MOLECULAR FINGERPRINTING OF MICROBIAL POPULATIONS IN SOILLESS CULTURE SYSTEMS

J. Postma, J.B.L. Lankwarden and J.D. van Elsas
Plant Research International,
PO Box 16, 6700 AA Wageningen,
The Netherlands
Email: J.Postma@plant.wag-ur.nl

Keywords: denaturing gradient gel electrophoresis, disease suppression, *Pythium aphanidermatum*, slow sand filtration

Abstract

The molecular fingerprinting technique (PCR-DGGE) of microbial populations was successfully adopted for soilless culture systems. Relatively low concentrations of micro-organisms in the nutrient solutions could be assessed. PCR-DGGE was applied in several experiments to compare the composition and diversity of the bacterial populations in different treatments, as well as at different locations and ages of the crop. Although numbers of culturable aerobic bacteria were similar in different rockwool treatments, the PCR-DGGE patterns showed that the bacterial composition and diversity differed. The bacterial composition in treatments which were suppressive to *Pythium aphanidermatum* (i.e. previously used rockwool and used rockwool that was recolonised after autoclaving) differed from that in autoclaved used rockwool with a high disease incidence. Additionally, a shift in the bacterial populations during plant growth was seen. Samples of the nutrient solution from different locations in a closed soilless culture system, i.e. drain, effluent, and solution in the rockwool slabs, showed different banding patterns. The results summarised in this paper showed that PCR-DGGE is a powerful tool to study the microflora under different conditions.

1. Introduction

In general, the strategy in glasshouse crops has always been to keep the growing systems as clean and aseptic as possible. Whereas in soil and peat the existence of a suppressive microflora towards root pathogens is generally accepted, suppressiveness in soilless systems has only been demonstrated recently. Artificial substrate suppressive towards fusarium wilt and pythium root rot could be created by growing consecutive infected crops reusing the nutrient solution or the substrate without disinfection (Rattink and Postma, 1996; Rattink unpublished). It was repeatedly demonstrated that a growing system is less sensitive for the development of pythium root rot if the autochthonous microflora is still present, as compared to a sterilised system (Postma *et al*., 2000). Remarkable were the results in NFT (nutrient film technique) systems with *Phytophthora* and *Pythium* in tomato, where closed systems showed less disease than run-to-waste systems (McPherson, 1998; Tu *et al*., 1999). These results show the potential of the microflora to inhibit root diseases in soilless growing systems. The recent research on slow (sand) filtration (van Os, this proceedings) is in line with these findings of the beneficial effects by the microflora. Slow filtration does not destroy the entire natural microflora and is expected to avoid unbalanced recolonisation, probably stimulating more stable and robust cropping systems as compared to a system where nearly the entire natural microflora is eradicated.

To stimulate microbiologically balanced growing systems, more knowledge on the microflora concerning specific bacterial and fungal groups, and on the presence of beneficial species is necessary. Several techniques to characterise the microflora are

available. Plate counts of specific bacterial and fungal groups on semi-selective media give information about population sizes of the culturable part of the population. The composition and diversity of total microbial populations can be studied with PCR-DGGE (polymerase chain reaction of 16S or 18S ribosomal RNA gene sequences, followed by denaturing gradient gel electrophoresis), a new and promising molecular fingerprinting technique (Muyzer *et al.*, 1993). The bands in the profile obtained most likely will correspond with dominant species present in the analysed sample. This technique has been applied to study the bacterial populations in several experiments carried out in different soilless culture systems.

Other techniques to characterise the microflora of closed systems are described elsewhere in this proceedings (Jung and Waechter Alsanius; Khalil *et al.*).

