A comparison of sole carbon source utilization patterns and phospholipid fatty acid profiles to detect changes in the root microflora of hydroponically grown crops

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Abstract: Sole carbon source utilization (SCSU) patterns and phospholipid fatty acid (PLFA) profiles were compared with respect to their potential to characterize root-inhabiting microbial communities of hydroponically grown crops. Sweet pepper (*Capsicum annum* cv. Evident), lettuce (*Lactuca sativa* cv. Grand Rapids), and four different cultivars of tomato (*Lycopersicon esculentum* cvs. Gitana, Armada, Aromata, and Elin) were grown in 1-L black plastic beakers placed in a cultivation chamber with artificial light. In addition to the harvest of the plants after 6 weeks, plants of one tomato cultivar, cv. Gitana, were also harvested after 4 and 8 weeks. The cultivation in this study was performed twice. Principal component analysis was used to analyze the data. Both characterization methods had the ability to discriminate between the root microflora of different plant species, cultivars, and one tomato cultivar at different ages. Differences in both SCSU patterns and PLFA profiles were larger between plant species than between cultivars, but for both methods the largest differences were between the two cultivations. Still, the differences between treatments were always due to differences in the same PLFAs in both cultivations. This was not the case for the SCSU patterns when different plant ages were studied. Furthermore, PLFA profiles showed less variation between replicates than did SCSU patterns. This larger variation observed among the SCSU data indicates that PLFA may be more useful to detect changes in the root microflora of hydroponically grown crops than the SCSU technique.

Key words: sole carbon source utilization (SCSU) patterns, phospholipid fatty acid (PLFA) profiles, *Lycopersicon esculentum*, *Lactuca sativa*, *Capsicum annum*, indigenous microflora.

Résumé : Les potentiels des motifs d'utilisation de sources de carbone uniques (USCU) et des profils d'acides gras phospholopidiques (AGPL), afin de caractériser les communautés microbienne nichant dans les racines de plantations hydroponiques, en ont été comparés. Le poivron (*Capsicum annum* cv. Evident), la laitue (*Lactuca sativa* cv. Grand Rapids), et quatre différents cultivars de tomate (*Lycopersicon esculentum* cvs. Gitana, Armada, Aromata, et Elin) ont été cultivés dans des béchers noirs de 1 L en plastique placés dans une chambre de culture sous une lumière artificielle. Les plants ont été récoltés après 6 semaines. De plus, les plants d'un cultivar de tomates, cv. Gitana, ont également été récoltés après 4 et 8 semaines. La culture a été effectuée deux fois dans cette étude. Des analyses en composantes principales ont permis d'analyser les données. Les deux méthodes de caractérisation ont réussi à discriminer la microflore des racines de plusieurs espèces végétales, de cultivars, et d'un cultivar de tomates à différents âges. Les différences retrouvées dans les motifs d'USCU ainsi que dans les profils d'AGPL étaient plus importantes entre les espèces de plantes qu'entre les cultivars; mais pour les deux méthodes, les différences les plus appréciables s'observaient entre les deux cultures. Néanmoins, les différences entre les traitements étaient toujours attribuables à des différences à l'intérieur des mêmes AGPLs dans les deux cultures. Ce ne fut pas le cas pour les motifs d'USCU lorsque nous avons étudiés de plantes à différents âges. Du reste, les profils d'AGPL ont démontré moins de variations entre les réplicatats que les motifs d'USCU. Cette plus grande variation observée dans les données d'USCU indiquent que les AGPL pourraient se montrer plus utiles que la technique d'USCU en vue de détecter des changements dans la microflore des racines de plantations hydroponiques.

