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Diversity of root-associated bacteria associated with field-grown canola (*Brassica napus* L.) and wheat (*Triticum aestivum* L.)

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Abstract

Little is known about the composition and diversity of the bacterial community associated with plant roots. The purpose of this study was to investigate the diversity of bacteria associated with the roots of canola plants grown at three field locations in Saskatchewan, Canada. Over 300 rhizoplane and 220 endophytic bacteria were randomly selected from agar-solidified trypticase soy broth, and identified using fatty acid methyl ester (FAME) profiles. Based on FAME profiles, 18 bacterial genera were identified with a similarity index >0.3, but 73% of the identified isolates belonged to four genera: *Bacillus* (29%), *Flavobacterium* (12%), *Micrococcus* (20%) and *Rathayibacter* (12%). The endophytic community had a lower Shannon-Weaver diversity index (1.35) compared to the rhizoplane (2.15), and a higher proportion of *Bacillus, Flavobacterium, Micrococcus* and *Rathayibacter* genera compared to rhizoplane populations. Genera identified in the endophytic isolates were also found in the rhizoplane isolates. Furthermore, principal component analysis indicated three clusters of bacteria regardless of their site of origin, i.e., rhizoplane or endophytic. In addition, the rhizoplane communities of canola and wheat grown at the same site differed significantly. These results indicate that diverse groups of bacteria are associated with field-grown plants and that endophytes are a subset of the rhizoplane community.

Keywords: Rhizosphere; Biodiversity; Endophyte; *Brassica napus*; *Triticum aestivum*

1. Introduction

Microorganisms in the rhizosphere of plants dominate the decomposition processes in soil and the cycling of nutrients in soil plant systems. These microorganisms are important for long-term sustainability [1]. Plant-bacteria associations can help plants to become established in degraded landscapes, protect plants from disease, or may even promote plant growth [2]. Understanding the diversity of plant-bacteria associations is important if these associations are to be manipulated to increase crop production, conserve biodiversity and sustain agro-ecosystems. Lavelle et al. [3] noted that many key pedological processes such as soil organic matter turnover and the maintenance of the soil structure are determined by the nature and efficiency of mutualistic associations between micro- and macroorganisms. Associations between soil organisms can have a significant influence on plant growth. For example, Nehl et al.

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[4] postulated that the activity of deleterious rhizosphere bacteria is the result of ecological interactions occurring at the root surface and that in some systems a bacterium may be beneficial, yet in others detrimental to plant growth. Inoculating plants with selected bacteria is known to alter microbial communities associated with plant roots and thus the mutualistic associations associated with soil organic matter turnover. For example, Gilbert et al. [5] found that inoculating *Bacillus cereus* UW85n1 altered the composition and/or diversity of rhizosphere communities in three of four experiments with soybeans. Investigating the ecology of microorganisms associated with plant roots is important for understanding what impact new agricultural technologies will have upon soil ecology, nutrient transformations and plant succession.

In addition to bacteria present on the root surface (rhizoplane) and in the rhizosphere, there are significant numbers of bacteria present in the root interior [6]. These endophytic bacteria can induce systematic resistance to plant disease or promote plant growth [7]. However, the relationship between the bacterial communities associated with the root surface and root interior is not fully understood. Endophytes in corn can arise from both seed and soil [6], suggesting that endophytic bacterial species may be either a subset of that found on the root surface or a distinct group. In this study we assessed the diversity of rhizoplane and endophytic bacteria associated with canola plants grown at three different field sites. In addition, we compared the rhizoplane communities of canola and wheat at one site to ascertain if plant type significantly influenced community composition in the same soil.

