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Comparison of VITEK 2 with ITS2-Fragment Length Polymorphism Analysis for Identification of Yeast Species

Caroline Massonet,[1]* Johan Van Eldere,[1,2] Mario Vaneechoutte,[3] Thierry De Baere,[3] Jan Verhaegen,[2] and Katrien Lagrou[2]

Department of Immunology and Microbiology, Rega Institute,[1] and Laboratorium geneeskunde,[2] U.Z. Gasthuisberg, B-3000 Leuven, and Department of Clinical Microbiology and Immunology, Ghent University Hospital, B-9000 Ghent,[3] Belgium

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A total of 61 clinical yeast isolates of *Candida*, *Cryptococcus*, *Blastoschizomyces*, and *Saccharomyces* spp. were used to compare two identification techniques, VITEK 2 and ITS2-fragment length polymorphism analysis (ITS2-FLP), with ID32C as the reference method. ID32C identified 58 isolates correctly. ITS2-FLP with Instagene DNA extraction identified 59 isolates. ITS2-FLP combined with boiling-freezing DNA extraction identified 55 isolates. VITEK 2 identified 41 isolates correctly.

The clinical impact of infections by yeast species such as *Candida*, *Cryptococcus*, *Blastoschizomyces*, and *Saccharomyces* spp. (especially in immunocompromised patients) has increased, and more antifungal agents have become available (7, 9, 11, 17). A rapid and correct identification of yeast species is important, because differences exist between species with regard to antifungal susceptibility (16). Conventional identification methods are time consuming (3, 17). Commercially available biochemical and molecular methods, which allow identification within several hours, have been developed and evaluated (1, 2, 4, 5, 7, 8, 9, 10, 13).

In this study we compared a commercial system, VITEK 2 (bioMérieux Vitek, Hazelwood, Mo.) and a PCR-based method, ITS2-fragment length polymorphism analysis (ITS2-FLP), with ID32C (bioMérieux, Marcy-l'Etoile, France) as the reference method.

We examined 61 isolates belonging to 10 different species. Of the 61 isolates, 57 (*Candida albicans* [*n* = 7], *Candida dubliniensis* [*n* = 1], *Candida glabrata* [*n* = 18], *Candida guilliermondii* [*n* = 3], *Candida krusei* [*n* = 1], *Candida parapsilosis* [*n* = 13], *Candida tropicalis* [*n* = 3], *Cryptococcus neoformans* [*n* = 3], *Blastoschizomyces capitatus* [*n* = 2], *Saccharomyces cerevisiae* [*n* = 4], and *Candida palmioleophila* [*n* = 1]) were from patients hospitalized in the University Hospital of Leuven (Leuven, Belgium) during a 5-month collection period. Additionally, two *C. krusei* isolates and two *C. neoformans* isolates from the culture collection of the University Hospital Leuven were included because of the small number of isolates recovered during the collection period. Isolates were grown at 37°C on Sabouraud chloramphenicol agar (Bio-Rad, Marnes-La-Coquette, France) for 24 to 48 h. For VITEK 2 assays, cultures were not older than 24 h (in accordance with the instructions by the manufacturer).

Inoculation, incubation, and reading of the ID32C strip were performed manually following the manufacturer's instructions. ATB Expression and ATB Plus software (version 2.0) (bio Mérieux) was used for interpretation of the results.

VITEK 2 uses fluorescence to monitor 47 metabolic reactions in the ID-YST card (yeast identification card). The system automatically fills, seals, and transfers cards into an incubator. The density of the yeast suspension was always checked and adapted to a 0.5 McFarland density.

After 15 h of incubation at 35°C, metabolic profiles are compared to the system's database.

ITS2-FLP is based on the amplification of the rRNA internally transcribed spacer region between the 5.8S and 28S rRNA genes followed by fragment length analysis (15). PCR of ITS2 and preparation of samples for capillary electrophoresis on an ABI Prism 310 capillary electrophoresis system (Applied Biosystems, Foster City, Calif.) were performed as described previously (4). The fragment length profile was compared to data in a self-compiled ITS2 size-based library that contains 240 profiles for 27 yeast species (4) (available at http://allserv.ugent.be/~mvaneech/Yeasts.txt). Two DNA extraction methods were evaluated: a boiling-freezing (BF) method (performed as described previously) (4) and an Instagene (I) extraction method (Bio-Rad, Hercules, Calif.) (performed following the manufacturer's instructions).

