A rapid molecular technique to distinguish *Fusarium* species

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The nuclear DNA (nDNA) of different isolates of three closely related, toxin-producing *Fusarium* species, *F. moniliforme*, *F. nygamai* and *F. napiforme*, was compared to ascertain the sensitivity of a molecular method to distinguish these three species. The nDNA of these strains was digested with the restriction enzyme EcoRI and Southern analysis performed with the 6·3 kb ribosomal DNA (rDNA) repeat unit of *Neurospora crassa* as probe. Distinct polymorphic fragment patterns, which distinguished between the different *Fusarium* species, were obtained.

Correct identification of *Fusarium* species is becoming increasingly important due to the toxin-producing capabilities of some of these species, especially the fumonisins producers (Marasas et al., 1986; Ross et al., 1991). Nelson and colleagues (1983) stated, in the introduction to their illustrated manual for the identification of *Fusarium* species, that workers interested in *Fusarium* species often encountered problems with the correct identification of *Fusarium* strains. This still remains a problem. Three closely related, toxin-producing *Fusarium* species, *F. moniliforme*, *F. nygamai* and *F. napiforme*, are currently distinguished on morphological characters such as the shape of macro- and microconidia, the presence of monon- and/or polyphialides, as well as the presence or absence of chlamydoconidia. Limited or questionable morphological data, however, prevent conclusive verification of the taxonomic position of these *Fusarium* species (Marasas et al., 1986, 1991).

Ribosomal DNA (rDNA) restriction fragment length polymorphisms (RFLPs) have been used in fungal rDNAs of *Aspergillus* (Moody & Tyler, 1990) and *Eutypnopaga* (Walsh et al., 1990) to demonstrate species-specific differences. To establish a reliable and relatively fast identification aid for toxin-producing *Fusarium* species, a clone of the *Neurospora crassa* rDNA repeat unit (Russell et al., 1984) was used as a probe to identify RFLPs of *Fusarium* rDNA genes. This paper presents the distinguishable EcoRI restriction patterns obtained for eleven *Fusarium* strains belonging to the above-mentioned toxine-producing *Fusarium* species.

Taxonomically well-characterized strains of *Fusarium moniliforme*, *F. nygamai* and *F. napiforme* were randomly picked from the culture collection of the South African Medical Research Council (MRC). The exception was *F. graminearum* MRC 10115 (included as a less related *Fusarium* control) which was obtained from the CSIR's culture collection. Lyophilized stock cultures of the *Fusarium* strains were revived on potato dextrose agar (PDA) slants at 25 °C. Spore suspensions (in sterile distilled water) were prepared from the PDA slants and used as inocula. The *Fusarium* strains were cultivated in 100 ml YM-liquid medium (0·3% malt extract, 0·3% yeast extract, 0·5% peptone and 1% glucose) for four days at 27°. The mycelia were harvested by filtration through sterile cheesecloth, washed with sterile water and stored at −20°.

The nuclear DNA (nDNA) was isolated from 0·2–0·4 g cell material according to the method of Hoffman et al. (1987). The polysaccharides associated with the nDNAs were removed with CTAB (cetyltrimethylammonium bromide) treatment, as described by Ausubel et al. (1988). The rDNAs were digested to completion with the restriction enzyme EcoRI (Boehringer Mannheim). The restriction fragments generated were separated by electrophoresis in 0·8% agarose gels and transferred onto nitrocellulose filters as described by Smith & Summers (1980). The *N. crassa* rDNA was purified from plasmid pMF2 (Russell et al., 1984) as a 6·3 kb Pst I fragment and labelled with [α-32P]dATP according to the random primer nick translation method of Feinberg & Vogelstein (1983). The rDNA hybridizations were performed in hybridization buffer described by Church & Gilbert (1984), and stringency washes done according to the method of Sambrook et al. (1989).

Three distinctive EcoRI restriction patterns were detected for the tandemly repeated rDNA segments of the different strains of *F. graminearum*, *F. napiforme* and *F. moniliforme*/*F. nygamai*, using the *N. crassa* rDNA probe (Fig. 1). All four of the *F. napiforme* strains had the same restriction pattern, with fragment sizes of approximately 5·3 and 3·7 kb. The restriction pattern for the rDNA repeat of one *F. moniliforme* strain, MRC 8, corresponded to that of *F. napiforme*. However, the other

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two _F. moniliforme_ strains (MRC 4363 and MRC 31) and the three _F. nygamai_ strains gave similar rDNA restriction patterns, which differed from that of _F. napiforme_. The approximate sizes for the _F. moniliforme//F. nygamai_ rDNA restriction fragments were 4.6 and 3.7 kb. The rDNA restriction pattern of the unrelated species, _F. graminearum_, differed noticeably from that of the other three _Fusarium_ strains. Two EcoRI rDNA fragments with sizes of approximately 4.6 and 3.1 kb were observed. The results indicated a closer relatedness between _F. moniliforme_ and _F. nygamai_.

Although it was previously thought that fumonisin production was restricted to isolates of _F. moniliforme_ and _F. proliferatum_, Thiel _et al._ (1991) recently reported the production of fumonisins by _F. nygamai_. In view of the similar rDNA patterns obtained in this study, their finding is not really surprising. The rDNA hybridization results cast some doubt on the identification of _F. moniliforme_ MRC 8, which shares a similar rDNA restriction pattern with the _F. napiforme_ strains. This technique therefore could be a valuable aid in clarifying the taxonomic position of _F. moniliforme_ MRC 8. The results obtained for the three toxin-producing _Fusarium_ species clearly indicated that the analyses of RFLPs in _Fusarium_ rDNA repeats could be a very helpful complementary tool to the existing taxonomic system for the identification of _Fusarium_ species.

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REFERENCES


