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Two xylanase genes of the vascular wilt pathogen *Fusarium oxysporum* are differentially expressed during infection of tomato plants

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Abstract Two genes encoding putative family F xylanases from the tomato vascular wilt pathogen *Fusarium oxysporum* f.sp. *lycopersici* have been cloned and sequenced. The two genes, designated *xyl2* and *xyl3*, encode proteins with calculated molecular masses of 33 and 39.3 kDa and isoelectric points of 8.9 and 6.7, respectively. The predicted amino acid sequences show significant homology to other family F xylanases. XYL3 contains a cellulose-binding domain in its N-terminal region. Southern analysis suggested that *xyl2* and *xyl3* homologs are also present in other formae speciales of *F. oxysporum*. Both genes were expressed during growth on oat spelt xylan and tomato vascular tissue in vitro. RT-PCR revealed that *xyl3* is expressed in roots and in the lower stems of tomato plants infected by *F. oxysporum* f.sp. *lycopersici* throughout the whole disease cycle, whereas *xyl2* is only expressed during the final stages of disease.

Key words *Fusarium oxysporum* · Pathogenicity · Tomato · Xylanase

Introduction

Fungal plant pathogens infect their hosts by penetrating the outer cell layer and invading different plant tissues. During infection, the pathogen encounters various polymers representing the structural components of the cell wall. Xylan is a heterogeneous carbohydrate composed of β-1,4-D-xylopyranosyl residues and represents a major constituent of plant cell walls. Microbial conversion of xylan to soluble products requires the combined action of several types of enzymes, such as endo-β-1,4-xylanases, β-xylosidases, α-L-arabinofuranosidases and α-glucuronidases (Biely 1985). Xylanases have been isolated from a wide variety of bacterial and fungal plant pathogens (Walton 1994). Multiple xylanase genes have been cloned and disrupted in two plant pathogenic fungi, *Cochliobolus carbonum* and *Magnaporthe grisea,* both of which cause foliar disease in cereals. In both fungi, the genes were found not to be essential for pathogenicity, probably due to the presence of additional xylanase genes (Apel-Birkhold and Walton 1996; Wu et al. 1997). Nevertheless, a number of studies suggest that xylanases may play an important role in other plant-pathogen interactions (Walton 1994).

Fusarium oxysporum Schlecht. is an economically important soil-borne plant pathogen with a worldwide distribution that causes vascular wilt disease on a wide variety of crops. The mechanisms of pathogenicity and symptom induction by this fungus are poorly understood despite many ultrastructural, biochemical, and genetic studies (Beckman 1987). The presence of xylanases in *F. oxysporum* has been reported (Jones et al. 1972; Alconada and Martínez 1994; Christakopoulos et al. 1996; Ruiz et al. 1997), but their production during infection and their role in pathogenicity has not been investigated. Here we report the isolation, sequencing and expression analysis of two putative family F xylanase genes of *F. oxysporum* f.sp. *lycopersici* and show that the two are expressed by the pathogen at different stages during the infection of tomato plants.

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Materials and methods

Fungal strains and culture conditions

F. oxysporum f.sp. *lycopersici* strain 42-87 (race 2) was obtained from J. Tello (Universidad de Almería, Spain), and stored as a microconidial suspension in 30% glycerol at −80° C. The pathotype of the isolate was periodically confirmed by plant infection assays in a growth chamber. For extraction of DNA, mycelium was ob-

