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Fusarium oxysporum f. sp. vasinfectum 5.8s rRNA gene and adjacent ITS1 and ITS2 regions

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Abstract

Fusarium oxysporum, Schlecht ex Fr. is a phytopathogenic fungus causing wilting or yellows disease on a variety of plant species throughout the world. It is categorized in *formae speciales* according to pathotypic variation and physiological character (Messiaen and Cassini, 1981 Fusarium:- Diseases, Biology and Taxonomy pp.427-445). The *F. oxysporum forma specialis vasinfectum* (Atk.) Snyder and Hansen is pathogenic on cotton (*Gossypium* spp.) on which it causes severe damage to susceptible races. We report here the DNA sequence of the 5.8S rRNA gene and flanking intergenic transcribed spacers of *F. oxysporum forma specialis vasinfectum*. DNA was isolated from mycelial cultures from three virulent isolates collected from single cotton plants from geographically distant sites in Bié, Cuanza Norte and Cuanza Bul regions of Angola (Ragazzi, 1992 J. Pl. Disease Protect. 99:499-504).

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Fusarium oxysporum f. sp. vasinfectum 5.8s rRNA gene and adjacent ITS1 and ITS2 regions

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Fusarium oxysporum, Schlecht ex Fr. is a phytopathogenic fungus causing wilting or yellows disease on a variety of plant species throughout the world. It is categorized in *formae speciales* according to pathotypic variation and physiological character (Messiaen and Cassini, 1981 Fusarium:- Diseases, Biology and Taxonomy pp.427-445). The *F. oxysporum forma specialis vasinfectum* (Atk.) Snyder and Hansen is pathogenic on cotton (*Gossypium* spp.) on which it causes severe damage to susceptible races. We report here the DNA sequence of the 5.8S rRNA gene and flanking intergenic transcribed spacers of *F. oxysporum forma specialis vasinfectum*. DNA was isolated from mycelial cultures from three virulent isolates collected from single cotton plants from geographically distant sites in Bié, Cuanza Norte and Cuanza Bul regions of Angola (Ragazzi, 1992 J. Pl. Disease Protect. 99:499-504).

Ribosomal DNA (rDNA) repeat units contain highly conserved DNA sequences which have been used to detect phylogenetic relationships between species, as well as more variable DNA sequence regions which have been used to detect genetic variation between related fungal species and strains (White et al, 1990 PCR Protocols: A Guide to Methods and Applications. pp.315-322. Academic Press; O'Donnell, 1992 Curr. Genet. 22:213-220). The intergenic transcribed sequence (ITS) comprises the transcribed region flanking the 5.8S gene and is located between the 3' of the 18S gene and the 5' of the following 28S gene. Amplification of this region by the polymerase chain reaction (PCR) using DNA primers specific for conserved 18S and 28S elements has been used to produce characteristic DNA fragments from yeasts and from filamentous fungi. Genetic relationships between fungal strains have been deduced from sequence variation found in the ITS1 and ITS2 intergenic transcribed spacer regions, or from characteristic RFLP maps resulting from differential restriction of the region (O'Donnell 1992 Curr. Genet. 22:213-220; Kasuga et al, 1993 Curr. Genet. 24:343-346). Notably, sequence variation in the ITS regions of fungi was often not associated with a restriction site and was not detected by RFLP mapping.

Results

Cuanza Bul and Cuanza Norte isolates of *F. oxysporum f.sp. vasinfectum* had identical ITS1 and ITS2 regions of 151 bp and 152 bp. respectively. The Cuanza Bul isolate varied from Bi, and Cuanza Norte isolates by a single T deletion in the ITS1 region at nucleotide 37 (Figure 1). Restriction polymorphism was reported to be absent from the rRNA gene repeat of many *forma speciales* of *F. oxysporum* (Kistler et al, 1987 Phytopath. 77:1289-1293). The variation reported here within *forma speciales vasinfectum* was detectable by DNA sequencing and does not occur within any known restriction site. This suggests that nucleotide variation undetected by restriction analysis may occur within the rRNA gene repeat other races of *F. oxysporum*. Alignment using the FASTA program (Pearson and Lipman, 1988 Proc. Natl. Acad. Sci. USA 85:2444-2448) showed the 5.8S gene of the *F. sambucinum* to be 100% homologous to the *F. oxysporum* gene. Examination of the sequence the ITS regions of races of *F. sambucinum*

(O'Donnell 1992 Curr. Genet. 22: 213-220) has also shown significant sequence diversity. The sequence of the flanking ITS1 and ITS2 regions surrounding the *F. oxysporum* 5.8S rRNA gene show marked sequence variation to the ITS regions of *F. sambucinum*, and also variation between the Angolan *F. oxysporum* isolates. The sequence of the 5.8S rRNA gene and ITS regions of *F. sambucinum* are shown for comparison in Figure 1.

