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**Molecular identification of *Fusarium* species complexes: which gene and which database
to choose in clinical practice?**

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ABSTRACT

Identification of *Fusarium* at the level of the species complex is difficult with phenotypic methods, so it is necessary to use molecular sequencing methods. This study presents, for 33 isolates distributed among the four major species complexes, the performance of five identification schemes involving ITS (internal transcribed spacer), EF1 α (translation elongation factor 1 alpha), RPB1 (largest subunit of RNA polymerase) and RPB2 (second largest subunit of RNA polymerase) genes and two databases: GenBank and *Fusarium* MLST (MultiLocus Sequence Typing). In our practice, the identification of the fungus from a culture is performed with EF1 α and from a primary sample with ITS, using in both cases the specific database *Fusarium* MLST.

Keywords:

Fusarium species complex; molecular identification; *Fusarium* MLST; EF1 α

INTRODUCTION

Fusarium species are molds that are widely distributed in the environment, including water and soil, and are significant plant pathogens (1). In immunocompetent patients, the genus *Fusarium* causes exclusively localized pathology; however, in immunocompromised hosts, principally burn and hematological patients, the infections are local or invasive (2). Clinical presentations of invasive fusariosis are: fungemia, cutaneous infection, pulmonary disease or other rare infections (endocarditis, osteomyelitis, etc.) (3–5). All drugs used in clinical practice have shown poor *in vitro* activity against *Fusarium*, which is why these pathologies have a high mortality rate (6,7). The genus *Fusarium* is divided into phylogenetic species complexes. Those most frequently involved in human infections are *Fusarium solani* species complex (FSSC), *Fusarium oxysporum* species complex (FOSC), *Fusarium dimerum* species complex (FDSC), *Gibberella fujikuroi* species complex (GFSC), and *Fusarium incarnatum* - *Fusarium equiseti* species complex (FIESC) (6). These different complexes have macroscopic and microscopic culture traits that are quite similar, making it difficult to identify species complexes with phenotypic methods (8). As a result, it is necessary to use molecular biology methods for the identification of species complexes. Currently, the recommended identification scheme is based on the sequencing of 3 genes: EF1 α (translation elongation factor 1 alpha), RPB1 (largest subunit of RNA polymerase) and RPB2 (second-largest subunit of RNA polymerase) (9). In daily laboratory practice, this scheme can be cumbersome. In current practice, the identification of species complexes can take place following isolation of *Fusarium* in culture, and also directly on primary samples (biopsies, for example) having benefited from a pan-fungal PCR in the context of an invasive fungal infection.

In this study, we compared the performance in terms of identification of four genes commonly used for identification (ITS, EF1 α , RPB1 and RPB2), as well as two databases (GenBank and *Fusarium* MLST), in order to propose an optimal methodology for identifying complexes of *Fusarium* species on culture or primary sampling.

MATERIALS AND METHODS

Isolates

Thirty-three clinical isolates of the genus *Fusarium* from different locations (nails, corneas or blood cultures) were studied, distributed among the four main species complexes: 19 (58%) FOISC; 10 (30%) FSSC; 3 (9%) GFSC and 1 (3%) FDSC.

Sequence analysis

After 5 days of culture on MALT medium, DNA extraction was carried out from the mycelium using the QIAamp DNA Mini Kit® (Qiagen) according to the manufacturer's recommendations.

The ITS (internal transcribed spacer) gene was sequenced by primers ITS1 and ITS4 (10) and the sequences obtained were analyzed with Blast, the GenBank database (<https://blast.ncbi.nlm.nih.gov>) and also with a specific database of the genus *Fusarium*, CBS-KNAW Fungal Biodiversity Centre's *Fusarium* MLST website (<http://www.westerdijkinstituut.nl/Fusarium>). The sequences of the 3 genes, EF1 α , RPB1 and

RPB2, were carried out according to the O'Donnell team protocols and analyzed with a specific database (9).

Performance indicators

In this work, 5 identification schemes were evaluated: ITS/GenBank; ITS/*Fusarium* MLST; EF1 α /*Fusarium* MLST; RPB1/*Fusarium* MLST and RPB2/*Fusarium* MLST.

