Molecular Identification of Microbial Communities in the Recycled Nutrient Solution of a Tomato Glasshouse Soil-Less Culture

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Abstract: This study aimed to assess microbial community diversity in recycled nutrient solutions used in soil-less glasshouse cultivation of tomato. One hundred bacterial strains, twenty oomycetes and 6 fungi were isolated and identified through genomic DNA isolation, PCR amplification of the ribosomal DNA region and database interrogations. Similarities of ITS regions with known species ranged from 95% to 100%. This artificial ecosystem was shown to be microbiologically diverse, since recovered isolate were close to 35 bacterial species, 11 oomycete species and 3 fungal species. Bacteria belonged almost exclusively to γ-Proteobacteria and Firmicutes, with most represented genera being *Bacillus*, *Acinetobacter*, *Klebsiella* and *Serratia*. A few bacterial sequences grouped with clones similar to plant and human pathogens, while other isolates could be protective bacteria such as *Pseudomonas fluorescens*. Oomycetes isolated mostly belonged to the genus *Pythium* (19 isolates) and were phylogenetically related to common cosmopolitan soil inhabitants or phytopathogenic *Pythium* species. The six fungal isolates were in 2 genera, *Rhizopus* and *Caesia*; *Rhizopus* isolates were closely related to the post harvest pathogen *Rhizopus stolonifer*. This original work adds to the efforts of assessing microorganism diversity in recycled nutrient solutions commonly used in glasshouse vegetable production; microbial diversity was high and included potential plant pathogens. This study demonstrated the existence of a wide cultivable microbial community in the nutrient solution before recycling and recirculation and supported the necessity for disinfecting nutrient solutions used in soil-less cultivation systems, during the recycling process, in order to ensure crop sanitation and avoiding plant disease spreading.

INTRODUCTION

Capturing and recycling irrigation runoff is now a common practice in the plant nursery industry, particularly in soil-less crop production, in the wake of environmental concerns and global water scarcity. In soil-less systems glasshouse crops are grown on artificial substrates that are preferred to soil-based media for economic reasons, and because of improved control over water, aeration, nutrition, and root distribution [1]. Traditionally, soil-less culture was operated as drain-to-waste or open systems, in which excess nutrient solutions were allowed to drain to the soil and into groundwater. Crops grown under these conditions were usually irrigated in excess in order to balance both variations in transpiration and nutrient demands of the individual plants and variations within the system in nutrient solution supply to the plants [1-2]. Up to 40% of the nutrient solution dosed per day might be in excess of crop requirements [3]. However, growers and advisory bodies now recognize that, for environmental and economic reasons, excess nutrient solutions should not be drained to waste, but collected and re-used within closed or re-circulating systems. Closed re-circulating

systems, in addition to promoting sustainability of glasshouse vegetable production, might economically reward growers, with savings in water and fertilizer costs.

Current water recycling methods, however, may return some economically important plant pathogens to irrigation reservoirs from where these organisms can spread further onto the crops. Pathogens of economic significance isolated from irrigation water include bacteria, fungi, nematodes and oomycetes [4]. Among oomycetes, *Pythium* spp. are more frequent than *Phytophthora* spp. in irrigation water [5]. Unlike *Phytophthora* pathogens, however, the potential impacts of *Pythium* species in irrigation water are poorly understood. This is due to the difficulty in identification of *Pythium* species and the large, undescribed diversity in this genus [6]. It was also reported in four soilless systems of tomato cultures that bacteria (*Pseudomonads*) predominated over fungi (*Fusarium* spp.) and *oomycota* (Pythium). [7]

There is growing public awareness that fresh or minimally processed fruit and vegetables can be sources of disease-causing bacteria, fungal toxins, viruses, protozoa and helminthes, inspiring new public health and food policies worldwide with an impact on harvesting practices in the soilless crop industry. Contaminated re-circulating nutrient solutions are one important way in which fruit and vegetables may become contaminated with food-borne pathogens or fungal toxins [8].

