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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 *Eco*RI 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 fumonisin 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 mono- and/or polyphialides, as well as the presence or absence of chlamydospores. 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 *Entomophaga* (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 *Eco*RI restriction patterns obtained for eleven *Fusarium* strains belonging to the above-mentioned toxin-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 nDNAs were digested to completion with the restriction enzyme *Eco*RI (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-^{32}P]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 *Eco*RI 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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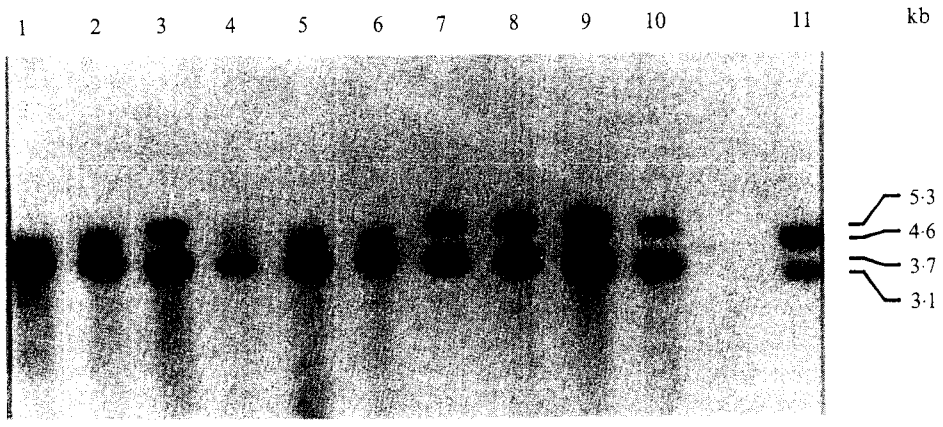

Fig. 1. rDNA hybridization patterns for *Eco*RI-digested nDNA of strains from four *Fusarium* species. *F. moniliforme* MRC 4363 (lane 1); MRC 31 (lane 2); MRC 8 (lane 3); *F. nygamai* MRC 4150 (lane 4); MRC 4373 (lane 5); MRC 4164 (lane 6); *F. napiforme* MRC 4131 (lane 7); MRC 4139 (lane 8); MRC 4144 (lane 9); MRC 4146 (lane 10) and *F. graminearum* MRC 10115 (lane 11).

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 *Eco*RI 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

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (1988). *Current Protocols in Molecular Biology*, **vol. 1.** Wiley: New York.

Church, G. M. & Gilbert, W. (1984). Genomic sequencing. *Proceedings of the National Academy of Sciences of the U.S.A.* **81**, 1991–1995.

Feinberg, A. P. & Vogelstein, B. (1983). A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Analytical Biochemistry* **132**, 6–13.

Hoffman, C. S. & Winston, F. (1987). A ten-minute DNA preparation from yeast efficiently releases autonomous plasmids for transformation of *Escherichia coli*. *Gene* **57**, 267–272.

Marasas, W. F. O., Thiel, P. G., Rabie, C. J., Nelson, P. E. & Toussoun, T. A. (1986). Moniliformin production in *Fusarium* section *Liseola*. *Mycologia* **78**, 242–247.

Marasas, W. F. O., Thiel, P. G., Sydenham, E. W., Rabie, C. J., Lübben, A. & Nelson, P. E. (1991). Toxicity and moniliformin production by four recently described species of *Fusarium* and two uncertain taxa. *Mycopathologia* **113**, 191–197.

Moody, S. F. & Tyler, B. M. (1990). Use of nuclear DNA restriction fragment length polymorphisms to analyze the diversity of the *Aspergillus flavus* group: *A. flavus*, *A. parasiticus*, and *A. nomius*. *Applied and Environmental Microbiology* **56**, 2453–2461.

Nelson, P. E., Toussoun, T. A. & Marasas, W. F. O. (1983). *Fusarium Species: An Illustrated Manual for Identification*. Pennsylvania State University Press: University Park.

Ross, P. F., Rice, L. G., Plattner, R. D., Osweiler, G. D., Wilson, T. M., Owens, D. L., Nelson, H. A. & Richard, J. L. (1991). Concentrations of fumonisin B_1 in feeds associated with animal health problems. *Mycopathologia* **114**, 129–135.

Russell, P. J., Wagner, S., Rodland, K. D., Feinbaum, R. L., Russell, J. P., Bret-Harte, M. S., Free, S. J. & Metzenberg, R. L. (1984). Organization of the ribosomal ribonucleic acid genes in various wild-type strains and wild-collected strains of *Neurospora*. *Molecular and General Genetics* **196**, 275–282.

Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular Cloning – A Laboratory Manual*. Cold Spring Harbor Laboratory Press: Cold Spring Harbor, N.Y.

Smith, G. E. & Summers, M. D. (1980). The bidirectional transfer of DNA and RNA to nitrocellulose or diazobenzyloxymethyl-paper. *Analytical Biochemistry* **109**, 123–129.

Thiel, P. G., Marasas, W. F. O., Sydenham, E. W., Shephard, G. S., Gelderblom, W. C. A. & Nieuwenhuis, J. J. (1991). Survey of fumonisin production by *Fusarium* species. *Applied and Environmental Microbiology* **57**, 1089–1093.

Walsh, S. R. A., Tyrrell, D., Humber, R. A. & Silver, J. C. (1990). DNA restriction fragment length polymorphisms in the rDNA repeat unit of *Entomophaga*. *Experimental Mycology* **14**, 381–392.

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