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Comparison of culturable Gram-negative bacterial community structures in the rhizosphere of three fruit plants

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Abstract: This research work was oriented to outlining the diversity of Gram-negative culturable portion of the bacterial community in three fruit plants rhizosphere. Rhizosphere samples were taken from European chestnut (*Castanea sativa* Mill), true service tree (*Sorbus domestica* L.) and cornelian cherry (*Cornus mas* L.) plants. Experiments were conducted for three years during the vegetation period, and the bacterial community structure was assessed with cultivation-dependent approach. Many Gram-negative isolates (n = 251) from the rhizosphere survived sub culturing and were identified by biochemical tests. A total of 57 species belonging to 29 genera were identified and assigned to four broad taxonomic groups (*Bacteroidetes*, *Alpha-*, *Beta-* and *Gamma-proteobacteria*). Several specific bacterial cluster communities were identified inside all the three rhizospheres. Most of the species belonged to the genera *Moraxella*, *Pseudomonas*, *Pantoea*, *Enterobacter* and *Acinetobacter*. In addition, while, using the plate count analysis, large discrepancies in numbers among physiological groups of bacteria cultured from three rhizosphere samples have not been revealed, more expressive distinctions among bacterial populations were obtained concerning the relative abundance of different genera, different taxonomic groups as well as different diversity indices. Furthermore, the number of cultured bacteria and their taxonomic distribution in the rhizosphere of all three plants changed not only explicitly during vegetation period but continually during the three years of investigation. It seems that rhizosphere bacterial populations of each plant are under the influence of the specific root-released materials.

Key words: Gram-negative bacteria; cultivation-dependent approach; bacterial diversity; root-borne bacterial community; fruit plants.

Introduction

One of the first steps in assessing an ecosystem is to describe the organisms inhabiting it. Different biotopes are characterized by bacterial assemblages with occurrence of the isolates harbouring different physiological properties. Plant roots influence the soil-borne microbial communities via several mechanisms, including excretion of specific organic compounds, competition for nutrients, and providing a solid surface for attachment. The nature of this influence is highly variable and depends upon both the amount and the composition of organic materials released by the plants (Griffiths et al., 1999). Since such root-released products can be highly specific for a given plant species or even a particular cultivar, plants are thought to selectively enrich their rhizospheres for microorganisms that are well adapted to utilization of specific organic compounds released (Lynch & Whipps, 1990; Bowen & Rovira, 1991). Since production of root-released materials can also vary during plant and root development (Swinnen et al., 1994), one might also expect microbial communities in the rhizosphere to be influenced by the developmental stage and age of a plant, as well as the location in particular parts of the root system. Thus, specific bacterial populations, including those that antagonize

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pathogen development, may be stimulated in the rhizosphere and be different in different plant species, genotypes (NEAL et al., 1970, 1973), plant developmental stages, or root parts – base versus tip (LILJEROTH et al., 1991). It should nevertheless be stated that either only limited information exist about bacterial structure of fruit plant rhizospheres or more often this information are quite omitted. It applies especially for the fruit plant species included into the group of the so called "neglected plant species". Fruit plant species in particular represent a special ecosystem influencing the microbial communities inhabiting it. There exist about twenty non-traditional, "neglected fruit plant species" in Slovakia, including European chestnut (*Castanea sativa* Mill), true service tree (*Sorbus domestica* L.) and cornelian cherry (*Cornus mas* L.).

The phytopathogenic microscopic fungi (*Phycomycetes*, *Ascomycetes*, *Basidiomycetes*, *Fungi imperfecti*) play an important role in the interactions among plants and microorganisms. They are potential agents causing different diseases of plants (JUHÁSOVÁ & HRUBÍK, 1984; JUHÁSOVÁ, 1999).

Information on interactions between rhizosphere of fruit plant species and bacterial assemblages are occurring only scarcely as well. The main aim of this work was therefore focused on characterizing the structure of bacterial communities occupying rhizosphere ecosystems of three fruit plant species, namely European chestnut, true service tree and cornelian cherry.

Material and methods

Rhizosphere samples

Bacterial strains were isolated from rhizospere of chestnut, service tree and cornelian cherry plants during the vegetation period (spring, summer, autumn) of three years. While the root samples of chestnut and service tree were taken from vineyards, rhizosphere of the cherry was sampled from a forest area. Both areas are located at the region of South-West Slovakia near the city of Nitra. Generally only tips of the roots taken from the studied plants were used in all experiments.

