Candida albicans is the most frequently isolated yeast pathogen, and candidiasis is increasingly a complication in both immunocompromised and immunocompetent individuals. Immediate identification of the pathogen is usually required, however, conventional methods such as the API 20C AUX and ID32C systems (bioMerieux Japan, Tokyo, Japan) take more than 24 to 48 hours to give results. Several researchers have attempted to develop rapid methods for the identification of C. albicans. Perry et al. and Dalton et al. reported rapid methods that make use of the fluorogenic substrate of β-galactosaminidase (EC 3.2.1.30). Perry et al. reported a modification of their previous method, which involves alteration of the test substrate and inclusion of a second substrate in one reaction tube and provides a colorimetric rather than a fluorometric reaction product.

Here, we report the evaluation of a newly developed identification kit, the RID Zyme CAS test, for C. albicans, 1136 C. albicans and 403 non-albicans Candida strains were tested. Distinction of medically important non-albicans strains, with the exception of C. dubliniensis, was obtained. These results show that this new kit is simple and effective for the identification of C. albicans in clinical samples. Furthermore, the one hour period for identification makes it very attractive.

Key words: Candida albicans, newly developed identification kit, RID Zyme CAS test

Abstract
To evaluate a newly developed identification kit, the RID Zyme CAS test for Candida albicans, 1136 C. albicans and 403 non-albicans Candida strains were tested. Distinction of medically important non-albicans strains, with the exception of C. dubliniensis, was obtained. These results show that this new kit is simple and effective for the identification of C. albicans in clinical samples. Furthermore, the one hour period for identification makes it very attractive.

Corresponding author: Reiko Tanaka
Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University
1-8-1 Inohana, Chuo-ku, Chiba, 260-8673 Japan

Candida albicans is the most frequently isolated yeast pathogen, and candidiasis is increasingly a complication in both immunocompromised and immunocompetent individuals. Immediate identification of the pathogen is usually required, however, conventional methods such as the API 20C AUX and ID32C systems (bioMerieux Japan, Tokyo, Japan) take more than 24 to 48 hours to give results. Several researchers have attempted to develop rapid methods for the identification of C. albicans. Perry et al. and Dalton et al. reported rapid methods that make use of the fluorogenic substrate of 4-methylumbelliferyl-N-acetyl-β-D-galactosaminide of β-galactosaminidase (EC 3.2.1.30). Perry et al. reported a modification of their previous method, which involves alteration of the test substrate and inclusion of a second substrate in one reaction tube and provides a colorimetric rather than a fluorometric reaction product.

Here, we report the evaluation of a newly developed identification kit, the RID Zyme CAS test, for C. albicans, 1136 C. albicans and 403 non-albicans Candida species and five serotypes of Cryptococcus neoformans.

Strains used in this study are listed in Tables 1 and 2. These strains are registered at the Research Center for Pathogenic Fungi and Microbial Toxicoses of Chiba University and were cultured on potato dextrose agar (PDA; Difco Laboratories, Detroit, MI, USA) at 25°C for 48 hours. Most of the strains were previously identified using the ID32C system (bioMerieux Japan) and/or the Candida Check system (Mitsubishi Kagaku Iatron, Inc., Tokyo, Japan), and were genotyped by PCR of 25S rDNA and the topoisomerase II gene. Some strains were purchased from ATCC: American Type Culture Collection (Manassas, VA, USA), CBS: Centraalbureau voor Schimmelcultures, (Delft, the Netherlands), IFO: Institute for Fermentation, Osaka (Osaka, Japan) or NBRC: National Institute of Technology and Evaluation Biological Resource Center (Chiba, Japan) as references.

The RID Zyme CAS test (Mitsubishi Kagaku Iatron, Inc.) was put out to the market in 2004. Although two of the enzymes C. albicans has are β-galactosaminidase and L-proline aminopeptidase, those substrates (MNGL, 4-methylumbelliferyl-N-acetyl-β-D-galactosaminide for β-galactosaminidase; fluorogenic and PRO, L-proline p-nitroanilide for L-proline aminopeptidase; chromogenic) are infused on a cotton swab in this kit. After picking up a single colony with the swab, one can determine within approximately 1 hour whether the isolate is Candida albicans. The procedure is very simple: pick-up of one colony with a swab, incubation for 1 hour at 37°C with one drop of accompanying buffer, then visualization with a UV lamp (366 nm) and addition of a color