ID32C was chosen as the reference system (1, 7, 8, 10). Discrepancies between VITEK 2, ITS-FLP, and the reference method were analyzed by repeated testing of the discrepant method and by additional tests (pigmentation of colonies on CHROMagar Candida [Chromagar Microbiologie, Paris, France] and morphology on Cornmeal-Tween 80 agar [OXOID, Hampshire, United Kingdom]) (11). Rapid trehalose and maltose tests were used as additional tests for the identification of *C. glabrata* (5, 6).

When discrepant results could not be resolved by these additional tests, the ITS2 region was sequenced. Preparation of the isolates and sequence analysis on the ABI 310 were performed as described previously (4).

ID32C has been used by several authors as a reference method because of its extensive database and accuracy (1). In our hands, all isolates (except for one isolate of *C. glabrata*, one isolate of *C. palmioleophila*, and one of the *C. albicans* isolates)

* Corresponding author. Mailing address: Department of Immunology and Microbiology, U.Z. Gasthuisberg, Herestraat 49, CDG 8ste, B-3000 Leuven, Belgium. Phone: 32-16-34 62 74. Fax: 32-16-34 62 75. E-mail: caroline.massonet@med.kuleuven.ac.be.

TABLE 1. Identification results with ID32C, VITEK 2, and ITS2-FLP

Organism	No. of isolates	System	No. of isolates with the following result:				Final identification established by:
			Identified	Low discrimination	Misidentification	Unidentified	
C. albicans	7	ID32C	6		1 (*C. sake*)		Green colonies on chromagar, morphology on cornmeal agar
		VITEK 2	7				
		ITS2-FLP–I	7				
		ITS2-FLP–BF	7				
C. dubliniensis	1	ID32C	1				
		VITEK 2		1			Weak growth at 42°C
		ITS2-FLP–I	1				
		ITS2-FLP–BF	1				
C. glabrata	18	ID32C	17	1			Pink colonies on chromagar, no pseudohyphae on cornmeal agar, T + M−[a]
		VITEK 2	5	4 (*C. magnoliae*)	3 (*C. magnoliae*)	5	Pink colonies on chromagar, no pseudohyphae on cornmeal agar, T + M−
				1 (*C. colliculosa*)			
		ITS2-FLP–I	18				
		ITS2-FLP–BF	16			2	
C. guilliermondii	3	ID32C	3				
		VITEK 2	3				
		ITS2-FLP–I	3				
		ITS2-FLP–BF	2			1	
C. krusei	3	ID32C	3				
		VITEK 2	1			2	Pink colonies on Chromagar plate
		ITS2-FLP–I	3				
		ITS2-FLP–BF	3				
C. parapsilosis	14	ID32C	14				
		VITEK 2	12			2	Morphology on cornmeal agar
		ITS2-FLP–I	13		1 (*Trichosporon mucoides*)		Morphology on cornmeal agar[b]
		ITS2-FLP–BF	13		1 (*T. mucoides*)		Morphology on cornmeal agar[b]
C. tropicalis	3	ID32C	3				
		VITEK 2	3				
		ITS2-FLP–I	3				
		ITS2-FLP–BF	3				
C. neoformans	5	ID32C	5				
		VITEK 2	5				
		ITS2-FLP–I	5				
		ITS2-FLP–BF	3			2	
B. capitatus	2	ID32C	2				
		VITEK 2	2				
		ITS2-FLP–I	2				
		ITS2-FLP–BF	2				
S. cerevisae	4	ID32C	4				
		VITEK 2	3	1 (*C. colliculosa*)			Purple colonies on chromagar, morphology on cornmeal agar
		ITS2-FLP–I	4				
		ITS2-FLP–BF	4				
C. palmioleophila	1	ID32C				1	
		VITEK 2				1	
		ITS2-FLP–I			1 (*C. guilliermondii*)		Sequencing[c]
		ITS2-FLP–BF			1 (*C. guilliermondii*)		Sequencing[c]
Total	61	ID32C	58		1	2	
		VITEK 2	41	7	3	10	
		ITS2-FLP–I	59		2		
		ITS2-FLP–BF	55		2	4	

[a] T + M−, trehalose positive, maltose negative.

