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Molecular characterization and PCR detection of the melon pathogen *Acremonium cucurbitacearum*

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Abstract

Acremonium cucurbitacearum is a soil-borne pathogen that causes collapse of muskmelon and watermelon plants. Cluster analysis based on RAPD patterns, obtained from use of 25 primers, divided isolates of *A. cucurbitacearum* from Spain and USA into two major groups. Most isolates from the USA fell into group 1, however, genetic similarity was not highly correlated with geographical origins or with previously established VCG groups. Analysis of 5.8S-ITS sequences showed very little sequence variation among isolates of *A. cucurbitacearum*, most had identical 5.8S-ITS sequence. *Nodulisporium melonis*, previously reported to cause a similar disease in Japan, had a 5.8S-ITS sequence that was identical to that of isolate A-419 proposed as the type strain of *Acremonium cucurbitacearum* suggesting that the two fungal pathogens should be considered a single species. Phylogenetic analysis, based on the 5.8S-ITS region, indicated that *Acremonium cucurbitacearum* is a monophyletic taxon more closely related to *Plectosphaerella cucumerina* than to other species of the genus *Acremonium*. Based on the 5.8S-ITS nucleotide sequence, a polymerase chain reaction was designed and used for specific detection of *A. cucurbitacearum* in diseased plants.

Introduction

Acremonium cucurbitacearum is the causal agent of 'Acremonium collapse', a soil-borne disease affecting muskmelon and watermelon in Spain (García-Jimenéz et al., 1994; Alfaro-García et al., 1996), California (Bruton et al., 1995; Gwynne et al., 1997; Aegerter et al., 2000), Texas (Bruton et al., 1996), Italy (Infantino et al., 2002) and Portugal (unpublished data). Symptoms of Acremonium collapse include the absence of root hairs and tertiary roots, development of corky brown lesions on roots, and a general decay of the radical system, yellowing of crown leaves and a general wilting and death of plants when fruits approach maturity (García-Jimenéz et al., 1989). In Japan, *Nodulisporium melonis* causes a similar disease (Sato et al., 1995; Watanabe and Sato, 1995). *Nodulisporium melonis* is morphologically similar to *A. cucurbitacearum* producing similar asexual spores and gross colony characteristics. *Nodulisporium melonis* differs from *A. cucurbitacearum* in that it produces conidiophores that are irregularly branched or subverticillate.

Genetic variability within isolates of *A. cucurbitacearum* collected from Spain was previously investigated by random amplified polymorphisms of DNA (RAPD) and vegetative compatibility group (VCG) analyses (Vicente et al., 1999). A total of 8 VCGs and 14 RAPD patterns revealed heterogeneity among isolates of *A. cucurbitacearum*. A VCG study of isolates collected from the USA revealed that the major VCGs (2 and 3) were present on both continents (Abad et al., 2000). However, European and North American isolates have not been compared using molecular or DNA analysis

techniques. In addition, no attention has been paid to the phylogenetic position of *A. cucurbitacearum*.

Ribosomal DNA contains one transcriptional unit with a cluster of the genes coding for the 18S, 5.8S and 28S rRNAs and two internal transcribed spacers, ITS1 and ITS2 (White et al., 1990). Previous work has demonstrated that the complex ITS regions (non-coding and variable) and the 5.8S rRNA gene (coding and conserved) are useful for measuring close genealogical relationships, since they exhibit far greater interspecies differences than the 18S and 28S genes (Lee and Taylor, 1992). Moreover, the variable and conserved regions within the same molecule can be exploited to develop specific probes that can be applied at different taxonomic levels (Gardes and Bruns, 1993; Levesque et al., 1994; Tisserrat et al., 1994; Faggian et al., 1999). The use of specific probes as selective amplification primers offers an alternative approach for rapid detection of the fungus.

One objective of this work was to assess genetic variability among isolates of *A. cucurbitacearum* from Europe and North America on the basis of data from analyses of mutiple loci (RAPD). A second objective was to determine the usefulness of nucleotide sequence data from the 5.8S rRNA gene and the two ITS regions (from this point referred to as 5.8S-ITS region) in revealing phylogenetic relationships among *A. cucurbitacearum*, *N. melonis*, other representative *Acremonium* species and other fungal species commonly associated with cucurbit roots. An additional aim was to design PCR primers for use in detecting the pathogen.