2. Materials and methods

2.1. Isolation of bacteria

Bacteria were isolated from the rhizosphere of 100–115-day-old canola (*Brassica napus*, cv. Westar), growing at three fields, each located in Allan, Bellevue and Watrous municipalities, Saskatchewan, and wheat (*Triticum aestivum* L.) plants growing at the Watrous site only. Soil characteristics were determined by EnviroTest Laboratories (Saskatoon, SK, Canada) Table 1. At each field location ($n = 3$), three sampling points were chosen. At these three points, four plants and their associated root material were harvested by removing a 10-dm^3 soil core. All samples from each field location were kept in plastic bags at 0°C for 12 h until processed in the laboratory. Roots from the four plants were separated with a sterile scalpel from shoots, pooled together and divided into two subsamples: one for isolation of rhizoplane bacteria and the other for endophytic bacteria, i.e., bacteria colonizing the root interior [7]. To isolate rhizoplane bacteria, root material (ca. 30 g) was soaked in sterile phosphate buffered saline (PBS, 0.01 M phosphate, pH 7.3) for 10 min to equilibrate osmotic pressure, chopped into small pieces (3 cm), mixed well and serially diluted (1/10) in PBS. For endophytic bacteria, root samples were surface sterilized by soaking roots in 95% (v/v) ethanol for 1 min followed by a 1-min soak in 0.1% (w/v) acidified $HgCl_2$, and then washed 10 times with sterile tap water [8]. Root material was suspended 1/10 (w/v) in PBS, triturated with a sterile mortar and pestle, and serially diluted 1/10 in PBS. Aliquots (0.1 ml) of appropriate dilutions were spread plated onto 0.3% (w/v) trypticase soy broth-TSB (Difco Laboratories) solidified with 1.5% (w/v) agar for total bacteria counts. Four inoculated plates per dilution were incubated at 28°C and bacterial colony-forming units (cfu) counted after 24, 48, and 72 h of incubation. Root material was dried at 60°C for 48 h and cfu expressed per gram oven-dried root. Plates containing 30–300 colonies, i.e., typically the 10^{-2} endophytic and 10^{-5} rhizoplane dilutions, were selected and each bacterial colony numbered. A random number table was consulted and approximately 50% of the colonies from a plate were isolated. For canola there were 117 isolates (44 endophytic and 73 rhizoplane) recovered from the Bellevue site, 119 isolates (49 endophytic and 70 rhizoplane) recovered from the Allan site and 283 isolates (122 endophytic and 161 rhizoplane) obtained from the Watrous site. For wheat there were 159 isolates obtained from Watrous. Isolates were streaked twice on the original medium, checked for purity and purified strains stored in a 1:1 mixture of TSB and glycerol (v/v) at −80°C.

2.2. Bacterial identification

Isolates were identified based on whole-cell cellular fatty acids, derivatized to methyl esters, i.e., FAMEs and analyzed by gas chromatography (GC), using the MIDI system (Microbial Identification System, Inc., Newark, NJ, USA). Isolates were grown on solidified TSB plates at 28°C for 24 h and bacterial cells (ca. 50 mg) collected. 1 ml of a methanolic NaOH solution (15% [w/v] NaOH in 50% [v/v] methanol) was added and cells were saponified at 100°C for 30 min. Esterification of fatty acids was performed with 2 ml of 3.25 N HCl in 46% (v/v) methanol at 80°C for 10 min. The FAMEs were extracted into 1.25 ml of 1:1 (v/v) methyl-*tert*-butyl ether-hexane, and the organic extract washed with 3 ml of 1.2% (w/v) NaOH before analysis by GC. The gas chromatograph (Hewlett-Packard 5890A) was equipped with a flame ionization detector and a capillary column Ultra 2-Hewlett Packard No. 19091B-102 (cross-linked 5% phenyl-methyl silicone; 25 m, 0.22 mm ID; film thickness, 0.33 μm; phase ratio, 150) with nytrogen as the carrier gas. FAME peaks were automatically integrated by a Hewlett-Packard 7673 integrator and bacterial isolates named using the MIDI Microbial Identification Software (Sherlock TSBA Library version 3.80; Microbial ID, Inc.). The FAME profile of *Xanthomonas maltophilia* ATCC 13637 was used as a reference for the MIDI determinations. Strains with a similarity index (SIM) of less than 0.3 were considered not conclusively identified.

2.3. Statistical analyses

The rhizoplane and endophytic communities were compared utilizing the Shannon-Weaver diversity index (H′) [9]. The Shannon-Weaver index combines measurements of richness with those of evenness.

