	100.00	*Betaproteobacteria*
S33	AY341128	Seed (1 day)	*Stenotrophomonas* sp. strain SAFR-173	DQ124701	94.76	*Gammaproteobacteria*
S34	AY341129	Root (1 wk)	*Methylophilus* sp. strain C2	AY436789	98.37	*Betaproteobacteria*
Straw						
T2	AY332595	Mix (0)	*Chryseobacterium formosense*	AJ715377	97.78	*Bacteroidetes*
T4	AY332596	Mix (0)	Uncultured *Bacteroidetes* clone CrystalBog5D8	AY792301	96.45	*Bacteroidetes*
T5	AY332597	Mix (0)	*Bacteroidetes* bacterium R2-Dec-MIB-3	AB126976	97.04	*Bacteroidetes*
T7	AY332598	Mix (0)	Uncultured compost bacterium 4b	AY489030	98.98	*Bacteroidetes*
T8	AY332599	Mix (0)	Uncultured bacterium PHOS-HE36	AF314435	93.73	*Chlorobi*
T9	AY332600	Mix (0)	Uncultured bacterium clone C7-K9	AJ421162	88.76	*Bacteroidetes*
T10	AY332601	Mix (0)	Uncultured bacterium clone Urania-2B-06	AY627565	98.91	*Betaproteobacteria*
T12	AY332602	Mix (0)	*Xanthomonas campestris* pv. vesicatoria	AM039952	96.91	*Gammaproteobacteria*
T15	AY332603	Mix (0)	Uncultured bacterium clone CYCU-NirS-16S-NH-123	DQ010317	98.51	*Bacteroidetes*
T16	AY341130	Seed (1 day)	*Sphingobacterium composta*	AB244764	96.68	*Bacteroidetes*
T17	AY341131	Seed (1 day)	*Rhizobium* sp. strain Kus-7	AF510381	98.66	*Alphaproteobacteria*
T18	AY341132	Seed (1 day)	Betaproteobacterium Ellin152	AF408994	97.99	*Betaproteobacteria*
T19	AY341133	Seed (1 day)	Uncultured bacterium clone B5	AB246720	98.17	*Firmicutes*

Continued on following page

TABLE 1—*Continued*

Treatment or organelle and band	Accession no.	Sample (time postsowing)	Most similar sequence (by BLAST analysis)			
			Organism	Accession no.	% Similarity	Phylum
T20	AY341134	Root (1 wk)	Uncultured bacterium HP1A92	AF502211	97.96	*Bacteroidetes*
T21	AY341135	Root (1 wk)	Uncultured *Bacteroidetes* strain BIti15	AJ318185	94.27	*Bacteroidetes*
T24	AY341136	Root (1 wk)	*Paenibacillus* sp. strain DS-1	DQ129555	99.63	*Firmicutes*
T26a	AY341137	Root (3 wk)	Marine bacterium MBIC1357	AB032514	92.79	*Bacteroidetes*
T26b	AY341138	Root (3 wk)	*Methylophilus* sp. strain C2	AY436789	99.45	*Betaproteobacteria*
T27	AY341139	Root (3 wk)	*Methylophilus* sp. strain C2	AY436789	97.99	*Betaproteobacteria*
T28	AY341140	Root (3 wk)	Uncultured bacterium clone 010B-B12	AY662047	98.13	*Betaproteobacteria*
Cucumber plastid	AY341141	NA[a]	*Cucumis sativus* chloroplast	AJ970307	97.85	Plastids

[a] NA, not applicable.

plastid sequence, 5′-GTT **C**GA TCC TGG CTC AG-3′; the mismatch is in boldface), was employed to reduce the otherwise pervasive PCR amplification of cucumber plastid sequences. Second, DNA extracts were also subject to PCR amplification with the "*Bacteroidetes*" primer set C319 (5′-GTA CTG AGA YAC GGA CCA-3′) (32)/907R (PCRs were conducted as described previously, with the exception that touchdown annealing temperatures were from 69°C to 65°C) and subsequently PCR amplified with the general bacterial for DGGE primer set (21). The resulting PCR products were then analyzed by DGGE.