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Understanding the microbial community structure in recycled nutrient solutions is essential for understanding microbial processes which underlie secondary contamination of the culture system. In order to reliably characterize the community structure, reliable and rapid qualitative methods, such as rRNA sequencing are required. Little is known of microbial diversity and population structures in recycled nutrient solutions; to date, however, no community structure studies have been carried out using such techniques, although the possibility that plant or human pathogens could contaminate recycled nutrient solution was recognized [9]. This paper reports a first assessment of the diversity of cultivable bacterial, fungal and oomycetes present in a recirculating nutrient solution system, identified using culture on selective media and sequence analysis of main representative isolates in these communities. This study was done in parallel with the evaluation of disinfecting systems of recycled nutrient solution [10]

MATERIALS AND METHODS

Glasshouse Trials

Experimental trials in closed soil-less systems were carried out at the Vegetable Crop section of the Professional Training Centre of Lullier (Jussy, Switzerland) during 2005. Tomato plants (hyb. Temptation [Enza Zaden BV, The Netherlands]), 30 days old were grown on Rockwool cubes (Rockwool, Mid-Glamorgan, UK) and transplanted into Rockwool substrate. Plants were maintained on concrete benches with a drip irrigation system (one drip nozzle per plant, flow rate: 2 L/h) in a soil-less culture. General information on water, fertilizers and the nutrient solution used was previously reported [11]. The ultrafiltration system used for disinfecting the recirculating nutrient solution was an ultrafiltration membrane (HYDRAcap, Hydranautics, USA) constituted of polyethersulfone capillaries (inside diam. of 0.8 mm; outside diam. of 1.2 mm; molecular weight cut-off about 100.000-150.000 Daltons) [10].

Chemical Analysis of Nutrient and Recycled Nutrient Solutions

Nutrient solution and recycled nutrient solution samples from tomato cultures were analyzed for total phosphorus (P), potassium (K), sulphur (SO_4), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), NH_4-N, NO_3-N, pH and electro conductivity (EC) at the beginning and after two months of culture Analytical methods used were inductively coupled plasma atomic emission spectroscopy (ICP-AES) for all ions except for NO_3-N which was assayed by the colorimetric method of Griss. Analysis of 1 L samples was carried out by SADEF-Pôle d'Aspach (France).

Evaluation of the Cultivable Microbiota in Irrigation Water

Bacteria, fungi and oomycete counts were obtained after 10-fold dilution plating on adequate culture media The cultivable bacterial population present in nutrient solution was obtained by passing used nutrient solution (5 L) through a 47 mm diam. Durapore®, 0.22 µm pore size membrane. Bacteria present on the filter were then released by vortexing the filter in 2 mL sterile water in an Eppendorf tube and serial dilution

plating on Rose Bengal agar in 90 mm diam. Petri dishes. Bacteria colonies were counted and checked visually for colony shape and morphology, and DNA was extracted from most bacteria observed in preparation for 16S rDNA PCR amplification and sequencing.

The cultivable fungal populations present in nutrient solution was obtained by passing 5 L of the same solution through a 47 mm diam. Durapore® membrane, 5.0 µm pore size [12]. Spores trapped on the membrane were re-suspended by vortexing in 2 mL sterile water in an Eppendorf tube for 30 s. Aliquots of 100 µL of the membrane washing were then spread evenly onto 20 mL of Potato Dextrose Agar (PDA) ampicillin in a 90-mm diam. Petri dish. Colonies recovered were counted and used in DNA extractions, ITS rDNA amplification and direct sequencing.

The cultivable oomycete population present in nutrient solution waste obtained by filtration of a 5 L sample of the used nutrient solution through a 5.0 µm pore sized membrane and trapped spores re-suspended in water, as described above [12]. Aliquots of 100 µL of the membrane washing were spread evenly on 20 mL of PARP-V8 agar [13], incubated at 23°C in the dark and examined daily with a dissecting microscope for mycelial growth. Developing colonies provisionally identified as *Pythiaceae* species were counted daily in each dish until no new colonies were found. Identities of these colonies were confirmed by examination of morphological traits, based on keys for identifying *Phytophthora* [13] and *Pythium* [14].

DNA Extraction and Molecular Identification of Recovered Isolates

Representative colonies recovered from nutrient solution runoff samples were chosen for DNA extraction, internal transcribed spacer of the ribosomal RNA (ITS rDNA) amplification and direct sequencing.

