Biotyping and Virulence Properties of Skin Isolates of
*Candida parapsilosis*

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The biotype and virulence of skin isolates of *Candida parapsilosis* were compared with blood isolates of the same fungus. Morphotype, resistotype, and electrophoretic karyotype determinations did not reveal any special cluster with a unique or dominant pathogenic feature among all of the isolates, regardless of their source. However, all cutaneous isolates had uniformly elevated secretory aspartyl-protease (Sap) activity, more than four times higher than the enzyme activity of the blood isolates. They were also highly vaginopathic in a rat vaginitis model, being significantly more virulent than blood isolates in this infection model. In contrast, skin isolates were nonpathogenic in systemic infection of cyclophosphamide-immunosuppressed mice, while some blood isolates were, in this model, highly pathogenic (median survival time, 2 days, with internal organ invasion at autopsy). Finally, skin isolates did not differ, as a whole, from blood isolates in their adherence to plastic. This property was associated with a morphotype, as defined by a colony with continuous fringe, which was present among both skin and blood isolates. While confirming the genetic heterogeneity of *C. parapsilosis*, our data strongly suggest that the potential of this fungus to cause mucosal disease is associated with Sap production and is substantially distinct from that of systemic invasion.

*Candida parapsilosis* is a human opportunistic fungus particularly involved in candidemic episodes in patients with underlying disorders of various types (15, 18, 21, 24, 26, 38). Over the last few years, its incidence has progressively increased relative to other *Candida* species in the settings of malignant hemopathetic patients to become, in some investigations (15), the most prevalent cause of candidemia. In contrast to *Candida albicans* and *Candida tropicalis*, studies on the virulence and experimental pathogenicity of *C. parapsilosis* are few and somewhat controversial (2, 15, 26, 35). We have previously highlighted the role of some biotypes of *C. parapsilosis* as aggressive agents of fungemia in central-venous-catheter-bearing leukemic patients (5, 15), as well as the vaginopathic potential of *C. parapsilosis* isolates from women with vaginal candidiasis (1, 10). These studies gave some experimental support to the notion that pathogenicity of *C. parapsilosis* is largely strain dependent, both for systemic and mucosal infections.

*C. parapsilosis* is also frequently isolated from the skin but, to our knowledge, no systematic comparative studies on the molecular characteristics and virulence of skin isolates have been performed. In view of the previously established importance of the isolation source for biotyping and virulence properties of *C. parapsilosis* (4, 5, 22, 29), we have now addressed the pathogenic potential of a number of *C. parapsilosis* isolates from the skin of human immunodeficiency virus-positive (HIV+) and HIV− subjects. This has been done in experimental systemic infections of either unmodified or cyclophosphamide-induced leukopenic mice (2) and in vaginal infection in ovariectomized estrogen-treated rats (9). In an attempt to identify potential virulence factors, we also evaluated plastic adherence properties, as well as secretion of aspartyl-proteinase, a putative virulence factor (8, 16, 31) that has been shown to be critical for mucosal infection by *C. albicans* (9, 11).

**MATERIALS AND METHODS**

**Yeast isolates and clinical source.** All available (total of 12) sequential skin isolates of *C. parapsilosis* from HIV+ and HIV− subjects were studied. No subject was affected by cutaneous candidiasis or was under treatment with antymycotics. For comparative purposes, nine isolates of the fungus from HIV− candidemic patients (representing seven strains sequentially isolated during the whole 1997 and not included in a previous study (5), plus two strains of microbial type collection) and strains of *C. albicans* with intact or deleted SAP2 gene were also examined. Table 1 lists the source and code of each *C. parapsilosis* isolate, as well as the status of each subject from whom the fungus was isolated.

