APPLIED AND ENVIRONMENTAL MICROBIOLOGY, June 1989, p. 1368–1374
0099-2240/89/061368-07$02.00/0

Vol. 55, No. 6

# Characterization of Bacteria That Suppress *Rhizoctonia* Damping-Off in Bark Compost Media by Analysis of Fatty Acid Biomarkers

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Received 28 September 1988/Accepted 28 February 1989

**Examination of cucumber roots (*Cucumis sativus* L.) grown in bark compost media and of the surrounding edaphic substrate showed profiles of polar lipid fatty acids commonly found in bacteria. The composition of fatty acids in these profiles differed significantly between roots grown in a medium naturally suppressive to *Rhizoctonia* damping-off and roots from a conducive medium. Cucumber roots from the suppressive medium had higher proportions of *cis*-vaccenic acid (18:1ω7*c*) and the iso-branched monoenoic fatty acid i17:1ω8 but lower proportions of several iso- and anteiso-branched fatty acids compared with roots from the conducive medium. The concentrations of the bacterial fatty acids were significantly lower in the surrounding media. However, the suppressive and conducive growth substrates had differences in the composition of the bacterial fatty acids similar to those found between the cucumber roots proper. These results suggest major differences in bacterial community composition between suppressive and conducive systems. Fatty acid analyses were also utilized to examine the effects on bacterial community composition of root colonization by *Flavobacterium balustinum* 299, a biocontrol agent. The concentration of the most prominent fatty acid in this bacterium, i17:1ω8, was increased on roots produced from inoculated seeds in a medium rendered suppressive by the treatment. This change was concomitant with a significant increase in the concentration of 18:1ω7*c*, not present in the lipids of the antagonist, indicating a shift in the microflora from a conducive to a suppressive bacterial community.**

Plant growth media amended with specific prepared composts suppress a variety of soil-borne plant diseases (21). Media amended with low-temperature composts from hardwood tree barks suppress *Rhizoctonia* and *Pythium* damping-off. The suppressive effects last for at least 1 year and are induced by microorganisms (9, 10, 27, 30, 31). However, plant growth media prepared with high-temperature composts from tree barks are conducive to *Rhizoctonia* damping-off for up to 4 weeks after preparation (26).

The relative contribution of fungal species in suppression of *Rhizoctonia* damping-off has been determined by examining the population density of various fungal taxa in suppressive and conducive compost media (27). Although a number of bacterial antagonists have been identified that can suppress *Rhizoctonia* damping-off in this system (28), the relative contribution of various bacterial groups has not been evaluated.

Most studies of microorganisms in the soil and rhizosphere have utilized direct counts or plate counts to estimate the biomass of microbes (e.g., see references 3, 40, and 50). However, direct microscopic counts of microorganisms in soil or on roots may give misleading values because the organisms often grow in microcolonies embedded in a network of extracellular polymers (1, 11, 39). Plate counts are complicated by selective growth of only viable cells on specific media and by the formation of a single colony from bacterial aggregates. Populations of specific rhizosphere bacteria have been monitored with antibiotic-resistant mutants (8, 28). This method suffers from the inability of many such marked bacteria to maintain their colonization potential (12).

To improve the situation, alternative methods have been developed to estimate the biomass and activity of microbes in natural environments. These are based on the analysis of chemical properties of microbial cells (51). Analysis of phospholipids has been shown to provide a reliable estimate of the biomass of microorganisms in environmental samples (2, 54). The composition of fatty acids varies widely among different bacteria (29), and isolation of bacteria from natural environments has shown that subsets of microbial communities contain specific signature fatty acids in their phospholipids (23, 35, 48). Quantitative recovery and analysis of these fatty acids have provided a reproducible and sensitive means to define the community structure of microbial assemblies in soils and sediments (14, 18, 34, 49). The fatty acid methods are free of the distortion associated with the requirements for quantitative removal of microbes from surfaces or the selectivity associated with growth on artificial media (52).

In this study, we used analysis of phospholipid fatty acids to characterize bacteria associated with cucumber roots grown in bark compost media either suppressive or conducive to *Rhizoctonia* damping-off. Furthermore, we demonstrated the use of this method to study the effects on the bacterial community composition of a biocontrol agent introduced by seed treatment in the rhizosphere of cucumber roots.