DNA was purified as previously reported [15] and DNA concentration was assessed in a NanoDrop miniaturized spectrophotometer (NanoDrop, USA). Reference identities of the tested bacterial, fungal and oomycete isolates were obtained by genotypic identification through DNA sequencing of target genes amplified by the polymerase chain reaction (PCR) in a Master Gradient thermocycler (Eppendorf, Germany). Bacterial strains were identified by 16S rDNA sequencing. 16S rDNA amplification of bacterial samples was carried out using the previously described primers fd1 and rp2 [16] (Table **1**). PCR conditions included an initial denaturation step of 95°C for 2 min followed by 35 cycles of 95°C (20 s), 42°C (30 s) and 72°C (2 min) terminated with a final 20 min chain elongation step. All oomycete isolates were identified by ITS1, 5.8S ribosomal gene and ITS2 gene sequencing. ITS amplifications of oomycete samples were carried out using universal primers ITS4 and ITS6 [17] (Table **1**). The reaction mixture contained 1x PCR buffer (75 mm Tris-HCl (pH 9.0), 50 mM KCl, 20 mM $(NH_4)_2$ SO_4), 0.1 mM dNTPs, 0.25 µM of each primer, 1.5 mM $MgCl_2$, 1 U of Taq Polymerase (Biotools, Spain) and 1 µL of mycelial DNA in a total volume of 50 µL and the following amplification program was applied: an initial denaturation step of 95°C for 2 min followed by 30 cycles including denaturation for 20 s at 95°C, annealing for 25 s at 55°C and extension for

Table 1.   PCR Primers Used in the Study: Oligonucleotide Sequences fd1 and rpe [16], ITS4, ITS5, ITS6 [17] and Location within Genomic DNA

Primer	Sense	Sequence (5'→ 3')	Location
ITS4	Reverse	TCCTCCGCTTATTGATATGC	28S gene
ITS5	Forward	GGAAGTAAAAGTCGTAACAAGG	18S gene
ITS6	Forward	GAAGGTGAAGTCGTAA	18S gene
fd1	Forward	AGAGTTTGATCCTGGCTCAG	16S gene
rp2	Reverse	ACGGCTACCTTGTTACGACTT	16S gene

50 s at 72°C. Amplification was terminated by a final extension step of 10 min at 72°C [17]. Fungal isolates were processed exactly as described for oomycetes, except that primer ITS5 [17] (Table **1**) was used in place of ITS4.

Aliquots of PCR products for all sample types were separated in 1% agarose (Biofinex, Switzerland) gels in 1x TBE, at 100 V for 1 h, stained with ethidium bromide (0.5 mg.L^{-1}) and visualized under UV light. PCR products were purified with the QIAquick PCR purification Kit (Qiagen, Hilden, Germany) and used for direct sequencing (Fasteris SA, Geneva, Switzerland). Ribosomal DNA sequences were compared using BLAST against the GenBank database (NCBI, Bethesda, USA). Identification of isolate to the species or genus level was based on the highest identity scores.

Nucleotide Sequence Accession Number

DNA sequences of the bacterial, fungal and oomycete isolates from recycled nutrient solutions were registered in GenBank under the following accession numbers DQ190274 – DQ190372 for 100 bacterial isolates, DQ195041 - DQ195059 and DQ195066 for 20 oomycete isolates and DQ195060 - DQ195065 for 6 fungi isolates.

Molecular Phylogenies

Molecular phylogenies of recovered eubacteria and stramenopiles were prepared using a previously published protocol [15- 18].

RESULTS

Recycled Nutrient Solution Constituents

Chemical analyses of the elements P, K, SO$_4$ Mg, Fe, NH$_4$-N, NO$_3$-N, pH and electro conductivity (EC) are shown in Table **2**. In tomato cultures, the recycled nutrient solution had higher concentrations of Ca, Mg, Fe and NO3-N and the solution pH decreased. EC values increased.

Molecular Identification of Cultivable Microbiota in Re-Circulating Nutrient Solution

Bacteria, fungi and oomycetes isolated from the recycling nutrient solution were identified by 16S rDNA sequencing for bacteria and ITS rDNA sequencing for eukaryotic organisms.