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Introduction

Cultivation of crops in closed hydroponic greenhouse systems may be used to minimize the environmental impact of plant production. The recirculation of the nutrient solution in such systems limits the release of nutrient-rich irrigation water from greenhouse crops and also allows for more efficient use of nutrients than in open cultivation systems (van Peer and Schipper 1988). In closed cultivation systems, however, the possible accumulation of organic compounds (Yu and Matsui 1993) and an increased risk of the spread of plant pathogens (Stanghellini and Rasmussen 1994; Vanachter et al. 1983) are matters of concern. Addressing these problems, several workers (McCullagh et al. 1996; van Peer and Schipper 1988; Waechter-Kristensen et al. 1994) have suggested that stabilization of the hydroponic system by biological means, using plant-growth-promoting rhizobacteria (PGPR), might be a fruitful approach. However, to use this efficiently it is important to be able to not only monitor the abundance of the added bacteria, but also follow the development of the total microbial community in the cultivation system. For example, in a study of the effect of adding a bacterium, antagonistic to plant pathogens, to a plant growth medium consisting of bark compost, it was found that the whole bacterial community was altered drastically, and that part of the antagonistic effect might be due to an altered bacterial community composition after the addition of the bacteria (Tunlid et al. 1989).

Monitoring entire microbial communities has traditionally been a very time consuming task, but the development of new, fast techniques that do not exactly give the species composition, but instead a fingerprint related to the microbial community structure, has made this type of study much easier. In the present study, two different methods for the characterization of microbial communities were compared to assess their suitability as tools to detect changes in the microbial community of roots of hydroponically grown plants. One of the methods is based on the determination of sole carbon source utilization (SCSU) patterns, an approach used to characterize the functional potential of microbial communities (Garland 1996*a*, 1996*b*; Waechter-Kristensen et al. 1998). The other method is based on the determination of phospholipid fatty acid (PLFA) profiles, which can provide information about the structure of the microbial communities (Federle 1986; Frostegård et al. 1993*a*; Vestal and White 1989; Waechter-Kristensen et al.1996).

Both these techniques have previously been shown to be applicable to hydroponic growth systems. For example, the rhizobacterial flora of hydroponically grown tomato showed differences in the PLFA profiles between roots treated with single- or multiple-strain inoculation of PGPR and untreated roots (Waechter-Kristensen et al. 1996). Furthermore, the study of the microbial communities from the rhizosphere of different plant species grown in hydroponic culture using SCSU patterns showed that this method is effective for detecting plant-dependent differences in rhizosphere communi-

ties and changes in response to plant developmental state (Garland 1996*a*).

The two characterization methods have previously been compared several times in different kinds of soil (Bååth et al. 1998; Ibekwe and Kennedy 1999). Although for the most part the two methods give similar results in differentiating between different soils or different treatments, the patterns from the SCSU technique often exhibit larger variations between replicates than the profiles from the PLFA technique, with consequently less power to detect significant differences, and thus the SCSU technique can be considered less sensitive than the PLFA technique. As an explanation for this difference, Bååth et al. (1998) suggested that only non-dormant, fast-growing copiotrophs will be able to contribute to the color development in wells of the SCSU plates and that this group of microorganisms is rare in soil. Thus, Buyer et al. (1999) suggested that the SCSU technique might perform better in studies of environments like the rhizosphere, where a larger part of the community is active than is the case in the bulk soil.

The objective of the present study was to compare the methods based on SCSU patterns and PLFA profiles to detect changes in the indigenous rhizosphere microflora of (*i*) different plant species (tomato, lettuce, and sweet pepper), (*ii*) different tomato cultivars (Gitana, Armada, Aromata, and Elin), and (*iii*) one tomato cultivar at different ages. The cultivation in this study was performed twice, because to use these techniques for larger scale monitoring the results must be reproducible. In this case it means not only that the techniques must differentiate between the different treatments (plant species, cultivars, or age) in the same way in both cultivations, but also that the differences between the two cultivations should not be too large. Furthermore, it is important that the differentiation between treatments is due to the same set of carbon sources or PLFAs each time because otherwise, one always has to include a standardized control treatment for comparison. This is not possible in most practical monitoring situations.