## MATERIALS AND METHODS

**Preparation of plant growth media.** A medium (pH 6.0 to 6.4) suppressive to *Rhizoctonia* damping-off was prepared by mixing bark composted in a windrow for a 4- to 5-month period with Canadian sphagnum peat and perlite (28). A conducive medium was prepared by heat treatment (5 days,

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60°C) of the suppressive medium in polyethylene bags in an oven. This treatment mimics the self-heating occurring in compost piles that destroys compost suppression of *Rhizoctonia* damping-off (32). The conducive medium was planted 24 h after the heat treatment. During this 24-h period, the medium was stored in the polyethylene bags to prevent recolonization of mesophiles (9).

**Experiments and cultivation of plants.** To compare the profiles of polar lipid fatty acids from cucumber roots and their surrounding edaphic substrate, we planted cucumber seeds (*Cucumis sativus* L. "Straight Eight;" eight seeds per pot, 90% germination) in the conducive and suppressive media in 400-ml pots. The plants were fertilized, watered, and incubated in growth chambers as described previously (9). Three weeks after planting, seedlings were harvested by carefully shaking adhering container medium from roots, which essentially removed all adhering particles from the growth medium. The roots were cut off at growth medium level and lyophilized. Three root systems from the seedlings in each pot were bulked and treated as one replicate. In a preliminary study, roots were rinsed with distilled water. However, fatty acid analysis revealed that the trends in composition of fatty acids of washed and unwashed roots were the same. After the cucumber roots were removed, samples of the growth substrate were transferred to polyethylene bags and lyophilized. These samples represent the edaphic growth substrate. Material from one pot was treated as one replicate.

To examine the effects of colonization of a biocontrol agent in the rhizosphere of cucumber roots, we treated cucumber seeds with *Flavobacterium balustinum* 299 ATCC 53198. This bacterium has been isolated from cucumber roots grown in the suppressive bark compost medium, and it has been shown that seed treatment with F-299 suppresses *Rhizoctonia* damping-off in the conducive bark compost medium (28). Cucumber seeds were soaked with a suspension of *F. balustinum* 299, and control plants were soaked in sterile distilled water as previously described (28). The seeds were planted in the conducive compost medium, and after 3 weeks, the roots were recovered and lyophilized as described above.

A completely randomnized design was used in the pot experiments. Samples from the different treatments were combined to give four replicates of cucumber roots grown in the suppressive medium, growth substrate from the suppressive medium, cucumber roots from the conducive medium, growth substrate from the conducive medium, and cucumber roots produced from the *F. balustinum* 299-treated seeds and grown in the conducive medium.

**Lipid extraction and fractionation.** The lyophilized samples were extracted with the single-phase mixture of chloroform-methanol-water (1:2:0.8, vol/vol/vol) by the method of Bligh and Dyer (4) as modified by White et al. (54). The solutions were split into two phases by the addition of 1 volume of water and chloroform, respectively. The organic phase was recovered, and the extracted lipids were fractionated on columns of silicic acid (Unisil, 100/200 mesh; Clarkson Chemical Co., Williamsport, Pa.) which were 1 cm in diameter and contained 1 g (dry weight) of adsorbent. Neutral lipids were eluted with 10 ml of chloroform, glycolipids were eluted with 40 ml of acetone, and polar lipids were eluted with 10 ml of methanol. The polar lipid fraction, containing phospholipids (24), was dried under a stream of nitrogen. The fractions containing neutral lipids and glycolipids were not used in this study.

**Mild alkaline methanolysis.** The polar lipid fractions were subjected to mild alkaline methanolysis, which yields the methyl esters of ester-linked fatty acids (54).

**GC.** The methyl esters were separated by capillary gas chromatography (GC) with a Hewlett-Packard 5880 GC equipped with a flame ionization detector. The column was a fused silica capillary column (50 m by 0.2 mm inner diameter) coated with a cross-linked nonpolar methylsilicone phase (Hewlett-Packard Co., Palo Alto, Calif.). The initial oven temperature was 80°C for 1 min, then increased at 10°C/min to 150°C, then at 3°C/min to 240°C, and finally at 5°C/min to 300°C. The injector temperature was 230°C, and the detector temperature 270°C. Hydrogen at a linear gas flow rate of 80 cm/s was used as the carrier gas. Injections were performed in the splitless mode (43).