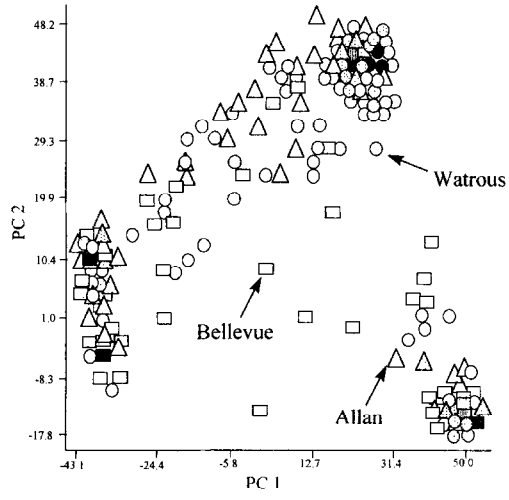

Fig. 1. 2-D plots of principal component analysis of FAME profiles of rhizoplane bacteria ($n = 302$) isolated from the roots of canola plants grown at three different field sites. Color of symbol indicates number of isolates: white = 1, 1 < gray < 8 and black > 8.

Therefore, we also compared the communities using Camargo's evenness index (E_{var}) [10]. We selected E_{var} on the basis of Smith and Wilson's [11] analysis of evenness indices in which they suggested that E_{var} was the best overall index. This index is an estimate of the variance in species abundance over the number of species, with 1 being the maximum evenness and 0 the minimum. A dendrogram analysis (centroid, single linkage) was used to differentiate at the genus level (Euclidean distance of 25) all of the rhizoplane and endophytic isolates. This approach allows the use of isolates regardless of their presence in the MIDI library.

Treatment comparisons of the number of genera isolated were made using the G test with William's correction [12]. The relationship between the number of identified genera and isolates was obtained by

Table 1
Selected characteristics of soils used in this study

Site	Soil type	Texture	pH[a]	Organic matter (%)	NO_3-N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})	SO_4^{2-} (kg ha^{-1})
Allan	Typic Kastanozem	Clay Loam	8.1	3.3	51	35	525	38
Bellevue	Chernozem	Loam	8.3	2.8	18	17	366	7
Watrous	Typic Kastanozem	Loam	7.5	4.5	11	28	573	64

[a]1:2 soil:water dilution.

randomly selecting increasing sample sizes from the results and determining the number of genera obtained [13]. This was repeated five times and the average relationship calculated utilizing CoSTAT's linearizable linear regression program. The principal component analysis (PCA) was performed by the statistical program packaged with the MIDI software.

3. Results

The sampling and isolation procedures on 1/10th strength TSA recovered approximately 10^7 cfu g^{-1} of oven-dried root for the rhizoplane samples and 10^4 cfu g^{-1} for endophytic samples with little difference ($< 10\%$) seen between sites. The MIDI system identified (SIM > 0.3) 41% (213 out of 522) of the bacteria isolated from the rhizoplane and root interior of canola and 45% (71 out of 159) of the bacteria isolated from the rhizoplane of wheat, for a total of

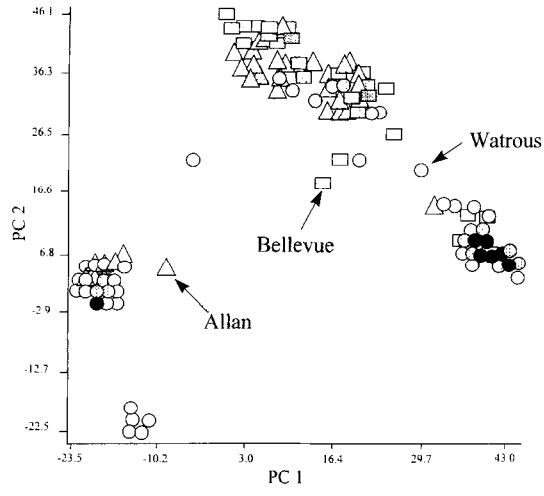

Fig. 2. 2-D plots of principal component analysis of FAME profiles of endophytic bacteria ($n = 220$) isolated from the roots of canola plants grown at three different field sites. Color of symbol indicates number of isolates: white = 1, 1 < gray < 8 and black > 8.