A comparison of the sequence of the PCR products from micro-organisms to those in the GenBank database revealed homologies with many previously characterized strains. In general, most sequences showed 95 - 100% similarity to the different groups identified (Table **3**).

After removal of suspected chimeral or poor quality sequences, 100 bacterial, 6 fungal and 21 oomycete sequences were registered in GenBank (Table **3**). Bacteria accounted for 79% and Eukaryota for 21% of the recovered isolates. Within the Bacteria, Proteobacteria represented 63% of isolates, Firmicutes 35%, Bacteroidetes 1% and Actinobacteria 1%. In the Eukaryota, Stramenopiles accounted for 80% and Fungi 20% of the recovered strains

Comparison of selected PCR products against the GenBank database showed that bacterial sequences almost exclusively clustered in two eubacterial groups: the γ-*Proteobacteria* (63 sequences) and the Firmicutes (33 sequences). One sequence grouped with the *bacteriodetes* and one with the *Actinobacteria*.

Most represented genera were *Bacillus* (30 isolates), *Acinetobacter* (28 isolates), *Klebsiella* (6 isolates) *and Serratia.*(6 isolates). Two main clusters of γ-*Proteobacteria* were detected (Fig. **1**), representing the *Acinetobacter* group and a group including *Enterobacter*, *Klebsiella*, *Serratia*, *Pectobacterium*, *Aeromonas* and *Pseudomonas*.

One main cluster *Bacillus* cluster was also detected in the Firmicutes (Fig. **1**).

Table 2.   Properties of Initial Nutrient Solution and Recycled Nutrient Solutions Used in the Experiments

Source	pH	EC (mS cm^{-1})	Phosphore (mg liter^{-1})	Potassium (mg liter^{-1})	Calcium (mg liter^{-1})	Magnesium (mg liter^{-1})	Iron (mg liter^{-1})	Manganese (mg liter^{-1})	N-No$_3$ (mg liter^{-1})	N-NH$_4$ (mg liter^{-1})	Sulfur (mg liter^{-1})
Nutrient solution	6.04	2.33	37.3	298	184	63	1.09	0.98	253	30.9	206
Recycled solution	4.7	3.1	41.7	359	292	100	3.55	1.44	369	30.4	326

Table 3.   Distribution of the rRNA Sequences 16S from Bacterial Isolates and ITS Sequences from Oomycete and Fungal Isolates Obtained in this Study. The Given Species or Subspecies Represents the Species Showing the Highest Similarity with the Isolated Organism in the Range 95-100% Identity

