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ORIGINAL ARTICLE

Candida albicans identification: comparison among nine phenotypic systems and a multiplex PCR

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Key words

C. albicans • Phenotypic identification • Multiplex PCR • Sensitivity • Specificity

Summary

Background. *Candida albicans is the most common fungal pathogen isolated from clinical samples and is also the most common yeast species carried as a commensal by healthy individuals although some non-C. albicans species account for an important number of infections.*
Objectives. *To compare nine phenotypic systems for C. albicans identification [API 20C AUX; RapID Yeast Identification panel (RYIP); Vitek2 ID-YST system; chromogenic media, CHROMagar, Oxoid Chromogenic Candida Agar (OCCA), Candida ID2, Candida Identification Agar, CandiSelect 4, and Chromalbicans Agar] with multiplex PCR.*
Patients/Methods. *A collection of 390 yeast strains was obtained by routine isolation from oral and vaginal swabs. All of the yeasts isolated were tested for germ tube formation, and then submitted to a multiplex PCR protocol tested in previous studies, and to nine phenotypical commercial methods, together with the reference ATCC strains. Comparison was limited to the ability of the tests to identify C. albicans.*
Results. *253 isolates were provisionally identified as C. albicans by germ tube, and their identities were further confirmed with the multiplex PCR. Sensitivity of phenotypical systems ranged from 81.9% (Vitek2) to 87.7% (Candida ID2 e CHROMagar). For specificity, the highest value was 96.8% for Candida ID2, and the lowest value (75.1%) was for Chromalbicans Agar.*
Conclusions. *Although with differences in discriminatory power, the methods tested showed overall acceptable levels of sensitivity and specificity respect to the multiplex PCR; therefore, all could be useful for C. albicans identification where molecular differentiation is not available.*

Introduction

Candida albicans is the most common fungal pathogen isolated from clinical samples [1-6] and is also the most common yeast species carried as a commensal by healthy individuals although some non-*C. albicans* species account for a number of infections [7].
Disseminated fungal infection is a significant cause of mortality in hospitalized patients [8]. *Candida* spp. account for 70-80% of fungal bloodstream infections, and collectively represent the fourth most common group of pathogens responsible for nosocomial bloodstream infection, with a mortality rate of approximately 50% [9]. Among those species that cause invasive infections, *C. albicans*, *C. parapsilosis*, *C. tropicalis* and *C. glabrata* typically account for between 80 and 90% of cases, although this can vary for a given clinical unit [8, 10].
As a result of intrinsic azole resistance of some of these species, diagnostic strategies that allow rapid and accurate species identification of isolates might be helpful for addressing therapeutic choices; identification might also be useful for studying their epidemiology, spread and modes of transmission [11].
Nowadays, a large variety of *Candida* spp. identification methods are commercially available, and they differ in principles, discrimination power and cost. Traditional microbiological procedures are based on macroscopic and microscopic analysis of colonies and cells (presumptive tests) and on biochemical characteristics of the yeasts (confirmative tests) [12]. Also, several molecular methods have been developed for the identification of the yeasts and they appear reliable and easy to use [13, 14].
In previous studies, we have found that multiplex PCR is an accurate, rather inexpensive, and easy-to-perform technique for the identification of *C. albicans* and other some species. By avoiding the need for preliminary DNA extraction and purification, this technique also can be carried out rapidly, therefore, it can be considered as a valid alternative to traditional phenotypic methods [13, 17].
In this study, we have compared nine phenotypic systems for *Candida* spp. identification with a multiplex PCR, here considered as reference method. The comparison was limited to the identification of the *albicans* species, the only one which can be identified by all the methods employed [15, 16].

Materials and methods

The study was carried out between 2003 and 2007. A collection of 390 yeast strains was obtained by routine isolation from oral and vaginal swabs in the laboratories

of the Hygiene Section, Department of Public, Clinical and Preventive Medicine of the Second University of Naples, the Microbiology Section, Department of Cellular and Molecular Biology and Pathology "L. Califano" and the Physiology and Hygiene Section, Department of Biological Sciences of the "Federico II" University of Naples. First cultures were obtained on Sabouraud–dextrose agar (SDA) and chloramphenicol after 48 h at 37°C.

Each isolate was stored at -70°C in glycerol; one aliquot was then subcultured as reported for the first isolation. All of the yeast isolates were analyzed for germ tube test and then submitted to identification by a multiplex PCR protocol, which was tested in previous studies [15, 17, 19], and nine phenotypic methods, together with the reference ATCC strains: *C. albicans* 90028, *C. albicans* 36082, *C. parapsilosis* 22017, *C. krusei* 6258, *C. neoformans* 1199 and *C. neoformans* 6852.