Table 2
Diversity of bacteria associated with canola and wheat

Genus	Number of isolates identified[a]		
	Canola		Wheat[b]
	Endophytic	Rhizoplane	Rhizoplane
Agrobacterium	0	0	2
Arthrobacter	3 (3)	2 (1)	4
Aureobacterium	0	4 (1)	3
Bacillus	49 (3)	13 (2)	45
Curtobacter	4 (2)	15 (3)	1
Enterobacter	0	4 (1)	0
Flavobacterium	22 (1)	4 (3)	3
Micrococcus	37 (2)	6 (2)	4
Microbacter	0	3 (1)	0
Pseudomonas	0	5 (3)	7
Psychrobacter	0	0	1
Rathayibacter	21 (3)	6 (2)	0
Staphylococcus	1 (1)	3 (2)	0
Variovorax	0	3 (2)	1
Others[c]	1	7	0
No library match	3	61	20
Unidentified[d]	79	166	68
Total	220	302	159

[a]Numbers in parentheses indicate the number of sites where bacterial genera were detected.
[b]For wheat, only the rhizoplane at the Watrous site was sampled.
[c]Others includes the genera: *Actinobacter*, *Clavibacterium*, *Comamonas*, *Corynebacterium*, *Nocardia* and *Xanthomonas*. These genera were only detected once or twice.
[d]Isolates named with a similarity index < 0.3.

20 different genera (Table 2). Furthermore, it assigned an additional 47% (245 out of 522) of the canola isolates and 43% (68 out of 159) of the wheat isolates a taxonomic name based on a similarity index of less than 0.3. The majority of them (62% or 189/313 isolates) were identified as belonging to the *Xanthomonas* genus. The MIDI system was significantly more successful in identifying endophytic compared to rhizoplane bacteria, with over 62% (138 out of 220) of endophytic isolates identified compared to only 25% (75 out of 302) of rhizoplane isolates.

The consistency with which rhizoplane genera were found at the different field sites was lower than that seen with endophytic genera. Fewer ($P < 0.25$) rhizoplane genera (17% or 3/18) compared to endophytic genera (38% or 3/8) were found at all three field sites. Furthermore, these genera were different between rhizoplane and endophytic communities. *Curtobacter, Flavobacterium* and *Pseudomonas* spp. were commonly found in the rhizoplane. In contrast, *Arthrobacter, Bacillus* and *Rathayibacter* spp. were frequently found in the endophytic community. In addition, more ($P < 0.15$) rhizoplane genera (56% or 10/18) compared to endophytic genera (25% or 2/8) were found at only one site. This suggests that the plant endophytic community is controlled to a greater degree by plant factors compared to the rhizoplane community.

No separation between rhizoplane isolates on the basis of field sites is apparent in PCA of FAME profiles (Fig. 1). There are approximately three groupings of rhizoplane bacteria regardless of field site, and no field site exerted a dominating influence on the bacterial diversity in the rhizoplane. Similarly, there are three groupings of endophytic bacteria (Fig. 2) which also did not cluster around field sites.

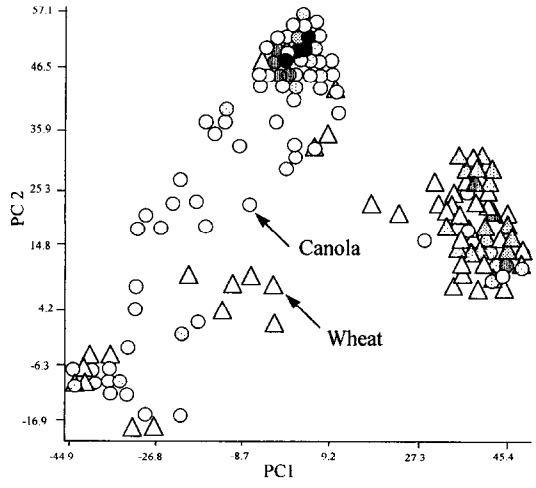

Fig. 3. 2-D plot of principal component analysis of FAME profiles of rhizoplane bacteria ($n = 461$) isolated from the roots of canola or wheat plants grown at the Watrous field site. Color of symbol indicates number of isolates: white = 1, 1 < gray < 8 and black > 8.