**Determination of the morpho-resistotype.** Morphotype on malt extract agar, were coded SV2N on Sabouraud-triphenyl-tetrazolium agar (smooth texture); P2 5 dark pink, corresponding to Pantone 1785C); V, violet color (V1 5 dark violet, corresponding to Pantone 262C); and N, no mycelial halo. Isolates that grew on boric acid, sodium chloride, and MacConkey agar, were coded as “a.” Growth on urea and citrate media was coded as b, on boric acid and sodium chloride as c, and no growth on any medium as d. For biotype delineation, we used a code based on a combination of the morphotyping and resistotyping codes. Therefore, isolates with the A1V2C code had no fringe on malt extract agar, were coded SV2N on Sabouraud-triphenyl-tetrazolium agar (smooth texture, dark violet, no mycelial halo surrounding the colony), and grew on sodium chloride and MacConkey media and on urea medium.

**Karyotype analysis by pulsed-field gel electrophoresis.** Cells of *C. parapsilosis* were grown to stationary phase in YPD medium (glucose, 2%; yeast extract, 1%; Bacto-Peptone, 2% [Difco, Detroit, Mich.]) overnight at 28°C. The cells were packed by centrifugation (3,000 rpm for 5 min) and washed in 1.2 M sorbitol solution containing 20 mM EDTA (pH 8.0). The pellet was resuspended to a cell
concentration of 10^6/ml in a solution of EDTA-sorbitol, as described above, but containing 20 mM mercaptoethanol and then incubated for 15 min at 37°C. The samples were then embedded in low-melting-point agar (L.M.; Bio-Rad, Richmond, Va.), and the spheroplast lysis method described by Vollrath and Davis (36) was used to prepare the chromosomal DNA. The cell-agarose mixture was transferred to plug molds. Solidified pellets were removed from the mold and placed in 1.2 M sorbitol solution containing 20 mM EDTA, 10 mM Tris-HCl (pH 7.5), and 100 µl of Zymoliase 100T (1 mg/ml) (100,000 U/g; Seikagaku, Tokyo, Japan). After incubation for 2 h at 37°C, the plugs were incubated in 1% sodium dodecyl sulfate solution containing 10 mM EDTA–10 mM Tris-HCl (pH 7.5) overnight at 37°C. The pellets were stored in 1% sarcosyl solution containing 20 mM mercaptoethanol and was then incubated for 15 min at 37°C. The samples were then embedded in low-melting-point agar (L.M.; Bio-Rad, Richmond, Va.), and the spheroplast lysis method described by Vollrath and Davis (36) was used to prepare the chromosomal DNA. The cell-agarose mixture was transferred to plug molds. Solidified pellets were removed from the mold and placed in 1.2 M sorbitol solution containing 20 mM EDTA, 10 mM Tris-HCl (pH 7.5), and 100 µl of Zymoliase 100T (1 mg/ml) (100,000 U/g; Seikagaku, Tokyo, Japan). After incubation for 2 h at 37°C, the plugs were incubated in 1% sodium dodecyl sulfate solution containing 10 mM EDTA–10 mM Tris-HCl (pH 7.5) overnight at 37°C. The pellets were stored in 1% sarcosyl solution containing 20 mM EDTA–10 mM Tris-HCl (pH 7.5) at 4°C.

The DNA samples in agarose inserts were resolved by contour-clamped homogeneous field electrophoresis (CHEF). CHEF analysis was performed with the CHEF-DR II apparatus (Bio-Rad). The operating conditions included three consecutive runs on each gel (14.5 by 20.5 cm; 1-cm-thick 1% agarose; Bio-Rad). The operating parameters for the CHEF-DR II apparatus (Bio-Rad) was used as a standard. The parameters for each run were at 150 V and 14°C for 24 h with 90-, 120-, and 180-s switches. Gels were then stained with ethidium bromide (0.5 µg/ml; 30 min), destained, and photographed under UV light.