[b] Due to a software problem this strain was misidentified. This problem has been resolved, and similar misidentifications are no longer possible.

[c] *C. palmioleophila* and *C. guilliermondii* have identical ITS2 lengths (323 to 324 bp). Differentiation is possible by restriction with RsaI, which yields a 157-bp restriction fragment for *C. guilliermondii* and no restriction for *C. palmioleophila*.

TABLE 2. VITEK 2 reactions leading to misidentification of the strains tested

No. of misidentified yeasts	Reference method	VITEK 2 identification	Possible cause of misidentification
1	*S. cerevisiae*	*C. colliculosa*	No assimilation of D-maltose
2	*C. krusei*	Unidentified	No cleavage of D-glucosidase-4MU
4	*C. glabrata*	*C. magnoliae*	Assimilation of D-mannitol and D-raffinose
5	*C. glabrata*	Unidentified	No assimilation of histidine arylamidase; extra assimilation test results were positive

were identified by ID32C (Table 1). ITS2-FLP–I identified 59 of 61 isolates correctly (Table 1). *C. palmioleophila*, a species not included in the database, was misidentified as *C. guilliermondii* (Table 1). *C. palmioleophila* is also absent from the ID32C and VITEK 2 database and remained unidentified by these two methods. The isolate was finally identified by sequencing of the ITS2 region. With ITS2-FLP–BF, only 55 out of 61 isolates were identified correctly: 4 isolates were not identified, and 2 were misidentified (Table 1). The better results with ITS2-FLP–I compared to ITS2-FLP–BF were probably due to more effective breakage of cell walls. This idea is supported by the absence of peaks on electropherograms of isolates unidentified with ITS2-FLP–BF. We found that ITS2-FLP performed better than VITEK 2. Others have found that the percentage of correct identifications with VITEK 2 is comparable with the results seen with other commercial techniques (9). In this study, VITEK 2 identified 41 isolates correctly: 10 were not identified, 3 were misidentified, and 7 isolates were identified with low discrimination (Table 1). Most problems were encountered with the identification of *C. glabrata*: five isolates remained unidentified and five isolates were identified with low discrimination (i.e., low discrimination between *C. glabrata* and *C. magnoliae* [4] or *C. colliculosa* [1]). A specific cause for these problems with *C. glabrata* was not found. In another recent study all *C. glabrata* isolates were correctly identified with VITEK 2 without supplementary tests (9). Table 2 contains the metabolic profiles of strains that were misidentified, identified with low discrimination, or unidentified with VITEK 2.

VITEK 2 has the advantage of speed compared to ID32C. The results of VITEK 2 are obtained after 15 h of incubation (9). With ID32C, most isolates were identified after 48 h. In general and compared to other techniques, ITS2-FLP offers superior speed, reproducibility, and sample volume throughput (12, 14). With ITS2-FLP it is possible to obtain results within 4 h, but it is more laborious and demands more expertise than ID32C and VITEK 2. Another advantage of ITS2-FLP is that the database of the ITS2-FLP, in contrast with VITEK 2 and ID32C, can be easily adapted and now contains *C. palmioleophila* (ITS2 fragment length, 324.21). The ease with which the ITS2 database can be adapted is a definite advantage (17).

A disadvantage of VITEK 2 is that cultures cannot be older than 24 h, which causes problems for identification of over-weekend cultures.

We conclude that ITS2-FLP is a very rapid and accurate method for the identification of medically important yeast isolates. VITEK 2 performed less well; particularly, identification of *C. glabrata* posed problems.

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