| Domain	Phyllum	Class	Order	Family	Genus	Species	Subspecies	Number of Strains
Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	rhizosphaerae		1
Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	calcoaceticus		9
Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	johnsonii		9
Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	genomosp. 3		1
Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	haemolyticus		3
Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	venetianus		1
Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	junii		3
Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	tandoii		1
Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	putida		1
Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	fluorescens		1
Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	monteilii		1
Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	veronii		1
Bacteria	Proteobacteria	Gammaproteobacteria	Aeromonadales	Aeromonadaceae	Aeromonas	hydrophila		3
Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Serratia	marcescens		5
Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Serratia	fonticola		1
Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Klebsiella	planticola		1
Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Klebsiella	oxytoca		3
Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Klebsiella	pneumoniae		2
Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacter	hormaechei		2
Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacter	aerogenes		4
Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacter	cancerogenus		1
Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacter	amnigenus		2
Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Pectobacterium	carotovorum	carotovorum	2
Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Pectobacterium	carotovorum	wasabiae	1
Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Pantoea	agglomerans		1
Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	Marinobacter	lipolyticus		2
Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Citrobacter	freundii		1
Bacteria	Actinobacteria	Actinobacteridae	Actinomycetales	Micrococcineae	Arthrobacter	nicotianae		1
Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	cereus		25
Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	thuringiensis		4
Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	subtilis		1
Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	Lysinibacillus	fusiformis		3
Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	sanguinis		1
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	disporicum		1
Bacteria	Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	Chryseobacterium	indologenes		1
Eukaryota	Stramenopiles	Oomycetes	Pythiales	Pythiaceae	Pythium	dissotocum		3
Eukaryota	Stramenopiles	Oomycetes	Pythiales	Pythiaceae	Pythium	spinosum		1
Eukaryota	Stramenopiles	Oomycetes	Pythiales	Pythiaceae	Pythium	pachycaule		1
Eukaryota	Stramenopiles	Oomycetes	Pythiales	Pythiaceae	Pythium	diclinum		8
Eukaryota	Stramenopiles	Oomycetes	Pythiales	Pythiaceae	Pythium	marinum		1
Eukaryota	Stramenopiles	Oomycetes	Pythiales	Pythiaceae	Pythium	coloratum		1
Eukaryota	Stramenopiles	Oomycetes	Pythiales	Pythiaceae	Pythium	lutarium		1
Eukaryota	Stramenopiles	Oomycetes	Pythiales	Pythiaceae	Pythium	helicoides		1
Eukaryota	Stramenopiles	Oomycetes	Pythiales	Pythiaceae	Pythium	aphanidermatum		1
Eukaryota	Stramenopiles	Oomycetes	Pythiales	Pythiaceae	Pythium	scleroteichum		1
Eukaryota	Stramenopiles	Phaeophyceae	Scytothamnales		Splachnidium	rugosum		1
Eukaryota	Fungi	Mucoromycotina	Mucorales	Mucoraceae	Rhizopus	stolonifer		4
Eukaryota	Fungi	Basidiomycota	Agaricomycotina	Polyporales	Postia	subcaesia		1
Eukaryota	Fungi	Basidiomycota	Agaricomycotina	Polyporales	Postia	caesia		1
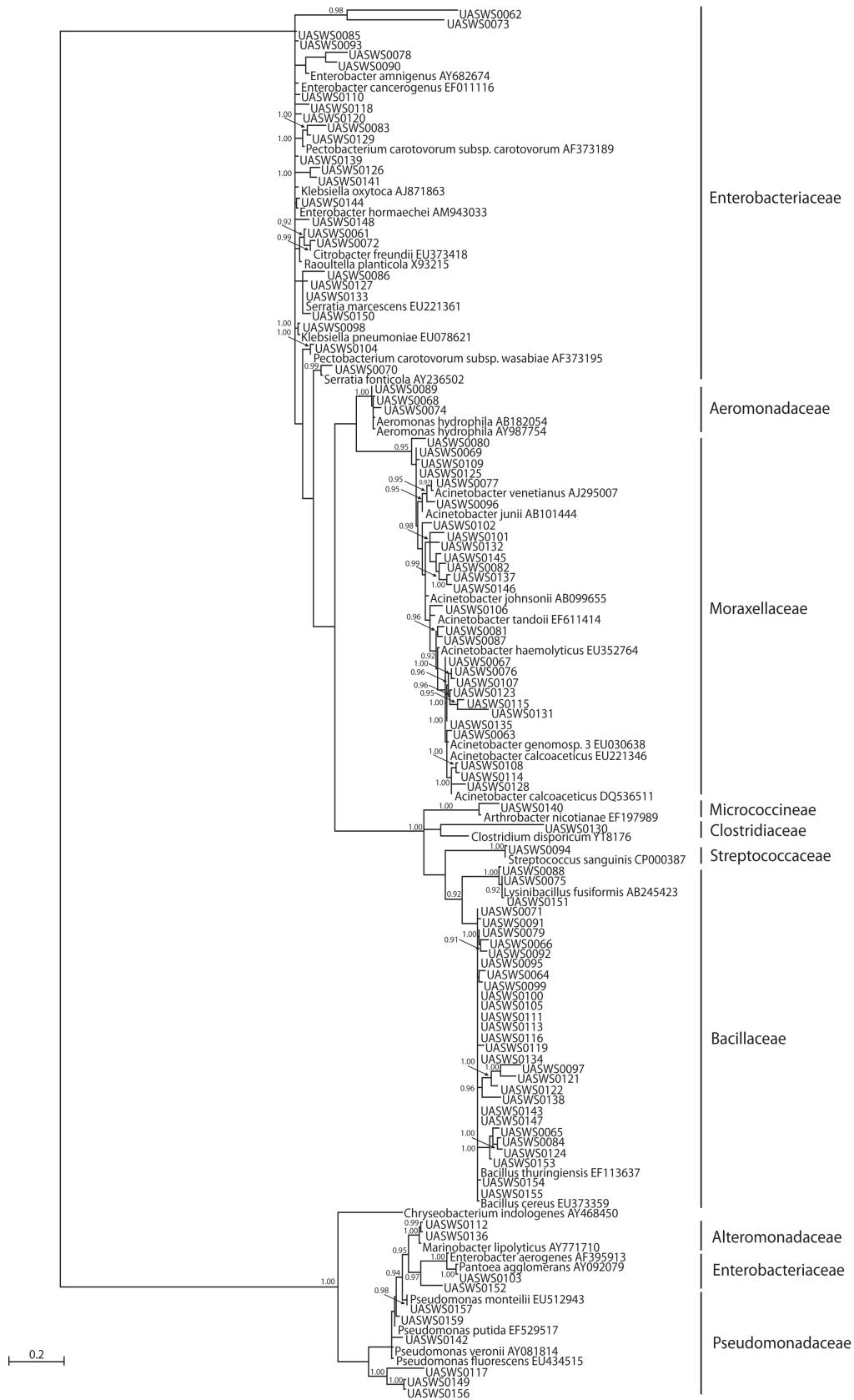
Fig. (1). Bayesian inference (BI) tree showing the relationships among the 16S rRNA sequences of the bacterial isolates recovered from recycled nutrient solution. The scale corresponds to 0.2 substitutions per nucleotide position. Values at the different nodes indicate bootstrap support (>0.90) from 2.000.000 generations.
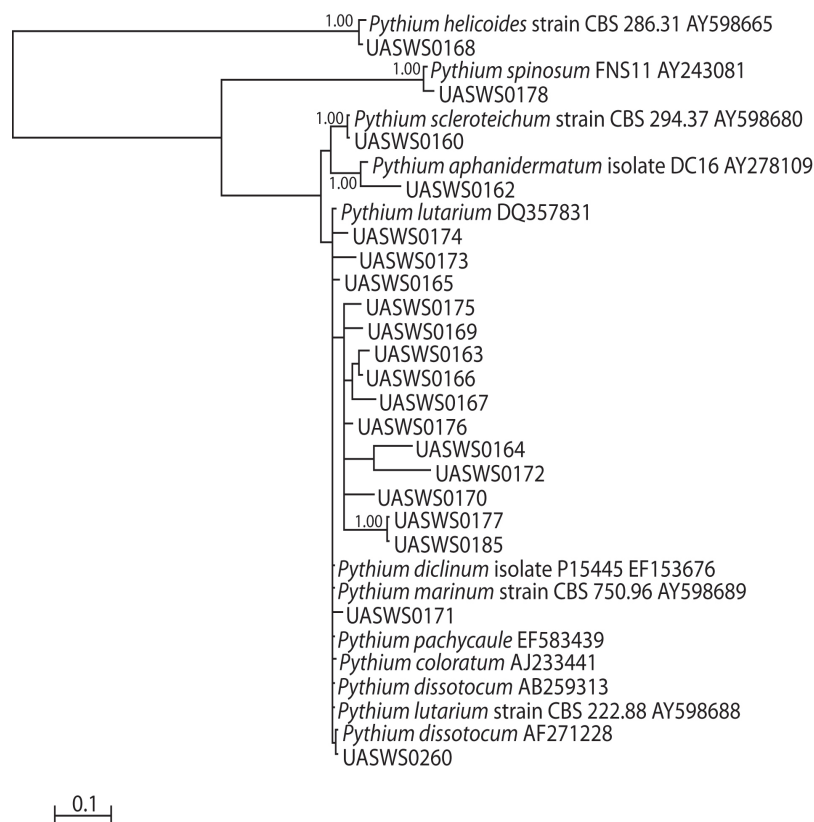
Fig. (2). Bayesian inference (BI) tree showing the relationships among the ITS sequences of Pythium isolates from the recycled nutrient solution. The scale corresponds to 0.1 substitutions per nucleotide position. Values at the different nodes indicate bootstrap (>0.90) support from 2.000.000 generations.