**TABLE 1. C. parapsilosis isolates used throughout this study**

<table>
<thead>
<tr>
<th>Isolate (lab code)</th>
<th>Source</th>
<th>Disease status and infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>2296</td>
<td>Skin</td>
<td>HIV+</td>
</tr>
<tr>
<td>2446</td>
<td>Skin</td>
<td>HIV+</td>
</tr>
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<td>Skin</td>
<td>HIV+</td>
</tr>
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<td>Skin</td>
<td>HIV+</td>
</tr>
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<td>Skin</td>
<td>HIV+</td>
</tr>
<tr>
<td>93179</td>
<td>Skin</td>
<td>HIV+</td>
</tr>
<tr>
<td>93585</td>
<td>Skin</td>
<td>HIV+</td>
</tr>
<tr>
<td>94259</td>
<td>Skin</td>
<td>HIV+</td>
</tr>
<tr>
<td>94260</td>
<td>Skin</td>
<td>HIV+</td>
</tr>
<tr>
<td>94864</td>
<td>Skin</td>
<td>HIV+</td>
</tr>
<tr>
<td>94915</td>
<td>Skin</td>
<td>HIV+</td>
</tr>
<tr>
<td>94938</td>
<td>Skin</td>
<td>HIV+</td>
</tr>
<tr>
<td>2057</td>
<td>Catheter</td>
<td>Catheter-related candidemia</td>
</tr>
<tr>
<td>2058</td>
<td>Catheter</td>
<td>Catheter-related candidemia</td>
</tr>
<tr>
<td>HEM-26</td>
<td>Blood</td>
<td>Catheter-related candidemia</td>
</tr>
<tr>
<td>HEM-27</td>
<td>Blood</td>
<td>Catheter-related candidemia</td>
</tr>
<tr>
<td>HEM-28</td>
<td>Blood</td>
<td>Catheter-related candidemia</td>
</tr>
<tr>
<td>HEM-29</td>
<td>Blood</td>
<td>Catheter-related candidemia</td>
</tr>
<tr>
<td>HEM-30</td>
<td>Blood</td>
<td>Catheter-related candidemia</td>
</tr>
<tr>
<td>HEM-31</td>
<td>Blood</td>
<td>Candidemia with deep-seated invasive infection</td>
</tr>
<tr>
<td>HEM-32</td>
<td>Blood</td>
<td>Candidemia with deep-seated invasive infection</td>
</tr>
</tbody>
</table>

a Neither HIV+ nor HIV− subjects were affected by cutaneous candidiasis.

b Catheter-related candidemia and deep-seated infection were defined as in reference 15.

**RESULTS**

**Molecular and phenotypic biotyping.** We analyzed the electrophoretic karyotype of both skin and blood isolates of C. parapsilosis by the CHEF technique, which gave an optimal resolution of the chromosome bands, particularly those between molecular sizes of 1.4 and 0.8 Mb. Common to almost all isolates were three well-defined chromosome-sized DNA molecules of relatively large molecular size (>3.0 to 1.9). In the lower-molecular-size range there were ample variations in the number and size of electrophoretic bands, and these constituted the source of the isolate distinctiveness. Overall, the numbers of chromosome-sized bands were seven (in 14 isolates) and eight (in 5 isolates).

Considering both the number and size of the chromosomal bands, there was some apparent relatedness among isolates. For instance, the skin isolates 2518, 94260, 2296, 93858, 94938, 94259, 93179, and 94915 and the blood strains HEM-23 and HEM-26 had identical seven-chromosome patterns (Fig. 1, lane 1). On the other hand, the skin isolate 93006 and the three blood isolates 2057, 2058, and HEM-28 also had the seven-chromosome pattern but the fifth chromosomal band had a smaller (~1.05 kb, first three isolates) or a higher (~1.18 kb, the latter isolate) molecular mass than the fifth band of the former group of isolates (~1.20 kb) (lanes 2 and 8, respectively, in Fig. 1). All other strains (with two exceptions, see below) had eight distinct chromosome-sized bands, with differences among them regarding the molecular size of the bands in the range of 1.6 to 0.8 (Fig. 1, lanes 3, 5 to 7, and 10 to 11).

Two blood strains (HEM-30 and HEM-31) had rather peculiar chromosomal profiles, characterized by two bands (or two doublets) in the 2.2- to 1.5-Mb section and, mostly, the absence of the prominent chromosomes in the 1.2- to 0.9-Mb size replacement, replaced by an apparent doublet or intense single-chromosome-sized band in a very low molecular mass region (ca. 0.65 Mb) where no other isolate of C. parapsilosis showed any band (Fig. 1, lanes 4 and 9).