Materials and methods

Fungal isolates and DNA preparation

Thirty-five fungal isolates were collected from muskmelon plants with symptoms of Acremonium collapse from different regions in Spain, Portugal and the USA, and previously identified on the basis of morphological studies as *A. cucurbitacearum* (García-Jimenéz et al., 1994; Alfaro-García et al., 1996). Two isolates of *N. melonis* (CBS 488.96 and CBS 489.96, the strain type) provided by Centralbureau voor Schimmelcultures (CBS, Utrecht, The Netherlands), were also included. Isolates of *A. charticola* (CBS 881.73), *A. crotocinigenum* (CBS 129.64), *A. kiliense* (CBS 122.29), *A. strictum* (CBS 310.85) and *A. sclerotigenum* (CBS 124.42) were included as reference species. Isolates of *Plectosporium tabacinum*, *Rhizoctonia solani, Macrophomina phaseolina, Fusarium oxysporum* f. sp. *melonis, Fusarium solani* f. sp. *cucurbitaceae*, *Verticillium dahliae*, *Monosporascus cannonballus*, *Rhyzopicnis vagum* and *Chaetomium* sp. were collected from muskmelon roots in Valencia and identified in our laboratory. The isolate of *Fusarium equiseti* (CECT 2149) was obtained from the Spanish Type Culture Collection (Valencia, Spain).

All isolates were grown on potato dextrose agar (PDA; Sigma) for 6–8 days in order to obtain spores, and transferred to 200 ml of potato dextrose broth (PDB; Sigma) in 500 ml Erlenmyer flasks. Total genomic DNA was isolated and purified according to the methods described elsewhere (Lee and Taylor, 1990), and it was diluted to a final concentration of 50–100 ng μl^{-1}.

RAPDs methods and analyses

Twenty-five random decamer primers OPA (1–20) and OPB (1, 2, 5, 8 and 10) (Operon Technologies, Alameda CA, EE.UU) were used. DNA amplifications were performed in a total volume of 25 µl containing 40–80 ng of genomic DNA, 0.2 µM primer, 0.1 mM dNTPs and 1U of DNA polymerase (Netzyme, Molecular Netline Bioproducts, N.E.E.D, SL, Valencia, Spain). The reaction mixtures were incubated in a thermalcycler (MJ Research Minicycler) during 40 cycles consisting of 1 min at 92 °C, 2 min at 36 °C and 2 min at 72 °C. The RAPDs-PCR products were separated on 1.4% agarose gels with 0.5× TBE buffer. After electrophoresis, gels were stained with ethidium bromide (0.5 mg ml^{-1}), and the DNA bands visualized under UV light. Sizes were estimated by comparison against a DNA length standard (100-bp molecular marker, Gibco BRI Life Technologies, Inc., Rockville, MD).

RAPDs bands obtained using the decamer primers were used to compare relatedness of *A. cucurbitacearum* isolates. The electrophoregrams were scanned and digitised using a LKB 2202 Ultrascan Laser Densitometer. The similarity distance between pairs of RAPD patterns were determined from the matrix of presence/absence of amplified fragments according to Dice's coefficient with the SimQual program of NTSYSpc version 1.8 (Exceter Software, Setauket, NY). This measure of similarity corresponds to the fraction of average

RAPD fragment differences between two patterns. These similarity values were used to infer a dendrogram through cluster analyses according to the UPGMA method (Sokal and Michner, 1958), using the SAHN and TREE programs in NTSYS.

Amplification, sequencing and phylogenetic analysis

The 5.8S-ITS region was amplified by PCR using the universal primers its5 and its4 (White et al., 1990). PCR products were cleaned with the GeneClean II Purification Kit (Bio 101, La Jolla, CA, USA) and directly sequenced using the Tag DyeDeoxy terminator cycle sequencing Kit (Applied Biosystems, Falmer, Brighton, UK), according to the manufacturer's instructions, in an Applied Biosystems automatic DNA sequencer (model 373A). The primers its5 and its4 were used to obtain the sequence of both strands.