tained from cultures grown in potato dextrose broth (PDB; Difco, Detroit, Mich.) in Erlenmeyer flasks on a rotary shaker at 150 rpm and 28° C. For analysis of gene expression, microconidia obtained from 4-day-old PDB cultures were germinated for 12 h in fresh PDB medium. Germlings were washed twice in sterile water and transferred to synthetic medium containing 0.2 g $MgSO_4 \cdot 7H_2O$, 0.4 g KH_2PO_4, 0.2 g KCl, 1 g NH_4NO_3, 0.01 g $FeSO_4$, 0.01 g $ZnSO_4$, and 0.01 g $MnSO_4$ per litre of distilled water, supplemented with 1% (w/v) of the appropriate carbon source(s), except for tomato vascular tissue which was added at 2.5% (fresh w/v). Larchwood xylan was obtained from United States Biochemical (Cleveland, Ohio), cellulose powder, carboxymethyl cellulose sodium salt, and D(+)-cellobiose were from ICN Biomedicals (Aurora, Ohio), oat spelt xylan and D(+)-xylose were from Sigma (St. Louis, Mo.), and pustulan from *Umbilicaria papullosa* was supplied by Calbiochem (La Jolla, Calif.). Tomato vascular tissue was collected from longitudinal sections of the stems of 3-month-old tomato plants, cv. Vemar (seeds were obtained from Sluis and Groot, Almería, Spain) by means of a sterile scalpel and stored at −80° C before use.

Isolation and characterization of *xyl2* and *xyl3*

A λEMBL3 genomic library of *F. oxysporum* f.sp. *lycopersici* strain 42-87 was screened using as a probe the *xylP* gene of *Penicillium chrysogenum* (Haas et al. 1993). Library screening under low-stringency conditions (56° C, two washes with 5 × SSC), subcloning and other routine procedures were performed using standard protocols (Sambrook et al. 1989). Sequencing was performed on double-stranded DNA using the Dyedeoxy Terminator Cycle Sequencing Kit (Perkin Elmer, Foster City, Calif.) on an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, Calif.). Analyses of sequence data were carried out using the Wisconsin Package, Version 8.3 (Genetics Computer Group, Madison, Wis.) and the Lasergene Programs (DNAStar, Madison, Wis.).

Nucleic acid isolation and analysis

Genomic DNA was extracted from *F. oxysporum* mycelium as described previously (Raeder and Broda 1985). Two micrograms of DNA were digested with appropriate restriction enzymes and subjected to Southern hybridization analysis as described in standard protocols (Sambrook et al. 1989), using the non-isotopic dig-dUTP labeling kit (Boehringer Mannheim, Germany) according to the instructions of the manufacturer. Total RNA was prepared according to the protocol of Chomczynski and Sacchi (1987). Five micrograms of total RNA were fractionated on a formaldehyde-1% agarose gel and transferred to positively charged nylon membranes (Boehringer Mannheim) by capillary transfer. For quantification, transferred RNA was stained on the membrane for 5 min in 0.02% methylene blue in 0.3 M sodium acetate pH 5.2. After destaining in 20% ethanol, filters were subjected to Northern hybridization analysis using the non-isotopic dig-dUTP labeling kit. Single-stranded antisense DNA probes corresponding to the *xyl2* coding region and to the *xyl3* cellulose-binding domain were generated following a standard protocol (Konat et al. 1994).

RT-PCR in infected tomato plants

Ten-day-old tomato seedlings (cv. Moneymaker) were inoculated with *F. oxysporum* f.sp. *lycopersici* strain 42-87 by dipping the roots for 30 min in an aqueous suspension containing 5×10^6 microconidia/ml. Control plants were immersed in water. Seedlings were planted in minipots containing vermiculite No. 3 and maintained in a growth chamber at 25° C with 14 h light and 10 h dark. Three, six, ten and fourteen days after inoculation, five plants from each treatment were sampled and total RNA was isolated from roots and the lower parts of the stems as described previously (Di Pietro and Roncero 1998). RT-PCR was performed essentially following the protocol of Tenberge et al. (1996), using gene-specific primers flanking the unique intron in *xyl2* and the first intron of *xyl3*. One microgram of total RNA was treated with RNase-free DNase (Boehringer Mannheim) and reverse-transcribed into cDNA with murine leukemia virus reverse transcriptase (Gibco BRL, Paisley, UK) using the *xyl2*-specific antisense primer 5′-ACTCTCGTGGGACCTAAACC-3′ or the *xyl3*-specific antisense primer 5′-GAGTTCCACCACCCTGAGTTGACG-3′. The entire RT reaction was used for PCR amplification with the *xyl2* sense primer 5′-AGAATGGCCTCAAGG TCCGA-3′ or the *xyl3* sense primer 5′-TTTTTCGGTTCTCCTCGCTCTCGC-3′and the antisense primers described above. PCR conditions were as follows: 36 cycles of denaturation at 94° C for 35 s, annealing at 62° C (*xyl2*) or 66° C (*xyl3*) for 35 s , and extension at 72° C for 90 s. An initial denaturation step of 2 min at 94° C and a final elongation step at 72° C for 6 min were included. Aliquots of the PCR products were analyzed on 2% agarose gels. The amplified fragments were cloned into the pGEM-T vector (Promega, Madison, Wis.) and sequenced in order to confirm their identity.