For each of the isolates and each scheme used, only the first 20 occurrences or identification responses were considered. Thus, several indicators were analyzed and compared: the percentage of minimum, median and maximum similarity, the number of different total responses (of the 20 occurrences) and the number of different responses of the first order (namely, the highest percentage of sequence similarity). Ideally, the optimal identification scheme should have high percentages of similarities, close to 100%, and the numbers of different responses close to 1.

RESULTS

For the 33 isolates studied, the five schemes described identified the same species complex with varying performance.

For the four species complexes studied, the performances of each of the genes studied/database pairs are shown in Table 1.

We observed that the identification performance of ITS, RPB1 and RPB2 genes depended on the species complex to be identified. For example, RPB2 analysis with *Fusarium* MLST was

indicative for FSSC, but much less so for FOSC or GFSC. In contrast, EF1 α was the most versatile gene; its performance was less variable, depending on the species complexes studied, with an average number of responses for the first 20 occurrences between 1 and 1.67. The numbers of different first order responses of the ITS gene with the GenBank database was high for the FSSC and GFSC complexes, respectively 2.80 and 4.33. In contrast, the numbers of different first-order responses for all genes with the specific *Fusarium* MLST base were close to 1. The numbers of different responses among the 20 occurrences were low: i) with EF1 α , regardless of the species complex (≤ 1.67); ii) with ITS and GenBank database for FOSC (1.21); iii) with ITS and the specific database for all the studied complexes (1.00 or 2.00) except FOSC (3.00). The numbers were elevated for all complexes with RPB1 and RPB2 genes (up to 9.90) (except FSSC and RPB2).

DISCUSSION

The universal approach for identifying a fungus with a molecular method relies on the sequencing of the ITS gene (11). However, using this target and the GenBank database, unambiguous identification cannot always be assured. Indeed, several identifications of species or species complexes can be proposed with the same high percentage of similarity. Also, partial identifications, such as *Fusarium* sp., are often reported. Finally, responses are rarely presented according to the complexes described. Thus, this scheme is not considered optimal for the identification of *Fusarium* species complexes.

As a result, specific genes and a database dedicated to the identification of *Fusarium* species complexes have been proposed (9). A multigene analysis makes it possible to identify the subtype within each complex. However, the analysis of a single gene leads to a complex.

RPB1 and RPB2 have a lower occurrence number for high percentages, suggesting a less-supplied database. EF1 α meets the expectations of our performance indicators. In addition, the smaller size of EF1 α allows one-piece sequencing for laboratories with modest equipment.

It is important to note that the performance of the ITS gene is improved when analyzing these sequences with the specific *Fusarium* MLST database.

This study establishes identification strategies in current practice that combine analytical performance and technical efficiency (time and cost). Thus, for the identification of the species complex of a *Fusarium* fungus obtained in culture, it is necessary to carry out a sequencing of the EF1 α gene and an analysis on the basis of the specific database *Fusarium* MLST, as recommended by the O'Donnell team (9). On the other hand, when the laboratory carries out a pan-fungal PCR on primary sampling (biopsy of cutaneous necrosis or ophthalmological sampling in the context of a keratitis), it is not necessary to resort to a second sequencing; the analysis of the ITS sequence with the specific database will allow a quality diagnosis of the species complex.

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Conflict of interest

None of the authors have any conflict of interest.