Multiplex PCR

The amplification reaction was carried out directly on colonies, without DNA extraction, as previously reported [12, 13, 17, 18]. We added a colony from fresh culture to a 50 µL reaction mix containing Taq Buffer 10 ×, dNTPs 10 mM, primers 5 µM and 0.5 µL Taq pol 5 u µL-1; the primers were ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3'), ITS2 (5'-GCT GCG TTC TTC ATC GAT GC-3'), CA3 (5'-GGT TTG CTT GAA AGA CGG TAG-3'), CA4 (5'-AGT TTG AAG ATA TAC GTG GTA G-3'), ITS1F (5'-CCA GCG CTT AAT TGC G-3'), ITS1K (5'-ATC GTC TGA ACA AGG CCT GC-3') and ITS2D (5'-GAG AAC CAA GAG ATC CGT TGT TG-3').

The mix was then submitted to a predenaturation at 95°C for 10 min, 40 cycles of 1 min at 95°C, 1 min at 50°C, 1 min at 72°C and a final extension at 72°C for 10 min. The presence and the length of the amplicons were analyzed in a 2% agarose gel with ethidium bromide (0.5 µg mL-1). The primers allows the identification of *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, *S. cerevisiae*, *C. parapsilosis*, *C. guillermondii*, *C. kefyr*, *C. famata*, *C. dubliniensis*, *C. lusitaniae*, *C. incospicua* [13, 17].

Phenotypic tests

Each strain was analyzed with these methods: API 20C AUX (bioMérieux S.p.A.); RapID Yeast Identification panel (RYIP; Dade Behring S.p.A.); Vitek2 ID-YST system (bioMérieux S.p.A.); chromogenic media (CHROMagar; AlfaWassermann S.p.A.), Oxoid Chromogenic Candida Agar (OCCA), Candida ID2 (bioMérieux S.p.A.), Candida Identification Agar (Biotest Italia S.r.l.), CandiSelect 4 (Bio-Rad), and Chromalbicans Agar (Biolife). All the procedures were performed as recommended by manufacturers. To avoid possible subjective variability, tests and interpretation of results were carried out by the same operators.

Elaboration of results

The results obtained with the phenotypic identification systems were compared with those of multiplex PCR, to determine sensitivity and specificity of each method in relation to the identification of *C. albicans*. Time needed for identification, handiness, and cost of each test were also evaluated.

Results

The reference strains were identified correctly by all the methods, on the basis of discriminatory power of each test. A total of 253 isolates were identified as *C. albicans* by multiplex PCR. All these isolates were germ tube-positive. The number and percentage of the identifications obtained with the different systems is reported in Table I. Chromogenic media Chromalbicans Agar, Candida ID2, Candida Identification Agar, and OCCA were not able to identify any species in 33 (13%), 23 (9.1%), 13 (5.1%), and 5 (2%) cases, respectively.

Sensitivity and specificity values of all the methods in comparison with the Multiplex PCR for identification of *C. albicans* are reported in Table II.

Tab. I. Results of the tests carried out with phenotypical systems on the 253 strains identified as *C. albicans* by multiplex PCR.

	API 20 C AUX	RYIP	Vitek2	CHROMagar	OCCA	Candida ID2	Candida Identification Agar	Candi Select 4	Chromalbicans agar
C. *albicans*	221 (87.3)	210 (83)	208 (82.2)	223 (88.1)	210 (83)	223 (88.1)	210 (83)	218 (86.2)	220 (87)
other *Candida* spp. identified	32 (12.7)*	43 (17)**	45 (17.8)^	30 (11.9)°°	38 (15)*^	7 (2.8)^^	30 (11.9)°	35 (13.8)*°	0
Candida spp. unidentifiable	0	0	0	0	5 (2)	23 (9.1)	13 (5.1)	0	33 (13)

* 15 *C. tropicalis*, 7 *C. glabrata*, 3 *C. krusei*, 3 *C. parapsilosis*, 2 *C. kefyr*, 2 *C. maris*, 1 *C. famata*, 1 *C. lusitaniae*
** 13 *C. glabrata*, 8 *C. parapsilosis*, 5 *C. tropicalis*, 2 *C. kefyr*, 2 *Cryptococcus neoformans*, 1 *C. krusei*, 1 *Pichia farinosa*, 1 *Saccharomyces cerevisiae*
^ 12 *C. glabrata*, 9 *C. parapsilosis*, 7 *C. tropicalis*, 4 *C. norvegensis*, 3 *C. dubliniesis*, 3 *C. famata*, 2 *C. krusei*, 2 *C. rugosa*, 1 *C. colliculosa*, 1 *C. sake*, 1 *C. sphaerica*, 1 *Rhodotorula glutinis*
^^ 6 *C. tropicalis*, 2 *C. kefyr*
*^ 18 *C. glabrata*, 14 *C. tropicalis*, 5 *C. parapsilosis*, 2 *C. kefyr*, 1 *C. krusei*
° 20 *C. glabrata*, 10 *C. tropicalis*, 3 *C. krusei*
°° 12 *C. glabrata*, 7 *C. tropicalis*, 6 *C. parapsilosis*, 4 *C. kefyr*, 2 *C. krusei*, 1 *S. cerevisiae*
*° 28 *C. glabrata*, 7 *C. tropicalis*, 2 *C. krusei*

Tab. II. Sensitivity and specificity values (%) of all the methods in comparison with the Multiplex PCR in the identification of *C. albicans*.