The 5.8S-ITS sequences from 15 isolates of *A. cucurbitacearum* representative of different VCGs, geographical origins and genetic variability based on RAPD patterns were obtained for phylogenetic analysis. Sequences from the two isolates of *N. melonis* and one isolate each of *A. crotocinigenum*, *A. sclerotigenum*, *A. charticola*, *A. kiliense* and *A. strictum* were also obtained. 5.8S-ITS sequences available in the EMBL data library from *Acremonium* species and from other fungal species with 5.8S-ITS sequence matches were also included in the analysis. Sequences were included from the following accessions: (AJ246154, U66732, L36640, AF132805, AF176952), *Plectosphaerella cucumerina*; AJ292396, *A. obclavatum*; U57672, *A. chrysogenum*; L07130, *A. lolii*; U57674, *A. alternatum*.

The 5.8S-ITS region sequences were aligned using the multiple-sequence alignment program CLUSTAL X. The genetic distances were calculated using the Jukes–Cantor model and the phylogenetic inference was obtained by the neighbour-joining (NJ) method (Saitou and Nei, 1987). The NJ tree and the statistical confidence of a particular group of sequences in the tree, evaluated by bootstrap test (1000 pseudoreplicates) (Hills and Bull, 1993), were performed using the computer program MEGA version 2.0 (Kumar et al., 2001).

The 5.8S-ITS sequence data of strains reported in the present study have been submitted to the European Molecular Biology Laboratory (EMBL) sequence data and accession no. AJ621754 to AJ621777, inclusive.

Primer design test for primer specificity

All the sequences were analysed, according to the previous phylogenetic analysis, searching for adjacent synapomorphic (shared derived) changes common to *A. cucurbitacearum*, in order to design specific PCR primers to identify this species. Compatibility between primer pairs was evaluated using the OLIGO program (National Biosciences, Plymouth, USA). The PCR primers designed for *A. cucurbitacearum* were acrecu1: d5′-GCC GCT GGG CGG GCT TTG G-3′ and acrecu2: d5′-TAG AGG CAG GAC GCC GAC-3′, located at ITS1 and ITS2 variable regions respectively. The specificity of each primer was tested against all DNA sequences available in the EMBL database with the WU-Blast2 program (available online at http://www.ebi.ac.uk/blast2/index.html). The oligonucleotides were synthesized by Amersham Pharmacia.

Primer specificity was evaluated against five *Acremonium* species (*A. crotocinigenum*, *A. sclerotigenum*, *A. charticola*, *A. kiliense* and *A. strictum*) and 10 fungal species of muskmelon rhizosphere (*P. tabacinum*, *R. solani*, *M. phaseolina*, *F. oxysporum* f. sp. *melonis* race1, *F. solani* f. sp. *cucurbitaceae*, *F. equiseti*, *V. dahliae, M. cannonballus*, *R. vagum* and *Chaetomium* sp).

PCR reactions using the specific primers acrecu1 and acrecu2 and universal primers its5 and its4 (White et al., 1990) were performed as before. The reaction mixtures were incubated in a thermocycler, for 35 cycles consisting of 1 min at 95 °C, 1 min at 50 °C and 1 min at 72 °C. PCR products were separated on 1% agarose gels with 0.5× TBE buffer. After electrophoresis, gels were stained with ethidium bromide (0.5 mg ml^{-1}), and the DNA bands visualized with UV light. DNA sizes were estimated by comparison with a DNA length standard (100-bp molecular marker, Gibco BRI Life Technologies, Inc., Rockville, MD).

PCR amplification from muskmelon plants

Muskmelon plants (cv. Piel de sapo PS1430) were grown in black plastic pots in a sterilized mixture of equal portions (v/v) of soil, sand and moss peat infested with *A. cucurbitacearum* as described previously by Armengol et al. (1998). Roots obtained from muskmelon plants with collapse symptoms collected from different locations (Almenara, Alboraia, Puçol) in Valencia (Spain) were also used for

both isolation and PCR detection of *A. cucurbitacearum*. For DNA extractions, roots from inoculated and non-inoculated plants were thoroughly washed with distilled water and surface disinfested in sodium hypochlorite (0.5% available chlorine) for 1.5 min. Non-inoculated plants were used as negative controls. Samples of root tissue (100 mg) showing brown lesions were placed into 1.5 ml Eppendorf tubes and homogeneized using Eppendorf micropestles (Eppendorf, Hamburg, Germany). DNA extractions were performed using the commercial kit EZNA (Omega bioteck, Doraville, USA) according to the manufacturer's instructions. Parameters of the PCR were as described above.