Tentative peak identifications before mass spectrometric (MS) analysis were based on comparison of retention times with data from standards obtained from Supelco, Inc. (Bellefonte, Pa.), Sigma Chemical Co. (St. Louis, Mo.), and NuChek Prep, Inc. (Elysian, Minn.) and from compounds previously identified in our laboratory. Peak areas were quantified by adding methyl nonadecanoate as an internal standard before GC injections. GC data were acquired and manipulated by a Nelson data system (Nelson Analytical, Inc., Paramus, N.J.).

**GC-MS.** GC-MS analyses were performed on a VG TRIO-3 GC/MS/MS instrument equipped with a Hewlett-Packard 5890 GC. The GC conditions were those described above, but helium was used as the carrier gas. The electron energy in electron impact ionization was 70 eV, and the source temperature was 220°C.

MS identifications of fatty acid methyl esters were based on comparison with spectra from standards or with spectra reported in the literature (6, 7, 41). The positions of double bonds were determined by preparing the dimethyldisulfide adducts (33).

**Fatty acid nomenclature.** Fatty acids are designated as the total number of carbon atoms:number of double bonds with the position closest to the aliphatic end of the molecule indicated with the geometry *c* for *cis* and *t* for *trans*. The prefixes i and a refer to iso and anteiso branching, respectively. Cyclopropane fatty acids are designated by the prefix cy. The prefix OH is used to designate hydroxyl groups, with the position from the carboxyl groups indicated.

**Statistical analyses.** Fatty acid profiles from the samples of cucumber roots and growth substrate from the suppressive and conducive media were analyzed by analysis of variance with the SAS/STAT subprogram GLM. Significant means were then compared by Tukey's wholly significant difference test with an error rate of 0.05. The effects of seed treatment with *F. balustinum* 299 were analyzed by comparing the patterns of fatty acids in treated versus nontreated samples, using a two-sided *t* test in the SAS/STAT subprogram TTEST.

## RESULTS

**Identification of bacterial fatty acids.** Data in Table 1 present all fatty acids identified in the polar lipids of cucumber roots and in plant growth substrates from the suppressive and conducive media. A great variety of fatty acids were detected, including normal saturated, monobranched, unsaturated, and cyclopropane fatty acids. Root samples had 30- to 40-fold-higher total amounts of fatty acids than medium samples. However, the common plant fatty acids palmitic acid (16:0) and linoleic acid and α-linolenic acid (18:2 and 18:3) (20) composed approximately 85 to 90 mol% of the total

TABLE 1. Ester-linked fatty acids from the polar lipids of cucumber roots and their surrounding edaphic substrate grown in suppressive or conducive bark compost medium

