

## 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-immunodepressed 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 hemopathic 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<sup>+</sup>) and HIV<sup>-</sup> 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 protein-

ase, 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<sup>+</sup> and HIV<sup>-</sup> subjects were studied. No subject was affected by cutaneous candidiasis or was under treatment with antimycotics. For comparative purposes, nine isolates of the fungus from HIV<sup>-</sup> 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 and on Sabouraud-triphenyltetrazolium agar, as well as the resistotype on MacConkey agar (Oxoid, Basingstoke, United Kingdom) or different media containing 1.8 g of boric acid, 110 to 130 mg of NaCl, 20 g of urea, and 25 mg of 5-fluorocytosine (Sigma Chemical Co., St. Louis, Mo.) per liter or a citrate buffer with a pH of 2.8 ± 0.1, was determined as previously described (5). Every isolate received a code depending on the characteristics of the colonies: A, no fringe; B, continuous fringe; and C, discontinuous fringe. On Sabouraud-triphenyltetrazolium agar the texture of the colonies, their color, and the presence or absence of a mycelial halo were recorded as follows: S, smooth texture; P, pink color (P1 = pale pink, corresponding to Pantone 176C; P2 = dark pink, corresponding to Pantone 1785C); V, violet color (V1 = pale violet, corresponding to Pantone 251C; V2 = dark violet, corresponding to Pantone 262C); and N, no mycelial halo. Isolates that grew on boric acid, sodium chloride, and MacConkey media were coded as "1." If they grew on sodium chloride and MacConkey media, they were coded as 2. Those that grew sodium chloride only were coded as 3. Code 4 was given to isolates that did not grow on any medium, and code 5 was used for any other further combination. A similar approach was used for resistotyping on the urea, citrate, and 5-fluorocytosine media. Isolates that grew on all three media were coded as "a." Growth on urea and citrate media was coded as b, on urea only 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 AIV2c 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

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

Isolate (laboratory code)	Source <sup>a</sup>	Disease status and infection <sup>b</sup>
2296	Skin	HIV <sup>+</sup>
2446	Skin	HIV <sup>+</sup>
2518	Skin	HIV <sup>+</sup>
2550	Skin	HIV <sup>+</sup>
93006	Skin	HIV <sup>-</sup>
93179	Skin	HIV <sup>+</sup>
93858	Skin	HIV <sup>+</sup>
94259	Skin	HIV <sup>+</sup>
94260	Skin	HIV <sup>-</sup>
94864	Skin	HIV <sup>-</sup>
94915	Skin	HIV <sup>-</sup>
94938	Skin	HIV <sup>-</sup>
2057	Catheter	Catheter-related candidemia
2058	Catheter	Catheter-related candidemia
HEM-26	Blood	Catheter-related candidemia
HEM-27	Blood	Catheter-related candidemia
HEM-28	Blood	Catheter-related candidemia
HEM-29	Blood	Catheter-related candidemia
HEM-30	Blood	Catheter-related candidemia
HEM-31	Blood	Candidemia with deep-seated invasive infection
HEM-32	Blood	Candidemia with deep-seated invasive infection

<sup>a</sup> Neither HIV<sup>+</sup> nor HIV<sup>-</sup> subjects were affected by cutaneous candidiasis.

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

concentration of 10<sup>9</sup>/ml in a solution of EDTA-sorbitol, as described above, but 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 molds and placed in 1.2 M sorbitol solution containing 20 mM EDTA, 10 mM Tris-HCl (pH 7.5), and 100  $\mu$ l of Zymolase 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 100 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-DR11 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) containing the agarose inserts of DNA immersed in running buffer (50 mM Tris-HCl, 50 mM boric acid, 1.5 mM EDTA; pH 8.2). *Saccharomyces cerevisiae* (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  $\mu$ g/ml; 30 min), destained, and photographed under UV light.