All isolates were also phenotyped by assessing the colony morphology and resistance to various chemicals in different media (a combination designated as a morpho-resistotype), according to a previously established, useful scheme (5, 17). By this method, all skin isolates were grouped into eight catego-
ries (A to H), the most numerous of which included only three isolates. Overall, there were more morpho-resistotypes (eight) than electrophoretic karyotypes (five) among all the skin isolates. Concerning the important feature of colonial morphology on malt extract (see also below), the isolates were equally distributed between those with no fringe colonies (A) and those with continuous fringe colonies (B) (six per group).

**Sap production.** Skin isolates of *C. parapsilosis* were assayed for Sap production by measuring BSA hydrolysis both on solid and liquid media. As shown in Table 2, both methods coherently indicated that all skin isolates, independently of their isolation from HIV+ or HIV− subjects, were high Sap producers, with top scores on BSA agar and uniformly elevated enzyme activity in BSA broth. In contrast, Sap production by the nine isolates of *C. parapsilosis* from candidemic patients showed low score in BSA-agar and averaged a score of 0.85 ± 0.07 (range, 0.77 to 1.02) in BSA broth (four times less than the Sap production by skin isolates). This confirms previous results showing the low Sap production by other blood isolates of this fungus (5) (Table 2).

**Plastic adherence.** Since the pathogenic potential of *C. parapsilosis* (mostly regarding the isolates from candidemic patients) has often been related to its adhesive properties (3, 38), we measured adhesion to plastic of all skin isolates of this fungus, again in comparison to the isolates from the candidemic subjects. The adhesion degree of skin isolates varied as much as from 0.1 to 3.4 with a mean (± the standard deviation [SD]) of 1.82 ± 1.16. The plastic adherence of the nine blood isolates also varied in the same range (0.1 to 3.6, with a mean ± SD of 1.78 ± 1.45). Thus, adherence to plastic was substantially similar between the two categories of the isolates. Interestingly, a comparison between the two basic morphotype categories of skin isolates (B, continuous fringe colonies; A, no fringe around colony), and plastic adherence values showed that the isolates of the first category were, as a group, significantly more adhesive (by >3-fold) to the plastic than those belonging to the no-fringe colony group (2.73 ± 0.5 versus 0.85 ± 0.9, respectively; *P* < 0.001, Student’s *t* test).

**Experimental pathogenicity in systemic infection of mice.** Skin isolates of *C. parapsilosis* were assessed for their experimental systemic pathogenicity in normal and in cyclophosphamide (Cy)-immunodepressed mice. The pathogenicity was evaluated both as MST and as the number of animals dead out of the total challenged. (D/T) over 30 days. The isolates of *C. parapsilosis* were collectively nonlethal upon intravenous challenge for both types of mice. Some occasional animal deaths were observed with isolate 94259, which killed one Cy-treated and one normal mouse (of the nine inoculated with 107 cells) and 2446 (one normal mouse died with 107 cells). The animals were not observed for organ invasion.

Eight of the nine hematologic isolates of *C. parapsilosis* were also tested in this model. Interestingly, two of them (HEM-31 and HEM-32) were highly pathogenic for leukopenic animals, with a median survival time of 2 days. The specific mortality was confirmed by the finding of high *Candida* burden in mice organs (kidney and heart) (not shown).

**Experimental vaginal infection in rats.** Ovariectomized, estradiol-treated rats were used to reproduce an experimental vaginal infection with *C. parapsilosis*. Seven fungal strains were randomly selected from all skin isolates and matched for their vaginopathic potential with similarly selected blood isolates. A high Sap producer strain of *C. albicans* and a Sap nonproducer mutant of the same species were also used as positive and negative controls, respectively, of the vaginopathic potential, as previously shown (9–11). Table 3 shows the cumulated viable counts of all fungal cells detected in the vagina over a 3-week period and the number of rats infected (>106 cells per ml of vaginal fluid) of the total after a vaginal challenge of 109 cells. Overall, all skin isolates of *C. parapsilosis* gave a sustained vaginopathic infection with a high number of viable fungal cells for 14 days, with general kinetics of fungal clearance that did not substantially differ from that typically obtained with vaginopathic strains of *C. albicans* (9, 11) (see also below). *C. parapsilosis* Sap was detected in the vaginal fluid of most of the rats tested in the first week of infection (data not shown). In contrast, the blood isolates were cleared from the vagina much earlier, by the first to the second week postchallenge (Table 3). One strain (HEM-27) was eliminated by day 5 postchallenge (not shown).