Preparation of root-borne bacterial suspension

A portion of 10 g (wet weight) of the root tips (mix of three samples from one tree) was mixed in a sterile 250 mL Erlenmeyer flask with 90 mL of 0.85 % (w/v) saline and incubated at 30 °C in a shaker incubator at 90 rpm for 2 h. The obtained suspensions were filtered through filter paper Whatman 1 (Merck, Germany) under sterile conditions, and pre-filtered bacterial suspensions were used for further work.

Plate count of physiological groups of bacteria

Colony forming units (CFU) of heterotrophs, sporulating, enteric, amylolytic and proteolytic bacteria, actinomyces and myxobacteria were determined by standard plate methods. The withdrawn sub-samples (1.0 mL) from root-borne bacterial suspension were serially diluted (in range: 10^{-1}–10^{-6}) and each dilution plated in triplicate on the appropriate solid agar medium as follows: heterotrophic vegetative and sporulating (differentiated by exposition in water bath at 80 °C for 20 min) colonies forming were counted on nutrient agar No. 2 (Biomark, India) after incubation at 22 °C for 72 h. To estimate the CFU of enteric bacteria, Endo-agar (Imuna, Slovakia) plates were inoculated and incubated at 37 °C for 24 h. The CFUs of amylolytic and proteolytic bacteria were counted on starch and milk agar respectively, both after incubation at 22 °C for 72 h. Actinomycetes colonies forming were counted on agar plates containing (per litre): 20.0 g soluble starch, 6.0 g KNO_3, 1.0 g K_2HPO_4, 20.0 g agar (pH 6.0±0.2) and incubated at 28 °C for 7 days. Myxobacteria were incubated at 28 °C for 28 days on Kopřivník champignon agar covered by filter paper (KOPŘIVNÍK & VYCHODILOVÁ, 1975), and only macroscopically different colonies forming were counted. CFU of individual physiological groups of bacteria inhabiting rhizosphere of three fruit plants were compared by one-way analysis of variance ($\alpha = 0.05$). Significant differences were determined by Tukey's test (Systat version 9.0).

Isolation of bacteria

The withdrawn sub-samples (1.0 mL) from root-borne bacterial suspension were serially diluted (in range: 10^{-1}–10^{-6}) and each dilution plated in triplicate on nutrient agar No. 2 (Biomark, India). Plates were incubated aerobically at 30 °C for 24–48 h and independently growing colonies were repeatedly inoculated by sterile bacteriological loops on new same medium. Several times pre-purified cultures were stained by Gram procedure and analysed by microscopy.

Biochemical identification of bacterial isolates

Gram-negative isolates were tested and characterized by oxi test (Oxoid, UK), TSI (Triple sugar iron agar, HiMedia, India) and OF basal medium (HiMedia, India) for basic differentiation of Gram-negative bacteria to fermentative (GNFR) or non-fermentative isolates (GNNFR).

For determination of *Enterobacteriaceae* and other fermentative Gram-negative rods the ENTEROtest 24 was applied.

In addition, NEFERMtest 24 was used for Gram-negative non-fermentative bacteria determinations. Besides, further 27 confirmation tests according to HOLMES et al. (1986) were used for the accurate non-fermentative bacteria determinations.

To confirm the correct bacterial isolate identification, control CCM strains (*Serratia marcescens* CCM 303, *Proteus vulgaris* CCM 1799, *Edwarsiella tarda* CCM 2238, *Citrobacter koseri* CCM 2535 for ENTEROtest 24, and *Pseudomonas aeruginosa* CCM 1960 and *Alcaligenes faecalis* CCM for NEFERMtest 24) were used.

Bacterial identification was achieved by referring to the Analytical Profile Index according to best likelihood and using the software TNW 0.5 from Czech Collection of Microorganisms (CCM, Brno, Czech Republic).

