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Dynamics of the indigenous microflora inhabiting the root zone and the nutrient solution of tomato in a commercial closed greenhouse system

Dynamik der Mikroflora an Tomatenwurzeln und in Nährlösung in einem geschlossenen Anbausystem mit Torf oder Steinwolle

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Summary

In a commercial closed hydroponic greenhouse system, the microflora inhabiting the root and the nutrient solution of peat and rockwool grown tomato were studied on the basis of viable count (colony forming units, CFU), functional diversity using sole carbon source utilization (SCSU) patterns and taxonomic diversity using phospholipid fatty acid (PLFA) profiles. Sampling was done during one growing season, from May to October. Viable count of bacteria inhabiting the roots showed that the number of general bacterial flora and of fluorescent pseudomonads was higher in rockwool than peat while the amount of general fungal microflora, actinomycetes and *Trichoderma* was higher in peat than in rockwool grown tomato plants. For the irrigation effluent, no significant differences were found with respect to the general bacterial flora. The number of fluorescent pseudomonads decreased during the observation period whereas the number of actinomycetes rose towards the end of the experiment. For the general fungal flora and *Trichoderma*, viable counts showed significantly lower CFU for the samplings in August and September compared with the other sampling incidents. Furthermore, SCSU patterns and PLFA profiles showed differences in the root microflora with respect to growing medium and to sampling incidents. A separation of the microflora inhabiting the nutrient solution between the sampling incidents could also be done. This indicates that all three methods showed that the composition and the function of the microflora inhabiting the root and the nutrient solution shifted during the growing season and were different with respect to the growing medium.

Zusammenfassung

In einem erwerbsmässigem Gewächshausbetrieb, in dem Tomaten in einem geschlossenen System mit Torf bzw. Steinwolle angebaut wurden, wurde die Dynamik der Mikroflora in der Wurzelzone und in der Nährlösung während der Vegetationsperiode untersucht. Die Veränderungen innerhalb der mikrobiellen Gesellschaften wurde durch Lebendzellzahlen auf semi-selektiven Nährböden, funktionelle Diversität anhand von Verwendungsmustern einzelner Kohlenstoffquellen (SCSU; Microlog GN-Panel) und taxonomische Diversität anhand von Phospholipidfettsäureprofilen (PLFA) charakterisiert. Der Untersuchungszeitraum erstreckte sich von Mai bis Oktober. In Bezug auf die allgemeine Bakterienflora und auf fluoreszierende Pseudomonaden waren die Lebendzellzahlen der Wurzelmikroflora höher in der Steinwollkultur als in der Torfkultur, während höhere Lebendzellzahlen für Aktinomyzeten, die allgemeine Pilzflora und *Trichoderma* in der Torfkultur festgestellt wurden. Keine Unterschiede konnten bezüglich der Lebendzellzahlen in der Nährlösung von Torf- und Steinwollbeeten gefunden werden. Die allgemeine Bakterienflora lag während des ganzen Untersuchungszeitraums auf dem gleichen Niveau. Die Zahl fluoreszierender Pseudomonaden nahm zum Ende des Untersuchungszeitraumes ab, während die der Aktinomyzeten anstieg. Sowohl SCSU als auch PLFA zeigten Unterschiede in der Zusammensetzung der Wurzelmikroflora in Hinblick auf das verwendete Pflanzsubstrat und auf den Probenentnahmezeitpunkt. Die mikrobielle Zusammensetzung der Nährlösung veränderte sich während der Vegetationsperiode in Hinblick auf funktionelle und taxonomische Diversität. Die Ergebnisse aller drei Methoden weisen auf jahreszeitlich- bzw. substratbedingte Veränderungen in der Zusammensetzung mikrobieller Gesellschaften in der Wurzelzone und der Nährlösung in geschlossenen Kulturen hin.

Introduction

Plant production in closed hydroponic greenhouse systems is considered to be environmentally friendly. The reuse of the irrigation effluent allows a more efficient use of nutrients compared to run-to-waste systems (Vanachter et al. 1983). However, an increased risk for spread of plant pathogens (Stanghellini and Rasmussen 1994) and accumulation of organic compounds (Yu and Matsui 1993) are sources of concern that have led to a certain reluctancy to use these systems on a broad commercial scale. A number of studies, however, indicate that disease suppressiveness towards major plant pathogens, such as *Pythium aphanidermatum* and *Phytophthora* spp. may establish in closed hydroponic systems (McPherson et al. 1995, Postma et al. 2000a, Stanghellini and Miller 1997). Furthermore, inocula-

tion of microorganisms stabilizing the root environment or nutrient solution may protect crops towards root disease (Hultberg et al. 2000, McCullagh et al. 1996, Paulitz 1997), phytotoxic effects (Caspersen et al. 2000) or have a positive growth effect on the crop (Alsanius and Gertsson 1998).

For an effective future commercial use of biological control agents, bca, in closed hydroponics, a deeper understanding of the composition and dynamics of the microflora in these systems is needed to enable to synchronize addition of bca with the status of the crop. Characterization of sole carbon source utilization (SCSU) patterns and phospholipid fatty acid (PLFA) profiles are increasely common in studies of microbial communities. These methods have earlier been assessed as tools to study the root microflora of hydroponically grown crops (Khalil et al. 2001). The SCSU patterns provide information about the function of the microbial community and are based on utilization of a suite of 95 different carbon sources where the utilization of each substrate is detected by the reduction of a tetrazolium dye, which results in a color change that can be quantified spectrophotometrically. PLFA profiles, on the other hand, provide more information about the community structure. Different subsets of a microbial community contain different compositions of PLFAs in their membranes (Tunlid and White 1992). The presence of such "signature" PLFAs enables the estimation of abundance of such subsets within the community. For example, bacteria contain certain PLFAs which do not exist in fungi and the actinomycetes have specific PLFAs (O´Leary and Wilkinson 1988) which do not exist in either fungi or bacteria. Within the bacteria groups, there are some PLFAs which are specific for the Gram-positive bacteria (O´Leary and Wilkinson 1988) and the Gram-negative ones (Wilkinson 1988).

In previous studies, the microbial status of the roots of five-week-old hydroponically grown tomato plants under controlled conditions as evaluated by SCSU patterns and PLFA profiles, were found to differ depending on the introduction of bacterial strains (Alsanius et al. 2001b) or of the pathogen *Pythium ultimum* (Khalil et al. 2000). Moreover, studies of the microbial status of older roots grown in closed greenhouse system also showed differences in the PLFA profiles due the bacterization treatment (Waechter-Kristensen et al. 1996).

The objective of the present study was to follow the development of the microbial community inhabiting the root zone and the nutrient solution in a commercial hydroponic greenhouse system using SCSU, PLFA and viable count in order to obtain an increased understanding of the dynamic of the microflora in such systems and to compare these results with the results of previous experiments performed under conditions of a growing chamber and a small scale closed greenhouse system.

Materials and Methods

Sample collection

Nutrient solution and plant roots were sampled from a commercial closed hydroponic greenhouse system (7800 m^2) with integrated slow filter, nearby Ystad, Scania in Southern Sweden (Ingeltorps trädgårdar; Johnny Nilsson) growing tomato (cv. Aromata) from January until the end of October. The irrigation effluent was reused from the start of May to the end of the growing season. The greenhouse complex was divided into 4 major units. In unit 1, peat was used as the growing medium and each tomato plant was cultivated in a 10 L bucket with individual drop irrigation. In units 2 to 4, tomato were grown in conventional rockwool slabs and supplied with nutrient solution. Plant density was 2–2.5 plants/m^{-2} in the whole greenhouse system. The effluents from the peat buckets and rockwool slabs were collected in a covered gutter between the rows and transported to a well by means of gravity. Each greenhouse unit was equipped with an individual well, each storing 100 L. Samples (5 L) were taken from the irrigation effluent of each greenhouse unit before it was released to the reservoirs and transported chilled to the laboratory. Root samples were collected from both rockwool and peat grown tomato plants at marked sampling sites with 4 replicates. Root samples and the nutrient solution were collected at five different occasions, from May until October 1999, with one sampling occasion each 6th week (sampling 1 in May, sampling 2 in June, sampling 3 in August, sampling 4 in September and sampling 5 in October).