Figure 2 shows the different vaginopathic potentials of rep-
TABLE 3. Vaginal infection by skin and blood isolates of \textit{C. parapsilosis}*

<table>
<thead>
<tr>
<th>Days</th>
<th>Mean CU (10&lt;sup&gt;3&lt;/sup&gt;) ± SE/ml of vaginal fluid</th>
<th>No. of rats infected/total&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Skin</td>
<td>Blood</td>
</tr>
<tr>
<td>1</td>
<td>&gt;100</td>
<td>92 ± 6</td>
</tr>
<tr>
<td>7</td>
<td>52 ± 20*</td>
<td>9 ± 7*</td>
</tr>
<tr>
<td>14</td>
<td>8 ± 2*</td>
<td>&lt;1*</td>
</tr>
<tr>
<td>21</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

* Cumulative results of randomly selected seven isolates from skin and seven isolates from blood. Each isolate was used to infect five rats, with an inoculum size of 10<sup>7</sup> fungal cells in 0.1 ml of saline. Sampling on day 0 (1 h postchallenge) showed that all rats had >10<sup>6</sup> cells/ml of vaginal fluid. 
<sup>b</sup> Rat infected, ×10<sup>3</sup> CU/ml of vaginal fluid.

The differences between each pair of values marked with an asterisk in the same row are highly significant (P < 0.01, two-tailed t test) according to the Mann-Whitney U test for CU and the \( \chi^2 \) test for rats infected/total number of rats.

representative skin and blood isolates of \textit{C. parapsilosis}, compared both to one another and to vaginopathic and nonvaginopathic strains of \textit{C. albicans}.

DISCUSSION

\textit{C. parapsilosis} is now recognized among the pathogenic species of the genus \textit{Candida}, and several authors have recently reviewed its increasing incidence in various disease settings, including nosocomial bloodstream infections and neonatal candidemia in intensive care units (15, 19, 21, 28, 37, 38). We have previously demonstrated a close clinical association between vaginal isolation of \textit{C. parapsilosis} from women with active vaginitis and the vaginopathic potential in an estrogen-dependent rat vaginitis model (1, 10), an observation that has subsequently been confirmed by others (34). These vaginopathic strains, at variance with blood isolates of the same fungus, were high producers of Sap and were equal to \textit{C. albicans}, a well-known cause of candidal vaginitis, in their experimental vaginopathic potential (1, 10). However, the low-Sap-producing blood isolates of the fungus included strains with rather remarkable pathogenicity in a systemic infection model of nonimmunodepressed mice (5, 15), a property that is usually possessed only by the most pathogenic \textit{Candida} species, such as \textit{C. tropicalis} and \textit{C. albicans} (2, 26).

Much less is known regarding skin isolates of \textit{C. parapsilosis} despite the fact that this species is rather commonly isolated from the skin, which has also been implicated in nosocomial transmission of fungemia, in particular from the hands of hospital personnel (20, 33). In addition, there is more than circumstantial evidence of the importance of the skin isolates in exogenously disseminated candidiasis in immunosuppressed patients (26, 37). On this basis, we have addressed biotyping and in vitro-in vivo virulence properties of various skin isolates of \textit{C. parapsilosis} and compared them with the properties of recent blood isolates of the same fungus.