Materials and methods

Cultivation of plants

Seeds of tomato (*Lycopersicon esculentum* cvs. Gitana, Armada, Aromata, and Elin), lettuce (*Lactuca sativa* cv. Grand Rapids), and sweet pepper (*Capsicum annum* cv. Evident) were sown on filter paper wet with 15 mL of nutrient solution (in mM): 3.5 KNO_3, 4.0 $Ca(NO_3)_2 \cdot 4H_2O$, 1.2 KH_2PO_4, 0.6 $MgSO_4$, 0.5 $Mg(NO_3)_2$, 0.02 FeEDTA, 0.01 $MnSO_4 \cdot 2H_2O$, 0.005 $ZnSO_4 \cdot 7H_2O$, 0.025 H_3BO_3, 0.00075 $CuCl_2 \cdot 2H_2O$, and 0.0005 $NaMoO_4 \cdot 2H_2O$. The pH of the solution was 5.6 ± 0.1, and the electrical conductivity (EC) was 1.5 mS·cm^{-1}. The seeds were left to germinate for 5 days in the dark at 23°C. The resulting seedlings were transferred to black polyethene foam mats (PEN 334, Åkesson & Örbo, Mönsterås, Sweden) and placed in 1-L black plastic beakers at a density of 4 plants per unit. There were 6 replicates of each treatment. The containers were placed in a cultivation chamber with an artificial light at 21°C and 83% relative humidity during the first week. During the second week the temperature was changed to 20°C and the rel-

ative humidity to 80%. During the third week and thereafter, the relative humidity was changed to 75% and the temperature to 19°C during the day and 18°C during the night. This regime was maintained until the end of the cultivation period. During the whole cultivation period the plants had a photoperiod of 17 h per day at a light intensity of 210 µmol·m^{-2}·s^{-1}.

The nutrient solution was continuously aerated and changed twice a week during the first 2 weeks of the cultivation period and three times a week during the last 3 weeks. The pH value was kept the same during the whole cultivation period while the EC value was increased for each week by increasing the concentration of the mineral nutrients of the solution. The EC value was 1.5 mS·cm^{-1} at week one, 2.3 mS·cm^{-1} at week two, 2.4 mS·cm^{-1} at week three, 2.5 mS·cm^{-1} at week four, and 2.7 mS·cm^{-1} at week five. All the plants were harvested after 6 weeks, except for a number of plants of the tomato cultivar Gitana, which were also harvested after 4 and 8 weeks.

The experiment was repeated once with the same design (cultivations *a* and *b*).

Collection of root microflora

The separation of bacteria from root tissues was performed as described by Waechter-Kristensen et al. (1996). Fresh roots (20 g), including both young and old root tissues, were macerated, washed in 50 mL of detergent solution, and shaken at 200 rpm for 20 min. After filtration (70 µm), the suspensions were centrifuged at $1250 \times g$ for 50 min. The supernatants were discarded and the pellets were resuspended with 5 mL of Ringer solution (primary suspension) and centrifuged at $1250 \times g$ for 30 min. The samples were washed once again with 5 mL of Ringer solution (secondary suspension). Finally, the pellets were suspended in 5 mL of sterile water (Elga Maxima ultra pure water), and the bacterial samples were centrifuged again at $1250 \times g$ for 30 min. The pellets were lyophilized and kept frozen at –8°C until phospholipid fatty acid analysis.

Sole carbon source utilization (SCSU) patterns

The sole carbon source utilization patterns were studied as described by Waechter-Kristensen et al. (1998). In short, 0.5 mL of the secondary suspensions was resuspended in 0.85% NaCl and adjusted to OD$_{405}$ 0.100. The suspensions were then inoculated in the wells of GN microtiter plates (Microlog, GN-panel, Biolog, Haywood, Calif.). The plates were incubated at 25°C, and utilization of the single compounds was measured spectrophotometrically by recording the absorption at 405 nm on a computer-integrated microtiter plate reader (Asys, Linz, Austria; Digiwin software) after 4, 8, 18, 24, 36, 48, 72, and 96 h.