Collection of root microflora

The separation of bacteria from root tissues was performed as described by Alsanius et al. (2000b). The root tissues (25 g fresh weight) were macerated in 50 ml of detergent solution and shaken at 200 rpm for 20 minutes. After filtration (70 µm), the suspensions were centrifuged at 1250 G for 50 minutes and resuspended with 5 ml of Ringer solution. The supernatants were discarded and the pellets were resuspended (primary suspension) and transferred to 15 ml test tubes. The samples were centrifuged for 30 minutes and washed once again with 5 ml of Ringer solution (secondary suspension). In the final step, the pellets were resuspended in 5 ml of sterile water (Elga Maxima ultra pure water) and centrifuged 30 minutes. Thereafter, the pellets were lyophilized and kept frozen at –80 °C until phospholipid fatty acids analysis.

Plate counts of microbial populations

For the preparation of the dilution series, 1 ml of the primary suspension from the collected samples of the root microflora and 1 ml of the nutrient solution, respectively, were serially diluted and 200 ml volumes spread, in duplicates, on the following media: (i) 0.1 Tryptic Soya Agar (TSA; DIFCO 0369-17-6) solidified by 1.5 % Bacto Agar (DIFCO) and R2A (DIFCO 1826-17-1) each complemented with cycloheximide (100 µg ml^{-1}) for enumeration of the general rhizobacterial flora and the general bacterial flora in the nutrient solution, respectively. (ii) King Agar B (KB, (King et al. 1954)) with cycloheximide (100 µg ml^{-1}) for enumeration of fluorescent pseudomonads, (iii) 0.5 % Malt extract agar (MA, DIFCO 0186-17-7) for enumeration of the general fungal flora, (iv) modified 0.25 % Potato Dextrose Agar (PDA, DIFCO 013-01-4) for selective enumeration of *Trichoderma* (Postma et al. 1999) and (v)

chitin oatmeal agar (COA) complemented with cycloheximide (100 µg ml^{-1}) for filamentous actinomycetes (Postma et al. 1999). For the latter one, the diluted nutrient solution and root suspension, respectively, was inoculated on sterile membrane filters (Optitran BA-S 83, 0.2 µm; Ø 82 mm; Schuell & Schleicher, Dassel Germany). The filters were removed and discarded after 5 days of incubation. Plate counts were done after additional 5 days of incubation.

Apart from KB plates, all the plates were incubated at room temperature. KB plates were incubated for 24 h at 27 °C, TSA and R2A plates for 72 h. MA and PDA plates were incubated for 7 days.

Sole carbon source utilization patterns

Sole carbon source utilization (SCSU) patterns were studied as described by Alsanius et al. (2001a). For the analysis of the nutrient solution, density was adjusted to an optical density of OD_{590} 0.010 using 0.85 % NaCl, and aliquots of 150 ml of the solution were directly inoculated into the microtitre plates (Microlog, GN-panel, Biolog, Haywood, CA, USA) while 0.5 ml of the secondary suspensions of the collected samples of the root microflora were resuspended in 0.85 % NaCl and adjusted to OD_{590} (0.100 ± 0.005). The suspensions were then inoculated in the wells of the GN panel. The plates were incubated at room temperature. Spectrophotometrical analyses (590 nm; DigiScan, Asys, Linz, Austria; software: Digiwin) of the utilization of sole carbon sources was performed on the basis of the color change of the redox dye tetrazolium blue to formazan, directly after inoculation and 4, 18, 24, 30, 36, 48, 72 and 96 h after inoculation.

Phospholipid fatty acids profiles

For the analysis of the nutrient solution by phospholipid fatty acid (PLFA), 3 L of the nutrient solution were membrane filtered (0.22 mm Cellulosa Acetate Filter; Satorius AG, Germany) and the collected samples on the filter paper and of the root microflora were subjected to phospholipid fatty acid analysis as described by Frostegård (1995) and Waechter-Kristensen et al. (1996). Briefly, the samples 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. A 4 ml aliquot of the lower, organic phase was dried under a gentle stream of nitrogen. The phospholipids were then isolated using silicic acid column chromatography. The fatty acid methyl esters 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, CA). Hydrogen was used as the carrier gas and injections were made in the splitless mode. The fatty acids are presented as the total number of carbon atoms: number of double bonds, followed by the position of the double bond from the methyl end (ω) of the molecule. Anteiso and iso are indicated by **a** and **i**, respectively. **Cy** refers to cyclopropane fatty acids. The PLFAs: i15:0, a15:0, 15:0, i16:0, 16:1w9, 16:1w7t, 16:1w5, i17:0, a17:0, 17:1w8, cy17:0, 17:0, 18:1w7 and cy19:0 were chosen to characterise the microflora of the root environment and the nutrient solution of hydroponically grown tomato plants. These PLFAs were previously identified as bacterial PLFAs and chosen to characterize the bacterial rhizosphere microflora in a hydroponic system (Khalil et al. 2000a).

Statistical analysis

The results from viable counts, expressed as mean ± SD after log transformation (Angle et al. 1996), were analysed using one-way ANOVA and LSD with $p < 0.05$ considered significant. For the SCSU data, the average well color development (AWCD), expressing the sum of absorbance units from all 95 wells containing single carbon sources divided by number of wells were calculated. SCSU patterns were analyzed using discriminant analysis (DA; software SAS, release 6.12). The PLFA data were expressed as the mol % of individual fatty acids. Principal component analysis (PCA) was used to separately analyse the data of PLFA profiles using the statistical program Minitab (release 12). The results from multivariate analysis were analysed by ANOVA and Tukey´s least significant differences, LSD, with $p < 0.05$ considered significant to determine statistically significant treatment differences.

Results

Viable count

The viable count of bacteria inhabiting the roots, enumerated on 0.1 TSA and KB, varied between the two growing media, rockwool and peat. The number of general bacterial flora and of fluorescent pseudomonads (CFU g^{-1} fresh weight roots) was higher ($p < 0.001$) in rockwool than in peat (Fig. 1 A and B). In both substrates no significant differences in the number of the general bacterial flora were found between the five sampling occasions. On the other hand, significant differences in the number of fluorescent pseudomonads were detected in both rockwool and peat grown plants between the sampling occasions in September and October compared with the first one in May. At all sampling occasions, the amount of general fungal flora, *Trichoderma* and actinomycetes was higher in peat compared with rockwool grown tomato plants (Fig. 1A and B). In both substrates, the number of the general fungal flora was higher later in the growing season (sampling 4 and 5) compared with the first sampling incident. In peat, high number of *Trichoderma* was also noticed in the last three sampling incidents compared with the first one, while in rockwool, their numbers were higher in the sampling incidents in June, August, September and October compared with the sampling incident in May. Moreover, actinomycetes, in peat grown plants, were higher in the last two sampling incidents compared with the first one, while on roots from rockwool