Results

Analysis of the similarities among A. cucurbitacearum *RAPD patterns*

Twenty-five primers were selected according to the clarity and reproducibility of the DNA bands that they generated among isolates of *A. cucurbitacearum*. Each of these primers generated from 4 to 17 reproducible bands, ranging in size from 0.3 to 1.9 kbp. In order to assure the reproducibility of the RAPD technique, two monosporic cultures from each strain were analysed. RAPD banding patterns were shown to be reproducible and identical for the monosporic cultures.

To determine the similarity of the different isolates from diverse origins, a cluster analysis of pairs of RAPDs patterns was performed using the combination of the data for all selected primers (Figure 1). According to this dendrogram all the isolates analysed, except isolates A-465, A-541 and A-542, can be grouped at a 65% level of similarity. Isolates of *A. cucurbitacearum* were highly variable. However, it was possible to observe two main groups. The first included 13 isolates from the USA and the two isolates of *N. melonis*. Within the USA isolates, a correspondence between the VCGs and their RAPD pattern similarities was observed. Thus, the isolates from California belonging to VCG3 clustered in a subgroup that was distinct from the isolates of VCG2. The second group contains the European

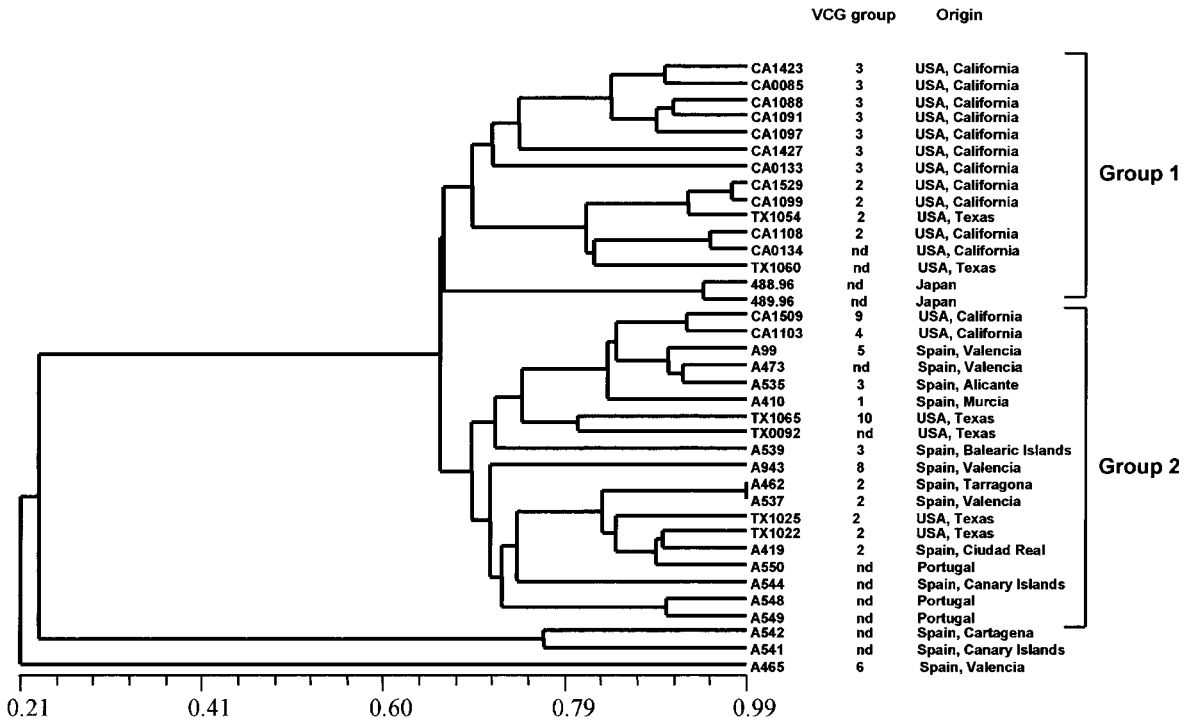

Figure 1. Generated dendrogram showing the clustering of isolates of *A. cucurbitacearum* based on the combination of all the RAPD data for the twenty-five selected primers used. The scale indicates the dissimilarity level (Dice coefficient × 100) as the factor of average fragment differences between RAPD patterns.

isolates from Spain and Portugal and five American isolates (CA1509, CA1103, TX1065, TX0092, CA1025 and TX1022). The isolate from Lanzarote (Canary Islands), which was obtained from watermelon, clustered in this second group. Within this group, Spanish and American isolates of VCG2 (A-462, A-537, CA1025, TX1022 and A-419) clustered together. Finally, the Spanish isolates A-542, A-541 and A-465 were not clustered in either of the two main groups.