Calculation of diversity indices

Number and frequency of species isolated from rhizosphere samples of three plant species were used to estimate the diversity of the different communities. Species richness was calculated as the number of different species, and the percentage coverage was calculated as the percentage of isolates that appeared at least two times in the same bacterial community. The Shannon-Weiner diversity index (H) was calculated using the equation $H = -\Sigma p_i(\ln p_i)$, where p_i is the frequency of the i^{th} species. A higher H value indicates greater diversity. Equitability (J) was calculated as

$J = H(\ln S)^{-1}$, where S is the total number of taxa. Equitability values vary from 0 to 1 and reflect the ratio of the observed diversity (H) to the maximum diversity within a sample, where maximum diversity equals 1. Simpson's index $= 1 - \Sigma(p_i)^2$ and measures "evenness" of the community from 0 to 1. Sorensen's index (S) was calculated using the equation $S = S_{12}/[0.5(S_1 + S_2)]$, where S_{12} is the number of species common to both sites and S_i is the number of species found at site i.

Results and discussion

Natural and agricultural ecosystems harbour a wide variety of microorganisms that play an integral role in plant health, crop productivity, and preservation of multiple ecosystem functions. Particularly plant rhizospheres represent a special ecosystem influencing the microbial communities inhabiting it. To characterize the bacterial rhizosphere ecosystems of three fruit plant species, European chestnut, true service tree and cornelian cherry, the structure analysis and diversity of bacterial communities occupying these ecosystems was carried out during vegetation period of three years.

Plate count analysis of physiological groups of bacteria in the roots of three plant species

Plate count analysis did not reveal a large discrepancy in the number of physiological groups of bacteria that could be cultured from three rhizosphere samples (Fig. 1). According to Tukey's test, there were not significant differences among heterotrophs, sporulating, amylolytic and proteolytic bacteria, actinomycetes and myxobacteria occupying the three root plants. A significant difference was observed only in the plate counts of enteric bacteria in chestnut root compared with the plate counts of same group of bacteria in service tree (22–fold decrease) and cherry (53–fold decrease) roots.

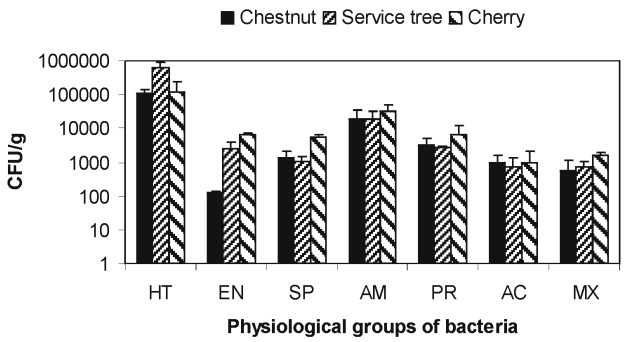

Fig. 1. Mean numbers of physiological groups of bacteria in the rhizosphere of three fruit plants. Data are presented as the average numbers of CFU per gram of dry weight of roots ($n = 9$) (with standard deviation). With the exception of enterobacteria, the histogram bars for the same physiological group of bacteria did not differ significantly (Tukey's test; $\alpha = 0.05$). Abbreviations: HT, heterotrophs; EN, enterobacteria; SP, sporulating bacteria; AM, amylolytic bacteria; PR, proteolytic bacteria; AC, actinomycetes; MX, myxobacteria.

That is, with exception of enterobacteria, the number of physiological groups of bacteria was not significantly affected by the rhizosphere of three different fruit plant species. However, plant roots influence the microbial communities in the rhizosphere via several mechanisms, such as the excretion of specific organic compounds, competition for nutrients, and providing a solid surface for attachment. The nature of this influence is highly variable and depends upon both the amount and the composition of organic materials released by the plants (Griffiths et al., 1999). With the exception of organotrophs, all investigated physiological groups of bacteria are specific for a given organic materials, such as cellulose, starch and proteins, which are widely considered to be of plant origin, but they do not represent specific substances. Thus, the number of physiological groups of bacteria and their distribution did not confirm whether the root-released products from chestnut, service tree and cherry enriched or suppressed their rhizospheres for bacterial growth.