2. Materials and methods

To analyse the diversity and structure of the bacterial communities in soilless culture systems, rockwool or nutrient solution was sampled. Two vertical cores (2.5 cm diameter) were cut out of the rockwool of each replicate, from which 2 g of rockwool was sampled. From the nutrient solution, 100 to 350 ml solution was concentrated on a sterilised 0.2 µm filter (47 mm diameter), depending on the bacterial concentration in the solution. The rockwool samples and filters were stored at –20 °C. DNA was extracted from the samples with a bead beater and purified with a Wizard DNA Clean-up Kit. A variable region between positions 968 and 1,401 on the eubacterial 16S rDNA was enzymatically multiplied in a touch down PCR with two primers to conserved regions. The PCR products were applied to DGGE gels with a 45 to 65 % denaturing gradient. Detailed descriptions of the DNA extraction, PCR and DGGE procedures are given by Postma *et al.* (2000).

Numbers of culturable aerobic bacteria were determined by plate counting on one tenth strength tryptic soy agar (Postma *et al.*, 2000).

3 Results

Although numbers of culturable aerobic bacteria were similar in the different rockwool treatments, PCR-DGGE patterns showed that the bacterial composition and diversity differed. The bacterial composition in rockwool which was suppressive to *Pythium aphanidermatum* (i.e. previously used rockwool and used rockwool that was recolonised after autoclaving) differed from that in autoclaved used rockwool with a high disease incidence (Fig. 1). The PCR-DGGE patterns of the three replicates taken from three separate closed culture systems were not identical. However, the larger variation between treatments than between replicates of treatments allows comparison of treatments with PCR-DGGE patterns. Especially in the initial stages of the experiment, PCR-DGGE patterns showed that the bacterial communities were different between the rockwool treatments. Interestingly, some bands occurred only in the disease suppressive treatments, i.e. nontreated and autoclaved recolonised used rockwool. The structures of the bacterial communities changed during the experiment (not shown). PCR-DGGE patterns of replicate samples of the same rockwool block were highly similar (Figure 1).

Samples of the nutrient solution were taken at different locations in a cucumber crop grown in a closed soilless culture system at IMAG. Samples of the drain, effluent, and nutrient solution in the rockwool slabs, showed different PCR-DGGE banding patterns (Fig. 2). Dominant bands in the solution of the rockwool slabs were often almost absent in the drain, and vise versa. The samples of the drain and the nutrient solution in the slabs from the four separate systems (replicates) were remarkably similar when the crop was 8 weeks old (Fig. 2). This was not yet the case when the crop was only 4 weeks old, i.e. some variation in PCR-DGGE patterns between the four replicate systems occurred (not shown).

In another experiment where different filter media were compared, the PCR-

DGGE patterns of the effluent of slow sand and glasswool filters was different from those in the influent. Although bacterial numbers were grossly similar in this experiment, the numbers of bands seen in the profiles of the influent was higher than in those of the effluent (approx. 10 % more). However, no clear differences due to the filter medium were visible (Postma *et al.*, 1999).

4. Discussion

Denaturing gradient gel electrophoresis applied to mixed amplicons generated by bacterial, fungal or otherwise specific PCR of community DNA allows genetic fingerprinting of bacterial, fungal or other populations (Muyzer *et al.*, 1993). With this technique the composition of the microflora (including non-culturable micro-organisms) and the genetic diversity of bacterial and fungal populations can be determined. PCR-DGGE applied to bacterial communities in soil and in the rhizosphere over a time course spanning the development to maturity of *Chrysanthemum* plants showed only few changes in bacterial composition, probably due to the dominance of recalcitrant nonculturable organisms in soil (Duineveld *et al.*, 1998). Our results in rockwool and nutrient solutions, however, showed that the PCR-DGGE patterns were distinctly different between treatments, locations of sampling and the age of the crop. In contrast with soil systems, the soilless culture systems start with low numbers of micro-organisms and do not have a long "microbial history". This makes the soilless systems sensitive to assessments by PCR-DGGE.

The PCR-DGGE patterns of drain and nutrient solution in the slabs from four separate systems (replicates) were remarkable similar in the 8-week old crop. Also PCR-DGGE patterns of replicate samples taken from the same rockwool block were similar, indicating the reliability of the technique. Therefore, different PCR-DGGE patterns between replicate treatments are expected to be the result of different bacterial communities in the replicates and not the result of variations due to the technique. Actually, the replicates were in all cases separate closed systems, and variations in bacterial communities can be expected.