Results

Isolation and sequencing of *xyl2* and *xyl3*

The *xylP* gene of *P. chrysogenum* was used as a heterologous probe to screen a λEMBL3 genomic library of *F. oxysporum* f.sp. *lycopersici.* under low-stringency conditions. Two recombinant phage clones were isolated and characterized by restriction digestion and Southern hybridization analysis. The results indicated that the two clones corresponded to different genomic regions, since they did not share common restriction fragments. A 5.0-kb *Eco*RI-*Hin*dIII fragment from the first clone and a 6.0-kb *Pst*I fragment from the second clone, which hybridized to the *P. chrysogenum xylP* gene, were cloned independently in Bluescript KS+.

The nucleotide sequences of a 1.8-kb region from the first clone and a 2.2-kb region of the second clone were determined. The two clones contained long ORFs designated *xyl2* and *xyl3*, respectively. The *xyl2* coding region consisted of an ORF of 984 bp encoding a 328 amino acids polypeptide (Fig. 1). One 52-bp intron was identified, based on alignment with other xylanase genes belonging to family F and on the presence of conserved splice site consensus sequences characteristic of filamentous fungi. The intron followed the 5′ GT/AG 3′ rule and contained putative lariat consensus sequences (Gurr et al. 1987). The calculated molecular weight and pI of the encoded mature protein were 33 kDa and 8.9, respectively. The first 23 residues of the putative protein are proposed to represent a signal peptide (von Heijne 1986). Although unusual, the presence of an Arg residue at the cleavage site has been described previously for the XYN22 xylanase from *M. grisea* (Wu et al. 1995) and for xylanases A and C from *Aspergillus kawachii* (Ito et al. 1992a,b). The TELD motif characteristic for the catalytic domain of family F xylanases was found at position 262. The 5′-flanking region of *xyl2* contained a putative CAAT box at position −58 followed by an AT-rich region. Putative consensus binding sites for the pH