REFERENCES

1. Nelson PE, Dignani MC, Anaissie EJ. Taxonomy, biology, and clinical aspects of *Fusarium* species. *Clin Microbiol Rev.* 1994 Oct;7(4):479–504.
2. Nucci M, Anaissie E. Emerging fungi. *Infect Dis Clin North Am.* 2006 Sep;20(3):563–79.
3. Sierra-Hoffman M, Paltiyevich-Gibson S, Carpenter JL, Hurley DL. *Fusarium* osteomyelitis: case report and review of the literature. *Scand J Infect Dis.* 2005;37(3):237–40.
4. Nucci F, Nouér SA, Capone D, Anaissie E, Nucci M. Fusariosis. *Semin Respir Crit Care Med.* 2015 Oct;36(5):706–14.
5. Kassab O, Charfi M, Trabelsi H, Hammami R, Elloumi M. *Fusarium solani* endocarditis in an acute leukemia patient. *Med Mal Infect.* 2016 Feb;46(1):57–9.
6. Guarro J. Fusariosis, a complex infection caused by a high diversity of fungal species refractory to treatment. *Eur J Clin Microbiol Infect Dis.* 2013 Dec;32(12):1491–500.
7. Debourgogne A, de Hoog S, Lozniewski A, Machouart M. Amphotericin B and voriconazole susceptibility profiles for the *Fusarium solani* species complex: comparison between the E-test and CLSI M38-A2 microdilution methodology. *Eur J Clin Microbiol Infect Dis Off Publ Eur Soc Clin Microbiol.* 2012 Apr;31(4):615–8.
8. de Hoog S, Guarro J, Gené J, Figueras M. *Atlas of Clinical Fungi.* 2011.
9. O'Donnell K, Sutton DA, Rinaldi MG, Sarver BAJ, Balajee SA, Schroers H-J, et al. Internet-Accessible DNA Sequence Database for Identifying *Fusaria* from Human and Animal Infections. *J Clin Microbiol.* 2010 Aug 4;48(10):3708–18.
10. Debourgogne A, Goehringer F, Umhang G, Gauchotte G, Hénard S, Boué F, et al. Primary Cerebral Alveolar Echinococcosis: Mycology to the Rescue. *J Clin Microbiol.* 2014 Feb;52(2):692–4.
11. van Diepeningen AD, Brankovics B, Iltes J, van der Lee TAJ, Waalwijk C. Diagnosis of *Fusarium* Infections: Approaches to Identification by the Clinical Mycology Laboratory. *Curr Fungal Infect Rep.* 2015;9(3):135–43.

FIGURES

Table 1: Comparison of the performances of the different identification schemes: gene/database.

For each identification condition and each species complex, the average percentage of similarity (maximum, median and minimum), the number of different responses from the first order and the total number of different responses are given.

		<i>Fusarium oxysporum</i> species complex (n=19)	<i>Fusarium solani</i> species complex (n=10)	<i>Gibberella fujikuroi</i> species complex (n=3)	<i>Fusarium dimerum</i> species complex (n=1)
ITS / GenBank	Maximum similarity (%)	100	100	100	99
	Median similarity (%)	100	99,6	99,67	99
	Minimum similarity (%)	100	99,6	99,67	99
	Number of different answers of the first order	1,21	2,80	4,33	1,00
	Number of different responses of the 20 occurrences	1,21	3,30	6,33	2,00
ITS / <i>Fusarium</i> MLST	Maximum similarity (%)	99,99	99,98	99,77	99,62
	Median similarity (%)	99,57	99,73	98,68	99,62
	Minimum similarity (%)	93,04	99,26	91,81	99,62
	Number of different answers of the first order	1,21	1,00	1,00	1,00
	Number of different responses of the 20 occurrences	3,00	1,00	2,00	1,00
EF1 / <i>Fusarium</i> MLST	Maximum similarity (%)	99,97	99,83	99,61	99,83
	Median similarity (%)	99,33	98,71	93,27	99,83
	Minimum similarity (%)	99,21	97,91	92,80	99,83
	Number of different answers of the first order	1,00	1,00	1,00	1,00
	Number of different responses of the 20 occurrences	1,05	1,00	1,67	1,00
RPB1 / <i>Fusarium</i> MLST	Maximum similarity (%)	99,56	97,63	99,35	99,73
	Median similarity (%)	96,66	85,85	95,87	80,55
	Minimum similarity (%)	95,04	84,41	94,79	79,93
	Number of different answers of the first order	1,00	1,00	1,00	1,00
	Number of different responses of the 20 occurrences	5,00	9,90	5,00	7,00
RPB2 / <i>Fusarium</i> MLST	Maximum similarity (%)	99,73	99,86	98,75	100,00
	Median similarity (%)	95,54	98,24	96,20	82,43
	Minimum similarity (%)	95,14	96,93	95,32	82,13
	Number of different answers of the first order	1,00	1,00	1,00	1,00
	Number of different responses of the 20 occurrences	6,00	1,00	4,67	2,00