In addition to comparing different field sites, we also determined the diversity of rhizoplane bacteria associated with two plant species at the same field site (Fig. 3). Wheat and canola grown at the same field site had significantly different rhizoplane communities. Furthermore, there were significantly ($P < 0.001$) more bacilli in the wheat rhizoplane (28% or 45/159) compared to the canola rhizoplane (6.2% or 10/161). This preponderance of one genus is reflected in the E_{var} with the wheat rhizoplane having

Table 3
Comparison of the diversity and evenness of endophytic and rhizoplane communities associated with canola and rhizoplane communities associated with wheat

Index[a]	Canola[b]		Wheat
	Endophytic	Rhizoplane	
Shannon-Weaver H′	1.35 (0.16)	2.15 (0.26)	0.851
E_{var}	0.538 (0.07)	0.480 (0.08)	0.187

[a]The Shannon-Weaver index measures species diversity with increasing diversity reflected in larger values. The E_{var} index measures species evenness with a maximum of 1 and a minimum of 0.

[b]Standard error of the mean in parentheses. Wheat was only sampled at one site, and therefore the variation associated with the indices can not be estimated.

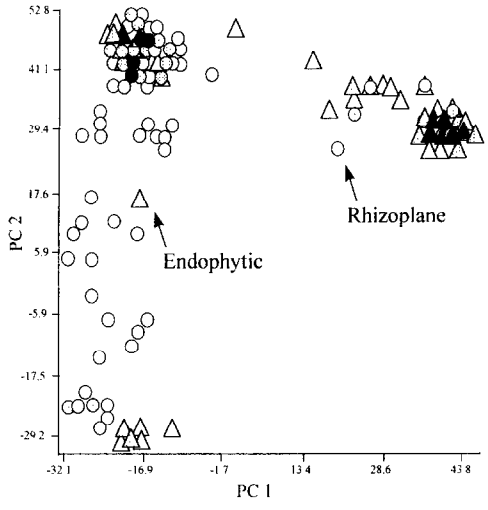

Fig. 4. 2-D plots of principal component analysis of FAME profiles of the rhizoplane and the endophytic bacteria ($n = 522$) colonizing canola roots. Color of symbol indicates number of isolates: white = 1, 1 < gray < 8 and black > 8.

an E_{var} of only 0.187 compared to 0.432 for the canola rhizoplane at the Watrous site (Table 3).

The composition of the canola endophytic population was markedly different than that of rhizoplane with the endophytic community dominated by Gram-positive genera and the rhizoplane approximately evenly split between Gram-positive and Gram-negative isolates (Table 2). The endophytic community was dominated by four genera: *Bacillus* (36% of identified isolates), *Micrococcus* (27%), *Flavobacterium* (16%) and *Rathayibacter* (15%), whereas the rhizoplane had no group of dominating bacteria. This becomes readily apparent when comparing the diversity and evenness of the two communities (Table 3). The endophytic community had lower species diversity ($P < 0.059$) compared to the rhizoplane community. However, despite these differences in community composition, no genera were detected in the root interior which were not present in the rhizoplane samples. Similarly, no separation between endophytic and rhizoplane bacteria were observed using PCA (Fig. 4). Surprisingly, no endophytic isolates were identified as pseudomonads and only 1.6% of the canola rhizoplane isolates were pseudomonads, whereas 4.4% of the wheat rhizoplane isolates were pseudomonads.

4. Discussion

Our results demonstrate that a diverse group of bacteria colonize the interior of canola roots. Few studies have investigated root-associated bacteria in canola, with potato and corn being the two crops most commonly investigated. Agarwhal and Shende [14] reported the presence of microorganisms inside the roots of *Brassica* species, but these bacteria were not isolated or identified. In our study, eight different genera were identified from 220 endophytic isolates of canola. In a recent study, McInroy and Kloepper [6] isolated 34 different bacterial genera in 1029 isolates from the root and stem interior of cotton and sweet corn. They postulated that cotton and sweet corn might have more diverse endophytic communities compared to other plants. In our study, the number of genera identified was related to the number of isolates by: #Genera = $-7.2+5.3 \times \ln$(#Isolates) with an $r^2 = 0.976$ and $P = 0.0016$. This suggests that only 30 genera would have been identified if a sample size similar to that of McInroy and Kloepper [6] had been used. In contrast, Lilley et al. [13] found 23 different genera in only 114 endophytic isolates of sugar beet (*Beta vulgaris*). Thus, it appears that the diversity of endophytic communities varies significantly between crop species.