DGGE analyses, band excision, cloning, and sequencing were conducted as described previously (21). Dominant bands from the general bacterial analyses of all samples were excised, and sequences recovered from these excised bands were submitted to the NCBI for BLAST analysis (2). Sequences were also examined by the CHECK_CHIMERA program located at the Ribosomal Database Project (14), and suspect sequences were removed from analyses.

Clustering analysis of DGGE profiles. The similarity of bacterial community PCR-DGGE profiles of replicates of samples was estimated by cluster analysis, as described previously (21). Normalizations and analyses of DGGE gel patterns were done with BioNumerics software version 3.0 (Applied Maths, Kortrijk, Belgium). The normalized banding patterns were used to generate dendrograms by calculating the Pearson product moment correlation coefficient (51) and by UPGMA (unweighted pair group method with arithmetic averages) clustering (47). This approach compares profiles based on both band position and intensity.

Sequence analyses. Sequences of excised bands were aligned to known bacterial sequences using the "green genes" 16S rRNA gene database and alignment tool (16; http://greengenes.lbl.gov/). Aligned sequences and close relatives were imported and manually refined by visual inspection in the Mega software package version 3.1 (28). Neighbor-joining phylogenetic trees were constructed on the basis of 397 (*Bacteroidetes*) or 508 (*Betaproteobacteria*) positions of the 16S rRNA gene by using the Kimura two-parameter substitution model with complete deletion of gapped positions. The robustness of inferred tree topologies was evaluated by 1,000 bootstrap resamplings of the data, and nodes with bootstrap values of >70% are indicated.

Nucleotide sequence accession numbers. Sequences of the excised DGGE bands were filed under GenBank accession numbers AY341108 to AY341141, AY561506, and AY561507 (Table 1). Dominant bands from the general bacterial analyses of the potting mixes were sequenced and filed under GenBank accession numbers AY332573 to AY332578 and AY332586 to AY332603 (21).

RESULTS

Triplicate DNA samples of potting mix from the time of sowing, seed, and 1- and 3-week roots were analyzed by PCR-DGGE using general bacterial primers. The similarity of PCR-DGGE profiles from replicate samples was assessed as previously described (21), and a representative analysis is presented in Fig. 1. The profiles of the replicate samples were found to be highly similar, with UPGMA Pearson correlation coefficients (r) of at least 92%, with most values higher. Due to the high similarity of the replicate profiles, a single representative sample from each time point and treatment was selected for further analysis.

For each representative DNA sample, a dual-primer-set, nested-PCR-DGGE analysis was performed to evaluate the bacterial community composition. Both general bacterial (11F/907R) and *Bacteroidetes*-specific (C319/907R) PCR amplicons were subject to nested PCR with the same general bacterial primers suitable for DGGE analyses (341FGC/907R). The resulting PCR products, approximately 500 to 550 bp in size, were analyzed by DGGE as described above (Fig. 2). Most of the visible bands detected by DGGE were excised and sequenced from the general bacterial analyses. The most similar sequences, by BLAST analyses, to those recovered are presented in Table 1, and phylogenetic analyses of *Oxalobacteraceae* and *Bacteroidetes* sequences are presented in Fig. 3 and 4, respectively.

The *Bacteroidetes*-specific PCR-DGGE analyses were highly specific to the phylum and did not amplify non-*Bacteroidetes* sequences. Bands detected in general bacterial PCR-DGGE

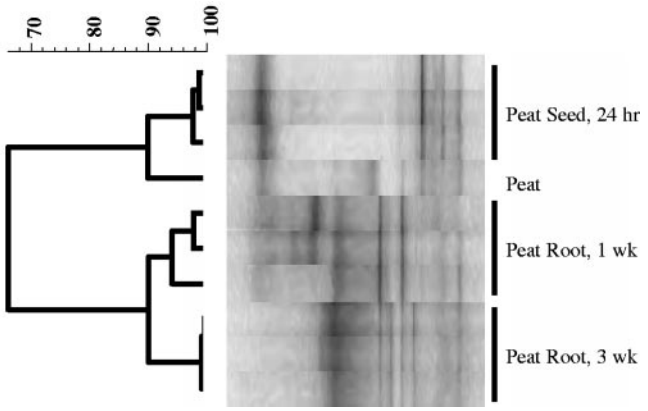

FIG. 1. A representative dendrogram depicting the similarity of profiles of bacterial communities generated from PCR-DGGE analyses of replicate samples of seed (24 h) and root (1 and 3 weeks) from cucumber grown in the peat-only potting mix. Bacterial community profiles were generated by PCR-DGGE analysis as described in the text. The UPGMA algorithm was applied to a similarity matrix of Pearson product moment correlation coefficients (r values) generated from the DGGE banding patterns.