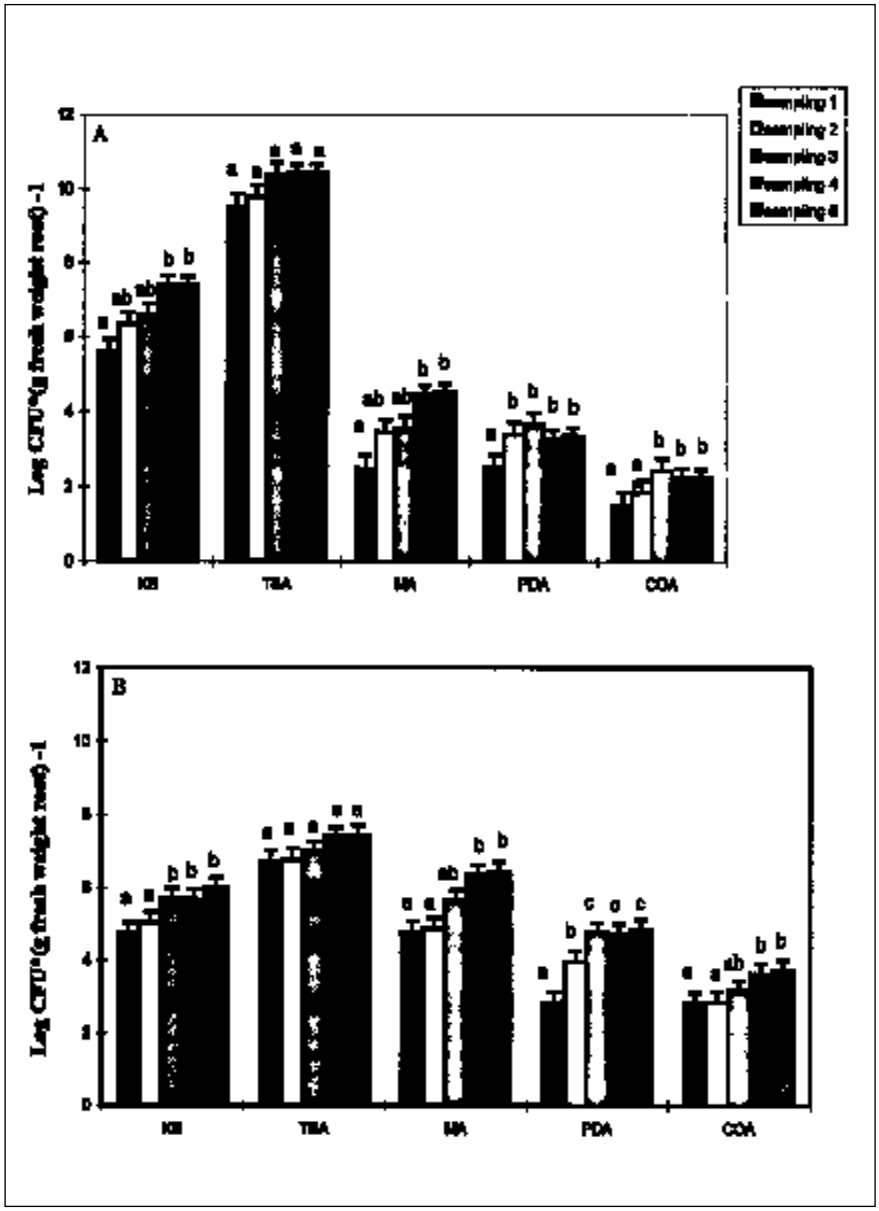


Fig. 1. Viable count of fluorescent pseudomonads, general bacterial flora, general fungal flora, *Trichoderma* and actinomycetes, measured on King Agar B (KB), 0.1 Tryptic Soya Agar (TSA), 0.5 Malt extract agar (MA), 0.25 Potato Dextrose Agar (PDA) and chitin oatmeal agar (COA) respectively, on the roots of hydroponically cultivated tomato. (A) Root samples collected from rockwool slabs and (B) root samples collected from peat buckets. The samples were collected during the growing season from May–October (sampling 1: May, sampling 2: June, sampling 3: August, sampling 4: September, sampling 5: October). Data were analyzed by ANOVA and LSD with $P < 0.05$ considered as significant and each column is the mean $\pm$ SE, $n = 4$.

Kolonisierung von Tomatenwurzeln aus einem geschlossenen erwerbsmässigen Kultursystem ausgedrückt als Lebendzellzahl der fluoreszierenden Pseudomonaden, gesamten Bakterienflora, Aktinomyzeten, gesamten Pilzflora und Trichoderma auf King Agar B (KB), 0.1 Trypto-Soya-Agar (TSA), 0.5 Malzextraktagar (MA), bzw. 0.25 modifiziertem Kartoffeldextroseagar (PDA), Chitin-Haferagar (COA). (A) Wurzelproben von Tomatenpflanzen aus einer Steinwollkultur und (B) Wurzelproben von Tomatenpflanzen aus einer Torfkultur. Die Proben wurden während der Vegetationsperiode von Mai bis Oktober entnommen (Probenentnahme 1: Mai, Probenentnahme 2: Juni, Probenentnahme 3: August, Probenentnahme 4: September, Probenentnahme 5: Oktober). Jede Säule repräsentiert den Mittelwert $\pm$ Standardabweichung aus vier Wiederholungen. Die Daten wurden varianzanalytisch verrechnet und ein Mittelwertvergleich mittels LSD-Test ($p < 0.05$) durchgeführt.

grown tomato their numbers were higher towards the end of the sampling period compared with the sampling incident in May (Fig. 1A and B).

With respect to microorganisms inhabiting the nutrient solution, no significant differences were obtained between the irrigation effluent from the units using peat compared to those using rockwool as a growing medium. Therefore these values are presented as pooled values in the further analyses. For the general bacterial flora, as evaluated on R2A, no differences could be obtained between the five incidents (Fig. 2). Fluorescent pseudomonads as enumerated on KB were significantly ($p < 0.001$) more abundant at the first sampling incident compared to all following ones; further the number of fluorescent pseudomonads differed significantly between the second and the last sampling incident. The general number of culturable fungi did not differ quantitatively when enumerated on MA or PDA. Therefore, we chose to present data from plate counts on 0.25 PDA, exclusively. Culturable fungi were significantly more abundant during the first two and the last sampling incidents compared to the third and fourth ones ($p < 0.001$). The same result was obtained for *Trichoderma*.

Sole carbon source utilization patterns

With respect to AWCD (Fig. 3), utilization of sole carbon sources (SCSU) patterns of the root inhabiting microflora gave significant differences both within and between the two growing media, peat and rockwool. In both substrates, carbon sources were utilized more rapidly in sampling on August compared to sampling on May, June, September and October. There were almost no differences between last two sampling incidents in SCSU by the microflora of peat grown tomato. However, the pattern differed in rockwool grown plants where substantial differences were obtained between the last sampling incident and the four preceding ones. Regarding multivariate analyses of sole car-

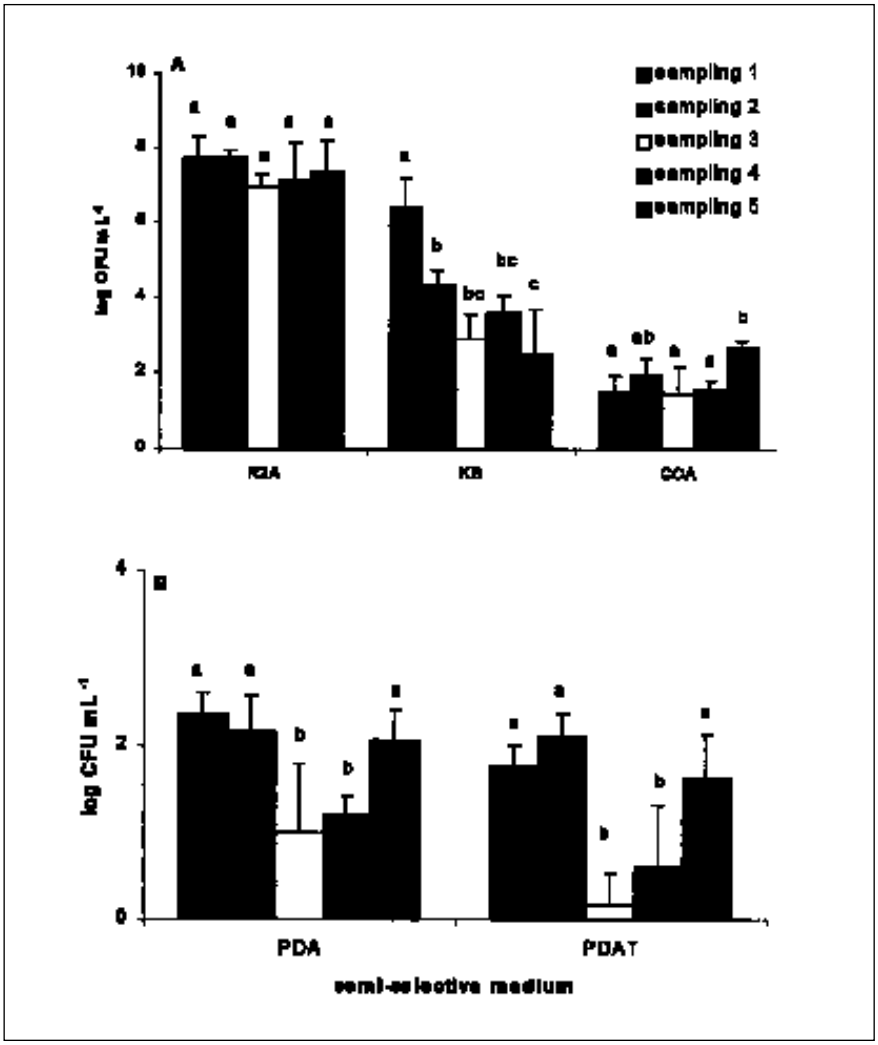


Fig. 2. Dynamics of the microflora in the irrigation effluent from a commercial hydroponic greenhouse system growing tomato. Samples were taken from the effluent irrigation water from 4 separate greenhouse compartments during the growing season from May-October (sampling 1: May, sampling 2: June, sampling 3: August, sampling 4: September, sampling 5: October). Enumeration of the microflora was performed for the both bacteria (A: general bacterial flora on R2A; fluorescent pseudomonads on King Agar B, KB; filamentous actinomycetes on chitin oatmeal agar, COA) and fungi (B: general fungal flora and *Trichoderma* on 0.25 modified potato dextrose agar, PDA and PDAT, respectively). (n = 4).