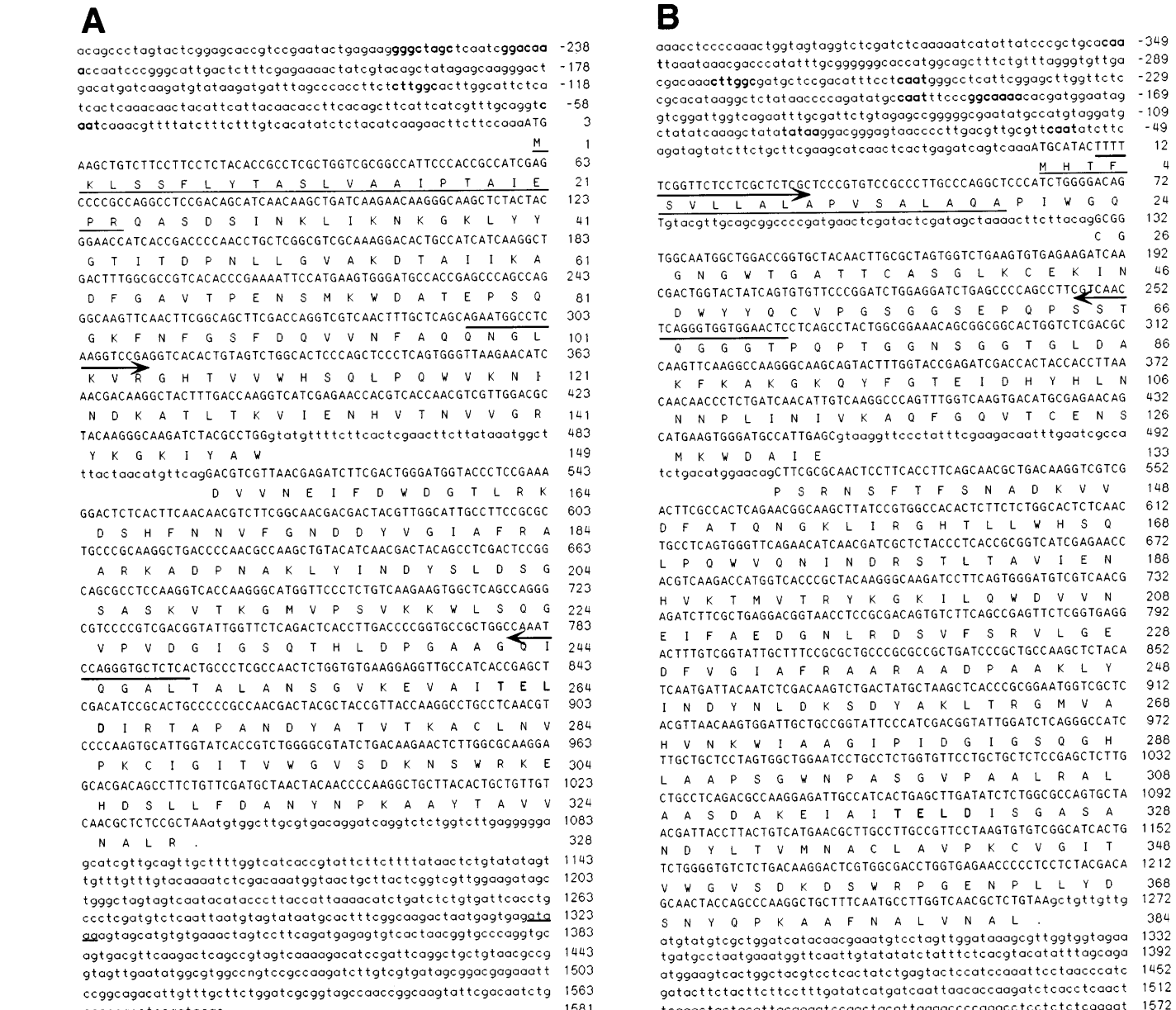

Fig. 1A, B Nucleotide sequences of the *F. oxysporum* f.sp. *lycopersici* genes *xyl2* (**A**) and *xyl3* (**B**). Introns are indicated in *lower case* within the coding region. Putative TATA and CAAT boxes, as well as putative PacC and XlnR binding sites are in *bold*. Putative polyadenylation signals are *underlined*. The deduced amino acid sequence is shown *below* the nucleotide sequence. The *underlined* amino acid region represents the putative signal peptide. The TELD motif is in *bold*. Positions and orientations of the primers used for RT-PCR are indicated by *arrows*. The nucleotide sequence data have been deposited in the EMBL and GenBank databases under accession numbers AF052583 (*xyl2*) and AF052582 (*xyl3*)

wide domain transcription factor PacC (CTTGGC; Tilburn et al. 1995) and the xylanase transcriptional activator XlnR (GGCTAAA; van Peij et al. 1998) were also detected. The sequence ATAAA, which resembles the AATAAA motif, was found 81 bp downstream of the stop codon and may represent the polyadenylation signal.

The *xyl3* coding region consisted of an ORF of 1152 bp encoding a polypeptide of 384 amino acids. The first 19 residues form the putative signal peptide, matching the (−3, −1)-rule (von Heijne 1986). Two short introns of 55 bp and 52 bp were identifed, containing putative lariat consensus sequences. The calculated molecular weight and pI of the encoded mature protein were 39.3 kDa and 6.7, respectively. The TELD motif was also present at position 319**.** The 5′-flanking region of *xyl3* contains putative CAAT boxes at positions −57 and −196, and putative TATA boxes at positions −91 and −106, as well as putative consensus binding sites for

PacC and XlnR. An AATAAA motif was found 415 bp downstream of the stop codon.