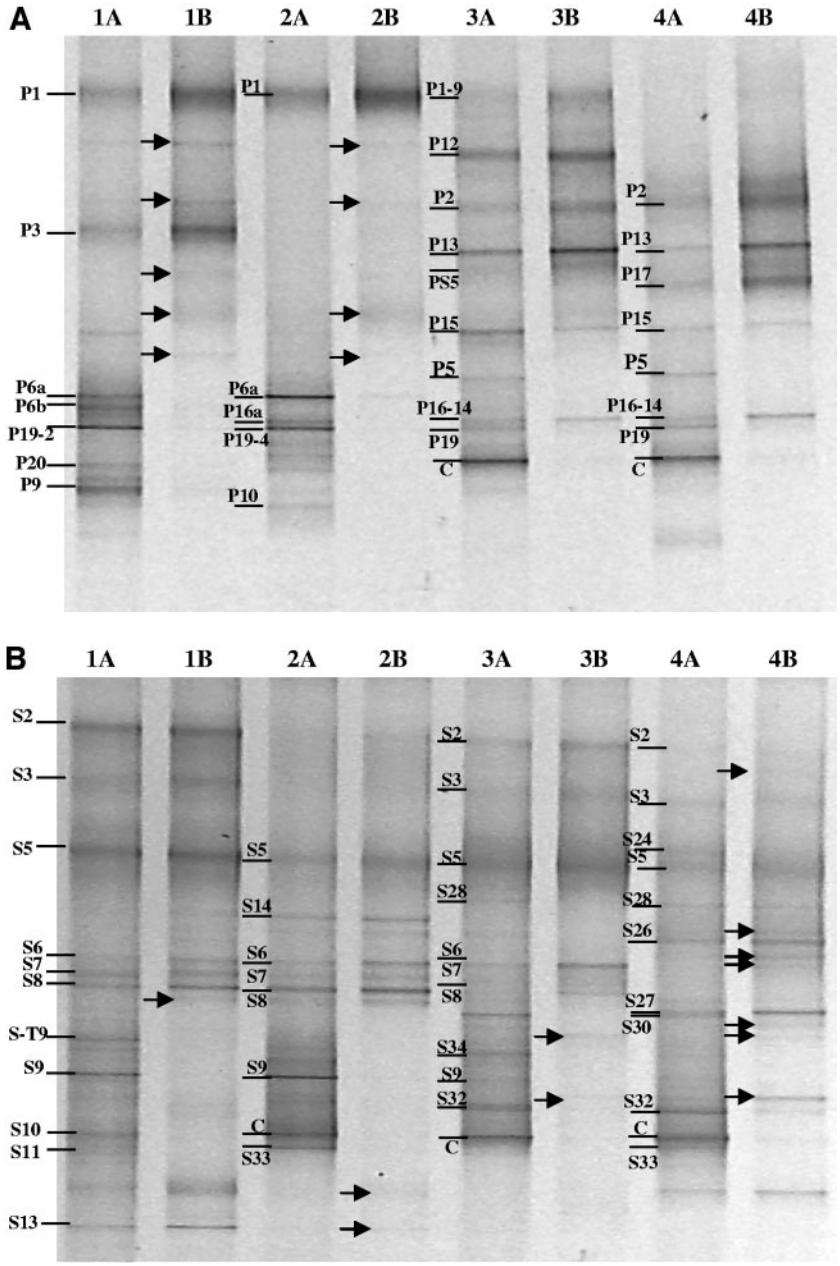

FIG. 2. PCR-DGGE analysis of partial 16S rRNA genes amplified from the peat-only (A), sawdust compost (B), and straw compost (C) treatments. For each treatment, PCR-DGGE profiles for potting mix from the time of sowing (lanes 1), seed surface after 24 h of incubation in the potting mix (lanes 2), roots after 1 week of growth in the potting mix (lanes 3), and roots after 3 weeks of growth in the potting mix (lanes 4) are shown. DNA samples were initially amplified with a general bacterial primer set (lanes A) or with a *Bacteroidetes* primer set (lanes B) and then subjected to nested PCR with general bacterial primers appropriate for DGGE analysis, as described in the text. Excised, cloned, and sequenced bands are labeled and are discussed in the text. Populations detected only in the *Bacteroidetes*-enhanced PCR-DGGE analyses are indicated by arrows.