Dynamik der Mikroflora in der abfliessenden Nährlösung einer erwerbsmässigen hydroponischen Tomatenkultur. Die Nährlösungsproben wurden in vier separaten Gewächshauseinheiten während der Vegetationsperioden von Mai bis Oktober entnommen (Probenentnahme 1: Mai, Probenentnahme 2: Juni, Probenentnahme 3: August, Probenentnahme 4: September, Probenentnahme 5: Oktober). Lebendzellzahlen wurden sowohl für Bakterien (A: allgemeine Bakterienflora auf R2A, fluoreszierende Pseudomonaden auf King Agar B, KB, Aktinomyzeten auf Chitin-Haferagar, COA) als auch für Pilze (B: allgemeine Pilzflora und Trichoderma auf 0.25 modifiziertem Kartoffeldextroseagar, PDA bzw. PDAT) ausgezählt . (n = 4).

bon source utilization on the basis of discriminant analyses, the carbon sources N-acetyl-D-galactosamine, cellobiose, D-psicose, turanose, methyl pyruvate, L-asparagine, L-leucine, L-proline, D-serine and γ-amino butyric acid discriminated between the five sampling incidents (Fig. 4A), whereas the carbon sources α-cyclodextrin, i-erythriol, m-inositol, methyl pyruvate, γ-hydroxybutyric acid, itaconic acid, succinamic acid, L-phenylalanine, thymidine and butanediol separated between the two treatments, peat and rockwool as a growing medium, respectively (Fig. 4B).

After direct inoculation of the nutrient solution into the wells of the microtitre plates, precipitation occurred in almost all plates but disappeared within the first three days. This affected the spectrophotometrical measurement of carbon utilization, initially, showing exaggerated values of absorbance units during this period of time (Fig. 5). The elevated absorbance levels could not be explained by color change of the redox dye. Discrimination analyses on the basis of the ten most discriminating sole carbon compounds showed a clear separation between the sampling incidents whereas no difference could be found between the growing media, peat and rockwool, using the same method (Fig. 6). Sampling sites differed on the basis of carbon source adonitol, cellbiose, i-erythritol, gentiobiose, D-melbiose, L-rhamnose, acetic acid, propionic acid, bromosuccinic acid and succinamic acid.

Phospholipid fatty acids profiles

The PLFA profiles of root microflora from both rockwool and peat grown plants, expressed as mol%, were analysed by principal component analysis (PCA), 36.7% of the total variation was explained by the first principal component (PC1) and 18.4% by the second one (PC2). In both substrates, PCA differentiated between the PLFA profiles of the root microflora on roots collected in May and June and those collected on August, September and October (Fig. 7A). PC1 differentiated between the PLFA profiles of the root microflora of plants grown in rockwool and sampled on May and June from those sampled at the last three sampling incidents. For the peat grown tomato plants, the differences between the first two sampling incidents and the last three ones were noticed to be contributed to PC2. No differences, on the other hand, could be found between the profiles of root samples collected in the last three incidents either in rockwool or in peat. Furthermore, differences in the PLFA profiles were also noticed between the root samples grown in rockwool and sampled on May and June and those grown in peat and

collected at the same sampling incidents. PCA of the individual PLFA (Fig. 7B) showed that PC1 had relatively high proportions of the fatty acid methyl esters a15:0, 16:1ω9, 16:1ω7t, i17:0 and cy19 while PC2 had relatively high proportions of the fatty acid methyl esters i14:0, 16:1ω5, cy17, 17:1ω8 and 18:1ω7.

When the PLFA data from the irrigation effluent collected from the rockwool slabs were subjected to PCA, 22.5% of the total variation could be explained by PC1 and 18.8% by PC2. The PCA loading plot showed that PC1 separated between the PLFA profiles of the microflora inhabiting the nutrient solution and collected on May from those collected on August, September and October (Fig. 8A). Furthermore, differences in PC2 could be noticed between the PLFA profiles of the microflora inhabiting the nutrient solution and sampled

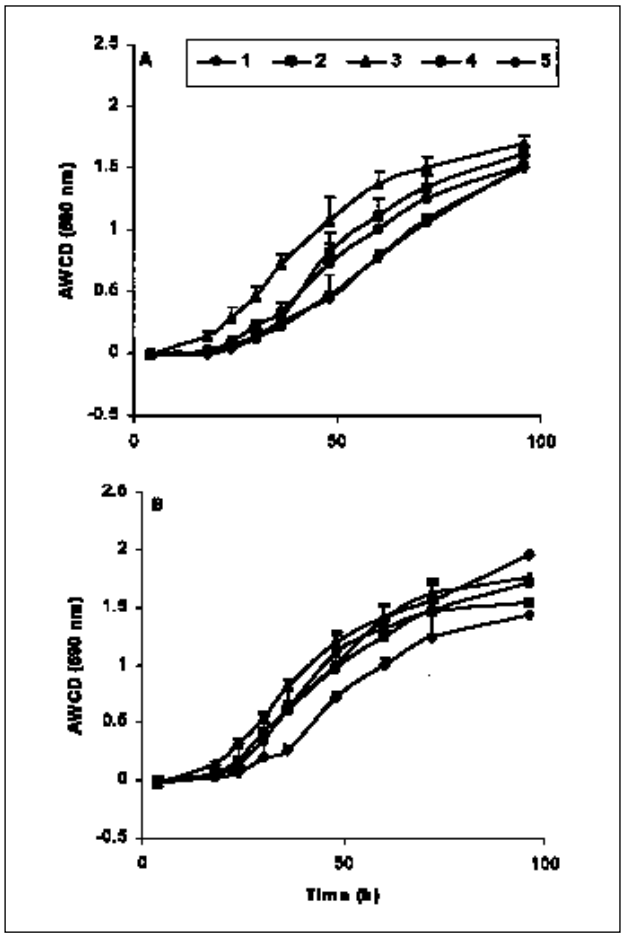


Fig. 3. Utilization of sole carbon sources by the root zone-inhabiting microflora from peat (A) and rockwool (B) grown tomato in a commercial closed hydroponic greenhouse system on the five sampling occasions (sampling 1: May, sampling 2: June, sampling 3: August, sampling 4: September, sampling 5: October). The analysis was performed on the basis of the Microlog GN-panel directly after inoculation and 4, 18, 24, 30, 36, 48, 72 and 96 h after inoculation. (n = 4). A,B: average well color development (AWCD) expressing the sum of absorbance of all 95 wells divided by number of wells at 590 nm by color change of the redox dye tetrazolium blue.

Verwendung einzelner Kohlenstoffquellen durch die wurzelkolonisierende Mikroflora von Tomaten aus einer kommerziellen hydroponischen Torf- (A) und Steinwollkultur (B) zu fünf Zeitpunkten während der Vegetationsperiode (Probenentnahme 1: Mai, Probenentnahme 2: Juni, Probenentnahme 3: August, Probenentnahme 4: September, Probenentnahme 5: Oktober). Die Analyse wurde auf der Basis von Microlog GN-panel durchgeführt und direkt nach der Inokulation, 4, 18, 24, 30, 36, 48, 72 und 96 h Stunden nach der Inokulation abgelesen. (n = 4). A, B: Die durchschnittliche Farbveränderungen der Vertiefungen (AWCD) drückt den Quotienten aus Summe der Absorption aller 95 Vertiefungen und Anzahl Vertiefungen bei 590 nm als Farbveränderung des Redoxfarbstoffs Tetrazoliumblau aus.

