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ScienceDirect
Crop Protection 27 (2008) 418–426
Crop Protection
www.elsevier.com/locate/cropro

Dynamics of rhizoplane bacterial communities subjected to physicochemical treatments in hydroponic crops

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Received 10 January 2007; received in revised form 10 July 2007; accepted 18 July 2007

Abstract

A highly manageable hydroponic system based on the nutrient film technique was designed to provide accurate regulation of the physicochemical and microbiological environment of roots to investigate the rhizoplane microbial dynamics of rose plants. Bacterial communities adhering to roots were subjected to pH and active free chlorine regulation, to determine up to what point they were established. PCR-single-strand conformation polymorphism (PCR-SSCP) fingerprinting showed that pH was a potentially strong stabilisation factor for root microbial communities. Active free chlorine had no quantitative effect when applied at a concentration of $0.15\,mg\,l^{-1}$, but did have a selective effect on well-established bacterial communities, which were resistant to this strong physicochemical perturbation. Thus, for the control of soil-borne diseases, the established community should be investigated, to determine its prior resistance to inoculation or to manage its physicochemical environment, increasing its robustness and excluding biological perturbation.

Keywords: Root microbial communities; Soil-less growing systems; PCR-SSCP; Biological control agent; Integrated pest management

1. Introduction

Greenhouse soil-less cropping systems provide an opportunity to combine physicochemical and biological methods for controlling plant pathogens in an environmentally friendly manner. The recycling of drainage water within a given system reduces fertiliser loss and conserves water, but may favour the dissemination of soil-borne pathogens (Poncet et al., 2001). Two different approaches can be used to reduce risks to plant health. Firstly, the drainage solution can be systematically disinfected by the physical or chemical methods developed and used in recent years by producers (heating, chlorination, filtration, etc.). Secondly, biological agents may be used to control soil-borne pathogens. Many studies have focused on the use of such agents in soil-less (Alsanius et al., 2001; Khalil and Alsanius, 2001; Postma et al., 2001; Spadaro and Gullino, 2005) and soil-grown (Whipps, 2001) crops, but the commercial development of such agents has not yet taken off (Ehret et al., 2001).

Exploitation of the full potential and durability of the various means of control requires improvements in our understanding of the structural diversity and dynamics of the microbial populations living in the root environment. The rhizosphere, the zone adjacent to the plant root, is of great importance to plant health. Molecular fingerprinting techniques are available to characterise its bacterial diversity. Denaturing or temperature-gradient gel electrophoresis (Duineveld et al., 1998; Smalla et al., 2001) and single-strand conformation polymorphism (SSCP) analysis (Schwieger and Tebbe, 1998; Stach et al., 2001) of 16S rDNA fragments amplified directly from the DNA in rhizosphere samples can be used to assess microbial diversity in diverse habitats, and can be applied to microbial lineages for which no known pure culture is available (Zumstein et al., 2000).

The soil rhizosphere is highly complex, as its composition depends on many factors, making it difficult to study. The most important factors are the plant itself (cultivar, stage of development, nutrient status) and the root

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0261-2194/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.cropro.2007.07.010

environment (Duineveld et al., 2001; Gomes et al., 2003; Kang and Mills, 2004; Wieland et al., 2001).

It is extremely difficult to study the effects of a single factor in isolation in soil. The aims of this study were therefore

(1) to implement a controllable cropping system in which bacterial communities adhering to the roots could be assessed in a dynamic and reproducible way and
(2) to investigate bacteria adhering to the roots, in the rhizoplane zone, in a soil-less system in which pH (a stabilising factor for bacterial community dynamics) and, conversely, the impact of a strong perturbation of the root environment due to the addition of chlorine were varied.

