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RESEARCH NOTE

High-affinity iron permease (*FTR1*) gene sequence-based molecular identification of clinically important Zygomycetes

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ABSTRACT

The clinical importance of zygomycosis, an emerging and frequently fatal mycotic disease, has increased during recent years. This report describes an identification method based on PCR amplification and sequencing of the high-affinity iron permease 1 gene (*FTR1*). Primers and amplification protocols were established and tested for the identification of *Rhizopus oryzae*, *Rhizopus microsporus* var. *rhizopodiformis*, *R. microsporus* var. *oligosporus*, *Rhizopus schipperae*, *Rhizopus niveus* and *Rhizopus stolonifer*. *Rhizomucor* and *Syncephalastrum* could be identified at the genus level. PCR–restriction fragment length polymorphism analysis of the amplified gene fragment using *Alu*I digestion distinguished three subgroups among the *R. oryzae* isolates.

Keywords Identification, iron permease 1 gene, Mucorales, PCR–restriction fragment length polymorphism analysis, *Rhizopus*, zygomycosis

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Zygomycetes have been reported to be agents of opportunistic mycoses that are frequently fatal [1]. The diagnosis of zygomycosis is based on the detection of hyphae in clinical specimens. Species determination is laborious and usually requires the expertise of a reference laboratory [2]; thus, clinical laboratories often identify such infections as zygomycosis only, without further species determination [3,4]. Although these mycoses are relatively rare, the associated high mortality, difficulty in diagnosis and resistance to the most widely used antifungal drugs emphasise the importance of developing new diagnostic assays [3,5]. Progress has already been made in the design of taxon-specific primer pairs based on 28S rDNA sequences [6], and Schwarz *et al.* [7] have reported an identification method based on internal spacer sequences and 5.8S rDNA regions.

The present study aimed to use a structural gene for molecular diagnostic purposes. A fragment of the high-affinity iron permease 1 gene (*FTR1*) was used to generate a sequence dataset in order to design PCR primer pairs for the rapid and accurate detection of Zygomycetes. Twenty-six strains, comprising *Rhizopus oryzae* (CBS 395.54, SZMC 8100, CBS 146.90, SZMC 0497, NRRL 2908, CBS 112.07, CBS 260.28, TJM 24B2, CBS 109.939), *Rhizopus schipperae* (CBS 138.95, UHF 3053), *Rhizopus microsporus* var. *rhizopodiformis* (CBS 220.92, CBS 102.277), *R. microsporus* var. *oligosporus* (NRRL 514), *Rhizopus niveus* (CBS 403.51), *Rhizopus stolonifer* (CBS 347.49, CBS 320.35), *Rhizomucor miehei* (CBS 360.92), *Rhizomucor pusillus* (WRLCN(M) 231), *Syncephalastrum racemosum* (SZMC 2011), *Mucor racemosus* (NRRL 3640), *Mucor circinelloides* (FRR 2109, CBS 277.49), *Mucor plumbeus* (ATCC 42423), *Mucor rouxii* (ATCC 24905) and *Backusella lamprospora* (NRRL 1422), were included in the study. As *Rhizopus* spp., particularly *R. oryzae*, are the predominant zygomycotic organisms, this study focused primarily on the members of this genus. Nine strains of *R. oryzae* were included, and the corresponding *FTR1* region of the clinical isolate 99–880 [8] was also added to the sequence analysis. Clinical isolates of *R. microsporus* var. *rhizopodiformis* and *R. schipperae*, a newly described species isolated exclusively from zygomycoses [9], were also investigated. *Rhizomucor miehei* and *Rhizomucor pusillus* were represented by isolates from human or animal mycoses. The strains of *Mucor*, *Backusella* and *Syncephalastrum* spp. were included in the study for comparison.

For DNA isolation, strains were grown in yeast extract–glucose medium (yeast extract 0.5% w/v, glucose 2% w/v) with continuous shaking at 200 rpm for 3 days. Genomic DNA was isolated as described by Iturriaga *et al.* [10]. *FTR1* fragments were amplified from the DNA samples using PCR and a degenerate primer pair designated as FTR-A (5′-GGTCTAGAGARGAYATHT GGGARGG) and FTR-B (5′-GGCTCGAGCC ANCCNARDATNGCRTTRAA). Primer design

Table 1. Oligonucleotide primers and annealing temperatures used for specific amplification of *FTR1* gene fragments from the species indicated, together with the corresponding sizes of the amplification products

PCR primer pairs (5′–3′)	Zygomycetes species identified	Size of PCR product (bp)	Annealing temperature (°C)
M1 GGGYCAAAAGATYGGWTTSAA *M2* GCAAMAGACTTCCACCKCGAT	*Backusella lamprospora* *Mucor plumbeus* *Mucor rouxii* *Mucor circinelloides* *Mucor racemosus*	215	63
Rhm1 GTATCACCATGCTTCGA *Rhm2* TGATGGATCCTGACTCCT	*Rhizopus microsporus* var. *oligosporus*	438	65
Rhr1 CTAGCACTGAAAAGACTGGCT *Rhr2* GGCAGAAATGTTTAATTCAGGAT	*R. microsporus* var. *rhizopodiformis*	431	68
Rsc1 CCTTCAAAGACAAACTCCAGAAG *Rsc2* CGTTTGTGTCAACATTCA	*Rhizopus schipperae*	417	60
Rho3 GATCATGATCACTGCCAT *Rho2* GCGGTWGAGACTCTGTARCYA	*Rhizopus oryzae*	465	68
Rhs1 GTCCAACTTYAAGGAAAAGAT *FTRB* GGCTCGAGCCANCCNARDATNGCRTTRAA	*Rhizopus stolonifer*	434	49
Rhn1 CGCAAGAGCGTTCTTCTTTCA *FTRB* GGCTCGAGCCANCCNARDATNGCRTTRAA	*Rhizopus niveus*	444	60
Sr1 GAAGACACTTAGCGCACGCA *Sr2* CAGCGCAGGGCAATCATAT	*Syncephalastrum racemosum*	273	64
R1 GGAAACCGATGCYTTGCA *R2* CRTCACCRCCTTCTTCGGC	*Rhizomucor miehei* *Rhizomucor pusillus*	432/441	68

R = A, G; Y = C, T; M = A, C; K = G, T; S = C, G; W = A, T; H = A, C, T; B = C, G, T; V = A, C, G; D = A, G, T; N = A, C, G, T.