It is known that \textit{C. parapsilosis} is genotypically heterogeneous, its karyotype extending from six to nine chromosome-sized bands in all different source groups (3, 4, 13, 22, 23, 29, 30). Variability in the chromosomal asset has been confirmed here with the skin isolates, although none of them had nine chromosomeosomal bands or showed the peculiar features of some blood isolates with the absence of a whole chromosome-sized region at less than 1.6 Mb or the presence of unusually low-molecular-weight bands. Overall, these variations support the idea of a possible separation of \textit{C. parapsilosis} isolates into three separate groups, or even species, as previously suggested (5, 22) and as more recently supported by DNA-relatedness studies (30). Noteworthy is that one of the two blood isolates with low-molecular-weight bands resembled other previously studied biotypes with the same peculiar karyotype (5), and was likewise highly pathogenic in systemic infection. Of interest is that no special electrophoretic karyotype was found among the skin isolates studied here, all of which originated from the same geographical region and clinical source. There was indeed a major cluster of eight isolates belonging to a single class, but two blood isolates also belonged to this group.

More association was previously found among morpho-resistotype, source, and experimental pathogenicity. In particular, the morpho-resistotype group BIV2c of blood isolates included the most pathogenic strains in experimental bloodstream infections (5). Interestingly, none of the \textit{C. parapsilosis} skin isolates belonged to this morpho-resistotype, and none was virulent for systemic mouse infection. On the other hand, no relationship could be found between any particular morpho-resistotype and vaginopathic potential since all skin isolates belonging to seven morpho-resistotype groups were equally vaginopathic. This suggests that, as for \textit{C. albicans} (12), the determinants of superficial infections by \textit{C. parapsilosis} may be very different from those of systemic or deep-seated infections, although it is not yet clear which specific virulence determinants or host-adaptation factors are differentially expressed on the mucosa or systemically.

Potential in vivo-inducible virulence factors of \textit{C. parapsilosis} are considered to include adherence and slime production, an attribute of special importance for adhesion to plastic and therefore for catheter-related candidemia. We have examined adhesion to plastic by our skin and blood isolates and found a great variation in this property among them, with adhesion values ranging from very low (0.09 ± 0.03) to high (3.42 ± 0.21), in both categories. These results are in agreement with previous data of others, who found rather large variations in the capability of \textit{C. parapsilosis} isolates from blood and catheter cultures to produce large amounts of viscid slime material in glucose-containing solutions (3). However, we have also observed that certain morpho-resistotypes, namely, those showing colonies on malt extract agar with continuous fringes and shared by skin and blood categories, are endowed with a higher capacity to adhere to polystyrene. These isolates grew on malt extract agar predominantly as pseudomyecelial cells, in contrast to their ability to adhere to plastic (2).

![FIG. 2. Kinetics of vaginal infection by representative, high (■) and low (▲) Sap producer strains of \textit{C. parapsilosis} isolated from the skin or blood, respectively, compared to a prototype \textit{C. albicans} strain (5314 [●]) and its \textit{SAP2} gene null mutant (□). For experimental details, see the text and references 9 to 11.](http://jcm.asm.org/Downloaded from http://jcm.asm.org)
to the no-fringe isolates, which grew as yeasts. Since in C. albicans the fringe is due to the growth in the filamentous phase (germ tubes) (17), which has increased capacity to adhere to polystyrene (32) and since cell length appears to increase in the more-adhesive strains of C. parapsilosis (27), it is tempting to speculate that the increased capacity to adhere to polystyrene shown by C. parapsilosis skin isolates belonging to biotypes with a continuous fringe is due to their ability to produce abundant pseudomycelium on that medium. However, since among the strains used for experimental vaginal infections, which were almost equally vaginopathic, adhesiveness to plastic varied over a wide range (5), it is clear that adhesion to plastic, as measured by the current methods, bears no direct and unequivocal relationship to vaginopathic potential or systemic infection, although adherence to cells in vivo may well be relevant for both kinds of infection.

What seems clear from our data is that the production of aspartyl proteinase (Sap) is associated with vaginopathic potential. All skin isolates of C. parapsilosis were uniformly high Sap producers, averaging more than four times the enzyme production by the blood isolates and significantly higher than previously studied vaginal isolates (10). High Sap production was common to all skin isolates of C. parapsilosis regardless of the variations in their electrophoretic karyotype and morpho-resistotype, suggesting that it was a common property and not was common to all skin isolates of C. parapsilosis, though adherence to cells in vivo may well be relevant for both sapotypes.

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REFERENCES