**Secretory aspartyl proteinase production.** The abilities to secrete aspartyl proteinase in bovine serum albumin (BSA) were assessed in solid and liquid media, as previously described (9, 10). Briefly, the yeast was pregrown in YPD medium and induced to proteinase secretion in BSA agar (1.17% yeast carbon base, 0.01% yeast extract, 0.2% BSA; Sigma) (pH 5.0). Enzyme activity was measured as the diameter of a lytic area on BSA and scored as – to ++, as previously described (9–11). For proteinase activity determination in liquid YPD medium by the spectrophotometric method culture, supernatants (0.1 ml) were added to 0.9 ml of 0.1 M citrate buffer (pH 3.2) with 0.2% BSA with or without the proteinase inhibitor pepstatin A (0.5 mg/ml; Sigma) and then incubated at 37°C. The reaction was terminated after 3 min by the addition of 5% trichloroacetic acid. The mixture was centrifuged, and the supernatant was read at 280 nm. Aspartyl proteinase (Sap) activity was expressed as the change in optical density per minute per milliliter of culture, as previously described (9–11).

**Adherence assay.** The adhesion of *C. parapsilosis* cells to polystyrene was quantified spectrofluorometrically. Cells in the stationary phase were inoculated into 350 ml of medium 199 (pH 6.7) (Gibco BRL/Life Technologies, Rockville, Md.) at a final concentration of 10<sup>6</sup>/ml, and the medium was incubated for 2 h at 37°C in 24-well polystyrene plates. After a washing, the plates were stained with 1% calcofluor white in saline for 20 min at room temperature. The plates were washed again, and the fluorescence was read in a spectrofluorometer (Fluorescan Ascent; Labsystem, Helsinki, Finland), with an excitation filter of 358 nm and an emission filter of 460 nm. Controls were used to examine possible interference from nonspecific binding and consisted of wells with medium 199. The results were expressed as the relative fluorescence, which was obtained by subtracting the reactivity obtained in the control wells from that shown by the test wells. All values are mean values derived from at least four independent assays.

**Systemic infection in mice.** For experimental systemic infections, yeasts were grown in YPD medium for 24 h at 28°C on a shaker at 200 rpm (New Brunswick Scientific Co.), centrifuged, washed, counted in a hemocytometer, and diluted in saline. Of this suspension, 0.2 ml containing either 10<sup>6</sup> or 10<sup>7</sup> cells of the *C. parapsilosis* isolates was injected intravenously into inbred male CDF1 mice (18 to 21 g; Charles River, Calco, Italy). Groups of nine mice each were pre-treated with cyclophosphamide (CY; Cytoxan; Sigma) 2 days before the infectious challenge. The drug was dissolved in sterile saline and injected intraperitoneally at a concentration of 150 mg/kg in a final volume of 0.2 ml. Survival was assessed over a period of 30 days and was expressed as the number of dead animals over the total number of animals infected (D/T) and as median survival time in days (MST).

**Experimental vaginal infection in ovariectomized rats.** For experimental vaginal infection, ovariectomized female Wistar rats (80 to 100 g) (Charles River) were injected subcutaneously with 0.5 mg of estradiolbenzoate (Benzatrone-Samil, Rome, Italy) every 2 days. At 6 days after the first estradiol dose, the animals were inoculated intravaginally with 10<sup>7</sup> cells (0.1-ml volume) of each isolate tested. Yeast cells had been grown in YPD medium at 28°C, centrifuged, washed, suspended in sterile saline, and injected into the vaginal cavity through a syringe with a multipurpose calibrated tip (Combipip PBI, Milan, Italy). Enumeration of *C. parapsilosis* in the vaginal cavity was achieved by taking 1- $\mu$ l samples by use of a calibrated plastic loop (Disponoic; PBI) from each animal every 2 days and culturing it on Sabouraud agar containing chloramphenicol (50  $\mu$ g/ml). Cultures were incubated at 28°C for 48 h.

**Statistics.** Differences between mean values of continuous variables were assessed by the Student's *t* test, whereas differences between proportions were assessed by the Fisher's exact test or the  $\chi^2$  method, as appropriate.