analyses (lanes labeled A in Fig. 2A to C) were inferred to represent bacteria from the phylum *Bacteroidetes* when a band in the adjacent lane to the right (lanes labeled B in Fig. 2A to C) migrated to the same vertical position. This is possible because (i) the PCR yields were derived from the same genomic DNA sample, (ii) the PCR fragment for DGGE was the same size and at the same location within the rRNA gene, (iii) the internal general bacterial primers 341F and 907R were checked in silico for potential bias against the *Bacteroidetes* and were found to perfectly match approximately 94% of *Bacteroidetes* sequences in the ARB database (substantially more than the *Bacteroidetes* primer C319) (see reference 32 for a description of primer targets), and (iv) all band sequences were recovered from the general bacterial analyses, not the *Bacteroidetes*-specific analyses, demonstrating that the bands in the general analyses migrating to the same vertical locations as

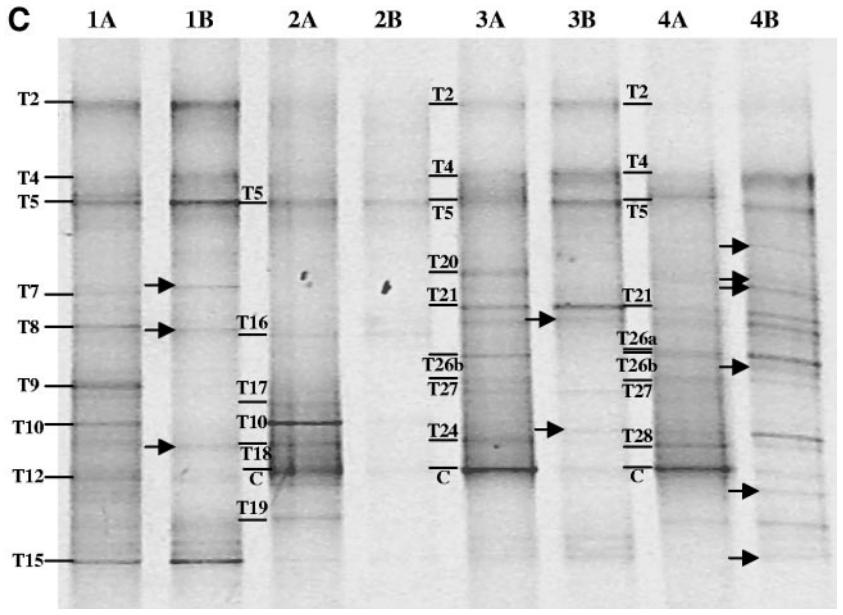

FIG. 2—*Continued.*

bands in the *Bacteroidetes* analyses were indeed *Bacteroidetes* and not merely comigrating DNA fragments (Table 1; Fig. 4). In this study, such inferences were highly reliable, as indicated by sequence analyses of bands excised and sequenced from the general bacterial DGGE analyses. However, due to the difficulty in designing a single primer to amplify rRNA gene sequences from all *Bacteroidetes* (32), some *Bacteroidetes* were detected with general bacterial analyses but not with the *Bacteroidetes*-specific analyses (e.g., bands ST-9 and T9 [Fig. 2B and C and 4]).

In all three treatments, the number of populations detected by PCR-DGGE analysis on the seed surfaces was lower than that detected in the potting mix prior to sowing. Many of the populations detected on the seeds were also detected in the potting mix from the respective treatments. Despite the differences in the compositions of the bacterial communities of the three potting mixes, particularly between the compost-amended and peat-only treatments (21), the seed surfaces in all treatments were colonized by bacteria from the genus *Chryseobacterium* (bands P1, S5, and T5) and by one or two populations belonging to the family *Oxalobacteraceae* (bands P6a, P19-4, S9, T10, and T18).