Different PCR-DGGE patterns were detected between treatments with different levels of suppression of *P. aphanidermatum*. By analysing the bands that specifically occur in such disease suppressive treatments, it might be possible to detect the bacterial species responsible for the disease suppression. The consequences of the bacterial shifts due to slow (sand) filtration for potential beneficial properties of the microflora are not yet clear. Experiments assessing the effect of slow filtration on the characteristics of the microflora in relation with the occurrence of suppressiveness towards root diseases are necessary.

In this paper it is shown that differences in bacterial communities can be visualised with PCR-DGGE. The next step in the research on the development of microbiologically balanced soilless systems is to relate the banding patterns with key organisms involved in suppression of plant diseases. In addition, group specific PCR-DGGE of actinomycetes, pseudomonads and other groups will be applied, allowing the characterisation of less abundant occurring sub-populations which are expected to play a role in disease suppression.

Acknowledgements

Results were obtained from different research projects, which were supported by the Dutch-Israeli Agricultural Research Program (DIARP), the Commission of the European Communities, Agriculture and Fisheries (FAIR), The Product Board for Horticulture in the Netherlands, and the Dutch Ministry of Agriculture, Nature Management and Fisheries.

References

Duineveld B.M., Rosado A.S., van Elsas J.D. and van Veen J.A., 1998. Analysis of the dynamics of bacterial communities in the rhizosphere of the chrysanthemum via denaturing gradient gel electrophoresis and substrate utilization patterns. Appl. Environ. Microbiol. 64: 4950-4957.

McPherson G.M., 1998. Root diseases in hydroponics - their control by disinfection and evidence for suppression in closed systems. In: Proceedings 7th International Congress of Plant Pathology, Edinburgh (Abstract 3.8.1S).

Muyzer G., de Waal E.C. and Uitterlinde A., 1993. Profiling of complex microbial populations using denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl. Environ. Microbiol. 59: 695-700.

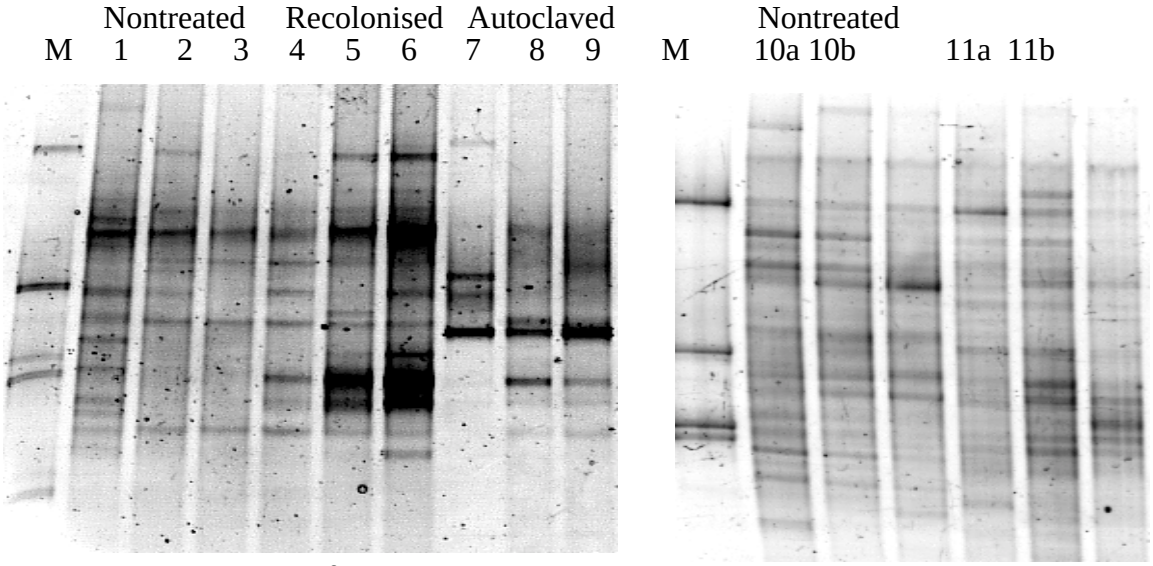
Postma J., van Os E.A. and Kritzman G., 1999. Prevention of root diseases in closed soilless growing systems by microbial optimization. Mededelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent 64/3b: 431-440.