Phylogenetic analysis

The length of the 5.8S-ITS region of *A. cucurbitacearum* isolates was determined to be 467 bp. Within isolates of *A. cucurbitacearum*, the 5.8S-ITS region exhibited very little sequence variation and most isolates had 5.8S-ITS regions whose sequences were identical to that of isolate A-419 proposed as the type strain (Alfaro-García et al., 1996). Exceptions were isolate A-99 that differed by 3 nucleotides (nt) and the American isolates TX-1060, TX-1054, CA-0133, CA-1103 and CA-1509 that differed by 1 nt. The two isolates of *N. melonis* also had 5.8S-ITS sequences that were identical to that of isolate A-419.

Based on the BLAST searches (National Center for Biotechnology Information, USA), *A. cucurbitacearum* was more similar to *P. cucumerina* isolates than to other *Acremonium* species. From the data of the present sequence analysis, the 5.8-ITS sequence of *A. cucurbitacearum* showed high homology with those from isolates of *P. cucumerina* (92%). In contrast, sequences of *A. cucurbitacearum* exhibited little homology (68–72%) to the sequences of other *Acremonium* species analysed.

The phylogenetic relationships among *A. cucurbitacearum*, *N. melonis*, *P. cucumerina* and other species of the genus *Acremonium* were analysed by cluster (neighbour-joining distance) analysis (Figure 2). All the *A. cucurbitacearum* isolates formed a monophyletic group, with high (99%) bootstrap support, in which were included the two isolates of *N. melonis*. Isolates of *P. cucumerina* clustered close to the isolates of *A. cucurbitacearum* and together formed a strongly supported group (100%). *Acremonium strictum*, *A. charticola*, *A. sclerotigenum*, *A. kiliense* and *A. crotocinigenum* together *Acremonium* sequences downloaded from the Genbank database appeared largely separated from *A. cucurbitacearum*.

Isolates A-541 and A-542, which based on the RAPDs analysis could not be grouped with the remaining isolates of *A. cucurbitacearum*, had almost identical 5.8S-ITS sequence as a sequence obtained from the Genbank database (AJ279474). This accession is from an unidentified Ascomycete (Wirsel et al., 2001). Isolates A-541 and A-542 previously identified on the basis of morphological studies as *A. cucurbitacearum* were assumed to be misidentified. Unfortunately, isolate A-465, which in the UPGMA dendrogram based on RAPD analysis could not be grouped with the remaining isolates of *A. cucurbitacearum*, was lost and could not be included in the phylogenetic analysis.

Primer design and PCR analysis

Based on the alignment of the 5.8-ITS sequences, primers specific for *A. cucurbitacearum*, designated acrecu1 and acrecu2, were designed. The predicted PCR product using these two primers with DNA from *A. cucurbitacearum* isolates as a template would be 475 bp in length. All the isolates of *A. cucurbitacearum* examined in this study were analysed by PCR using the primers acrecu1 and acrecu2. Two isolates of *N. melonis* were also analysed. To test the primer specificity, five other *Acremonium* species and ten fungal species isolated from muskmelon rhizosphere were also analysed.

The electrophoretic analysis of PCR fragments showed the expected fragment of 475 bp (Figure 3A). The same PCR product was obtained with all the isolates of *A. cucurbitacearum* and the two isolates of *N. melonis*. No amplification products were detected using DNA templates from other *Acremonium* species and other fungi analysed (Figure 3A). In contrast, the conserved ITS primer pair its5 and its4, included as positive control, amplified DNA from each of the species (Figure 3B).