The root bacterial community profiles differed significantly from the initial potting mix and seed surface community profiles in all treatments (Fig. 2A to C). Within each treatment, root communities had many populations in common (represented by bands P2, P5, P13, P15, P16-14, and P19; bands S2, S3, S5, S30, and S32; and bands T2, T4, T5, T21, T26b, and T27 for peat-only, sawdust compost, and straw compost treatments, respectively), but these populations were generally not detected in potting mix and seed samples. Within treatments, those bacteria that were detected in potting mix, seed, and root samples belonged to the genus *Chryseobacterium* and the family *Oxalobacteraceae*. For example, in the peat-only treatment, of the two *Oxalobacteraceae* populations detected on the seed surface (represented by bands P6a and P19-4), a band at the position of P19-4 was detected on the roots at 1 and 3 weeks. This band was confirmed to be a member of the *Oxalobacteraceae* (data not shown). Likewise, in the sawdust and straw compost treatments, *Chryseobacterium* populations (bands S5 and T5) were detected in all samples from potting mix to root surface at 3 weeks, and other *Chryseobacterium* populations (bands S2, T2, and T4) were detected in the potting mix and on the roots at 1 and 3 weeks. As with the peat-only treatment, *Oxalobacteraceae* populations were also detected on the root surfaces in compost-amended treatments. In the sawdust compost treatment, two *Oxalobacteraceae* populations were detected (bands S9 and S32). Band S9, detectable on the seed surface and the root at 1 and 3 weeks, migrated to the same position on the DGGE gel as bands P6a and T10, while band S32, detected only on the root surface at 24 h and 3 weeks, migrated to the same position as P19 and T28 (data not shown). In the straw compost treatment, two *Oxalobacteraceae* populations (bands T10 and T18) were detected on the seed, while only a single population (band T28) was detected on the root at 3 weeks.

In this study, 60 sequences (including cucumber plastid) were obtained from bands excised from DGGE gels. Based on sequence analyses, 44 of these sequences were 95% or more similar, and 2 were less than 90% similar (bands ST-9 and T9, *Bacteroidetes* by phylogenetic analyses), to published sequences. The inferred bacterial populations were unevenly distributed among five taxa, i.e., *Bacteroidetes* (34 sequences), *Proteobacteria* (19 sequences), *Firmicutes* (4 sequences), *Acidobacteria* (1 sequence), and *Chlorobi* (1 sequence). Sequences affiliated with the phylum *Bacteroidetes* were the most frequently recovered and revealed the presence of a large diversity of bacteria belonging to this phylum (Fig. 4). The application of *Bacteroidetes*-specific analyses revealed the presence of additional diversity within some of the samples (Fig. 2A to C). Interestingly, the presence of additional *Bacteroidetes* diversity was observed primarily in the potting mix and seed surface in the peat-only