Out of 20 Stramenopile species isolated, one belonged to the genus *Phaeophyceae* and 19 to the the *Pythium* genus (*Oomycota*). Most abundant isolates showed a 99% sequence homology with known *Pythium*, slightly root pathogens as P. dissotocum or known cosmopolitan soil or water inhabitants such as *P. marinum, P. dissotocum, P. aquatile, P. diclinum* and *P. lutarium*. At last one *Pythium* species was found with homology to *P. aphanidermatum*, one isolate had a very strong sequence (> 99%) homology with *Pythium helicoides* and another one with *Pythium scleroteichum*. Phylogeny of found Pythium is shown on Fig. (**2**).

Fungi were the less represented organisms and fungal species identified comprised 4 isolates of *Rhizopus* and 2 of *Postia*. The *Rhizopus* isolates grouped with *Rhizopus stolonifer*, whereas *Postia* isolates grouped with *Postia caesia*.

DISCUSSION

Analyses of the recycled nutrient solution compared with the initial nutrient solution used in the soil-less cultivation of tomato in this work demonstrated clear changes in the concentrations of the different ions investigated, before the disinfected nutrient solution be adapted and recirculated. Such analyses were complemented by measurement of electro conductivity and pH of the solution. They showed that metal and sulphate concentrations increased in the used nutrient solution. The amounts of total P, K, SO_4, Ca, Mg, Fe, Mn, and N as NH_4 or NO_3, pH and EC showed considerable variation, which is routinely adapted to optimal nutritional conditions in culture by additions of nutrients to the filtered recycles nutrient solution. The diversity of microbial communities is known to be influenced by the medium composition [19]; it is possible, therefore, that increasing amounts of the measured elements here could impact on microbial community development in soil-less culture systems, favouring more tolerant organisms over sensitive species. This aspect should be further investigated with adequate replications, not carried out in of the course of the present study.

The water from the nutrient recycling system storage tank contained 1.04×10^5 CFU bacteria mL^{-1}. Some microorganisms could be well adapted to this environment, as supported by other studies demonstrating that metal stress did not always reduce overall productivity or biomass [20].

However, nutrient solution microbiota may have been overlooked in the past, as species discrimination was usually considered a difficult and was mainly conducted by colony morphology observations on selective media. Moreover, opportunistic pathogens may grow slowly or not grow at all on routinely used culture media, consequently escaping detection. Some highly discriminatory technologies, such as mass sequencing of ribosomal RNA, are well suited to profiling microbial populations in re-circulating nutrient solutions and could allow assessment of non cultivable organisms. Direct detection of pathogens by PCR circumvents problems with the morphological identification of the organisms and the production of sexual structures on standard microbiological media. Results obtained here differed from

previous observations. For instance, in tomato production in four different soilless systems (rockwool, coco peat; and nutrient film technique) [7], bacteria and fungi were assessed by simple colony forming units (cfu) counting. Bacteria were largely predominant over fungi (with *Pythium* considered as fungi in this study) and Pseudomonads detected by fluorescence represented 20-50% of counted bacteria. In all systems but the deep flow technique system, *Fusarium* fungi dominated over *Pythium* species. In comparison in our coco peat system, bacteria dominated, but *Pseudomonas* were very rare while prominent genera were *Bacillus* (30 isolates) and *Acinetobacter* (28 isolates. *Pythium* ssp. came second in isolates numbers and fungi were the rarest organisms in the nutrient solution. No *Fusarium* isolates were recovered. Colony morphology and selective media were also used for isolating fungi (mainly *Fusarium* and *Trichoderma*) and oomycete from Gerbera cultures in open and closed soilless systems [21] Other methods have been developed: PCR-DGGE (density gradient gel electrophoresris was used for evaluating bacteria in soilless systems [22]. It proved useful for identifying aerobic cultivable bacteria at low concentrations and showed that bacterial composition and diversity differed between rockwool treatments. Most interesting observations included a shift in the bacterial populations during plant growth and also an important variation in banding pattern, thus in bacterial composition between nutrient solution samples taken from different locations in a closed soilless culture system (drain, effluent, and nutrient solution in rockwool slabs)