Structure of cultured bacterial communities occupying roots of three plant species

Within culturable bacterial assemblages ($n = 299$), Gram-negative bacteria (84 %) predominated in the rhizosphere of all three fruit plant species, although Gram-positive bacteria, with cocci predominating, were also observed. Many Gram-negative isolates ($n = 251$) from the rhizosphere survived sub-culturing and were identified by biochemical tests. Due to the differences obtained for number of identified isolates (n) from rhizosphere of individual plant species, n differed for individual root-borne isolates. In total, 72 bacterial isolates were identified from rhizosphere of chestnut, 65 from service tree and 114 from root of cherry, respectively (Table 1). A total of 57 species belonging to 29 genera were identified from rhizosphere of three plants and assigned to four broad taxonomic groups (*Bacteroidetes, Alpha-*, *Beta-* and *Gamma-proteobacteria*). Furthermore, 45.6% of identified species assigned to the group of fermentative bacteria belong to the family *Enterobacteriaceae* which formed heterogenous cluster within *Buttiauxella*, *Cedecea*, *Edwardsiella*, *Enteric* group, *Enterobacter*, *Escherichia*, *Ewingella*, *Hafnia*, *Pantoea*, *Photorhabdus*, *Serratia*, *Xenorhabdus* and *Yersinia* species, and predominated (61.9%) within group of *Gammaproteobacteria*. The structure of cultured Gram-negative bacterial communities inhabiting rhizosphere of three fruit plants with *Gammaproteobacteria* predominating (72.1%) is not surprising. Low occurrence of *Alpha-* (11.5%) and *Beta-proteobacteria* (14.8%), and absence of *Deltaproteobacteria* is a common phenomenon in plated communities, probably because the members of these groups are generally slow-growing (Mitsui et al., 1997), or they may have specific physiological requirements and therefore may have been lost during the sub-culturing (Ellis et al., 2003).

The isolates from rhizosphere of chestnut were sub-

Table 1. Diversity of bacterial communities in rhizosphere samples of three plant species.

Plant species	No. of isolates[a]	% Coverage[b]	Richness[c]	Simpson[d]	Shannon[e]	Equitability[f]
European chestnut	72	82	26	0.93	2.94	0.90
True service tree	65	77	27	0.93	2.96	0.90
Cornelian cherry	114	82	41	0.95	3.29	0.89

[a] The number of individual bacterial isolates. [b] The percentage of isolates that were at least duplicated in the bacterial communities. [c] The number of different species. [d] The Simpson diversity index. [e] The Shannon-Weiner diversity index. [f] Equitability was calculated from the Shannon-Weiner diversity function.

divided into 26 species belonging to 18 genera; those of the service tree were differentiated into 27 species belonging to 19 genera; and those from cherry were differentiated into 41 species belonging to 22 genera, respectively (Table 1). While 6 bacterial species belonging to 6 genera (*Brevundimonas*, *Buttiauxella*, *Enterobacter*, *Escherichia*, *Photorhabdus*, *Pseudomonas*) have represented exclusively chestnut root-borne bacteria, 7 bacterial species belonging to 5 genera (*Bordetella*, *Cedecea*, *Edwardsiella*, *Oligella*, *Yersinia*) were unique for service tree rhizosphere and 21 bacterial species belonging to 13 genera (*Achromobacter*, *Acidovorax*, *Acinetobacter*, *Afipia*, *Enteric* group, *Enterobacter*, *Escherichia*, *Hafnia*, *Kingella*, *Moraxella*, *Pseudomonas*, *Serratia*, *Xenorhabdus*) were unique for rhizosphere of cherry bacterial community. In addition, 16 bacterial species belonging to 15 genera (*Acidovorax*, *Acinetobacter*, *Agrobacterium*, *Brevundimonas*, *Burkholderia*, *Buttiauxella*, *Eikenella*, *Enteric* group, *Enterobacter*, *Ewingella*, *Ochrobactrum*, *Pantoea*, *Pseudomonas*, *Serratia*, *Yersinia*) occupied two of three plant roots and 10 bacterial species belonging to 8 genera (*Achromobacter*, *Acinetobacter*, *Enterobacter*, *Moraxella*, *Myroides*, *Pantoea*, *Pseudomonas*, *Psychrobacter*) have represented bacterial communities inhabiting rhizosphere of all three plant species. Whereas some of isolates have been specific for a certain plant species, another has occurred in root bacterial communities either of two or even of all three plant species. It seems that inside of all three bacterial communities some specific bacterial clusters have been created. For the rhizosphere of chestnut, *Photorhabdus* spp. has appeared as a unique representative; the rhizosphere of service tree has been characterized by the representatives of bacteria clustered to the genera *Bordetella-Cedecea-Edwardsiella-Oligella*, and the rhizosphere of cherry was represented by the bacterial cluster of the genera *Afipia-Hafnia-Kingella-Xenorhabdus*. However, the majority of isolates were related to the most abundant bacterial genera associated with the rhizosphere either of two or of all three plants, such as *Moraxella*, *Pseudomonas*, *Pantoea*, *Enterobacter*, *Acinetobacter*, although there were observed differences in species distribution within these bacterial genera inhabiting individual plant roots. Furthermore, Sorensen's index showed relatively small similarity between rhizospheral bacterial communities of all three plants as follows: $S = 0.53$ for comparing chestnut and service tree, $S = 0.46$ for comparing chestnut and cherry, and $S = 0.45$ for comparing service tree and cherry. In fact, these results suggested discrepancies between structures of bacterial communities inhabiting the rhizosphere of the three plants and showed that the bacterial structure of cherry rhizosphere is the most differed, while the bacterial community compositions of chestnut and service tree root systems are more similar, but not significantly.