Phospholipid fatty acid (PLFA) profiles

The extraction method followed as described by Frostegård et al. (1991). Briefly, the collected samples of the root microflora were extracted with a one-phase mixture of chloroform, methanol, and citrate buffer (0.15 M, pH 4.0) in the proportions 1:2:0.8 v/v/v. After centrifugation, the pellet was washed once with the one-phase mixture, and the supernatants were combined and split into two phases by adding chloroform and citrate buffer. An aliquot of the lower, organic phase was dried under a gentle stream of nitrogen. The polar lipids were then isolated using silicic acid column chromatography. The fatty acids 13:0 and 19:0 were added as internal standards. The phospholipid fraction was subjected to a mild alkaline methanolysis, and the resulting fatty acid methyl esters were separated by a Hewlett Packard 5890 gas chromatograph (GC) equipped with a flame ionization detector. The column used was a 50-m HP5 capillary column (phenylmethyl silicone; Hewlett Packard Co., Palo Alto, Calif.). Hydrogen was used as a carrier gas, and injections were made in the splitless mode. The injector

temperature was 230°C and the detector temperature was 270°C. The temperature was initially held at 80°C (1 min), increased by 20°C/min to 160°C and then by 5°C/min to the final temperature of 270°C as described by Frostegård et al. (1993*b*).

Fatty acid nomenclature and determination of bacterial fatty acids

The fatty acids are written as (the total number of carbon atoms):(the number of double bonds), followed by the position of the double bond from the methyl end (ω) of the molecule. Anteiso- and iso-configurations are indicated by a and i, respectively, and cy refers to cyclopropane fatty acids.

PLFAs of axenic plants were determined to separate the bacterial PLFAs from the PLFAs found in roots. Seeds of tomato, sweet pepper, and lettuce were immersed in 10% H$_2$O$_2$ for 30 min and then washed with sterile, ultrapure water (Elgastat maxima, Elga Ltd.). The surface-sterilized seeds were placed on 10% Tryptic Soya Agar (TSA) for 1 week. The seedlings were then subjected to the phospholipid fatty acid analysis procedure (as described in the previous section). The following PLFAs were not found in roots and were considered as bacterial: i15:0, a15:0, 15:0, i16:0, 16:1ω9, 16:1ω5, 16:1ω7t, i17:0, a17:0, 17:1ω8, cy17:0, 17:0, 18:1ω7, and cy19:0.

Statistical analyses

Statistical analyses were conducted using the statistical program Minitab (version 10.2, 1994). Principal component analysis (PCA) was used to analyze the data of the SCSU patterns and PLFA profiles. For the SCSU, data from the 36-h reading were used in the PCA. The average well color development (AWCD) was calculated and both original data and data standardized by dividing by AWCD were used for the PCA. Individual PLFAs were converted to mole percent of the total PLFAs before being subjected to PCA. Both PLFA and SCSU were further normalized to unit variance. The PCA for SCSU resulted in a first principal component (PC1) that was strongly correlated with the AWCD (see Results). The second and third principal components (PC2 and PC3) were used to compare the effect of different treatments on the SCSU pattern without confounding with effect of different AWCDs. Thus, PC1 for PLFA profiles and PC2 for SCSU data reflected the different plant species or the age of the plants. The resulting principal components (PCs), PC1 and PC2 for the PLFA data and PC2 and PC3 for the SCSU data, were used in ANOVA with Tukey's LSD, with $P < 0.05$ considered significant, to determine statistically significant differences between treatments. The scores of PC1 from PLFA profiles were plotted against the scores of PC2 from SCSU patterns, and Spearman's correlation coefficient was calculated to study if the relative separation between plant species or plant age was similar for the two techniques.

Results

Sole carbon source utilization patterns

Average well color development (AWCD) differed strongly ($F = 164.0$, $P < 0.001$) between the two cultivations. To avoid including effects of different AWCDs in further calculations it is recommended that the data be standardized by dividing by the AWCD (Garland and Mills 1991). However, when doing this and then subjecting the data to a principal component analyses (PCA), a correlation between the scores of PC1 and AWCD was still found (data not shown). This confounded the effects of the different treatments on the SCSU patterns with the differences in AWCD. Thus, we chose not to standardize the data before the PCA. This resulted in a first principal component that was almost totally correlated

Fig. 1. Utilization pattern of sole carbon sources after 36 h of incubation by the root microflora of lettuce (L), sweet pepper (P), and tomato cvs. Gitana (G), Aromata (Aro), Armada (Arm), and Elin (E) using principal component analysis. Each value is the mean ± SE of six replicates. Solid and open circles indicate data from experiments *a* and *b*, respectively. PC2 and PC3 are the second and the third principal components, respectively, since PC1 only reflected differences in AWCD between the replicate cultivations.