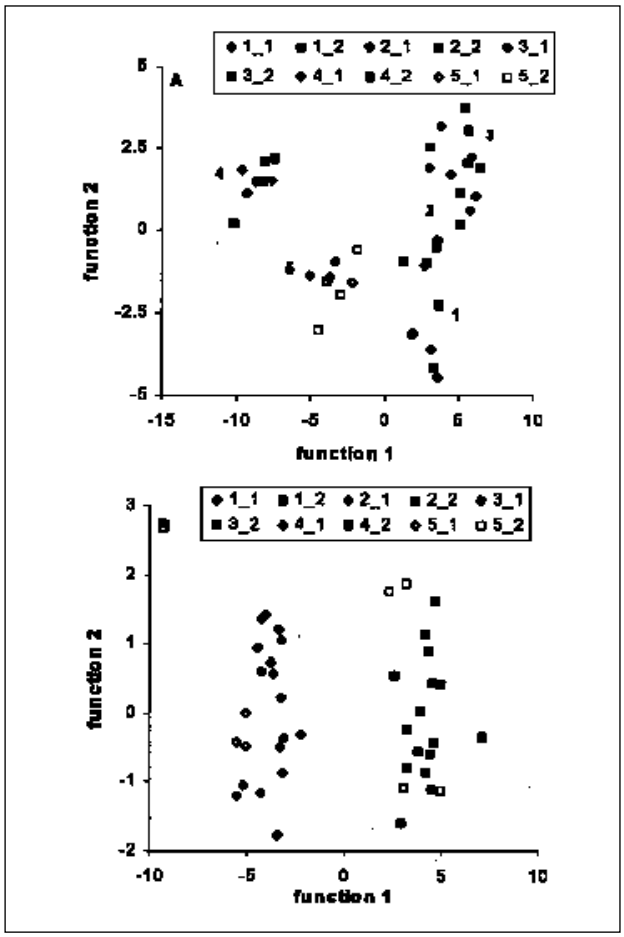


Fig. 4. Functional diversity of the microflora inhabiting the root zone during the growing season 1999 on the basis of the Microlog GN-panel (Biolog Inc. Hayward CA, USA), using discriminant analysis, standardized to ten decisive carbon sources. (n = 4). Sampling incidents were (1) May (2) June, (3) August, (4) September and (5) October. A: with respect to the sampling incident and B: with respect to the treatment.

Funktionale Diversität der wurzelkolonisierenden Mikroflora während der Vegetationsperiode 1999 auf der Basis des Microlog GN-panels (Biolog Inc. Hayward CA, USA). Die funktionelle Diversität wurde mit Hilfe von auf 10 determinierenden Kohlenstoffquellen standardisierter Diskriminantanalyse berechnet. (n = 4). Die Probenentnahmezeitpunkte waren (1) Mai, (2) Juni, (3) August, (4) September und (5) Oktober. A: Analyse in Bezug auf Probenentnahmezeitpunkte. B: Analyse in Bezug auf Behandlung.

on June and those sampled at the last three incidents. The PCA loading plot of the individual PLFA (Fig. 8B) showed that the fatty acids methyl esters i14:0, i16:0, 17:0 and 18:1ω7 had relatively high proportions in PC1 while the fatty acid methyl esters i15:0, 15:0, 16:1ω9, 16:1ω7t, 16:1ω5 and cy19 had relatively high proportions in PC2. For the irrigation effluent collected from peat grown tomato, no differences in the PLFA profiles could be found between the five sampling incidents (Table 1). In all sampling incidents the fatty acid methyl esters i15:0, a15:0, 16:1ω5, 18:1ω7 and cy 19 had relatively high amount compared with the other fatty acid methyl esters.

Discussion

In several studies, microbial analyses have been conducted by enumeration of the cultural aerobic heterotrophic microflora colonizing different parts of the system, such as root zone, nutrient solution, substrate and system devices (tubes, gutters etc.) using viable count (CFU) (Berkelmann 1992, Strayer 1991, Strayer 1994). These studies are in some cases also followed by selective isolation, transfer to pure culture and identification, using chemical (Berkelmann 1992, Brand 2000) or biochemical methods (Berkelmann 1992, Brand 2000, Strayer 1991). However, only 1 to 10 % of the microflora may be recovered by techniques based on a plate culture approach. During recent years, these studies have been complemented with characterization of microbial communities, using chemical (Alsanius et al. 2000b, Khalil et al. 2000a and b, Waechter-Kristensen 1996), biochemical, such as functional diversities (Alsanius et al. 2000a and b) and molecular (Postma et al. 2000a and b) methods, respectively.

In the present study, viable counts indicate that in rockwool, the microflora inhabiting the roots shifted to a higher number of fluorescent pseudomonads and

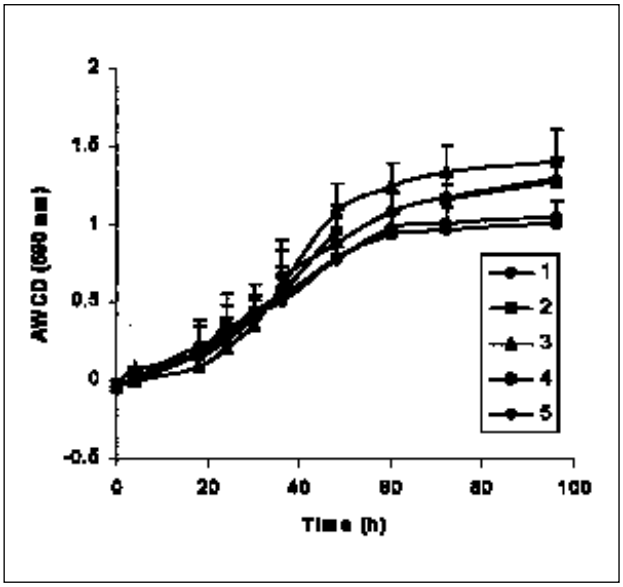


Fig. 5. Utilization of sole carbon sources by the microflora inhabiting the irrigation effluent of a commercial tomato culture expressed as average well color development, AWCD. Samples from the effluent irrigation water were collected at five different occasions (sampling 1: May, sampling 2: June, sampling 3: August, sampling 4: September, sampling 5: October). The analysis was performed on the basis of Microlog GN-panel. AWCD expresses sum of absorbance of all 95 wells divided by number of wells at 590 nm by color change of the redox dye tetrazolium blue. Readings were conducted directly after and 4, 18, 24, 30, 36, 48, 72 and 96 h after inoculation. (n = 4).
Verwendung einzelner Kohlenstoffquellen durch nährlösungkolonisierende Mikroflora einer erwerbsmässigen Tomatenkultur in geschlossenem Kultursystem, ausgedrückt als durchschnittliche Farbveränderung der Vertiefungen (AWCD). Die Proben wurden aus der abfliessenden Nährlösung zu fünf Zeitpunkten während der Vegetationsperiode entnommen (Probenentnahme 1: Mai, Probenentnahme 2: Juni, Probenentnahme 3: August, Probenentnahme 4: September, Probenentnahme 5: Oktober). Die Analyse wurde auf der Basis von Microlog GN-panel durchgeführt und direkt nach der Inokulation, 4,18,24, 30, 36, 48, 72 und 96 h Stunden nach der Inokulation abgelesen. (n = 4). A, B: Die durchschnittliche Farbveränderungen der Vertiefungen (AWCD) drückt den Quotienten aus Summe der Absorption aller 95 Vertiefungen und Anzahl Vertiefungen bei 590 nm aus.

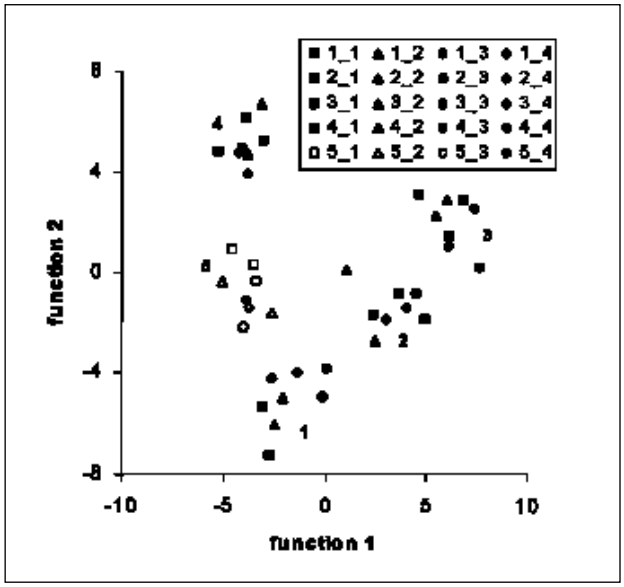


Fig. 6. Functional diversity of the microflora inhabiting the nutrient solution during the growing season 1999 on the basis of the Microlog GN-panel, using discriminant analysis, standardized to ten decisive carbon sources. (n = 4). Sampling incidents were. (1) May, (2) June, (3) August, (4) September and (5) October.
Funktionale Diversität der nährlösungskolonisierenden Mikroflora während der Vegetationsperiode 1999 auf der Basis des Microlog GN-panels (Biolog Inc. Hayward CA, USA). Die funktionelle Diversität wurde mit Hilfe von auf 10 determinierenden Kohlenstoffquellen standardisierter Diskriminantanalyse berechnet. (n = 4). Die Probenentnahmezeitpunkte waren (1) Mai, (2) Juni, (3) August, (4) September und (5) Oktober.