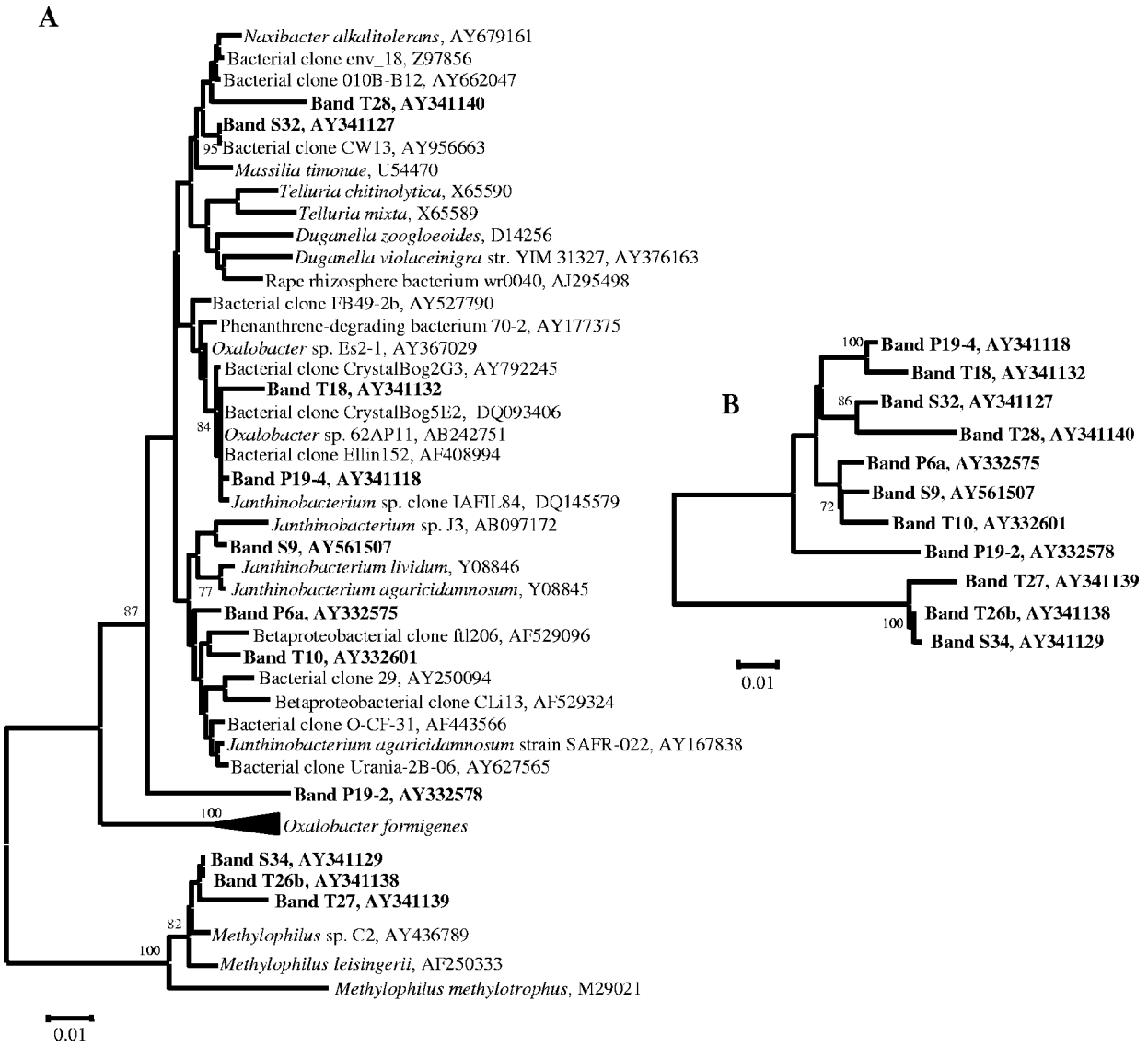

FIG. 3. Neighbor-joining phylogenetic trees of detected betaproteobacterial populations. Bootstrapped neighbor-joining trees were generated with 1,000 resamplings, and nodes with bootstrap values of greater than 70% are indicated, as described in the text. Band numbers refer to bands isolated from DGGE analyses. The scale bars represent 0.01 substitution per nucleotide position. A: Phylogenetic tree of betaproteobacterial sequences recovered from DGGE bands and most similar sequences as identified by BLAST. B: Phylogenetic tree of betaproteobacterial sequences recovered from DGGE bands alone.

treatment and on the root samples from the compost treatments. In the peat-only treatment, the bacterial community profiles of roots at 1 and 3 weeks, as determined by the general bacterial DGGE analysis, were nearly completely composed of *Bacteroidetes*, except for the cucumber plastid, a *Bacteriovorax* population (band P5), and an *Oxalobacteraceae* population (band P19).

The detected *Betaproteobacteria* were predominantly from the family *Oxalobacteraceae*, and phylogenetic analyses of *Oxalobacteraceae* revealed three groupings of bands recovered from all three treatments (bands P6a, S9, and T10; P19-4 and T18; and S32 and T28). With the exception of the grouping of P19-4 and T18, bootstrap values above 70% could not be established initially for these groups due to the short sequence length and high similarity of the sequences (Fig. 3A). When the band sequences were analyzed alone, strong support was given for the clustering of band P19-4 with T18, S32 with T28, and P6a with S9 and T10 (Fig. 3B).