PCR using acrecu1 and acrecu2 primers was capable of detecting *A. cucurbitacearum* directly from infected plant material, and the presence of the pathogen could be detected 10 days after infection. At this stage, the plants were still symptomless. To determine the optimal quantity of root samples for template DNA extraction, root samples (10–300 mg) from artificially infected muskmelon plants were used. In addition two different DNA dilutions (10^{-1} and 10^{-2}) were used for PCR amplification. As shown in Figure 4A, amplifica-

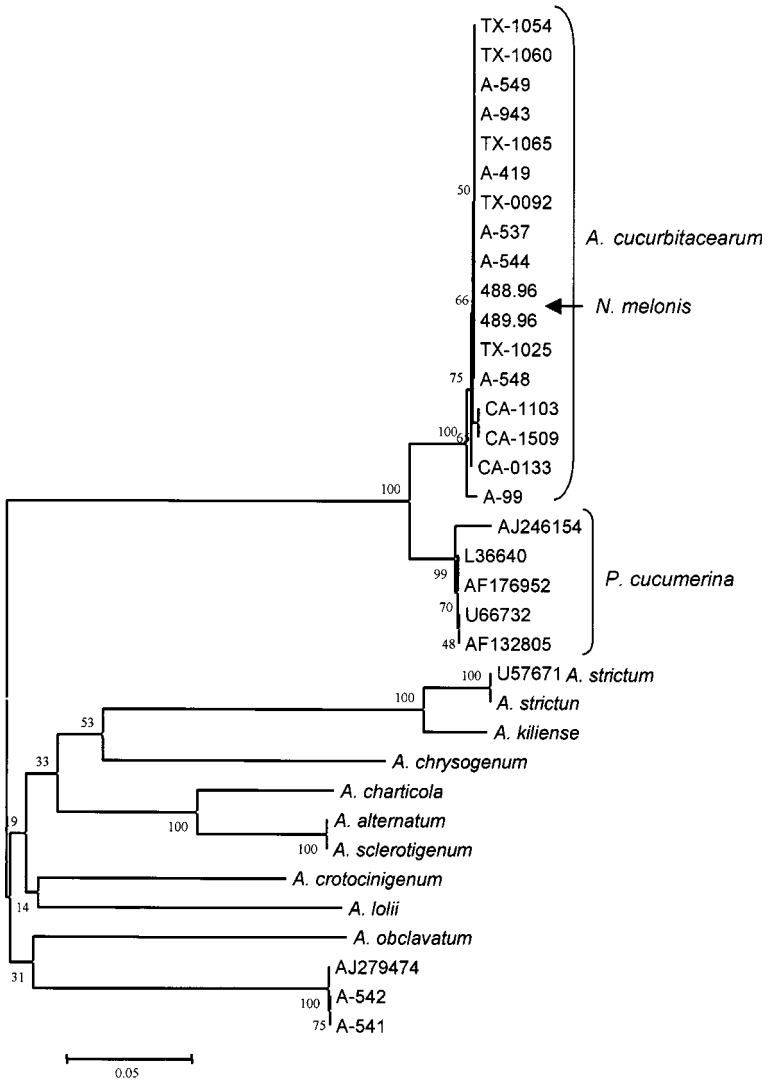

Figure 2. Neighbour-Joining tree based on nucleotide divergences, estimated according to Jukes–Cantor model, from the 5.8S-ITS sequences. The numbers on the nodes are the frequency (in percent) with which a cluster appears in a bootstrap test of 1000 runs. The phylogenetic tree shows the relationships among *A. cucurbitacearum*, *Acremonium* species and other fungal species closely related.

tion of the expected PCR fragment was observed using DNA from all root samples with the exception of sample 6 (300 mg) using the 10^{-1} DNA dilution. By contrast, PCR product was produced using the same sample 6 when DNA from 10^{-2} dilution was included in the amplification reaction indicating possible inhibition of PCR by tissue extract components. No amplification product was obtained from non-inoculated control plants.

The results indicated that the PCR procedure was also capable of detecting *A. cucurbitacearum* in muskmelon plants under field conditions. DNA extractions were made from field samples with characteristic collapse symptoms. Extractions were made from 100 mg of root tissue and were diluted 10^{-1} for amplification. The PCR assay using the primers acrecu1 and acrecu2 with DNA purified from diseased melon roots produced an amplicon at 475 pb (Figure 4B). The PCR failed to detect the pathogen in three samples in which *A. cucurbitacearum* was not isolated on culture media.