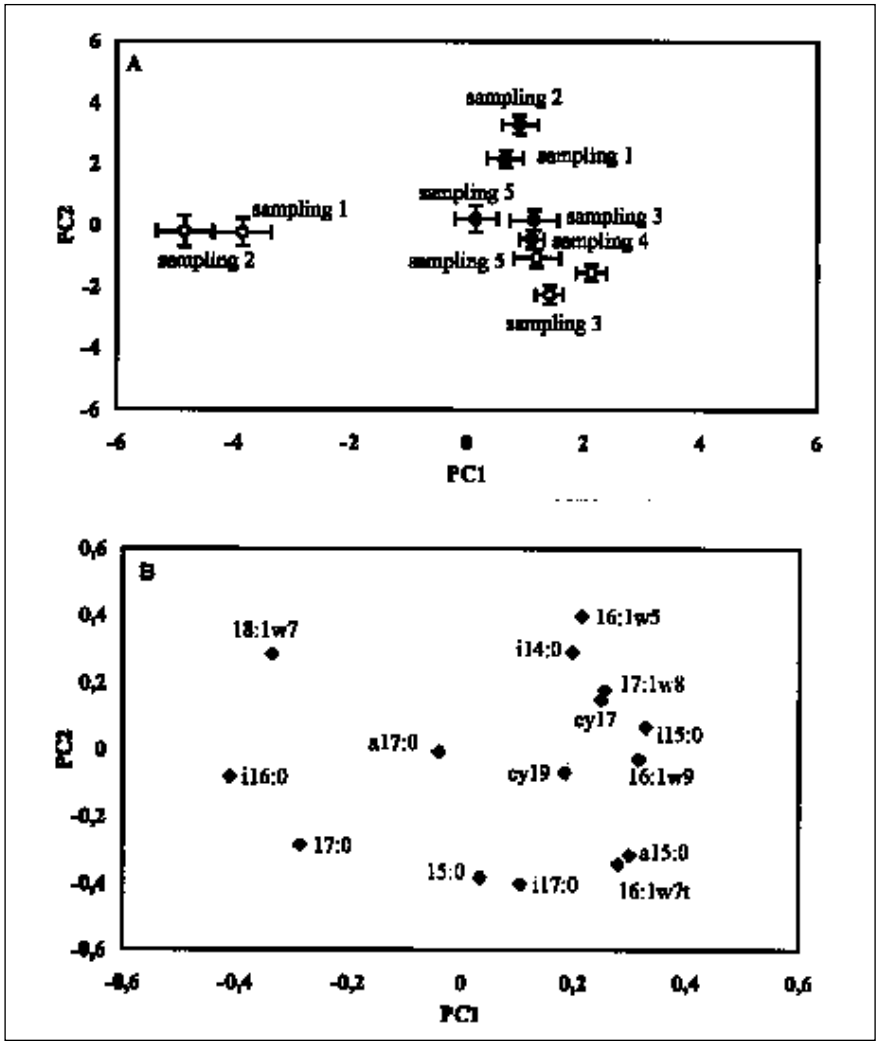


Fig. 7. (A) Principal component analysis (PCA) of phospholipid fatty acid (PLFA) profiles of the root microflora of hydroponically grown tomato roots collected from two different substrates, rockwool ( ) and peat ( ), at five different occasions; (sampling 1: May, sampling 2: June, sampling 3: August, sampling 4: September, sampling 5: October). Each value is the mean ± SE of four replicates. PC1 and PC2 are the first and the second principal component respectively. (B) Loading values of individual PLFA from the principal component analysis.

(A) Hauptkomponentenanalyse (PCA) der Phospholipidfettsäurenprofile (PLFA) der wurzelkolonisierenden Mikroflora einer erwerbsmässigen Tomatenkultur in geschlossenem Kultursystem. Die Tomatenpflanzen wurden auf zwei Substraten angebaut, Steinwolle ( ) und Torf ( ). Die Proben wurden zu fünf Zeitpunkten entnommen (Probenentnahme 1: Mai, Probenentnahme 2: Juni, Probenentnahme 3: August, Probenentnahme 4: September, Probenentnahme 5: Oktober). Jeder Wert repäsentiert den Mittelwert ± Standardfehler von vier Wiederholungen. PC1 und PC2 sind der erste bzw. zweite Faktor. (B) Faktorladungen der individuellen Phospholipidfettsäuren aus der PCA.

general bacterial microflora, whereas the root microflora in peat shifted to a higher number of *Trichoderma* and actinomycetes with increasing length of the growing period. In previous studies, Gertsson et al. (1994) observed an increase in number of actinomycetes in the nutrient solution of a closed DFT (deep flow technique) system with increasing growing period length. Postma *et al.* (2000a) found an interaction between actinomycetes and disease suppressiveness towards *Pythium aphanidermatum* in reused rockwool slabs from healthy plants. However, no qualitative analysis of *Trichoderma* and actinomycetes isolates were conducted. In future studies this should be performed in order to evaluate the role of these groups in disease suppressiveness, especially in the light of plant pathogenicity of certain *Trichoderma* spp. Furthermore, for a further analyses of the microbial status in the studied commercial greenhouse, investigations on the antagonistic potential of the root and nutrient solution inhabiting microflora would be an interesting approach.

Evaluation of SCSU has been used as an indicator of the functional diversity of the microflora in different habitats (Garland and Mills 1991). With respect to tomato growing in soilless systems, average well color development and number of positive wells accelerated in the root microflora of *Pythium* attacked plants (Alsanius et al. 2000a). There, α-D-glucose, D-trehalose, Cis-aconitic acid, D-saccharic acid, D-gluconic acid and L-asparagine were found to be the decisive C-sources. In the present study, AWCD (Fig. 3 A, B) differed between different sampling incidents. Discrimination of the root microflora with respect to sampling incidents (Fig. 4A) was enabled by a selection of predominantly sugars and amino acids (N-acetyl-D-galactosamine, cellobiose, D-psicose, turanose, methyl pyruvate, L-asparagine, L-leucine, L-proline, D-serine and γ-amino butyric acid). Interestingly, L-asparagine is congruent with the study of Alsanius et al. (2000a). On the basis of growing media (Fig. 4b) discriminating compounds: α-cyclodextrin, i-erythriol, m-inositol, methyl pyruvate, γ-hydroxybutyric acid, itaconic acid, succinamic acid, L-phenylalanine, thymidine and butanediol), both methyl pyruvate and i-erythriol were found among the 10 C-sources discriminating between the two growing media, peat and rockwool. These compounds were previously found to discriminate between bacterized, with PGPR *P. fluorescens* strain, 5.014, and its mutant 5-2/4, or non-bacterized tomato plants exposed to *Pythium* (Khalil et al. 2000b). On the basis of these results, it would be favorable to get further information on the implication of