| Fatty acid[a] | pmol/g (dry wt) (mean and SD) | | | |
|---|---|---|---|---|
| | Suppressive medium | | Conducive medium | |
| | Roots | Substrate | Roots | Substrate |
| Br13:0** | 436 (45) | ND[b] | 568 (258) | ND |
| Br14:0*** | 351 (72) | ND | 283 (167) | ND |
| 14:0* | 1,092 (465) | 18 (25) | 1,134 (672) | 16 (32) |
| Br15:1*** | 402 (126) | 13 (26) | 300 (105) | ND |
| i15:0*** | 3,034 (864) | 317 (312) | 3,322 (680) | 196 (193) |
| a15:0** | 1,672 (507) | 140 (129) | 1,701 (417) | 98 (99) |
| 15:1** | 727 (159) | ND | 909 (294) | ND |
| 15:0*** | 6,333 (1,264) | 113 (73) | 7,291 (1,654) | 64 (52) |
| i16:0*** | 1,915 (412) | 950 (366) | 6,490 (1,141) | 2,337 (768) |
| 16:1ω9*c** | 357 (60) | 46 (41) | 402 (110) | 49 (30) |
| 16:1ω7*c**** | 5,323 (813) | 600 (173) | 5,759 (419) | 209 (65) |
| 16:1ω7*t**** | 1,719 (209) | 23 (19) | 2,265 (231) | 5 (4) |
| 16:1ω5*c** | 744 (76) | 218 (55) | 657 (50) | 53 (13) |
| 16:0*** | 186,800 (4,014) | 1,900 (447) | 244,700 (2,001) | 1,794 (368) |
| Br17:0*** | 447 (25) | 68 (22) | 747 (148) | 69 (32) |
| i17:1ω8*** | 1,467 (291) | 177 (33) | 1,691 (132) | 54 (12) |
| 10Me16:0*** | 115 (14) | 100 (13) | 149 (9) | 421 (46) |
| i17:0*** | 2,284 (373) | 900 (126) | 4,527 (916) | 1,641 (174) |
| a17:0*** | 1,469 (261) | 973 (126) | 4,314 (1,034) | 2,186 (232) |
| cy17:0*** | 1,691 (309) | 431 (135) | 2,645 (493) | 260 (15) |
| 17:0*** | 2,431 (136) | 215 (18) | 4,335 (716) | 995 (94) |
| 10Me17:0* | 622 (30) | 265 (24) | 656 (247) | 404 (40) |
| 18:2 and 3[c] | 270,900 (11,540) | 2,368 (271) | 373,000 (28,180) | 847 (190) |
| 18:1ω9*c**** | 8,951 (551) | 1,468 (87) | 11,765 (1,813) | 508 (55) |
| 18:1ω7*c**** | 8,276 (530) | 2,005 (82) | 9,518 (798) | 874 (87) |
| 18:1ω7*t**** | 309 (35) | 113 (12) | 356 (27) | 69 (16) |
| 18:0*** | 14,340 (1,297) | 865 (56) | 20,110 (1,686) | 1,377 (166) |
| Br19:0a***[d] | 1,142 (130) | 447 (25) | 1,085 (211) | 169 (63) |
| 10Me18:0*** | 365 (94) | 334 (25) | 878 (331) | 580 (79) |
| Br19:0b*** | 209 (62) | 181 (10) | 7,511 (218) | 521 (61) |
| cy19:0*** | 4,056 (445) | 2,044 (178) | 4,883 (804) | 1,695 (262) |
| Bacterial acids***[e] | 33,630 (4,169) | 8,500 (868) | 50,250 (4,824) | 10,950 (1,186) |
| Total*** | 531,100 (25,480) | 18,000 (1,681) | 718,200 (49,640) | 17,710 (1,604) |

[a] Asterisks indicate significant differences between means by analysis of variance (*, $0.01 < P \leq 0.05$; **, $0.001 < P \leq 0.01$; ***, $P \leq 0.001$).
[b] ND, Not detected.
[c] Peaks for 18:2 and 18:3 coeluted.
[d] Br19:0a and Br19:0b designate two different, unidentified branched $C_{19}$ fatty acids.
[e] Sum of the following fatty acids: i15:0, i16:0, i17:0, a17:0, 10Me18:0, 18:1ω7*c*, 18:1ω7*t*, i17:1ω8, cy17:0, cy19:0, 15:0, and 17:0.

fatty acids in the root samples. A more sensitive way to examine differences was therefore to compare the amounts and patterns of a few, more specific bacterial fatty acids. These included methyl-branched fatty acids, monounsaturated acids with the double bond in the ω7 position, cyclopropane fatty acids, and normal-chain fatty acids with an odd number of carbon atoms in the chain (Table 2). Data in Fig. 1 and 2 show the concentration, expressed as picomoles per gram of dry weight, of the bacterial fatty acids, and data on the proportions, expressed in moles percent, of these fatty acids are presented in Tables 2 to 5.

**Fatty acids in cucumber roots.** The amount and composition of bacterial fatty acids differed significantly between cucumber roots grown in the suppressive and conducive media. Roots produced in the suppressive medium had a lower total concentration of bacterial fatty acids. Concentrations of most of the individual bacterial fatty acids were also lower than in roots from the conducive medium (Fig. 1; Table 1). Comparing the composition of fatty acids in these profiles, roots from the suppressive medium had a lower proportion of the methyl-branched fatty acids i16:0, i17:0, a17:0, and 10Me18:0 but higher proportions of the monounsaturated fatty acids 18:1ω7*c* (*cis*-vaccenic acid) and i17:1ω8*c* than roots recovered from the conducive medium (Tables 2 and 3).

**Fatty acids in suppressive and conducive growth substrates.** The growth substrates surrounding the cucumber roots had considerably lower total concentrations of the bacterial fatty acids than either type of cucumber root (Table 1; Fig. 1). Furthermore, the composition of bacterial fatty acids recovered from the growth substrates differed from the fatty acid profiles of the cucumber roots. The growth substrates had higher proportions of the branched acids i17:0, a17:0, and 10Me18:0 but lower proportions of i17:1ω8, cy19:0, and 15:0 than the cucumber roots proper (Tables 2 and 3).