To the best of our knowledge, the genus *Rathayibacter* has not been previously isolated from root interiors. In this study, *Rathayibacter* comprised approximately 10% of the isolates recovered from canola roots and was found at all three sampling sites. However, this may be because this genus is a new genus designed to accommodate Gram-positive, aerobic, coryneform bacteria previously placed in the genus *Clavibacter* [15] which has been previously identified in the root interior of cotton [16].

In our study the MIDI system identified only 60% of isolates from canola and wheat while McInroy and Kloepper [6] found that MIDI identified 95% of isolates and Lilley et al. [13] found that MIDI identified 80% of all isolates. These differences may be due to different SIM standards being used, 0.1 in [6], unknown in [13] and 0.3 in this study. If our study included those isolates identified with a SIM < 0.3, then the MIDI system identified 85% of isolates which is consistent with the other studies. However, isolates identified as a species with a

SIM < 0.3 indicates that these isolates are not present in the MIDI database and the indicated species is the most closely related species [20]. In contrast, isolates identified as a species with a SIM between 0.3 and 0.5 can be considered an atypical strain of that species whereas isolates identified with a SIM > 0.5 can be considered a good match [20].

The community composition of the rhizoplane of canola was not dominated by any one group of bacteria. Similarly, Lilley et al. [13] found that no single genus dominated sugar beet roots with *Bacillus* comprising 14% of isolates, *Arthrobacter* 12% and *Pseudomonas* 11%. In our study, we found that pseudomonads comprised a small proportion of the isolates obtained from canola (2.5%) and wheat (4%) roots. Thus, the widespread use of pseudomonads as crop inoculants might not be appropriate for all plant species.

The canola and wheat bacterial communities associated with roots were markedly different. The wheat rhizoplane was dominated by *Bacillus* (63% or 45/71 identified isolates), whereas the canola rhizoplane had a more even distribution. Similarly, Sato and Jiang [17] found that one genus, i.e., *Arthrobacter*, comprised 50% of the rhizoplane population of wheat. The differences between our study and theirs may be attributed to differences in plant growth conditions or isolation media. Our study investigated field-grown wheat and used 1/10 TSA as an isolation medium. In contrast, Sato and Jiang [17] investigated growth chamber grown wheat and albumin agar as an isolation medium. Differences in isolation media have been shown to influence the composition of the isolated community [18]. Our comparison between wheat and canola should be considered preliminary due to the differences in rhizoplane sample size with 302 isolates for canola and 159 for wheat. In addition, wheat was only sampled at one site whereas canola was sampled at three sites.

These results indicate that canola plants play a large role in controlling the diversity of root-associated bacteria. The three sites studied were located in two different soil climatic zones, yet bacterial diversity was not different between them. Hence, it appears that soil factors played a minor role in controlling the bacterial diversity in this study. Other investigators, however, have found that soil factors play a large role in determining the composition of fluorescent pseudomonad populations in the rhizosphere of flax (*Linum usitatissinum* L., cv. opaline) or tomato (*Lycopersicon esculentum* Mill. cv. H63-5) [19]. This suggests that the relative influence of soil and plant factors on bacterial diversity may be dependent upon the plant species being investigated. Therefore, while our results indicate that rhizoplane colonizers are largely affected by plant and not soil factors, further studies are necessary to determine if this is limited to canola or occurs for other plant species as well.

The results from this study suggest that the endophytic community is a subset of the rhizoplane community. Genera found in the rhizoplane community were present in the endophytic community and vice versa. Similarly, Lilley et al. [13] found that only two of 114 endophytic isolates belonged to genera (i.e., *Phyllobacterium rubiacearum* and *Brochothrix campestris*) not observed in the rhizosphere. Hence, we postulate that bacteria move from the root exterior to the interior, and that these processes to a large part are controlled by the plant. Supporting this, we found that differences in endophytic community composition between field sites were less than those seen for the rhizoplane community. Furthermore, endophytic communities between plant species vary widely (i.e., compare [13] and [6]). This suggests that plants play a dominating role in determining the composition of the endophytic community. Such additional selection pressure would explain the lower diversity and greater evenness observed in endophytic compared to rhizoplane communities observed in this study.