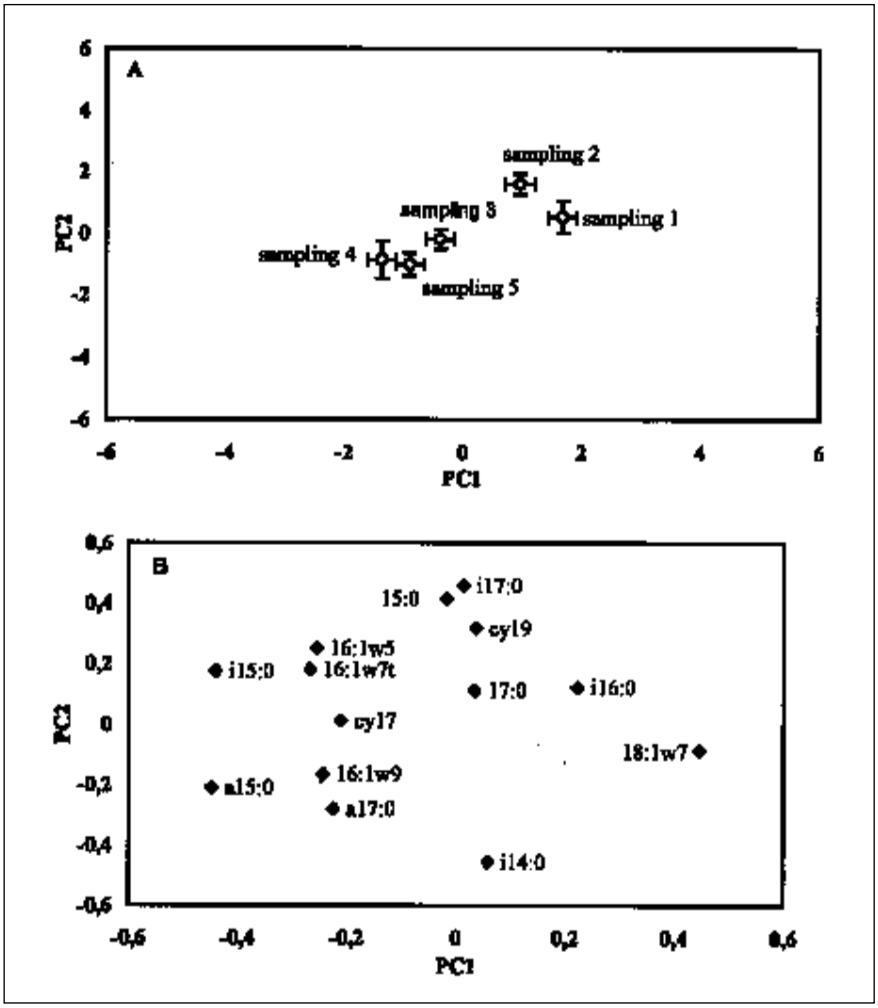


Fig. 8. (A) Principal component analysis (PCA) of phospholipid fatty acid (PLFA) profiles of the microflora inhabiting the irrigation effluent in a commercial closed greenhouse system with tomato. Rockwool was used as a growing medium. The nutrient solution was collected at five different occasions; (sampling 1: May, sampling 2: June, sampling 3: August, sampling 4: September, sampling 5: October). Each value is the mean ± SE of three replicates. PC1 and PC2 are the first and the second principal component respectively. (B) The loading values of individual PLFA from the principal component analysis.
(A) Hauptkomponentenanalyse (PCA) der Phospholipidfettsäurenprofile (PLFA) der nährlösungskolonisierenden Mikroflora einer erwerbsmässigen Tomatenkultur in geschlossenem Kultursystem. Steinwolle wurde als Substrat verwendet. Die Nährlösung wurde zu fünf Zeitpunkten während der Vegetationsperiode entnommen (Probenentnahme 1: Mai, Probenentnahme 2: Juni, Probenentnahme 3: August, Probenentnahme 4: September, Probenentnahme 5: Oktober). Jeder Wert repäsentiert den Mittelwert ± Standardfehler von vier Wiederholungen. PC1 und PC2 sind der erste bzw. zweite Faktor. (B) Faktorladungen der individuellen Phospholipidfettsäuren aus der PCA.

these compounds as indicators for disease suppressiveness. This will, however, also require further knowledge of abundance of PGPR forming metabolites with antibiotic action (such as 2,4-diacetyl phloroglucinol, phenazine-1-carboxylic acid, pyrrolnitrin, pyoluteorin) as well *in situ* metabolite concentration.

Evaluation of irrigation effluents on the basis of functional diversity has not been published before. For explaining the data, comparison has to be done with data from microbial communities inhabiting the root. Discrimination of the sampling incidents on the basis of the irrigation effluent was enabled by predominantly sugars and organic acids (adonitol, cellobiose, i-erythritol, gentiobiose, D-melbiose, L-rhamnose, acetic acid, propionic acid, bromosuccinic acid and succinamic acid), showing small similarity with sampling incidents of the root microflora (cellobiose). However, some similarity was found with the results presented by Khalil et al. (2000b) where both adonitol, i-erythriol, D-melbiose, acetic acid were found to be among the ten decisive C-sources. Analyses of the SCSU as a technique may be used to discriminate microbial populations in different habitats. However, for meaningful interpretation in complex plant production situations, it is necessary to have further knowledge on the load of discriminating compounds and to broaden the spectrum of parameters also to other known indicators of microbial interactions and disease suppressiveness.

The PLFA profiles indicated differences in the community composition, in the first two sampling incidents, between roots grown in rockwool and those grown in peat. These differences disappeared in the last three sampling incidents. This might indicate that the composition of the root microflora differed only at the beginning of the cultivation period and becomes more conservative towards the end of the cultivation period. The PLFA composition of the root microflora of tomato plants grown in rockwool and collected on May and June was characterised by high proportions of the fatty acids methyl esters i16:0 and a17:0, typical for the Gram-positive bacteria (O´Leary and Wilkinson 1988), and the saturated fatty acid methyl ester 17:0. Meanwhile, PLFA composition of the root microflora of tomato grown in peat and collected in the first two sampling incidents was characterized by high proportions of the fatty acid methyl esters 16:1ω5, 17:1ω8, cy17 and 18:1ω7, typical for the Gram-negative bacteria (Wilkinson 1988) and the fatty acid methyl ester i14:0, typical for the Gram-positive bacteria (O´Leary and Wilkinson 1988). Furthermore, the composition of the root microflora in the last three sampling incidents, in both substrates, was characterized by high proportions of the fatty acids methyl esters 16:1ω9, 16:1ω7t and cy19, typical for Gram-negative bacteria (Tunlid et al. 1989, Wilkinson 1988) and the fatty acid methyl esters a15:0 and i17:0 which are abundant in wide variety in Gram-positive bacteria (O´Leary and Wilkinson 1988).

Table 1. Amount of phospholipid fatty acid (PLFA) profiles of the microflora inhabiting the irrigation effluent in a commercial closed greenhouse system with tomato expressed as mol %. Peat was used as a growing media. PLFAs are listed after retention times in a 50-m HP5 capillary column.
Phospholipidfettsäuremenge (mol %) der mikrobiellen Gesellschaften in der abfliessenden Nährlösung eines erwerbsmässigen, geschlossenen Kultursystems mit Tomaten. Torf wurde als Substrat verwendet. Die Phospholipidfettsäuren sind nach ihrer Retentionszeit in einer kapillären 50-m-HP5-Kolonne aufgeführt.

PLFA	Sampling 1[a]	Sampling 2	Sampling 3	Sampling 4	Sampling 5
i14:0 b	5.1	4.1	4.7	5.4	6.3
i15:0	14.3	13.1	12.0	13.5	12.1
a15:0	10.4	9.8	10.5	9.2	8.0
15:0	4.7	3.9	4.2	3.4	4.3
i16:0	5.6	4.2	5.0	4.5	4.7
16:1ω9	5.1	4.2	4.3	5.6	4.3
16:1ω7t	2.8	1.4	2.7	1.5	3.0
16: ω5	7.5	9.1	8.5	9.5	8.0
i17:0	5.1	4.9	6.3	6.6	5.11
a17:0	3.1	5.5	4.3	3.8	4.3
17:1ω8	1.6	3.2	2.4	3.7	2.2
cy17:0	5.3	4.9	3.1	5.6	4.4
17:0	3.9	4.5	4.0	5.2	5.8
18:1ω7	18.2	20.2	19.5	16.3	20.5
cy19:0	7.3	7.1	8.5	6.4	7.1

[a] Sampling occasions during the growing season; (sampling 1: May, sampling 2: June, sampling 3: August, sampling 4: September, sampling 5: October).
[b] Fatty acids are expressed as total number of carbon atoms: number of double bonds, followed by the position of the double bond from the methyl end (ω) of the molecule. Anteiso and iso are indicated by a and i, respectively. Cy refers to cyclopropane fatty acids.
[a] Probenentnahmezeitpunkte während der Vegetationsperiode (Probenentnahme 1: Mai, Probenentnahme 2: Juni, Probenentnahme 3: August, Probenentnahme 4: September, Probenentnahme 5: Oktober).
[b] Fettsäuren ausgedrückt als (Gesamtanzahl von Kohlenstoffatomen): (Anzahl von Doppelbindungen), gefolgt von der Position der Doppelbindung des Methylrests des Moleküls (*). Anteiso und iso wurden durch a bzw. i gekennzeichnet. Cy bezieht sich auf Cyklopropanfettsäuren.