	Sensitivity (%)								
	API 20 C AUX	RYIP	Vitek2	Candida ID2	OCCA	Candida Identification Agar	CHROMagar	Candi Select 4	Chromalbicans agar
C. albicans	86.9	87.3	81.9	87.7	82.4	82.7	87.7	85.8	86.5
	Specificity (%)								
	API 20 C AUX	RYIP	Vitek2	Candida ID2	OCCA	Candida Identification Agar	CHROMagar	Candi Select 4	Chromalbicans agar
C. albicans	94.0	92.0	93.6	96.8	92.0	93.6	93.7	78.7	75.1

Among the phenotypic systems, Candida ID2 and CHROMagar gave the same highest sensitivity (87.7%), while Vitek2 showed the lowest value (81.9%). For the specificity, the highest value was 96.8% for Candida ID2, and the lowest value (75.1%) was for Chromalbicans Agar.

Discussion

Candida infections are still an important problem, especially for immunosuppressed individuals [18]. Several investigators have reported that the incidence of infections caused by species other than *C. albicans* has risen, although among the *Candida* genus, *C. albicans* is still the most common species that causes nosocomial infections [11, 18, 20]. Therefore, the rapid and accurate identification of this yeast and its correct differentiation from intrinsically azole-resistant species, such as *C. krusei* or *C. glabrata*, remains a fundamental goal of microbiology laboratories, mainly in hospital settings and for critically ill patients [21].

We evaluated six chromogenic media and three biochemical methods for *C. albicans* identification, in comparison to multiplex PCR.

The API 20C AUX, a manual system, requires great precision during test implementation, experience in the interpretation of turbidity level, and sometimes, long incubation times, but it is reliable, easy to use and inexpensive.

The Vitek2 and RYIP systems are both automated, rapid, and able to identify a large number of species, owning great discriminatory power, as demonstrated by other studies [24-26]. Thus, they are considered excellent for use in microbiology laboratories. In our experience, however, Vitek 2 showed the lower sensitivity, although this value was acceptable (81.9%). Moreover, both automated systems are expensive and require periodic maintenance.

Candida ID2, OCCA, and Candida Identification Agar, with different rates, gave no identifications, that is colonies that did not appear with typical colors and aspects identified by manufacturers as indicative of one species. In particular, with Candida ID2 media, 24 strains (9.3%) of *Candida* were not identified. Nevertheless, Candida ID2 is the identification system with the highest percentage sensitivity (the same as CHROMagar) and specificity in comparison with the Multiplex PCR for identification of *C. albicans*, as also demonstrated by other authors [27, 28].

Chromalbicans Agar is a chromogenic media that identify only the *albicans* species, so it showed the highest number of not identified clinical isolates and the lowest specificity.

Chromogenic media were simple and rapid to use and economically advantageous. Their main limitations were the lower discrimination power compared with automated systems, and a chromatic gradient that was not always easy to interpret. Moreover, the color of yeast colonies on chromogenic media may vary after storage and subculture, as demonstrated for *C. dubliniensis* [29]. Therefore, their use is particularly advisable for screening and preliminary assays [17, 27, 30].

In the present study, phenotypic systems showed overall good levels of sensitivity and specificity respect the results of multiplex PCR; therefore, all of them could be useful for yeasts identification, although limits exist about the discriminatory power of some of them, especially chromogenic media. However, in some cases the combination of two phenotypic methods, eg. a chromogenic medium with a biochemical test, can increase the sensitivity of the identification.

As previously reported, the multiplex PCR method assayed here was accurate for identification, simple and quick to implement, and did not require toxic and expensive chemical reagents [13, 17, 19, 31, 32].

Over the past few years, molecular techniques have enhanced greatly the identification of causal agents of infectious diseases, including *C. albicans* [15, 22], but these methods are not yet routine because of the high initial equipment costs and the need to have trained personnel available [23].

However, during epidemiological studies, and when the diagnosis of recurrent infections or the differentiation between *C. dubliniensis* and *C. albicans* are needed, molecular methods are irreplaceable to accurately identify *Candida* spp., and particularly *C. albicans*, which account for the majority of candidoses [33].

Thus, the choice among the available methods depends on the objectives that the laboratory intends to pursue.

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