2. Materials and methods

2.1. Trial

The trial took place at the *Unité de Recherches Intégrées en Horticulture* (URIH), INRA, Sophia-Antipolis, France. Six rows of 35 "Red France" rose plants (Rosaplant Co.) were installed in NFT gutters, with their roots in direct, continuous contact with a nutrient solution, which was recycled through a two-tank system (120 l each) with volumetric pumps (flow: 300 l h^{-1}). This made it possible to measure physicochemical factors, such as the temperature, electroconductivity, pH and active free chlorine concentration of the root environment in which bacterial communities evolve. Nutrient solution was produced from water subjected to reverse osmosis and had the following composition: 7 meq l^{-1} NO_3, 7.1 meq l^{-1} $H_2PO_4^-$, 2.4 meq l^{-1} SO_4^{2-}, 0.7 meq l^{-1} Cl, 3.5 meq l^{-1} K^+, 4.5 meq l^{-1} Ca^{2+}, 2.4 meq l^{-1} Mg^{2+}, 0.7 meq l^{-1} Na^+, 1 mg l^{-1} Fe, 0.063 mg l^{-1} Cu, 0.226 mg l^{-1} Zn, 0.50 mg l^{-1} Mn, 0.027 mg l^{-1} Mo and 0.265 mg l^{-1} B. The composition of the solution in the tanks was checked and corrected weekly to keep it constant. The six rows, which were installed 10 months before the treatments, were organised into two plots: A and B. The three rows in each plot (A1, A2, A3 and B1, B2, B3) corresponded to three replicates of the same trial. The pH varied between 5.8 and 7.5 before T0, but was adjusted to 6.2 at T0. The effect of active free chlorine in this system was investigated by adding sodium hypochlorite to the nutrient solution of plot B (rows B1, B2, B3) via a peristaltic pump (Bioblock Scientific). From T0 onwards, active free chlorine concentration was maintained at 0.15 mg l^{-1} (standard deviation: 0.01 mg l^{-1}) by means of a Dulcotest D2C system connected to a CLE 0.5 (Prominent) probe. Sodium ion concentration was maintained at equivalent levels in the non-chlorinated plot A (rows A1, A2, A3) by adding sodium chloride. Plots A and B experienced identical physicochemical conditions, with the exception of active free chlorine concentration, throughout the study. Temperature varied each day from 12 to 24 °C, with similar variations in each row.

2.2. Microbial community analysis

2.2.1. Sampling of bacterial cells from the nutrient solution and roots

Samples of nutrient solution were taken from plots A and B and samples of plant root tips were taken from the six rows on days T0−15, T0, T0 + 8, T0 + 15, T0 + 20 and T0 + 25. Rhizoplane bacteria were defined as cells adhering to the roots that could be removed by the following protocol. Samples from white plant root tips were cut into small pieces (1−2 cm in length, with 5 g of wet root material suspended in 5 ml of isotonic sterile water). The rhizoplane cells were removed from the washed root material by shaking for 10 min at 150 rpm in a small, custom-made rotary shaker. The suspension obtained was filtered to eliminate debris (pore size: 0.7 mm). Viable plate counts on LPGA medium were used to evaluate the numbers of bacteria in the nutrient solution and in the rhizoplane. There were seven dilutions and three replicates for each sample.

2.2.2. DNA extraction

Rhizoplane bacterial suspensions were centrifuged (15 min at 8000*g*). Supernatants were discarded and cell pellets were resuspended in 575 µl of lysis buffer (500 µl of 4 M guanidine thiocyanate in 0.1 M Tris–HCl pH 7.5 and 75 µl of 10% *N*-lauroyl sarcosine). A 250 µl aliquot of cell suspension was transferred to a 2 ml screw-cap tube, frozen and stored at −20 °C. For total DNA extraction, 500 µl of 5% *N*-lauroyl sarcosine in 0.1 M phosphate buffer, pH 8 was added to each tube and the mixtures were incubated for 1 h in a 70 °C water bath. The tubes were then subjected to sonication for 30 s to complete cell lysis. DNA was extracted as described by Delbès et al. (2000). The extracted DNA was purified on Qiamp DNA minikit columns (Qiagen).

2.2.3. PCR-SSCP amplification of total DNA

For PCR-SSCP analysis, the target DNA was the variable V3 region of bacterial 16S rRNA genes, corresponding to a fragment of about 200 bp in *Escherichia coli* (position 331–533) (Brosius et al., 1981). The bacterial primers used in this study, corresponding to conserved sequences bordering this region, were w49 (5′-ACGGTCCAGACTCCTACGGG; forward, *E. coli* position 330) and w34 (5′-TTACCGCGGCTGCTGGCAC; reverse, *E. coli* position 533) (Zumstein et al., 2000). The w34 primer was labelled with 5′-fluorescein phosphoramidite (TET, Applied Biosystems, Perkin-Elmer). Both primers were obtained commercially (MWG, Biotech). Amplifications were performed with a GeneAmp 9700 thermocycler (PE Applied Biosystems). The PCR mixture contained 2.5 ng µl^{-1} of each primer, 0.2 mM of each deoxyribonucleotide triphosphate (dNTP), 1 × cloned *Pfu* turbo DNA polymerase reaction buffer (Stratagene), about 50 ng of genomic DNA and 1.25 U of cloned *Pfu* turbo DNA polymerase (Stratagene), in a total volume of 50 µl.