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Table 1. Characteristics of the 5.8S rRNA gene from *F. oxysporum f. sp. vasinfectum*
Organism:
Fusarium oxysporum Schlecht ex Fr. *forma specialis vasinfectum* (Atk.) Snyder and Hansen. Single spore mycelial cultures from Bié, Cuanza Norte and Cuanza Bul regions of Angola.
Sequence Source:
Direct PCR products amplified from genomic DNA templates. The amplification products were sequenced directly after isolation from low melting agarose gels. Four PCR primers served as sequencing primers for both strands of the ITS region, ITS1, ITS2, ITS3 (White et al, 1990 PCR Protocols: A Guide to Methods and Applications. pp.315-322. Academic Press) and 58S (5'-GGGCGCAAGGTGCGTTCAAA). The genes were located by comparison to yeast and filamentous fungal genes and by reference to the likely secondary structure (Nazar et al, 1975 J. Biol. Chem. 250:8591-8597).
Sequence infomation:
The representitive 5.8S rRNA gene, ITS1 and ITS2 regions are shown in Figure 1. The 5.8S gene sequence was 168 bp long and was identical in all three Angolan isolates of *F. oxysporum*. The ITS1 region was 150bp or 151bp, and the ITS2 region was 152 bp in all isolates.

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  ITS1 -->
              10         20         30         40         50
Foxv  CCGAGTTTACAACTCCCAAACCCCTGTGAACATACCTTACTTGTTGCCTC
      |||||||||||||||||||||||||||||||||||||||   |||||||||
Fsam  CCGAGTTTACAACTCCCAAACCCCTGTGAACATACCTTTA-TGTTGCCTC

      51       60         70         80         90        100
Foxv  GGCGGATCAGCCCGCTCCCGGTAAAACGGGACGGCCCGCCAGAGGACCCC
      |||||||||| | | |||        |||||||||||||   |||| |||
Fsam  GGCGGATCAGTCTG-TCC------TTCGGGACGGCCCGCCGCAGGA-CCC

      101    110        120        130        140        150
Foxv  TAAACTCTGTTTCTATA-TGTAACTTCTGAGTAAAACCAT-AAATAAATC
      ||||||||||  | || || ||||||||||||||  |  |||||||||
Fsam  TAAACTCTGTT--TTTAGTGGAACTTCTGAGTAAAA-AAACAAATAAATC

  5.8S -->
      151    160        170        180        190        200
Foxv  AAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAG
      ||||||||||||||||||||||||||||||||||||||||||||||||||
Fsam  AAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAG

      201    210        220        230        240        250
Foxv  CAAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATC
      ||||||||||||||||||||||||||||||||||||||||||||||||||
Fsam  CAAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATC
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      251    260       270       280       290       300
Foxv  TTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTTCGA
      ||||||||||||||||||||||||||||||||||||||||||||||||||
Fsam  TTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTTCGA

      ITS2 -->
      301    310       320       330       340       350
Foxv  GCGTCATTTCAACCCTCAAGCACAGCTTGGTGTTGGGA-CTCGCGTTAAT
      |||||||||||||||||||| ||||||||||||||| ||  |||
Fsam  GCGTCATTTCAACCCTCAAGCCCAGCTTGGTGTTGGGAGCTGTCGT---C

      351    360       370       380       390       400
Foxv  TCGCGTTCCTCAAATTGATTGGCGGTCACGTCGAGCTTCCATAGCGTAGT
      |  |  ||| |||||  ||||||||||||||||||||||||||||||||
Fsam  TGACACTCCCCAAATACATTGGCGGTCACGTCGAGCTTCCATAGCGTAGT

      401    410       420       430       440       450
Foxv  AGTAAAACCCTCGTTACTGGTAATCGTCGCGGCCACGCCGTTAAACCCCA
      | |  |  | ||||||||||||||||||||||||| ||||||| ||||
Fsam  AATTTACACATCGTTACTGGTAATCGTCGCGGCCACG-CGTTAAA-CCCA

      451    460       470
Foxv  ACTTCTGAATG
      ||||||||||||
Fsam  ACTTCTGAATG
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Figure 1. Nucleotide sequence of the antisense strand of the 5.8S rRNA gene of the *F. oxysporum f.sp. vasinfectum* (Foxv) from Angola. Representitive sequences are shown in comparison with *F. sambucinum* (Fsam) (EMBL: X65482) (O'Donnell 1992 Curr. Genet. 22: 213-220). Bold symbols indicate the 5.8S rRNA gene. The nucleotide 37 (***bold italic***) in the ITS1 region of Cuanza Bul and Cuanza Norte isolates is absent in Bié isolate. EMBL Accession Nos. for the sequences reported in this article are: X78258, X78259, X78260.