Comparison of the amino acid sequences deduced from *xyl2* and *xyl3* revealed significant homology with fungal family F xylanases. The highest degrees of identity to the *xyl2* product (60%, 57%, and 55%, respectively) were detected the proteins encoded by *xynA* from *A. kawachii* (Ito et al. 1992a), *xylP* from *P. chrysogenum* (Haas et al. 1993) and *xyn33* from *M. grisea* (Wu et al. 1995). The product of *xyl3* was 98% identical to that of a cDNA encoding a putative endo-β-1,4-glucanase from *F. oxysporum* (Sheppard et al. 1994), 58% identical to XynA, and 57% identical to the products of *xyl2* and *xylP*. Residues 20–52 encoded by *xyl3* were homologous to cellulose-binding domains from microbial cellulases and xylanases (Gilkes et al. 1991).

Occurrence of *xyl2* and *xyl3* in different formae speciales of *F. oxysporum*

The occurrence of *xyl2* and *xyl3* homologs in *F. oxysporum* isolates belonging to different formae speciales was studied by Southern hybridization analysis. Genomic DNA from fourteen isolates (Table 1) was digested with *Pst*I, blotted onto nylon membrane, and sequentially hybridized under high-stringency conditions to the following probes: a 415-bp PCR fragment containing part of the coding region of *xyl2*; a 590-bp internal PCR fragment corresponding to the *xyl3* catalytic domain; and a 260-bp PCR fragment encompassing the cellulose-binding domain of *xyl3*. With the *xyl2* probe, a strongly hybridizing 4.2-kb band was detected in all the isolates studied (Fig. 2A). The two *F. oxysporum* f.sp. *niveum* isolates showed an additional hybridizing band of 2.8 kb that may correspond to a homologous gene. Longer exposures revealed a number of additional, faintly hybridizing bands in most isolates (results not shown). Hybridization with the *xyl3* catalytic domain probe in *F. oxysporum* f.sp. *lycopersici* isolates gave a strongly hybridizing band of 8 kb corresponding to *xyl3* and three additional hybridizing bands of 4.2 kb (probably due to cross-hybridization with *xyl2*), 6.5 kb and 12 kb (Fig. 2B). In most of the other formae speciales, a single strongly hybridizing band of 4.2 kb was observed that was possibly due to cross-hybridization with *xyl2*. The probe from the *xyl3* cellulose-binding domain revealed a 17-kb band, in addition to the 8-kb band corresponding to *xyl3*, in *lycopersici* (Fig. 2C). Again, in most formae speciales other than *lycopersici*, a 4.2-kb band was observed instead of the 8-kb band. As a DNA loading control, the filter was stripped and hybridized to the *nit1* gene of *F. oxysporum* f.sp. *lycopersici* (García-Pedrajas and Roncero 1996) producing clear hybridizing bands of approximately the same size in all isolates (results not shown).

In vitro expression of *xyl2* and *xyl3*

Expression of *xyl2* and *xyl3* in culture was examined by Northern hybridization analysis. Total RNA obtained from mycelia of *F. oxysporum* f.sp. *lycopersici* grown for 12 h in synthetic medium supplemented with different carbon sources was probed under high-stringency conditions with the *xyl2* coding region and with a fragment corresponding to the cellulose-binding domain only present in *xyl3*. A single transcript of 1.2 kb (*xyl2*) or 1.4 kb (*xyl3*) was detected in mycelia grown on oat spelt xylan and tomato vascular tissue (Fig. 3). No transcript was detected in mycelia grown on the other carbon sources tested.

Expression of *xyl2* and *xyl3* during infection of tomato plants

To determine whether *xyl2* and *xyl3* are expressed by *F. oxysporum* f.sp. *lycopersici* during infection of its host,