## 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 (~1,050 kb, first three isolates) or a higher (~1,180 kb, the latter isolate) molecular mass than the fifth band of the former group of isolates (~1,200 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 section, 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-

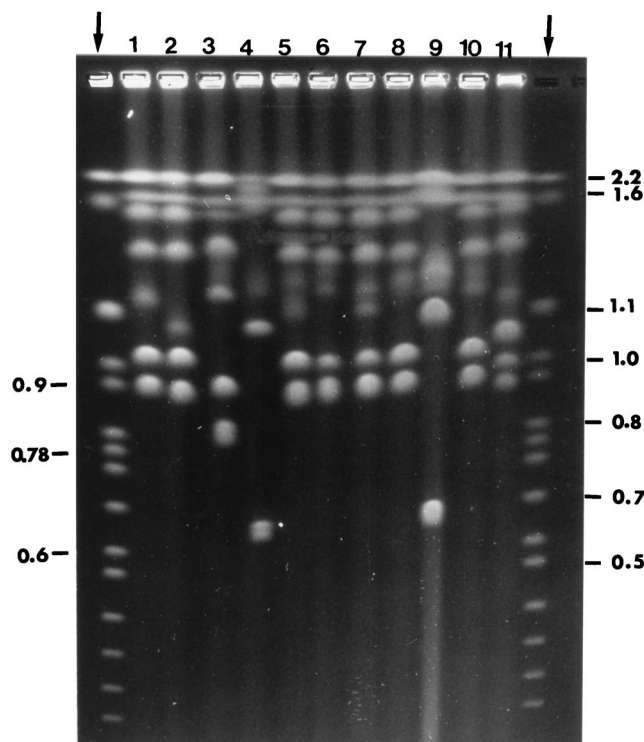


FIG. 1. Representative electrophoretic karyotype patterns of *C. parapsilosis*. Each numbered lane corresponds to a cluster of overlapping karyotypes, as defined in the text. Arrowed lanes at left and right are *S. cerevisiae* chromosomal markers, and their molecular size is expressed in megabases. For other details, see the text.

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<sup>+</sup> or HIV<sup>-</sup> 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 cate-

gories 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 10<sup>7</sup> cells) and 2446 (one normal mouse died with 10<sup>7</sup> 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 (>10<sup>3</sup> cells per ml of vaginal fluid) of the total after a vaginal challenge of 10<sup>7</sup> cells. Overall, all skin isolates of *C. parapsilosis* gave a sustained vaginal 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 during 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 2. Sap production by skin and blood isolates of *C. parapsilosis*

Source (n)	HIV status	Sap production on:			
		BSA agar <sup>a</sup>		BSA broth <sup>b</sup> (mean U ± SD)	<i>P</i> <sup>d</sup>
		No. ++/total	<i>P</i> <sup>c</sup>		
Skin (7)	HIV <sup>+</sup>	7/7		3.84 ± 0.15	
Skin (5)	HIV <sup>-</sup>	5/5		3.80 ± 0.17	
Blood (9)	HIV <sup>-</sup>	0/9	<0.01	0.85 ± 0.07	<0.01

<sup>a</sup> Measured as the diameter of BSA clear zone (0, none; +, 1 to 2 mm; ++, 3 to 5 mm) (see also references 9 and 10).

<sup>b</sup> Mean ± SD units, from the spectrophotometric determination (see references 9 to 12).

<sup>c</sup> *P* < 0.01 (Fisher exact test), comparing Sap production by skin isolates (only HIV<sup>+</sup> or else HIV<sup>+</sup> plus HIV<sup>-</sup>) to that by blood isolates.

<sup>d</sup> *P* < 0.01 (Student's *t* test), comparing Sap production as in footnote c.

TABLE 3. Vaginal infection by skin and blood isolates of *C. parapsilosis*<sup>a</sup>

Days	Mean CFU ( $10^3 \pm$ SE/ml of vaginal fluid)		No. of rats infected/total <sup>b</sup>	
	Skin	Blood	Skin	Blood
1	>100	92 $\pm$ 6	35/35	35/35
7	52 $\pm$ 20 <sup>*c</sup>	9 $\pm$ 7*	35/35	35/35
14	8 $\pm$ 2*	<1*	13/35*	0/35*
21	<1	<1	3/35	0/35

<sup>a</sup> 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  $10^7$  fungal cells in 0.1 ml of saline. Sampling on day 0 (1 h postchallenge) showed that all rats had  $>10^5$  cells/ml of vaginal fluid.