Fig. 2. Utilization pattern of sole carbon sources after 36 h of incubation by the root microflora of 4-, 6-, and 8-week-old to-mato plants, cv. Gitana (G), using principal component analysis. Each value is the mean ± SE of six replicates. Solid and open circles indicate data from cultivations *a* and *b*, respectively. PC2 and PC3 are the second and third principal components, respectively, since PC1 only reflected differences in AWCD between the replicate cultivations.
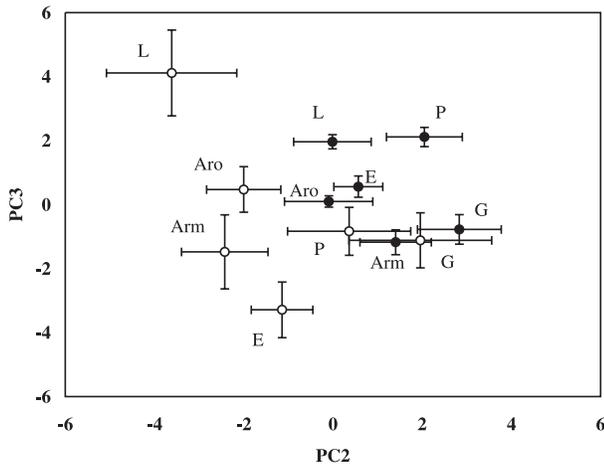
with the AWCD ($r^2 = 0.98$, $n = 70$ for the plant cultivar data), whereas no correlation with the AWCD was found for PC2 ($r^2 = 0.007$). This procedure thus resulted in variation due to the AWCD being removed by the first principal component, while changes in the SCSU pattern were found in PC2 and PC3. Because there were no correlations between AWCD and the different treatments, this procedure did not affect the separation of the treatments.

The scores of the different plant species and cultivars are given in Fig. 1. ANOVA of PC2 indicated significant differences between the cultivations ($F = 12.8$, $P < 0.001$), but also a significant plant effect ($F = 3.9$, $P < 0.01$), with no statistical interactions between these two variables ($F = 0.59$, $P = 0.71$). Scores from cultivation *a* were found to the right along PC2, and those from cultivation *b* to the left. Lettuce differed significantly from pepper and tomato cv. Gitana, and the latter differed from the three other tomato cultivars. The mean standard error from the ANOVA of PC2 was 0.81. Lettuce was the plant species differing the most from the others along PC3.

The interpretation of the SCSU data for the time series was more difficult (Fig. 2). Although significant differences between the utilization pattern of the root microflora from 4-week-old plants and older plants were found for both cultivations along PC2, the main variation was between 4-week-old plants from the two cultivations. This was indicated by a significant statistical interaction between cultivations and plant age ($F = 63.8$, $P < 0.001$). The explanation for this is that different carbon sources differentiated between the treatments (plant age) for the two cultivations. Thus, in cultivation *a*, the 4-week-old plants had a bacterial community characterized by relatively high values for gentibiose and lactulose, and relatively low values for L-histidine, L-pyroglutamic acid, and phenyl-ethylamine, whereas the op-