DISCUSSION

Compost amendment to soils and potting mixes can significantly modify plant-associated microbial communities of plants grown in such media (25, 49). In addition to shifts associated with plant development, plant-associated microbial communities can be influenced by the chemical, physical, and biological properties of soils and potting mixes amended with compost. In this study, seed surfaces were colonized largely by bacterial populations detectable in the potting mixes at the time of sowing. In all three treatments, the seed-colonizing bacterial communities

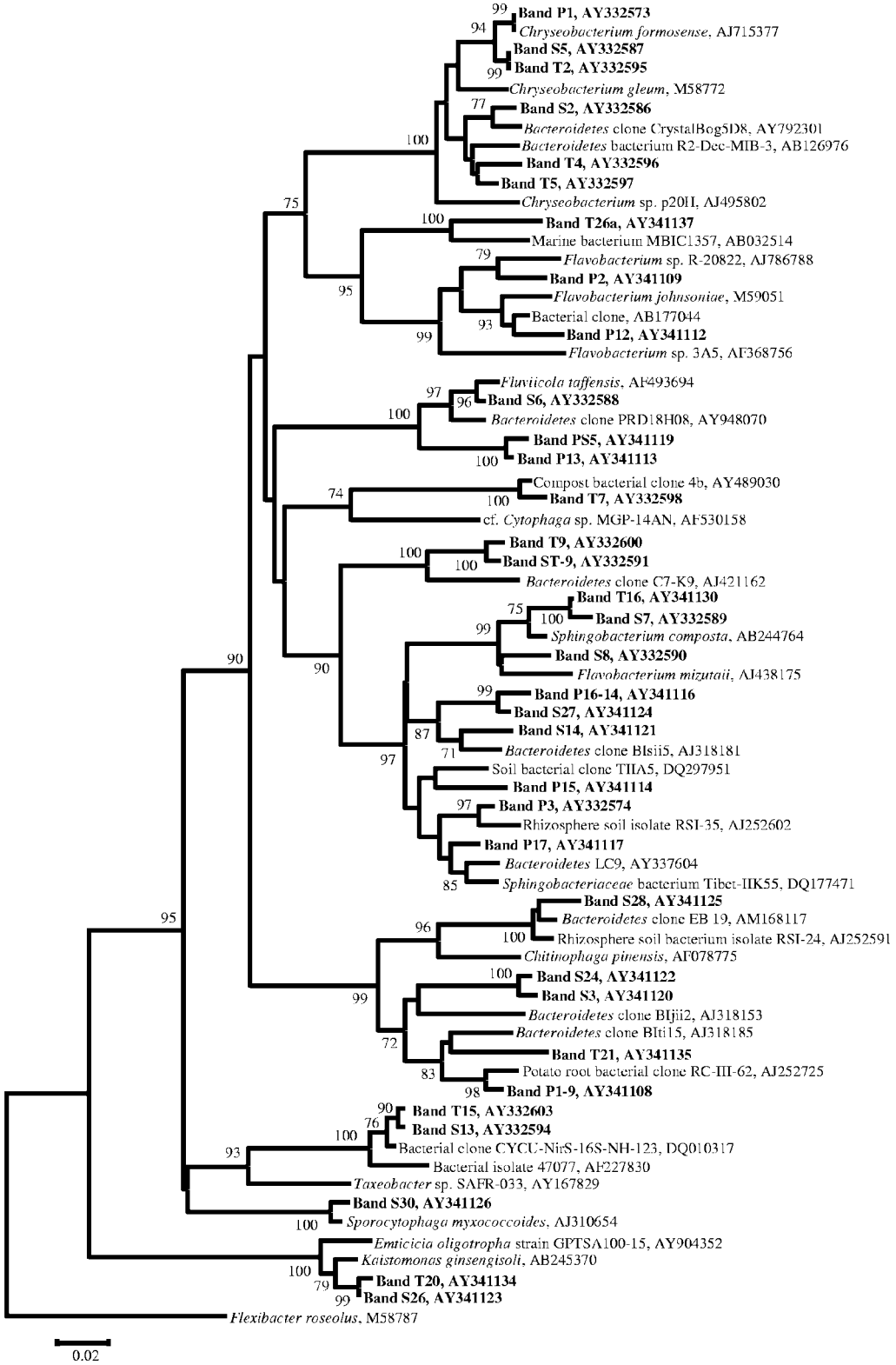

FIG. 4. Neighbor-joining phylogenetic tree of *Bacteroidetes* populations detected in the study. Bootstrapped neighbor-joining trees were generated with 1,000 resamplings, as described in the text. Band numbers refer to bands isolated from DGGE analyses. Nodes with bootstrap values of greater than 70% are labeled. The scale bar represents 0.02 substitution per nucleotide position.