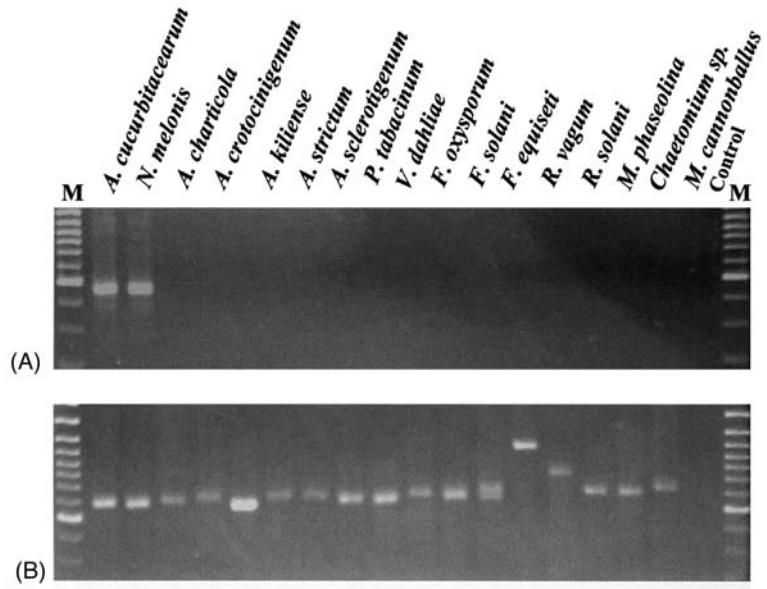

Figure 3. PCR amplification products using the *A. cucurbitacearum* primers acrecu1 and acrecu2 and conserved internal transcribed primers its5 and its4. (A) PCR amplification products obtained with the primer pair acrecu1 and acrecu2. (B) PCR amplification products obtained with the universal primers its5 and its4. Lane 1 *A. cucurbitacearum*, lane 2 *N. melonis*, lanes 3–17 correspond to other *Acremonium* species and several fungi from muskmelon rhizosphere. Lanes M correspond to the 100 bp molecular weight marker (Gibco BRL).

Discussion

Cluster analysis based on RAPD patterns obtained from the use of 25 primers showed two main groups within *A. cucurbitacearum* isolates. Within this division, some clustering according to geographical origin and VCG group was observed. With the exception of the two Japanese isolates of *N. melonis*, group 1 comprised only USA isolates that belonged to VCG2 and VCG3 groups. However, group 2 was comprised of isolates responsible for muskmelon collapse in Europe, including Canary and Balearic Islands, and USA. In addition, this second group included isolates previously grouped in different VCGs (Vicente et al., 1999; Abad et al., 2000) including the major groups VCG2 and VCG3. Genetic similarity measured by analysis of RAPD patterns was not totally correlated to either VCGs or differences in geographical origins and isolates of *A. cucurbitacearum* appeared continually distributed.

The phylogenetic analysis, based on sequence data of the 5.8S-ITS region, confirmed the genetic similarity among isolates of *A. cucurbitacearum* and indicated that *A. cucurbitacearum* is a monophyletic taxon not closely related to other *Acremonium* species. This finding is in agreement with previous phylogenetic analyses of *Acremonium*, where the genus was shown to be a polyphyletic taxon with affiliations to at least three ascomycetous orders based on sequences of a portion of the 18S rDNA (Glenn et al., 1996). In contrast, *A. cucurbitacearum* was closely related to *P. cucumerina*, a fungal pathogen that has been reported from many hosts and isolated very frequently as a component of the rhizosphere, mostly under the name *Plectosporium tabacinum*. This fungus was differentiated from *Acremonium* species on the basis of morphological characters (Palm et al., 1995). However, the molecular phylogeny based on 5.8S-ITS sequence analysis indicates that *A. cucurbitacearum* and *P. cucumerina* are closely related. This finding together with the high level of sequence divergence (28–32%) between *A. cucurbitacearum* and the other *Acremonium* species analysed in this study suggests that *A.cucurbitacearum* and *P. cucumerina* should be included in the same genus. However we prefer to amass more evidence of their interrelationships before taking this step. Additional morphological studies and sequence data from other genes (probably based on conserved regions) are