posite was the case for 4-week-old plants from cultivation *b*. This was also seen when separate PCAs were made for each cultivation, when different carbon sources were responsible for the main differentiation between plant age for the two cultivations (data not shown). The mean standard error in the ANOVA of scores from PC2 was 0.30.
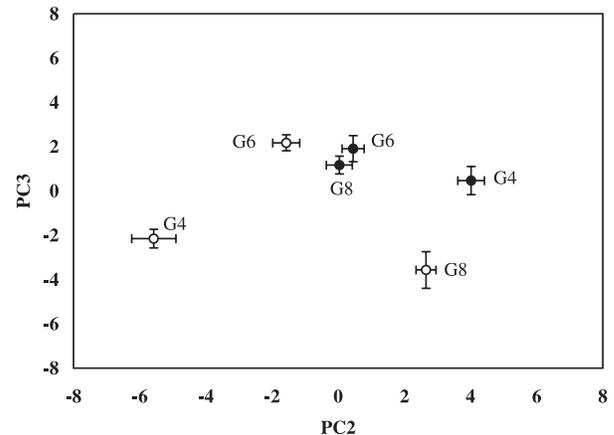
Phospholipid fatty acid profiles

When the PLFA data of the different plant species and cultivars were subjected to a principal component analysis, 29.9% of the total variation could be explained by PC1 and 17.8% by PC2 (Fig. 3A). ANOVA for PC1 indicated significant differences between the two cultivations ($F = 229.8$, $P < 0.001$) and also between the plant species and cultivars ($F = 30.4$, $P < 0.001$). However, contrary to what was found for the SCSU patterns, ANOVA revealed statistical interactions between these two variables ($F = 10.9$, $P < 0.001$).

The scores from cultivation *a* were found in the upper right of Fig. 3A, whereas those from cultivation *b* were found in the lower left of Fig. 3A. Lettuce differed significantly from sweet pepper and tomato, and sweet pepper showed some similarities with tomato. Similarities were also noticed between the tomato cultivars except cv. Aromata in cultivation *a* (Fig. 3A). The mean standard error in the ANOVA of scores from PC1 was 0.10. The loading plots of the individual PLFAs (Fig. 3B) showed that PC1 was characterized by a relatively high proportion of the PLFAs cy17:0 and cy19:0 and low proportion of the PLFAs i17:0, 16:1ω9, 18:1ω7, 16:1ω5, 15:0, and 17:0.

For the time series (Fig. 4A), PC1 accounted for 38.5% of the total variation and PC2 for 24.5%. ANOVA of PC1 showed significant differences between age of the plants ($F = 96.5$, $P < 0.001$). However, statistical interactions between the plant age and the two cultivation occasions were found ($F = 24.8$, $P < 0.001$). In both cultivations, differences in the PLFA profiles could be found between the 4-week-old

Fig. 3. (A) Principal component analysis of phospholipid fatty acid (PLFA) profiles of the rhizosphere microflora of 6-week-old hydroponically grown crops. Each value is the mean ± SE of six replicates. PC1 and PC2 are the first and second principal components, respectively. Abbreviations and symbols as in Fig. 1. (B) Loading plots for individual PLFA from the principal component analysis of data from both cultivations.
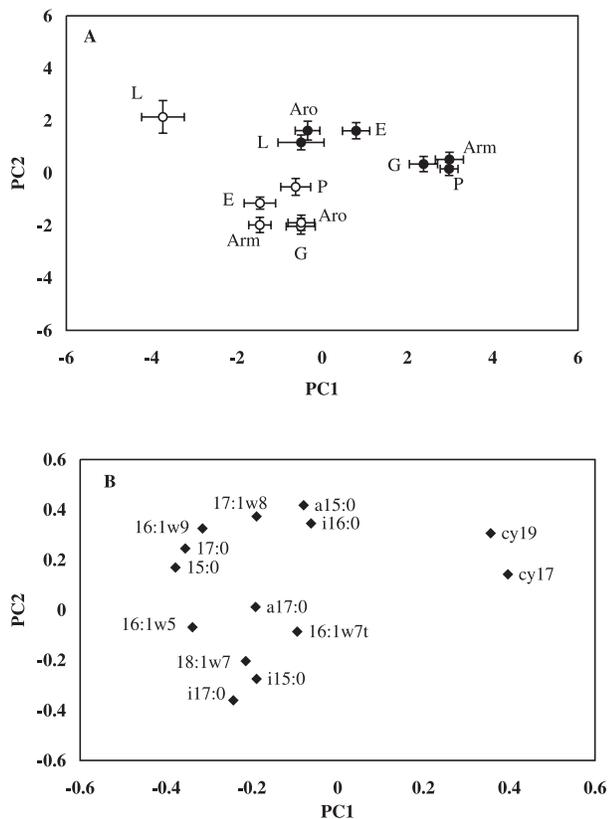
roots and the older roots and also between the 6-week-old and 8-week-old roots. The mean standard error from the ANOVA was 0.12. The PLFAs cy19:0 and 17:1ω8 showed relatively high proportions in PC1, while the PLFAs cy17:0 and 15:0 had lower proportions (Fig. 4B), indicating that the latter were more common in young roots.