Short Report
Evaluation of a Newly Developed Identification Kit, RID Zyme CAS Test, for Candida albicans

Reiko Tanaka, Junko Ito, Ayaka Sato, Kazuko Nishimura
Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University
1-8-1 Inohana, Chuo-ku, Chiba, 260-8673 Japan

(Received: 26, October 2004. Accepted: 28, December 2004)
chromogen turns the swab purple

fluorescence is produced, and the PRO Organism No. tested MNGL PRO

If the isolate is > bombi stellatoidea savonica lipolytica sake
lusitaniae chiropterorum melinii cylindracea famata krusei

+, positive; -, negative.

MNGL, 4-methylumbelliferyl-β-d-galactosaminide; PRO, L-proline p-nitroanilide.

Table 1. Specificity of RID Zyme CAS test

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. tested</th>
<th>MNGL</th>
<th>PRO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida albicans (serotypes A, B)</td>
<td>1136</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C. dubliniensis</td>
<td>21</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C. stellatoidea</td>
<td>5</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>54</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C. guillermondii</td>
<td>15</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>C. kefyr</td>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C. krusei</td>
<td>29</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>138</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>107</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Cryptococcus neoformans (serotypes A, B, C)</td>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cx neoformans (serotypes D, AD)</td>
<td>2</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Species distinguishable from C. albicans by RID Zyme CAS test

<p>| |
||</p>
<table>
<thead>
<tr>
<th>Candida albicans (serotypes A, B)</th>
<th>1136</th>
</tr>
</thead>
<tbody>
<tr>
<td>MNGL Proline</td>
<td>4-Methylumbelliferyl-β-D-galactosaminide</td>
</tr>
</tbody>
</table>

Results of the RID Zyme CAS test with nine clinically important Candida species and two C. neoformans species are shown in Table 1. All of the 1136 strains of C. albicans tested, including development reagent (p-dimethylaminocinnamaldehyde). If the isolate is C. albicans, MNGL fluorescence is produced, and the PRO chromogen turns the swab purple (Fig. 1).
serotype B strains, were positive in both tests (MNGL and PRO tests). A recently classified atypical C. albicans species, C. dubliniensis, was also positive in both tests. C. stellatoidea, about which the taxonomy difference with C. albicans has been argued and which now is recognized as a synonym of C. albicans, was only positive in the MNGL test. Clear distinction from C. albicans was obtained for C. parapsilosis, C. guilliermondii, C. kefyr, C. glabrata, C. tropicalis, C. krusei, and C. neoformans. With the exception of eight species (C. catenulata, C. dubliniensis, C. kruisii, C. maltaosa, C. rugosa, C. sake, C. savonica, C. suecica), distinction from C. albicans was obtained with other non-albicans species (Table 2). Four of the eight indistinguishable species (C. kruisii, C. sake, C. savonica, C. suecica) showed no growth at 37°C, and four species (C. catenulata, C. dubliniensis, C. maltaosa, C. rugosa) showed no distinction from C. albicans with the kit. However, C. maltaosa has been reported as non-pathogenic for mice, and distinction of C. catenulata and C. rugosa from C. albicans can be made microscopically. Summarizing these data, the sensitivity of this kit was 100% and the specificity was 97.6%. On the other hand, Crist et al. and Heelan et al. compared four methods (MUREX C. albicans, Albicans-Sure, BactiCard Candida and the germ tube test), when the operation time of BactiCard Candida and Albicans-Sure was being emphasized as 5 minutes. Although the operation time of the RIDzyme CAS test is 1 hour, it is superior to those two methods in that one colony is sufficient for the inoculation. The specificity of those four methods apparently was higher than the RIDzyme CAS test since C. dubliniensis was not taken into consideration. According to these results, the RID Zyme CAS test kit is effective in the identification of C. albicans from clinical samples. Unfortunately, C. dubliniensis, which has been increasingly reported in recent years, was indistinguishable from C. albicans.

This study was performed as part of the Frontier Studies and International Networking of Genetic Resources in Pathogenic Fungi and Actinomycetes (FN-GRPF) through the Special Coordination Funds for Promoting Science and Technology from the Ministry of Education, Culture, Sports, Science and Technology of Japan (2003), and Grant-in-Aid for Scientific Research (C-16510176) from Japan Society for the Promotion of Science.

REFERENCES