<sup>b</sup> Rat infected,  $>10^3$  CFU/ml of vaginal fluid.

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

representative skin and blood isolates of *C. parapsilosis*, compared both to one another and to vaginopathic and nonvaginopathic strains of *C. albicans*.

## DISCUSSION

*C. parapsilosis* is now recognized among the pathogenic species of the genus *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 *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 *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 *Candida* species, such as *C. tropicalis* and *C. albicans* (2, 26).

Much less is known regarding skin isolates of *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 *C. parapsilosis* and compared them with the properties of recent blood isolates of the same fungus.

It is known that *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 chromosomal 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 *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 *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 *C. albicans* (12), the determinants of superficial infections by *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 *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 adherence to plastic by our skin and blood isolates and found a great variation in this property among them, with adherence values ranging from very low ( $0.09 \pm 0.03$ ) to high ( $3.42 \pm 0.21$ ), in both categories. These results are in agreement with previous data of others, who found rather large variations in the capability of *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 pseudomycelial cells, in contrast

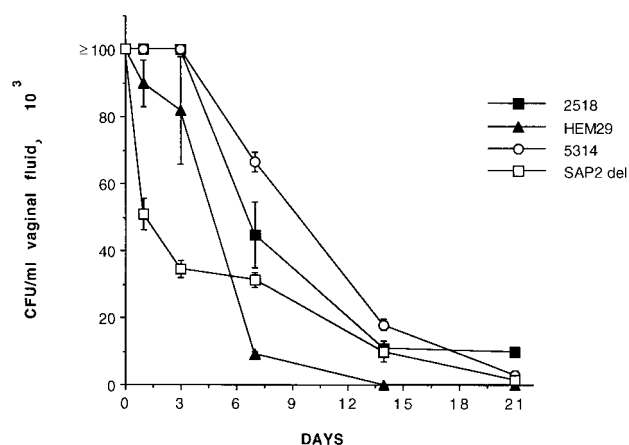


FIG. 2. Kinetics of vaginal infection by representative, high (■) and low (▲) Sap producer strains of *C. parapsilosis* isolated from the skin or blood, respectively, compared to a prototype *C. albicans* strain (5314 [○]) and its *SAP2* gene null mutant (□). For experimental details, see the text and references 9 to 11.

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 morphoresistotype, suggesting that it was a common property and not a particular feature of some special biotypes of the fungus. Interestingly, high Sap production did not confer upon the strains any appreciable capacity for systemic infection, even in strongly leukopenic, cyclophosphamide-immunodepressed mice. Thus, among all the putative virulence features of this fungus, only Sap production appeared to reflect the vaginopathic property of the skin isolates. In their study of *C. parapsilosis* complex, Lin et al. (22) showed the variability of Sap production by isolates of *C. parapsilosis* from various sources, and this is here confirmed with regard to a comparison between blood and skin isolates of the fungus. Importantly, of the 10 isolates with the highest Sap score, 7 were from a source directly or indirectly bound to the skin or mucosa, such as vaginal fluid, ear, leg wound, or vitreous fluid (22).

*C. parapsilosis* possesses two *SAP* genes, which are partly homologous to the aspartyl proteinase of *C. tropicalis* (14, 16, 25). The vaginal environment has several properties that favor the induction and expression of this activity inclusive of the acidic pH reached under estrogen treatment and the abundance of proteinaceous secretions. Studies of *C. albicans* with deleted *SAP* genes have clearly demonstrated the role of some of these enzymes in vaginitis (11). Moreover, Sap inhibitors, inclusive of HIV-protease inhibitors (6, 7), have exerted a therapeutic effect in experimental vaginitis. The role of Sap in superficial candidiasis may be somewhat advantageously studied in *C. parapsilosis* since this fungus does not seem to possess other penetrant virulence attributes, has a less varied clinical spectrum of diseases, and apparently possesses fewer Sap enzymes than those secreted by *C. albicans*.

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