included bacteria from the genus *Chryseobacterium* and the family *Oxalobacteraceae*. Since the seeds are the first plant surfaces to be colonized, there is a great deal of interest in the provenance of such organisms and their eventual persistence and colonization of growing root surfaces. However, Normander and Prosser (39) observed a disparity between seed and root microbial communities and proposed that this difference was an indication that emerging plant roots are colonized by soil-borne, rather than seed-borne, microorganisms. In our study, the persistence of seed-colonizing populations varied by taxon and with potting mix treatment, although, overall, many of the seed-colonizing populations were not detected in root samples. For example, root communities in the peat-only treatment shared only a single population with the seed (an *Oxalobacteraceae* population).

Oxalobacteraceae populations were present in seed and root samples in the compost treatments as well. Phylogenetic analyses revealed that although all the recovered sequences were more than 95% similar, two different clades were detected on seed surfaces while a third clade was detected in root samples. The detection of phylogenetically distinct, but closely related, populations on seeds and roots suggests a physiological difference that may explain their environmental distribution. Furthermore, these *Oxalobacteraceae* were either absent or only faintly detectable in general bacterial analyses of potting mix samples taken at the later time points, suggesting that their persistence was a result of rhizosphere competence rather than abundance in the potting mix (data not shown). Members of the *Oxalobacteraceae* are aerobic, flagellated, root- or soil-dwelling bacteria that are capable of degradation of a variety of organic molecules, including chitin, and are easily mistaken for pseudomonads (8, 9, 48, 52). These characteristics may explain their persistence in the root environment.

In addition to *Oxalobacteraceae*, bacteria belonging to the genus *Chryseobacterium* were detected on seed surfaces 1 day after sowing in all treatments. This genus (family *Flavobacteriaceae*, phylum *Bacteroidetes*) consists of bacteria that are nonmotile, aerobic, pigmented, and capable of saprophytic or parasitic growth (7). In this study, the distribution of *Chryseobacterium* varied significantly with treatment; in the peat-only treatment they were not detected in root samples, while in root samples from compost treatments certain *Chryseobacterium* spp. were among the most persistent. In contrast to the case for the *Oxalobacteraceae* spp., we observed that the detection of *Chryseobacterium* spp. on plant surfaces largely mirrored their detection in potting mix samples from the same time points (data not shown). While motility has been shown to be important for root colonization by pseudomonads, the persistence of the nonmotile *Chryseobacterium* spp. on root surfaces may be a result of a reservoir of organisms maintained in the compost-amended potting mix, although transport via plant growth or water percolation may also have played a role (17, 35). The provenance and persistence of the *Chryseobacterium* spp. and the *Oxalobacteraceae* are currently being further investigated to determine if composts are sources and factors for maintenance of these organisms in the root environment.

Overall, molecular analyses revealed a surprising dominance and diversity of *Bacteroidetes*. *Bacteroidetes* are known for their utilization of macromolecules, including proteins and polysaccharides such as cellulose and chitin (6, 26, 32, 41). *Bacteroidetes*, including *Chryseobacterium* spp., have been previously detected in composted materials (1, 12, 21, 37), in soil environments (52, 54), and in association with plant surfaces (22, 26, 27, 29, 30, 33, 36, 40, 44, 45). We have consistently recovered *Chryseobacterium* sequences directly from these and other cow manure composts produced in the same location (Ohio Agriculture Research and Development Center, Ohio State University, Wooster). Certainly, cultivation-independent molecular techniques often reveal a greater abundance and diversity of *Bacteroidetes* than cultivation-based analyses of the same samples, perhaps due to the difficulty of isolating some members of this phylum (27). The application of general bacterial and *Bacteroidetes* primer sets, nested with the same internal general primer set, proved to be a rapid and reliable technique for detection of bacteria from this phylum. Since *Bacteroidetes* can be large contributors to nutrient cycling in plant environments via production of degradative enzymes (26), we believe that this technique will prove to be a useful tool in plant and other environmental studies.

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