Table 1 *Fusarium oxysporum* isolates used in this study

No.	Isolate	Forma specialis	Race	Host plant	Source
1	8503	*ciceris*	5	Chickpea	I.A.S., Córdoba, Spain[a]
2	699	*conglutinans*	2	Cruciferae	I.N.I.A., Madrid, Spain[b]
3	2869	*gladioli*	n.d.[b]	Gladiolus	I.N.I.A., Madrid, Spain[b]
4	2159	*lini*	n.d.	Flax	C.E.C.T, Valencia, Spain
5	218	*lycopersici*	1	Tomato	I.N.I.A., Madrid, Spain[b]
6	2715	*lycopersici*	1	Tomato	I.N.I.A., Madrid, Spain[b]
7	42–87	*lycopersici*	2	Tomato	I.N.I.A., Madrid, Spain[b]
8	281	*lycopersici*	2	Tomato	I.N.I.A., Madrid, Spain[b]
9	4887	*lycopersici*	2	Tomato	I.N.I.A., Madrid, Spain[b]
10	18 M	*melonis*	1	Muskmelon	I.N.I.A., Madrid, Spain[b]
11	1127	*melonis*	2	Muskmelon	I.N.I.A., Madrid, Spain[a]
12	275	*melonis*	1,2w	Muskmelon	C.I.D.A., Almeria, Spain[c]
13	G-60301	*niveum*	1	Watermelon	I.P.O., Wageningen, Netherlands
14	311	*niveum*	2	Watermelon	I.P.O., Wageningen, Netherlands

[a] Provided by R. Jimenez Díaz
[b] Provided by J. Tello
[c] Provided by J. Gomez

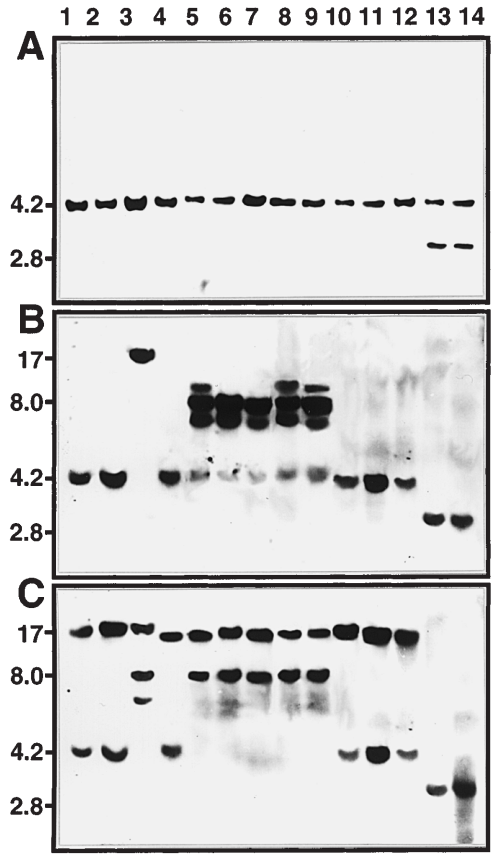

Fig. 2A–C Southern analysis of genomic DNA from various *F. oxysporum* isolates. Lane numbers refer to the isolates listed in Table 1. DNA was digested with *Pst*I and probed with the following DNA fragments labeled with digoxigenin-dUTP: a 415-bp PCR fragment containing part of the coding region of *xyl2* (**A**); a 590-bp internal PCR fragment of *xyl3* corresponding to the catalytic domain (**B**); a 260-bp PCR fragment encompassing the cellulose-binding domain of *xyl3* (**C**). Sizes are indicated in kb