The bacteria genera found in the present study belonged mainly to γ-Proteobacteria (63 isolates), with 3 main genera *Acinetobacter* (28 isolates), *Klebsiella* (6 isolates) and *Serratia*, and to the Firmicutes (33 isolates of which 30 belonged to *Bacillus*). Lots of these bacteria grouped with bacteria previoulsy observed in lake water and old glacial ice [23-24], coastal marine sediment, sea floor sediment and subsurface and deep sediment [25], activated sewage sludge [26], chemostat enrichments [27], contaminated sediments and soils [28] and the rhizosphere of diverse plants [29]. These cultivable bacteria could be very common constituents of the recycled nutrient solution and might have importance in the ecology of the recycled nutrient solution. Very little are potential pathogens for plants (*Pectobacterium carotovorum subsp carotovorum, Arthrobacter nicotianae*) or animals (*Aeromonas hydrophyla*) or humans (*Citrobacter freundi, Klebsiella oxytoca, Klebsiella pneumoniae, Raoultella planticola, Serratia marcescens* and *Streptococcus sanguinis*). Potential humans pathogens could certainly be brought into the glasshouse by workers. *Acinetobacter* which was represented by 28 isolates is a very little known genus of ubiquist bacteria colonising soils, waters, plants, healthy skins of humans and animals [30]. Most unknown *Bacillus* isolates were close to the entomotoxin producing *B. thuringiensis* and to *B. cereus*, an ubiquist telluric bacterium known for its implication in food poisoning [31].

The association of some recycled nutrient solution clones with clones observed in polluted sites could suggest that some bacterial species in the recycled nutrient solution are related to a tolerance of high concentration of metal ions. The monitoring of the growth of such species could only be determined by the design and use of specific oligonucleotide probes.

Interestingly α-, *β-*, δ-, ε-*Proteobacteria* or *Cyanophyceae* were not retrieved in the course of this study, which might be explained by the limited coverage of the present probe set [19] which assessed only cultivable organisms.

The experiment revealed a clear abundance of γ-*Proteobacteria* and Firmicutes, a pattern which could result from changes in the metal concentration, or other environmental factors, for example, changes in solution temperatures in different parts of the recycling system, or the presence of other organisms reliant on the bacteria as a food source. A previous report suggested that γ-*Proteobacteria* were positively correlated to total metal concentrations in a fresh water environment [19]. Other environmental factors, although not studied here, such as the abundance of viruses [32], the protistan grazing pressure [33], or nematode grazing [34], or the plant roots exudates are known to influence the structure of microbial communities and might explain the distribution of the eubacterial groups identified in the present study. As for *Pythium* spp recovered in this study, most isolates were unknown species close to *P. lutarium* and *P. marinum,* cosmopolitan species found in soils and waters. Only a few isolates displayed a high similarity with known plant pathogens *P. aphanidermatum, P. helicoides, P. scleroteichum or P. dissotocum*. The rare fungal isolates were allocated for half to the plant pathogen *Rhizopus stolonifer.* Many environmental variables could thus be implicated in the composition of recycled nutrient solution microbial communities, and thus make it difficult to compare between studies carried in different conditions (substrate, systems, cultivated plants) and to then assess the impacts of all possible variables.

CONCLUSION

Root diseases and the potential for large scale dissemination of plant pathogens are major limiting factors affecting the use of recycled nutrient solutions. Disinfecting recycling solutions through different filtering systems is known to be effective for removing most microorganisms from recycling solutions [10]. Identification of micro-organisms present in recycled nutrient solutions supported that disinfection prior to the recycling and recirculating process would be required in order to produce quality plants and plant products.

This assessment of the microbial community in a recycled nutrient solution from a tomato culture showed a wide diversity of cultivable micro-organisms. Most of the isolated species were not known before. A few of them however showed a high similarity to some plant or animal pathogens highlighting the necessity for disinfecting recycled nutrient solutions. A few others could be advantageous and protecting organisms. This work could be further enhanced by studying the evolution of total microbiota species, cultivable and not cultivable, in course of culture and in function of the evolution of the chemical composition of the recycled solution.

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