The suppressive and conducive growth substrates differed significantly in the patterns of bacterial fatty acids. The suppressive substrate had lower concentrations of i16:0, i17:0, a17:0, and 17:0 but a higher concentration of 18:1ω7*c* than the conducive substrate (Table 1; Fig. 1). Similar differences were found between the suppressive and conducive substrates when we compared the composition of the bacterial fatty acid profiles. The suppressive substrate had lower proportions of i16:0, i17:0, a17:0, 10Me18:0, and 17:0

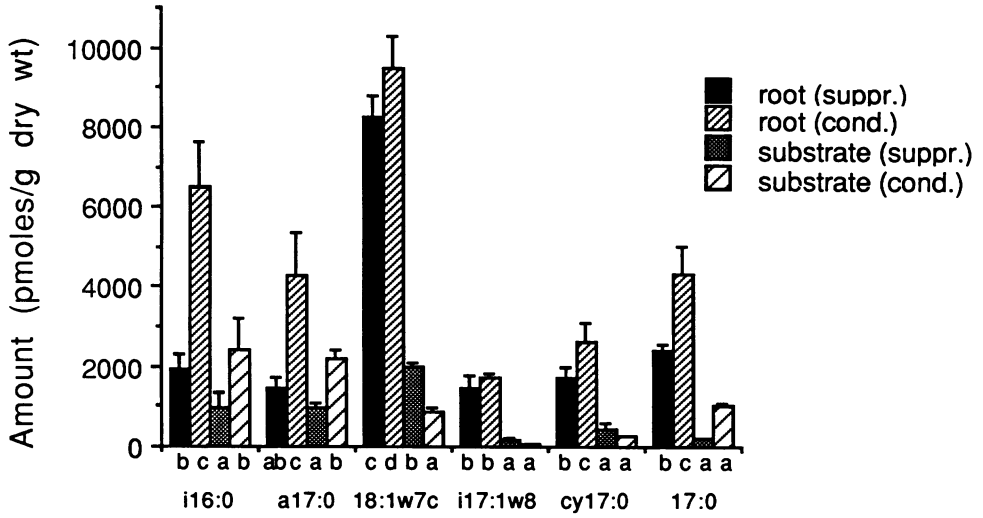


FIG. 1. Concentration of some bacterial fatty acids from the polar lipids of cucumber roots and their surrounding growth substrates grown in suppressive or conducive bark compost medium. Vertical bars show 1 standard deviation ($n$ = 4). Mean values with the same letters are not significantly different (Tukey's wholly significant difference test, $P < 0.05$).

but higher proportions of 18:1ω7*c*, i17:1ω8, and cyclopropane fatty acids than the conducive substrate (Tables 2 and 3).

**Fatty acids in *F. balustinum* 299.** The ester-linked fatty acids in the polar lipids from *F. balustinum* 299 contained mainly odd-numbered fatty acids (Table 4). The major component was identified as i17:1ω8. Among the major components in the fatty acid profile of *F. balustinum* 299 there was also an uncommon hydroxy acid with a chain length of $C_{15}$. The electron impact spectrum of this acid showed molecular ions at *m/z* 272. Ions at *m/z* 213, formed by 1,2 cleavage with loss of the methoxycarbonyl group, at *m/z* 227 (M-$CH_2$ CHOH + H) and at *m/z* 90 [$CH_2OC(OH)CH(OH)$] are characteristic for 2-hydroxy esters (41). The relative reten-

TABLE 2. Bacterial fatty acids from the polar lipids of cucumber roots and their surrounding edaphic substrate grown in suppressive or conducive bark compost medium