Besides other genera, representatives of the genus *Pseudomonas* were previously related to such bacterial genera which were associated with the rhizosphere (DUINEVELD et al., 2001). But there exists either only limited information or those about the interaction between fruit plant species and bacterial assemblages are quite omitted. More information exists about the interactions between plants and phytopathogenic microscopic fungi, probably because the bacterial diseases of plants are less prevalent than diseases caused by fungi and viruses (VIDAVER, 2002). From this point of view, the occurrence of some representatives of the genera *Pseudomonas*, *Agrobacterium* and *Burkholderia* in the rhizosphere of fruit plant species is interesting since some species of these genera are considered to be phytopathogenic bacteria. Mainly *Pseudomonas aeruginosa*, which occupied rhizosphere of cherry, is not only phytopatogenic but some strains of this species are assigned to the human pathogens. Another representative of this genus, *Pseudomonas syringae* pv. *syringae* strain FF5 is a phytopathogen associated with a rapid die off on ornamental pear trees (PENALOZA-VAZQUEZ et al., 2004). In addition, presence of *Escherichia coli* within chestnut rhizosphere microbial community and presence of several representatives of the genus *Enterobacter* in the roots of all three plant species also indicated possible occurrence of pathogens.

On the other hand, the rhizosphere microflora can be augmented with disease suppressive bacteria that are directly antagonistic to root pathogens (SANFORD, 1946; WEINDLING, 1946). In this manner, general disease suppression may involve competition by non-pathogenic bacteria and fungi that compete for the same growth substrates used by the pathogen. Specific bacterial strains and fungi may also promote stasis of the disease by creating an environ-

Table 2. Mean relative abundance distribution of bacterial genera within the collection of isolates (n=251) from rhizosphere samples of three plant species.

Taxonomic group	Bacterial genus	Mean abundance, % in plant species		
		European chestnut	True service tree	Cornelian cherry
Bacteroidetes		0.4	0.4	0.4
	Myroides spp.	0.4	0.4	0.4
Proteobacteria				
α-Proteobacteria		1.6	0.8	3.6
	Afipia spp.	0.0	0.0	2.4
	Agrobacterium spp.	0.8	0.0	0.4
	Brevundimonas spp.	0.8	0.4	0.4
	Ochrobactrum spp.	0.0	0.4	0.4
β-Proteobacteria		1.2	1.6	4.8
	Achromobacter spp.	0.4	0.4	0.8
	Acidovorax spp.	0.0	0.4	1.2
	Bordetella spp.	0.0	0.4	0.0
	Burkholderia spp.	0.4	0.0	0.8
	Eikenella spp.	0.4	0.0	1.2
	Kingella spp.	0.0	0.0	0.8
	Oligella spp.	0.0	0.4	0.0
γ-Proteobacteria		25.5	23.1	36.7
	Acinetobacter spp.	1.6	1.2	1.6
	Buttiauxella spp.	4.0	0.0	0.4
	Cedecea spp.	0.0	2.4	0.0
	Edwardsiella spp.	0.0	1.6	0.0
	Enteric group spp.	0.0	0.4	4.0
	Enterobacter spp.	2.0	2.0	1.6
	Escherichia spp.	0.4	0.0	0.4
	Ewingella spp.	0.4	0.8	0.0
	Hafnia spp.	0.0	0.0	1.2
	Moraxella spp.	7.1	7.1	12.3
	Pantoea spp.	2.8	2.0	0.8
	Photorhabdus spp.	0.4	0.0	0.0
	Pseudomonas spp.	5.2	2.4	11.2
	Psychrobacter spp.	0.4	0.4	0.4
	Serratia spp.	0.8	2.0	1.2
	Xenorhabdus spp.	0.0	0.0	1.6
	Yersinia spp.	0.4	0.8	0.0