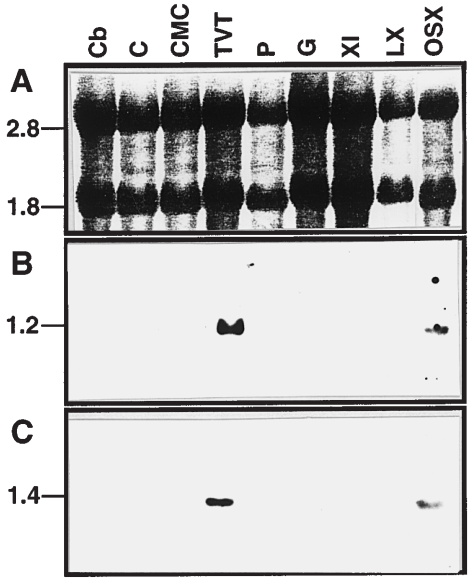

Fig. 3A–C Northern hybridization analysis of *xyl2* and *xyl3* transcript accumulation in *F. oxysporum* f.sp. *lycopersici* mycelium grown for 12 h in synthetic medium containing various carbon sources. Cb, 0.1% cellobiose; C, 1% cellulose; CMC, 1% carboxymethyl cellulose; TVT, 2.5% tomato vascular tissue; P, 0.5% pustulan; G, 1% glucose, Xl, 0.1% xylose; LX, 1% larchwood xylan, OSX, 1% oat spelt xylan. Total RNA was blotted onto a nylon membrane and stained with 0.02% methylene blue (**A**). The filter was then destained and hybridized with a digoxigenin-dUTP-labeled, 415-bp internal PCR fragment of *xyl2* (**B**) or a 260-bp PCR fragment encompassing the cellulose-binding domain of *xyl3* (**C**). Sizes are indicated in kb

RT-PCR with gene-specific primers (see Materials and methods) was used to detect their transcripts in total RNA extracted from roots and lower stem sections of tomato plants at different times after infection. Control reactions were performed with total RNA from uninfected plants, and genomic DNA was used as template to compare the sizes of the amplified fragments with or without introns (505 and 453 bp, respectively, for *xyl2* and 261 and 206 bp, respectively, for *xyl3*). Electrophoretic analysis of the RT-PCR products obtained with the *xyl2*-specific primers showed a fragment of the expected size only from extracts of stems at 14 days after inoculation, corresponding to the final stages of the disease (Fig. 4). Conversely, expression of *xyl3* was detected both in roots and stems, at all the time points sampled. Both amplified fragments were absent in the uninoculated controls. Cloning and sequencing of the bands confirmed that their sequences completely matched those of the genomic *xyl2* and *xyl3* clones except for the absence of introns.

Discussion

The main role of xylanases in fungal pathogenicity to plants is likely to involve degradation of cell walls during host colonization (Walton 1994). The soil-borne vascular wilt pathogen *F. oxysporum* enters the plant through the roots, proceeds to the vascular system, and colonizes the host by spreading upward through the xylem vessels. Secretion of lytic enzymes is thought to be the major mechanism by which *F. oxysporum* disrupts primary physical barriers and induced structural defenses of the host (Beckman 1987). Xylanases are part of the arsenal of cell wall-degrading enzymes produced by *F. oxysporum* (Jones et al. 1972; Cooper and Rankin 1978; Christakopoulos et al. 1996; Ruiz et al. 1997) but their role in pathogenicity is at present unknown. In this work, two putative xylanase genes from *F. oxysporum* f.sp. *lycopersici* have been isolated. Their nucleotide and deduced amino acid sequences show homology to fungal xylanases of family F , as expected since the probe used was obtained from a gene for a family F xylanase from *P. chrysogenum*. Since neither of the protein sequences deduced from the two genes encoded the N-terminal sequence empirically determined from the previously purified family F endoxylanase XYL1 (Ruiz et al. 1997), the genes were named *xyl2* and *xyl3*. While the structure

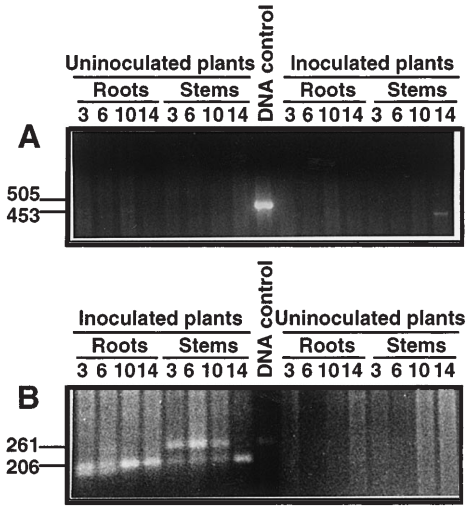

Fig. 4A, B RT-PCR products showing the expression pattern of *xyl2* (**A**) and *xyl3* (**B**) during infection of tomato plants by *F. oxysporum* f.sp. *lycopersici*. First-strand cDNAs generated from total RNA isolated at the indicated time points (days after inoculation) from roots and stems of infected or uninfected plants were used as templates for PCR with gene-specific primers (see Materials and methods). Aliquots of the PCR products were run on a 2% agarose gel with a 100 bp ladder marker. DNA control refers to PCR with genomic DNA as the template