| Fatty acid[a] | mol%[b] (mean and SD) | | | |
|---|---|---|---|---|
| | Suppressive medium | | Conducive medium | |
| | Roots | Substrate | Roots | Substrate |
| Branched | | | | |
| i15:0** | 8.91 (0.34) | 3.51 (3.09) | 6.69 (1.80) | 1.69 (1.56) |
| i16:0*** | 5.66 (0.55) | 10.92 (3.02) | 12.85 (1.29) | 20.90 (5.04) |
| i17:0*** | 6.82 (0.99) | 10.54 (0.47) | 8.96 (1.16) | 14.94 (0.68) |
| a17:0*** | 4.37 (0.56) | 11.39 (0.48) | 8.52 (1.47) | 19.90 (0.97) |
| 10Me18:0*** | 1.09 (0.28) | 3.96 (0.49) | 1.72 (0.56) | 5.32 (0.88) |
| Unsaturated | | | | |
| 18:1ω7*c*** | 24.73 (1.50) | 23.74 (2.83) | 18.96 (0.38) | 7.98 (0.68) |
| 18:1ω7*t* | 0.94 (0.22) | 1.34 (0.19) | 0.71 (0.07) | 0.63 (0.13) |
| il7:1ω8*** | 4.35 (0.30) | 2.07 (0.16) | 3.37 (0.11) | 0.49 (0.06) |
| Cyclopropane | | | | |
| cy17:0*** | 5.00 (0.31) | 5.07 (0.17) | 5.24 (0.59) | 2.38 (0.18) |
| cy19:0*** | 12.10 (0.94) | 24.33 (3.51) | 9.68 (0.84) | 15.66 (3.44) |
| Saturated | | | | |
| 15:0*** | 18.73 (2.06) | 0.71 (0.51) | 14.71 (4.30) | 1.00 (0.57) |
| 17:0*** | 7.30 (0.99) | 2.53 (0.11) | 8.59 (0.76) | 9.10 (0.90) |

[a] Asterisks indicate significant differences between means by analysis of variance (*, $0.01 < P \leq 0.05$; **, $0.001 < P \leq 0.01$; ***, $P \leq 0.001$).
[b] Calculated from the total amount of the bacterial fatty acids.

TABLE 3. Significant difference map (Tukey's wholly significant difference test) of the bacterial fatty acids from Table 2[a]

| Fatty acid | Lowest | | Highest | |
|---|---|---|---|---|
| Branched | | | | |
| i15:0 | SUB(c) | SUB(s) | ROOT(c) | ROOT(s) |
| i16:0 | ROOT(s) | SUB(s) | ROOT(c) | SUB(c) |
| i17:0 | ROOT(s) | ROOT(c) | SUB(s) | SUB(c) |
| a17:0 | ROOT(s) | ROOT(c) | SUB(s) | SUB(c) |
| 10Me18:0 | ROOT(s) | ROOT(c) | SUB(s) | SUB(c) |
| Unsaturated | | | | |
| 18:1ω7*c* | SUB(c) | ROOT(c) | SUB(s) | ROOT(s) |
| i17:1ω8 | SUB(c) | SUB(s) | ROOT(c) | ROOT(s) |
| Cyclopropane | | | | |
| cy17:0 | SUB(c) | ROOT(s) | SUB(s) | ROOT(c) |
| cy19:0 | ROOT (c) | ROOT(s) | SUB(c) | SUB(s) |
| Saturated | | | | |
| 15:0 | SUB(s) | SUB(c) | ROOT(c) | ROOT(s) |
| 17:0 | SUB(s) | ROOT(s) | ROOT(c) | SUB(c) |

[a] Designations used: ROOT(s), cucumber roots grown in the suppressive medium; SUB(s), edaphic substrate from the suppressive medium; ROOT(c), cucumber roots grown in the conducive medium; SUB(c), edaphic substrate from the conducive medium. Treatments that are not joined by lines indicate significant ($P < 0.05$) differences, with the gradient from lowest to highest value indicated from left to right.

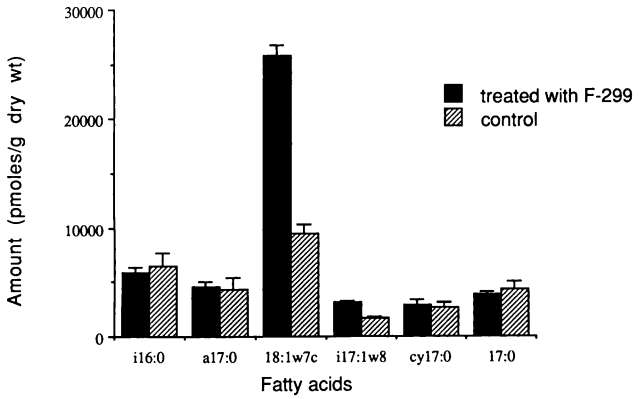


FIG. 2. Concentration of some bacterial fatty acids from the polar lipids of cucumber roots treated with *F. balustinum* 299 and grown in the conducive compost medium. Control plants were produced from seeds not treated with *F. balustinum* 299 and grown in the conducive compost. Vertical bars show 1 standard deviation ($n = 4$).

tion time of this compound to 19:0 was lower than predicted for a straight chain 2-OH 15:0, suggesting a branched 2-OH 15:0.