Evaluation of irrigation effluent, from the rockwool slabs, on the basis of PLFA profiles indicates differences in the composition of the microflora between samples collected in May and June and those collected in August, September and October. On the first two sampling occasions, the composition of the microflora was characterized by high proportions of the fatty acid methyl esters i16:0 and i17:0, typical for Gram-positive bacteria, and the fatty acid methyl esters 16:1ω7c, 18:1ω7 and cy19, typical for the Gram-negative bacteria. The composition of the microflora in the last three sampling incidents was characterized by high proportions of the fatty acid methyl esters i15:0, a15:0 and a17:0, found in Gram-positive bacteria and the fatty acids methyl esters 16:1ω9, 16:1ω7t, 16:1ω5 and cy17 found in Gram-negative bacteria. The composition of the microflora inhabiting the irrigation effluent collected from the peat buckets were characterized by high proportion of the fatty acid methyl esters i15:0 and a 15:0 typical for the Gram-positive bacteria and the fatty acid methyl esters 16:1ω5, 18:1ω7 and cy19 usually found in Gram-negative bacteria.

In conclusion, the present study indicates shifts in the microbial communities during the growing season and due to the growing media when evaluated by viable count, SCSU patterns and PLFA profiles. With regard to root microflora, no common trends in community shift was found for the two studied growing media using viable counts or SCSU patterns. PLFA profiles, on the other hand, indicate differences in the composition of the root microflora only at the beginning of the growing season in both peat and rockwool. Regarding shifts in the irrigation effluent viable count and SCSU patterns did not indicate differences between the growing media while PLFA profiles indicate differences in the composition of the microflora inhabiting the irrigation effluent collected from the rockwool slabs in the first two sampling incidents. No differences in the composition of the microflora inhabiting the irrigation effluent and collected from the peat buckets could be found. Furthermore, analysis of the fate of microbial groups with importance of disease suppressiveness, such as fluorescent pseudomonads, Trichoderma, actinomycetes and some Bacillus species should be investigated.

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References

Alsanius B.W. and U.E. Gertsson 1998: Einfluss der Bakterisierung von Tomatenpflanzen auf Zuwachs und Ertrag in geschlossenen Kultursystemen. BDGL-Schriftenreihe 16, 148.

Alsanius, B.W., M. Hultberg, and S. Khalil 2001a: Microorganisms – tools for closed hydroponic greenhouse systems? Acta Hort 548, 207–214.

Alsanius, B.W., S. Khalil, M. Hultberg, J.-E. Englund and P. Sundin 2001b: Assessment of the microbial status of roots from hydroponically grown tomato. Revised; Soil Biol. Biochem.

Angle, J.S., J.V.Gagliardi, M.S. McIntosh and M.A Levin 1996: Enumeration and expression of bacterial counts in the rhizosphere. p.233–251. *In*: Soil Biochemistry (Stotzky G. and Bollag J.-M. Eds.). Marcel Dekker, Inc. N.Y.

Berkelmann, B. 1992: Charakterisierung der Bakterienflora und des antagonistischen Potentials in der zirkulierenden Nährlösung einer Tomatenkultur (*Lycopersicon esculentum* MILL.) in Steinwolle. *Geisenheimer Berichte* 10.

Brand, T. 2000: Beurteilung der Bakterienflora von Langsamfiltern als biologische Filtrationskomponente in geschlossenen Bewässerungssystemen des Gartenbaus. *Geisenheimer Berichte* 41.

Caspersen, S., B.W Alsanius and P. Sundin 2000: Bacterial amelioration of ferulic acid toxicity to hydroponically grown lettuce. Soil Biol. Biochem. 32, 1063–1070.

Frostegård, Å. 1995: Phospholipid fatty acid analysis to detect changes in microbial community structure. PhD Thesis, Department of Ecology, University of Lund.

Garland, J.L. and A.L. Mill 1991: Classification and characterisation of heterotrophic microbial communities in the basis of patterns of community-level sole-carbon-source utilisation. Appl. Envir. Micrbiol. 57, 2351–2359.

Gertsson, U.E; I. Hansson, B. Waechter-Kristensen, S. Lundqvist, G. Svedelius and R. Weich 1994: Tomato grown in circulating nutrient solution using rockwool and as hydroponics. Acta Hort. 361, 237–244.

Hultberg, M., B. W. Alsanius and P. Sundin 2000: Suppression of *Pythium* induced Damping-off in tomato seedlings by *Pseudomonas fluorescens* 5.014 and 5–2/4. Biol. Cont. 19, 1–8.

Khalil, S., E. Bååth, B.W. Alsanius, J.-E. Englund, P. Sundin, , P. Jensén and U.E. Gertsson 2001: A comparison of sole carbon source utilization patterns and phospholipid fatty acid profiles to detect changes on the root microflora of hydroponically grown crops. Can. J. Microb. 47, 302–308.

Khalil S., M Hultberg., B.W Alsanius and P. Sundin 2000: The influence of *Pythium ultimum* and introduced *Pseudomonas* isolates on the rhizobacterial community of hydroponically grown tomato. Submitted to FEMS Microbiol. Ecol.

King, E.O., Ward, M.K. and Raney, D.E. 1954: Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. and Clin. Med. 44 (2), 301–307.

McCullagh, M., R. Utkhede, J.G. Menzies, Z.K. Runja and T.C. Paulitz. 1996: Evaluation of plant growth promoting rhizobacteria for biological control of *Pythium* root rot of cucumbers grown in rockwool and effects on yield. Europ. J. of Plant Path. 102, 747–755

McPherson, G.M., M.R. Harriman and D. Pattisson 1995: The potential for spread of root diseases in recirculating hydroponic systems and their control with desinfection. Med. Fac. Landbouww. Univ. Gent 60/2b, 371–379.

O'Leary W.M. and S.G. Wilkinson 1988: Gram-positive bacteria. p. 117–201. *In*: Microbial Lipids (C. Ratledge and S.G. Wilkinson, Eds). Academic Press, London.

Paulitz T.C. 1997: Biological control of root pathogens in soilless and hydroponic systems. Hort Science 32, 193–196.

Postma, J., E.A van Os and G. Kritzman 1999: Prevention of root diseases in closed soilless growing systems by microbial optimization. Med. Fac. Landbouww. Univ. Gent 64 (3b): 431–440.

Postma, J., M.J.E.J.M. Willemesen-de Klein and J.D. van Elsas 2000a: Effect of the indigenous microflora on the development of root and crown rot caused by *Pythium aphanidermatum* on cucumber grown rockwool. Phytopath. 90, 125–133.

Postma, J., J.B.L. Lankwarden and J.D. van Elsas. 2000b: Molecular fingerprinting of microbial populations in soilless culture systems. Acta Hort. 548, 537–541.

Stanghellini, M.E. and S.L. Rasmussen 1994: Hydroponics – a solution of zoosporic pathogens. Plant Dis. 78 (12), 1129–1138.

Stanghellini, M.E. and R.M. Miller R.M. 1997: Biosurfactants – their identity and potential efficacy in the biological control of zoosporic plant pathogens. Plant Dis. 81, 4–12.

Strayer, R.F. 1991: Microbiological characterization of the biomass production chamber during hydroponic growth of crops at the controlled ecological life support system (CELSS) breadboard facility. Regenerative Life Support Systems and Processes (SP-873), SAE Technical paper series 911427, pp. 35–48.

Strayer R.F. 1994: Dynamics of microorganism populations in recirculating nutrient solutions. Adv. Space Res. 14, 357–36.

Tunlid, A., H.A.J. Hoitink, C. Low and D.C. White 1989: Characterization of bacteria that suppress Rhizoctonia damping- off in bark compost media by analysis of fatty acid biomarkers. Appl. Envir. Microbiol. 55, 1368–1374.

Tunlid, A. and D.C. White 1992: Biochemical analysis of Biomass, comunity structure, nutritional status, and metabolic activity of microbial communities in soil. p. 229–262. *In:* Soil Biochemistry (Stotzky G. and Bollag J.-M. Eds.). Dekker, Inc. N.Y.

Vanachter, A., E. van Wambeke and C. van Assche 1983: Potential danger for infection and spread of root disease of tomatoes in hydroponics. Acta Hort. 133, 119–127.

Waechter-Kristensen, B., S. Khalil, P. Sundin, J.-E. Englund, U.E. Gertsson and P. Jensén 1996: Study of the microbial dynamics in the root environment of closed, hydroponic cultivation systems for tomato using phospholipid fatty acid profiles. Acta Hort. 440, 139–189.

Wilkinson, S.G. 1988: Gram-negative bacteria. pp. 299–489. *In*: Microbial Lipids (C. Ratledge and S.G. Wilkinson, Eds). Academic Press, London.

Yu, J.Q. and Y. Matsui 1993: Extraction and identification of phytotoxic substances accumulated in nutrient solution for the hydroponic culture of tomato. Soil Scien. Plant Nutr. 39, 691–700.

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