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

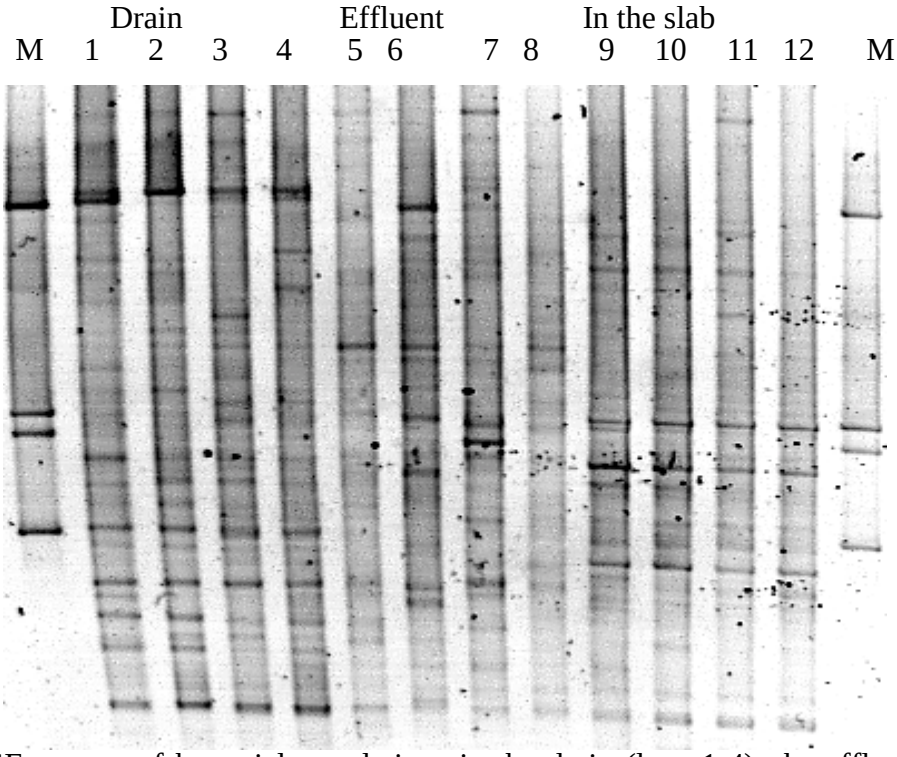
Rattink H. and Postma J., 1996. Biological control of fusarium wilt in carnations on a recirculation system by a nonpathogenic *Fusarium oxysporum* isolate. Mededelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent 61/2b: 491-498.

Tu J.C., Papadopoulos A.P., Hao X. and Zheng J., 1999. The relationship of a pythium root rot and rhizosphere micro-organisms in a closed circulating and an open system in rockwool culture of tomato. Acta Horticulturae 481: 577-583.

Figures



1. PCR-DGGE pattern of bacterial populations in used rockwool which was nontreated (lane 1-3), autoclaved and recolonised (lane 4-6), or autoclaved (lane 7-9) 7 days after planting. Lane 10a & 10b, and 11a &11b are duplicate samples taken from one rockwool block 35 days after planting. M = bacterial marker.



2. PCR-DGGE pattern of bacterial populations in the drain (lane 1-4), the effluent just below slow sand filters (lane 5-8), and the nutrient solution in the rockwool slabs (lane 9-12). Samples were taken from an 8-week old cucumber crop grown in closed soilless culture systems at IMAG. M = bacterial marker.