**PRIMER NOTE**

**Isolation of eight polymorphic microsatellite loci, using an enrichment protocol, in the phytopathogenic fungus *Fusarium culmorum***

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**Abstract**

We report the development of eight microsatellite markers in the haploid filamentous fungus *Fusarium culmorum*, a pathogen of numerous cereal crops. An enrichment protocol was used to isolate microsatellite loci, and polymorphism was explored with isolates of *Fusarium culmorum* and *F. graminearum* from natural populations collected from several French locations.

**Keywords:** enriched library, filamentous fungus, *Fusarium graminearum*, Giberella spp.

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The genus *Fusarium* (anamorphs Giberella, Ascomycete) contains numerous phytopathogenic species, *F. culmorum* and *F. graminearum* being particularly important pathogens of cereal crops in many areas of the world. The fungus causes head and seedling blight of small grains such as wheat and barley, ear and stalk rot of corn, and stem rot of carnation (Nelson et al. 1975; Cook 1981; Kommedahl & Windels 1981). These diseases cause yield reduction in many crops, and many *Fusarium* species also produce trichothecenes, which are highly toxic to both plants and animals, including humans (Desjardin et al. 1993). Molecular markers are needed to study the relationships between the different species of the *Fusarium* complex, and to identify trichothecenes-producing isolates. The molecular markers that have been used to date either were not polymorphic enough or exhibited problems of paralogous sequences (O’Donnell & Cigelnik 1997; Aoki & O’Donnell 1999). This prompted a search for microsatellite loci in *F. culmorum*.

A microsatellite enriched-library of *F. culmorum* was built according to (Dutech et al. 2000) using biotin-labelled microsatellite oligoprobes [(TG)\(_{10}\) and (AAG10)] and streptavidin-coated magnetic beads. Minor modifications were as follows: (i) total genomic DNA was extracted from the strain L2 of *F. culmorum* (isolated in 1996 from wheat in France) using the method of (Möller et al. 1992); (ii) the DNA fragments generated by RsaI digestion of the total genomic DNA were not selected for their sizes before ligation to the adaptors; (iii) the polymerase chain reaction (PCR) fragments obtained after enrichment were cloned using TOPO TA Cloning Kit (Invitrogen K450641); and (iv) recombinant colonies were transferred onto charged Nylon membranes (Hybond N+, Amersham-Pharmacia) and screened by hybridization of dioxigenine-labelled oligoprobes [(TG)\(_{10}\) and (AAG10)]. A total of 800 clones were screened and 132 gave a positive response. Despite the lack of a size-selecting step, inserts were of an appropriate size, i.e. mainly between 300–700 bp. Of the 33 clones that were sequenced all contained microsatellite loci. Approximately half of the clones contained microsatellite motifs with too low a number of repeats, i.e. less than eight repeats. PCR primers were designed for eight loci, using the computer program Oligo™ (Macintosh version 4.0, National Bioscience). Each locus was screened for variation and cross-amplification using a panel of 29 *Fusarium* spp. isolates (Table 1). PCR amplifications were performed using a Biometra thermal cycler, with 35 cycles of 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 30 s. Each reaction (10 μL) contained 1 μL of 10x reaction buffer (50 mM KCl, 0.1% Triton X-100, 10 mM Tris-HCl, pH 9.0), 75 μM of dCTP,
dGTP, dTTP, 6 µM of dATP, 0.02 µL of 33P dATP, 0.2 µg/L BSA, 1.5 mM MgCl2, 2.5 pmol of each primer, 0.25 U of Taq DNA polymerase (Promega), and approximately 10 ng of sample DNA. PCR products were analysed in 6% polyacrylamide gels and visualized by autoradiography. Alleles were scored by length in bp.

The eight loci successfully amplified fragments of appropriate size in *F. culmorum* and *F. graminearum* (Table 2). Only
the locus F10 failed to cross-amplify in *F. graminearum*. The other loci seem to differentiate the two species, as few alleles were shared between *F. graminearum* and *F. culmorum*. Despite the low number of individuals genotyped in each of the two species, the microsatellite loci characterized here proved to be highly polymorphic, and this opens opportunities to study the *Fusarium* species complex.

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**References**