To further study the similarities between the SCSU patterns and the PLFA profiles, we plotted the data of PC1 from the PLFA profiles against the data of PC2 from the SCSU patterns (Fig. 5). PC1 and PC2 were chosen because the main differences between treatments were found with these principal components. The results showed a significant linear relationship ($r^2 = 0.657$, $P = 0.02$) in the plot for the plant species and cultivars (Fig. 5A), indicating that the SCSU patterns and PLFA profiles of rhizosphere bacteria differentiated between the plant species and the tomato cultivars in a similar way. On the other hand, no significant relationship ($r^2 = 0.086$, $P = 0.87$) could be found in the plot for the tomato cultivar Gitana at different ages (Fig. 5B), indicating that the SCSU patterns and PLFA profiles differentiated between the root microflora at different ages in different ways.

Discussion

In general, the size and composition of the rhizosphere microbial community are plant dependent, since the root exudates will have a selective stimulatory effect on microorganisms, which in turn will affect the community composition (Rovira 1965; Curl and Truelove 1986; Bowen and Rovira 1991; Garland 1996*a*; Grayston and Campell 1996; Grayston et al. 1998; Ibekwe and Kennedy 1998).

In the present study, it was shown that both sole carbon source utilization (SCSU) patterns and phospholipid fatty acid (PLFA) profiles can be used to indicate differences in the rhizosphere microbial community due to both plant species and cultivars of hydroponically grown crops (Figs. 1 and 3). Furthermore, both methods indicated a similar order of differences between the different treatments, indicated by a significant correlation between the scores from the PCAs (Fig. 5A). The PLFA profile is a measure of the presence of all organisms and species composition (often called community structure), whereas the SCSU theoretically is a measure of the degrading capacity of the community (often called functional potential). However, it is not surprising that the two techniques give similar results because the degrading capacity of a community of bacteria to a certain extent has to reflect the species composition. It has also been found that the two techniques differentiate between microbial communities in a similar way in soil affected by different metals (Bååth et al. 1998). Less evident is that a technique reflecting all cells (PLFA) will correlate with a technique reflecting only a minor part of the community (fast-growing cells, capable of growth under the conditions of SCSU determination). The fact that no correlation between PLFA and SCSU was found in the time series (Fig. 5B) might indicate that this will not always be the case.

The reason for selecting both different plant species and different cultivars within one species was that we expected that there would be larger differences between species than between cultivars. This was also the case for both methods, where lettuce and pepper differed most, especially using the PLFA measurements (Fig. 5A).

To compare and evaluate the two methods, however, it is not enough to state that they gave similar and expected results. The sensitivity is also of importance. Like earlier studies (Bååth et al. 1998; Ibekwe and Kennedy 1998), we found that the SCSU technique had higher variation between replicates (mean standard error from PC2 is 0.81 and 0.30 for plant species and time series, respectively) than the PLFA technique (mean standard error from PC1 is 0.10 and 0.12 for plant species and time series, respectively). The lower variation for the latter technique meant not only higher significant values for differences between treatments, but also significant interactions between replicate cultivations and plant species. Thus, the PLFA technique was sensitive enough to detect that the two presumably identical cultivations did not give exactly the same results, whereas this was not the case using the SCSU technique.