The importance of endophytic bacteria in promoting plant growth and combating plant diseases is well recognized [7]. The results from our study demonstrate that the plant exerts a dominating influence on the composition of the endophytic community and that this community is intimately related to the rhizoplane community. If the plant plays a dominating role in controlling the ecology of the root-associated microbial community, then this suggests that the appropriate plant growth-promoting bacterial inoculants may work in a variety of environments. Furthermore, practices that affect plant diversity may also alter plant-associated bacterial diversity. The long-term effects of this are currently unknown.

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References

[1] Ditchfield, J. (1993) We can't live without them – soil microorganisms. Global Biodiversity 3, 6–11.
[2] Glick, B.R. (1995) The enhancement of plant growth by free-living bacteria. Can. J. Microbiol. 41, 109–117.
[3] Lavelle, P., Lattaud, C., Trigo, D. and Barois, I. (1995) Mutualism and biodiversity in soils. In: The Significance and Regulation of Soil Biodiversity (Collins, H.P., Robertson, G.P. and Klug, M.J., Eds.), pp. 23–33. Kluwer, Dordrecht.
[4] Nehl, D.B., Allen, S.J. and Brown, J.F. (1997) Deleterious rhizosphere bacteria: an integrating perspective. Appl. Soil Ecol. 5, 1–20.
[5] Gilbert, G.S., Clayton, M.K., Handelsman, J. and Parke, J.L. (1996) Use of cluster and discriminant analyses to compare rhizosphere bacterial communities following biological perturbation. Microb. Ecol. 32, 123–147.
[6] McInroy, J.A. and Kloepper, J.W. (1995) Survey of indigenous bacterial endophytes from cotton and sweet corn. Plant Soil 173, 337–342.
[7] Chanway, C.P. (1996) Endophytes: they're not just fungi! Can. J. Bot. 74, 321–322.
[8] Somasegaran, P. and Hoben H.J. (1995) Handbook for Rhizobia: Methods in Legume-*Rhizobium* Technology. Springer-Verlag, New York.
[9] Ludwig, J.A. and Reynolds, J.F. (1988) Statistical Ecology: A Primer on Methods and Computing. John Wiley and Sons, New York.
[10] Camargo, J.A. (1993) Must dominance increase with the number of subordinate species in competitive interactions? J. Theor. Biol. 161, 537–542.
[11] Smith, B. and Wilson, J.B. (1996) A consumer's guide to evenness indices. Oikos 76, 70–82.
[12] Sokal, R.R. and Rohlf, F.J. (1995) Biometry: The Principles and Practice of Statistics in Biological Research, 3rd edn. W.H. Freeman and Company, New York.
[13] Lilley, A.K., Fry, J.C., Bailey, M.J. and Day, M.J. (1996) Comparison of aerobic heterotrophic taxa isolated from four root domains of mature sugar beet (*Beta vulgaris*). FEMS Microbiol. Ecol. 21, 231–242.
[14] Agarwhal, S. and Shende, S.T. (1987) Tetrazolium reducing microorganisms inside the root of *Brassica* species. Curr. Sci. 56, 187–188.
[15] Zgurskaya, H.I., Evtushenko, L.I., Akimov, V.N. and Kalakoutskii, L.V. (1993) *Rathayibacter* new genus, including the species *Rathayibacter rathayi* new combination, *Rathayibacter tritici* new combination, *Rathayibacter iranicus* new combination, and six strains from annual grasses. Int. J. Syst. Bacteriol. 43, 143–149.
[16] Misaghi, I.J. and Donndelinger, C.R. (1990) Endophytic bacteria in symptom-free cotton plants. Phytopathology 80, 808–811.
[17] Sato, K. and Jiang, H.-Y. (1996) Gram-positive bacterial flora on the root surface of wheat (*Triticum aestivum* L.) grown under different soil conditions. Biol. Fertil. Soils 23, 121–125.
[18] Germida, J.J. and Theoret, C. (1997) Do enumeration media affect estimates of bacterial diversity in soil? In: Annual Meeting of the Canadian Society of Microbiology, June 15–19, Quebec City, Canada, p. 65.
[19] Latour, X., Corberand, T., Laguerre, G., Allard, F. and Lemanceau, P. (1996) The composition of fluorescent pseudomonad populations associated with roots is influenced by plant and soil type. Appl. Environ. Microbiol. 62, 2449–2456.
[20] MIDI Inc. (1993) Microbial identification system: Operating Manual Version 4 using HP3365 ChemStation, pp. 7–8.