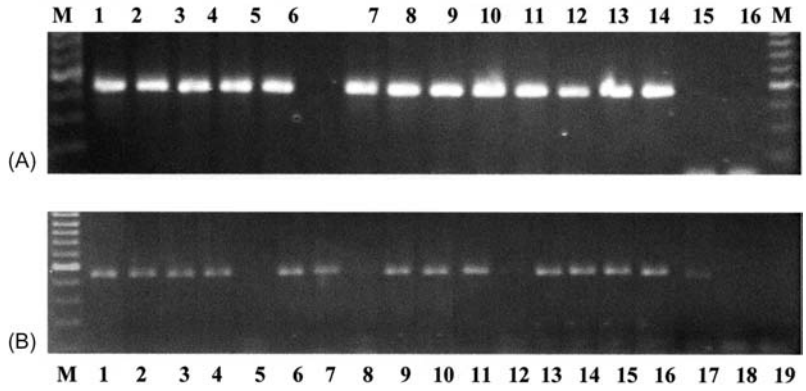

Figure 4. PCR amplification products using the *A. cucurbitacearum* specific primers acrecu1 and acrecu2 from root samples.(A) PCR amplification products from root samples experimentally infected with *A. cucurbitacearum*. Lanes 1–6 PCR amplification products obtained with DNA extractions from root samples 10, 20, 50, 100, 200 and 300 mg and the dilution 10^{-1}. Lanes 7–12 PCR amplification products obtained with DNA extractions from root samples 10, 20, 50, 100, 200 and 300 mg and the dilution 10^{-2}. Lane 13 and 14 positive controls with DNA from mycelia of *A. cucurbitacearum*. Lanes 15 and 16 negative controls with healthy muskmelon plant and water. (B) PCR amplification products (475 pb) from field samples using 100 mg of root tissue for DNA extraction and the dilution 10^{-1}. Lanes 18 and 19 negative controls with healthy muskmelon plant and water. Lanes M correspond to the 100 bp molecular weight marker (Gibco BRL).

required in order to examine the taxonomic relatedness.

The two isolates classified as *N. melonis* (Sato et al., 1995; Watanabe and Sato, 1995) had identical 5.8S-ITS sequence to the isolate of *A. cucurbitacearum* A-419, proposed as the type strain (Alfaro-García et al., 1996). ITS regions are generally viewed as conserved within a species and variable among species (Bruns et al., 1991). However, there are cases in which different species have identical ITS (Kuhls et al., 1996). In addition to the sequence data, the two isolates of *N. melonis* clustered within *A. cucurbitacearum* isolates in the RAPD dendrogram. Thus, results from this study agree with the similar morphology of the fungi and similarities in symptoms described for the disease in Japan (Sato et al., 1995; Watanabe and Sato, 1995). The identical rDNA sequence and similarities in RAPD analysis, morphology and symptoms could be an indication that both fungi represent the same species. The evidences presented here suggest that the taxonomic status of *A. cucurbitacearum* and *N. melonis* should be revised.

By using the alignment of 5.8-ITS sequences, species-specific primers for detection of *A. cucurbitacearum* were designed. The specificity of the primers was predicted from comparison (alignment) of the 5.8-ITS sequences analysed in the present study. PCR with template DNA from all the *A. cucurbitacearum* isolates studied, two isolates of *N. melonis*, five different species of *Acremonium* and ten other rhizosphere fungi confirmed the specificity of the primers. This indicated that acrecu1 and acrecu2 primers could be used for the specific identification and detection of *A. cucurbitacearum*.

The PCR method detected fungal DNA in the roots of symptomless infected melon plants in early stages of the disease. The PCR assays were also effective in detection of *A. cucurbitacearum* under field conditions. This PCR procedure would circumvent problems associated with isolation of *A. cucurbitacearum* in roots, were the presence of the soil-borne pathogens can make its isolation difficult. In addition, it may be used as a tool for rapid, large-scale diagnosis of *A. cucurbitacearum*, since a small piece of root tissue is sufficient for analysis. The PCR procedure may be easily adapted for the detection of the pathogen in soil, as has been described for other soil-borne pathogens (García-Pedrajas et al., 1999). The evaluation of soil infestation in plots would help to prevent melon collapse by aiding management and efficient crop rotation procedures.

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