To be able to use these techniques as monitoring tools, it is important not only that there are low variations within replicate treatments, but also that there are not large variations between replicate cultivations. If this is not the case, one would always have to include a control or a standard treatment as a comparison, which would be impossible under

Fig. 4. (A) Principal component analysis of the PLFA profiles of the rhizosphere microflora of 4-, 6-, and 8-week-old hydroponically grown plants of the tomato cv. Gitana (G). Each value is the mean ± SE of six replicates. PC1 and PC2 are the first and the second principal components, respectively. Symbols as in Fig. 2. (B) Loading plots for individual PLFA from the principal component analysis of data from both cultivations.

Fig. 5. (A) Correlation between scores from the first principal component (PC1) from the PLFA data and the second principal component (PC2), reflecting differences between treatments, from the SCSU data of the rhizosphere microflora of hydroponically grown tomato cvs. Gitana, Aromata, Armada, and Elin, sweet pepper, and lettuce (*n* = 6). Symbols and abbreviations as in Fig. 1. (B) Correlation between scores from the first principal component (PC1) from the PLFA data and the second principal component (PC2) from the SCSU data of the rhizosphere microflora of 4-, 6-, and 8-week-old hydroponically grown tomato cv. Gitana (*n* = 6). Symbols as in Fig. 2.

commercial growing conditions. However, with both techniques the largest differences were between the cultivations and not between plant species (Fig. 5A). Since the experiment was replicated using the same growth conditions and still showed such different rhizosphere communities, as judged from the two techniques used, it is unlikely that they could be easily applied as a standard monitoring tool.

Even more important is that the differences between treatments are due to the same set of individual PLFAs or carbon sources for the SCSU techniques in both cultivations. This was also the case in the experiment with different plant species for both techniques, but not when different ages of a tomato cultivar were studied using the SCSU technique. For example, in both cultivations of the plant species experiment the rhizosphere bacteria of lettuce were characterized by a lower proportion of the PLFAs cy17:0 and cy19:0 compared with pepper (Fig. 3B), whereas for the cultivation of the time series the 4-week-old tomato plants had proportionally more cy17:0 but less cy19:0 than older plants in both cultivations (Fig. 4B). However, using the SCSU technique on different ages of plants resulted in a separation between the rhizobacterial community of 4-, 6-, and 8-week-old plants for both cultivations (Fig. 2), but the largest differences were between the 4-week-old plants of the cultivations. This shows that the separation between treatments was due to dif-

ferent sets of carbon sources for the two cultivations, and that one would not be able to use the SCSU pattern to directly indicate the treatment. This is a major disadvantage because if this also holds, for example, in a situation with added rhizobacteria, one would not be able to judge directly from the SCSU pattern whether this has affected the rhizosphere community without having a control without the added bacteria.

Although the bacterial inoculum used for the SCSU technique was standardized to the same OD, there were large differences in AWCD, especially between the different replicate cultivations. This indicates that standardization using OD is not optimal. Garland and Lehman (1999) stated that the ideal enumeration technique would account for only those organisms which respond in the Biolog plates, and plate counts would probably seem a more appropriate enumeration technique compared with total microscopical counts. In our case, OD readings might be more similar to total counts than to plate counts. However, it will be difficult to use plate counts as a standardization technique in a monitor-

ing situation, since this involves several days of incubation of the plates.

In conclusion, the results demonstrated that both SCSU patterns and PLFA profiles are useful techniques to detect changes in the microbial community of hydroponically grown crops. Both methods were effective in discriminating between the root microflora of different plant species, cultivars, and cultivars at different ages of the plants. The PLFA profiles were more sensitive in detecting changes than the SCSU patterns and showed better reproducibility, in that the treatment differentiation was determined by the same variables in the two identical cultivations performed. This suggests that PLFA profiles are a better tool to study the microbial dynamics in hydroponic systems, but the SCSU technique can still provide useful information in support of other assays. However, differences between the two presumably identical cultivations raise questions about the reproducibility of the microbial communities in hydroponic systems, and thus about the possibility of using either of the methods as monitoring tools. This might affect SCSU patterns more than PLFA profiles, since PLFA measures the entire community, whereas SCSU patterns measure only a fraction of the community.

Acknowledgements

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