**Colonization of cucumber roots by *F. balustinum* 299.** Cucumber roots produced from seeds treated with *F. balustinum* 299 and grown in the conducive growth medium had a significantly higher concentration of i17:1ω8 than control samples, i.e., roots produced from seeds not treated with *F. balustinum* 299 (two-sided *t* test, $P < 0.001$) (Fig. 2). This change was concomitant with a significant increase in the concentration of 18:1ω7*c* (two-sided *t* test, $P < 0.001$) (Fig. 2). The shifts in the fatty acid profile from the samples treated with *F. balustinum* 299 also included a decreased proportion of i16:0 and 17:0 compared with the control samples (Table 5).

The peak for 2-OH Br15:0 from *F. balustinum* 299 coeluted with the peak for 16:1ω7*c* and could not be analyzed with the chromatographic system used.

## DISCUSSION

Examination of the bacterial fatty acid profiles indicated significant differences in the composition of the bacterial community in systems that are suppressive compared with those that are conducive to *Rhiozoctonia* damping-off. The suppressive environments had lower proportions of several methyl-branched fatty acids but higher proportions of *cis*-vaccenic acid and i17:1ω8 than the conducive environments.

Iso- and anteiso-branched fatty acids are common in the lipids of gram-positive bacteria but are rare in gram-negative bacteria (22). Tuberculostearic acid (10Me18:0) is characteristic for bacteria within the actinomycetes (25). Monounsaturated fatty acids with omega 7 *cis* unsaturation indicate the activity of the anaerobic desaturase pathway (42). This pathway is found in all strict anaerobic bacteria but is also common in most gram-negative aerobes. However, in general, gram-positive aerobes (including bacilli, micrococci, corynebacteria, and actinomycetes) utilize the $O_2$-dependent pathway for unsaturated fatty acid biosynthesis (15). This system generally produces unsaturation in the delta 9 position, but there are a great variety of unsaturated fatty acids synthesized within this group (15). The branched unsaturated fatty acid i17:1ω8 was the most prominent fatty acid identified in the polar lipids from *F. balustinum* 299. This species has been isolated from cucumber roots grown in suppressive bark compost medium (28). Iso-branched monoenoic fatty acids have so far only been identified in *Desulfovibrio* sp. and in certain *Pseudomonas* and *Flavobacterium* strains (5, 36, 37).

The above facts suggest that the bacterial community associated with cucumber roots and their surrounding media from the suppressive bark compost medium had lower proportions of gram-positive bacteria and actionomycetes but a higher proportion of certain gram-negative bacteria including *Flavobacterium* sp. than the bacterial community from the conducive medium. Although the relative population density of bacterial antagonists isolated from the suppressive bark compost medium has not been determined, gram-negative bacteria were the predominant biocontrol agents (28). Other studies have shown that the most common bacteria isolated from high-temperature composts are ther-

TABLE 4. Ester-linked fatty acids in the polar lipids of *F. balustinum* 299

| Fatty acid[a] | Proportion (mol%) |
|---|---|
| i15:0 | 26.8 |
| a15:0 | 6.1 |
| 15:0 | 0.5 |
| i16:1 | 0.9 |
| i16:0 | 3.4 |
| 2-OH Br15:0 | 14.6 |
| 16:0 | 2.2 |
| i17:1ω8 | 42.2 |
| a17:1 | 1.5 |
| i17:0 | 1.2 |
| a17:0 | 0.6 |

[a] Total amount was 42,300 pmol/mg (dry weight).