of the *xyl2* product matched that of typical family F xylanases, the N-terminal cellulose-binding domain encoded by *xyl3* is unusual. Cellulose-binding domains have been reported in a number of bacterial xylanases (Gilkes et al. 1991) but so far only in one fungal xylanase – an enzyme purified from *F. oxysporum* (Christakopoulos et al. 1996). The deduced product of *xyl3* is 98% identical to that of a cDNA clone previously isolated from *F. oxysporum* (Sheppard et al. 1994), indicating that both originate from the same gene. These authors classified the product of their cDNA clone as a putative family F cellulase, in spite of the fact that most members of family F are actually xylanases and not cellulases (Gilkes et al. 1991). Our results provide strong evidence that *xyl3* indeed encodes a β-1,4-xylanase, as suggested by sequence alignments of the catalytic domain and induction of *xyl3* expression on oat spelt xylan but not on cellulose or carboxymethyl cellulose.

Southern hybridization analysis indicated that *xyl2* is widely distributed and structurally conserved among different formae speciales of *F. oxysporum*, as observed previously for an endopolygalacturonase gene from this fungus (Di Pietro et al. 1998). The banding pattern obtained with the *xyl3* probes was polymorphic among different formae speciales: the 8-kb *Pst*I fragment was only detected in forma specialis *lycopersici*, whereas in most other formae speciales a strongly hybridizing fragment of the same size as *xyl2* was observed. Thus, either the *xyl2* homolog in these formae speciales contains a region with similarity to the *xyl3* cellulose-binding domain, or *xyl2* and *xyl3* are clustered on the same *Pst*I restriction fragment. The detection of additional hybridizing bands with the *xyl3* probes suggests the occurrence of a small xylanase gene family in *F. oxysporum*.

Both *xyl2* and *xyl3* are induced on oat spelt xylan but not on larchwood xylan. Although these two commercial xylan preparations may differ substantially in their carbohydrate composition, this result indicates a considerable degree of specificity in the regulation of *xyl2* and *xyl3* expression. The monomer xylose was not an effective inducer of *xyl2* and *xyl3*, an observation reported also in other fungi (Hrmová et al. 1986). Significantly, expression of *xyl2* and *xyl3* was activated by tomato vascular tissue, indicating that the host tissue preferentially colonized by *F. oxysporum* f.sp. *lycopersici* can induce production of these enzymes in vitro. Secretion of xylanase activities during culture growth on host plant tissue has been reported previously in *F. oxysporum* (Jones et al. 1972; Ruiz et al. 1997) and other plant pathogens (Apel-Birkhold and Walton 1996; Wu et al. 1995).

Expression of fungal xylanase genes in planta during pathogenesis has been poorly studied. Due to the great diversity of mechanisms of infection and symptom expression developed by fungal plant pathogens, such expression studies must be conducted case by case in different pathogen-host systems. Recently, expression of two genes encoding a family F and a family G xylanase from *Claviceps purpurea* in rye ovarian tissue was reported during all infection stages (Giesbert et al. 1998). In the vascular wilt pathogen *F. oxysporum* f.sp. *lycopersici*, we found that *xyl2* and *xyl3* are expressed differentially during infection of tomato plants. Transcription of *xyl3* was observed at all the time points sampled, indicating that the gene is expressed throughout the entire disease cycle, from penetration of the root through colonization of the vascular bundles to expression of wilt symptoms. In contrast, *xyl2* transcripts were only detected during the last stages of infection coinciding with the inset of plant death (Beckman 1987). Differential expression of *xyl2* and *xyl3* during pathogenesis may indicate that the two genes may carry out different functions in the life cycle of *F. oxysporum*. Expression of *xyl2* seems to be associated with saprophytic growth of the fungus on the moribund plant tissue, whereas *xyl3* may play an active role during plant infection and disease establishment. Specific inactivation of *xyl3* should allow the assessment of its contribution to pathogenicity of *F. oxysporum* f.sp. *lycopersici*.

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