ment that is hostile for growth and survival of the pathogenic bacteria. In addition, specific suppression has been shown for various strains of fluorescent pseudomonads that produce antibiotics (Cook et al., 1995; Raaijmakers & Weller, 2001; Landa et al., 2002) including *Pseudomonas cepacia* as well (Toyota et al., 1994). Naseby & Lynch (1999, 2001) illustrated the variability in efficacy of *Pseudomonas fluorescens* biocontrol agents with soil pH. Among the other bacteria which have been shown to be effective to control the root pathogens the representatives of the genus *Agrobacterium*, *Bacillus*, *Cytophaga*, *Enterobacter* and *Polyangium* belong, with certain *Bacillus* strains being the most effective (Cook et al., 1995; Hessenmueller & Zeller, 1996). It is suggested that the occurrence of some representatives of the genera *Agrobacterium*, *Enterobacter* and mainly fluorescent pseudomonads, such as *Pseudomonas fluorescens* which belong to the most abundant species within root-borne bacterial communities of all three plant species, and *Pseudomonas putida* inhabiting exclusively rhizosphere of chestnut, could participate on disease suppression.

Diversity of cultured bacteria in the roots of three plant species

Structural discrepancies in the rhizosphere bacterial communities of three fruit plant species suggested certain degree of diversity among them. The relative abundance of the bacterial genera and different taxonomic groups is shown in Table 2. It can be seen that there were large differences between rhizosphere samples in the relative abundance of the different genera. However, larger differences between rhizosphere bacterial populations were obtained in the relative abundance of the different taxonomic groups. These differences indicated the relationship to the rhizosphere of different fruit plant species. In particular, *Alpha-* and *Betaproteobacteria* (mainly *Afipia* spp. and *Kingella* spp., respectively) had a higher relative abundance in the cherry root system, whereas *Bacteroidetes* (*Myroides* spp.) and *Gammaproteobacteria* (mainly *Moraxella* spp., *Pseudomonas* spp., *Pantoea* spp., *Enterobacter* spp. and *Acinetobacter* spp.) increased in relative abundance in all three rhizosphere samples. The coverage of bacterial isolates in chestnut, service tree and cherry

Table 3. Diversity of bacterial communities in rhizosphere samples of three plant species during spring, summer and autumn of three years.

Plant species	Period	No. of isolates[a]	% Coverage[b]	Richness[c]	Simpson[d]	Shannon[e]	Equitability[f]
European chestnut	spring	34	85	11	0.85	2.11	0.88
	summer	23	57	12	0.81	2.06	0.83
	autumn	15	53	9	0.79	1.90	0.86
True service tree	spring	21	57	13	0.89	2.38	0.93
	summer	26	73	12	0.81	2.09	0.84
	autumn	18	67	11	0.89	2.29	0.96
Cornelian cherry	spring	48	77	18	0.88	2.47	0.85
	summer	35	77	16	0.88	2.46	0.89
	autumn	31	48	22	0.94	2.97	0.96

[a] The number of individual bacterial isolates. [b] The precentage of isolates that were at least duplicated in the bacterial communities. [c] The number of different species. [d] The Simpson diversity index. [e] The Shannon-Weiner diversity index. [f] Equitability was calculated from the Shannon-Weiner diversity function.

Table 4. Diversity of bacterial communities in rhizosphere samples of three plant species during first, second and third year of the vegetation period.