TABLE 5. Bacterial fatty acids from the polar lipids of cucumber roots produced from seeds treated with *F. balustinum* 299[a]

| Fatty acid[b] | mol% (mean and SD)[c] | |
|---|---|---|
| | Treated | Control[d] |
| Branched | | |
| i15:0 | 5.51 (0.41) | 6.69 (1.80) |
| i16:0** | 8.56 (0.07) | 12.85 (1.29) |
| i17:0* | 6.85 (0.23) | 8.96 (1.16) |
| a17:0 | 6.69 (0.16) | 8.52 (1.47) |
| 10Me18:0 | 1.18 (0.05) | 1.72 (0.56) |
| Unsaturated | | |
| 18:1ω7*c**** | 37.98 (0.80) | 18.96 (0.38) |
| 18:1ω7*t* | 0.42 (0.14) | 0.71 (0.07) |
| i17:1ω8*** | 4.59 (0.08) | 3.37 (0.11) |
| Cyclopropane | | |
| cy17:0 | 4.18 (0.54) | 5.24 (0.59) |
| cy19:0 | 8.22 (0.45) | 9.68 (0.84) |
| Unsaturated | | |
| 15:0 | 10.14 (0.43) | 14.71 (4.30) |
| 17:0** | 5.68 (0.33) | 8.59 (0.76) |

[a] The plants were grown in the conducive bark compost medium.

[b] Asterisks indicate significant differences between means of the treated sample and the control. Two-sided *t* tests. *, $0.01 < P \leq 0.05$; **, $0.001 < P \leq 0.01$; ***, $P \leq 0.001$.

[c] Calculated from the total amount of the bacterial fatty acids.

[d] Cucumber roots produced from seeds not treated with *F. balustinum* 299 and grown in the conducive medium.

mophilic *Bacillus* spp. (13, 46) and that the species composition varies with the composting temperature (47). The higher concentrations of the bacterial fatty acids on the roots compared with the surrounding substrates are probably due to the stimulation of bacterial growth by root exudates (3).

The community composition of the suppressive microbiota can be further characterized by the application of more extensive biochemical analysis. The proportion of gram-negative and gram-positive bacteria in the community can be estimated by analysis of cell wall amino acids (19). Gram-negative bacteria could be further characterized by their covalently bound lipopolysaccharide fatty acids (38), and gram-positive bacteria could be characterized by examination of their teichoic acid (17). Fungal biomass could be estimated by the analysis of specific sterols (45, 53). Furthermore, the nutritional status of bacteria in the rhizosphere can be examined by determining the ratio of the storage polymer poly-β-hydroxybutyrate to cellular biomass (49).

Treatment of cucumber seeds with the bacterial antagonist *F. balustinum* 299 induced a significant increase in the amount and proportion of i17:1ω8 in the polar lipids recovered from produced roots grown in the conducive medium. Previous studies with rifampin-resistant mutants have shown that *F. balustinum* 299 can colonize cucumber roots after seed treatment (28). However, the most noticeable change in the fatty acid profiles was the increase in the concentration and proportion of 18:1ω7*c*, which indicates an increased proportion of gram-negative bacteria. This shift parallels the change of the system from being conducive to become suppressive for *Rhizoctonia* damping-off (28). The data therefore suggest that seed treatment with *F. balustinum* 299 induced a change in the community structure of the rhizosphere microflora. This change might be important for the long-term induced suppression of *Rhizoctonia* damping-off; previous work has shown that the suppressive effect continues to last a long time after the population of an antibiotic-resistant mutant of *F. balustinum* 299 declines in the system (28). The specificity of *F. balustinum* 299 in inducing these shifts on the community composition compared with other gram-negative seed inoculants (28) is not known.

Suppression of certain plant diseases owing to the activity of microorganisms has been reported for various soils and composts (21, 44). An effort is being made to identify the microbes responsible for the suppressiveness and to introduce them as biological control agents in disease-conducive systems (16). However, in many cases, a sustained disease suppression is probably maintained by the activity of an assortment of microorganisms (21, 44). We think that analyses of phospholipid fatty acids as demonstrated in this report can provide a useful tool to study how biotic and abiotic factors affect the ecology of such antagonistic consortia of microorganisms. With this knowledge, it should be possible both to predict and to manipulate physical and chemical factors in soil to sustain a long-term soil suppression (W. Chen, H. A. J. Hoitink, and L. V. Madden, Phytopathology, in press).

## ACKNOWLEDGMENTS

This work was supported in part by grants from the Office of Naval Research (N00014-87-0012) (to D. C. White) and by grants from the Swedish Natural Science Research Council (B-Bu 8564-300) (to A. Tunlid). The VG MS was purchased by grants from the Department of Energy and the Army Research Office (to D. C. White).

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