Plant species	Year	No. of isolates[a]	% Coverage[b]	Richness[c]	Simpson[d]	Shannon[e]	Equitability[f]
European chestnut	1st	35	66	17	0.89	2.51	0.89
	2nd	7	29	6	0.82	1.75	0.98
	3rd	30	83	11	0.86	2.14	0.89
True service tree	1st	10	70	8	0.68	1.36	0.85
	2nd	24	46	17	0.92	2.71	0.96
	3rd	31	65	15	0.84	2.28	0.84
Cornelian cherry	1st	54	81	21	0.90	2.69	0.88
	2nd	20	40	15	0.91	2.58	0.95
	3rd	40	68	18	0.86	2.38	0.82

[a] The number of individual bacterial isolates. [b] The percentage of isolates that were at least duplicated in the bacterial communities. [c] The number of different species. [d] The Simpson diversity index. [e] The Shannon-Weiner diversity index. [f] Equitability was calculated from the Shannon-Weiner diversity function.

rhizospheral environments was calculated to be 82%, 77% and 82%, respectively (Table 1).

Differences in diversity among the bacterial rhizosphere samples of investigated plant species were even more emphasized by diversity indices (Table 1). Bacterial community inhabiting cherry root systems suggested the highest level of diversity in almost all counts. Richness, the Shannon's and Simpson's diversity indices (which both emphasize phylotype richness but also measure structure), were all higher in root system of cherry than those calculated for the two remaining rhizosphere bacterial communities. These two communities, inhabiting rhizosphere of chestnut and service tree, showed very similar indices suggesting that their rhizospheral environment was nearly similar diverse. However, the equitability of all three rhizosphere bacterial communities was similar indicating that the frequency distribution in all three bacterial communities was similarly skewed and also diverse. Unlike physiological groups of bacteria, these results suggested the specific effect of root-released materials from individual plant species. It seems that while the cherry root enriched most the bacterial community inhabiting it, diversity of cultured bacteria in rhizosphere of chestnut and service tree was decreased by root-released materials and was more similar for both plant root systems (Tables 1,2). Thus, while some of released specific substances may be stimulating for a bacterial community, most phenolic substances of plant origin are toxic to microorganisms (MOHAN & MAHADEVAN, 2003), and the low tannin dose of chestnut significantly decreased bacteria count as well (SLIWINSKI et al., 2002). In addition, the ethyl acetate soluble fraction of the same tree was shown to have pronounced antibacterial effect against seven of the eight strains of Gram-positive and Gram-negative bacteria (SORBO et al., 2000).

Furthermore, the number of cultured bacteria and their taxonomic distribution in the rhizosphere of all three plants changed not only during vegetation period (Table 3) but within the time course as well (Table 4). Since production of root-released materials can also vary during plant and root development (SWINNEN et al., 1994), one might also expect microbial communities in the rhizosphere to be influenced by the developmental stage and age of a plant, as well as the location in particular parts of the root system. There were thus found more stable bacterial assemblages as well as bacterial populations which altered their taxonomic distribution in the rhizosphere of different plants.

In this work, we have tried to outline the diver-

sity of Gram-negative culturable portion of the bacterial community in the rhizosphere of three fruit plants. We were aware that the Gram-negative culturable bacteria present only a small part of bacterial assemblages inhabiting rhizosphere of plants, and therefore we cannot do any extensive conclusions. In addition, it should be noted that diversity indices used in this work do not take into account phylogenetic distances among the community members. This means that while the cherry rhizosphere-associated community appears to be the most diverse of those studied here, the observation that nearly all bacterial isolates belonged to a limited number of lineages in the *Proteobacteria* (Table 2) is not taken into account. Furthermore, we have used culture-dependent approaches to analyse the bacterial assemblages which are often criticized for their selectivity. The use of nucleic acid-based methods for estimating the community diversity is generally regarded as more appropriate in comparison with the classical culture-dependent methods (Ovreĺs & Torsvik, 1998), mainly for their greater complexity (Yang et al., 2001). However, the traditional microbiological methods possess the important characteristics that directly provide the live bacteria, and not only a "molecular strain". It appears that the culture-dependent technique may be a suitable method for determining the diversity of microbial communities in the rhizosphere of plants. The classical bacterial isolation can guarantee further studies able to properly characterize the rhizospheral bacterial structures and understand the interactions between bacterial community and rhizosphere of plants.

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