

## Expression of Plasma Coagulase among Pathogenic *Candida* Species

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***Candida* coagulase production was assessed by the classical tube test. All *Candida krusei* strains were coagulase negative, but most *C. albicans* and *C. tropicalis* strains can produce coagulase. Some strains agglutinated the Pastorex Staph-Plus reagent, probably because of antigen similarities to coagulase produced by *Staphylococcus aureus* that may cause mistakes in clinical laboratories.**

The pathogenesis of candidosis involves several factors, among which may be counted germ tube and hypha formation (10), adhesion factors (13), phenotypic switching (11), and thigmotropism (9), as well as the production of different enzymes. Hydrolytic enzymes such as lipases (4) and proteases like the secreted aspartyl proteinases, phospholipases, esterases, and phosphatases are also among the putative virulence factors of *Candida albicans* (1, 2). This diversity of pathogenic attributes may facilitate adaptation to distinct stages or types of infection, acting synergistically to enhance fungal survival (12). We have noticed at our routine laboratory that some yeast strains were able to react positively with the reagent of the Pastorex Staph-Plus coagulase test (Bio-Rad, Marnes-La-Coquette, France). We decided to evaluate the expression of the enzyme coagulase in *Candida* isolates by using both the classical methodology (clot formation from plasma) and the Pastorex latex test kit.

A total of 161 clinical *Candida* isolates (isolated from blood, respiratory secretions, genital secretions, stool, and urine) representing six different species (*C. albicans* [70 isolates], *C. tropicalis* [23 isolates], *C. glabrata* [25 isolates], *C. parapsilosis* [29 isolates], *C. krusei* [11 isolates], and *C. guilliermondii* [3 isolates]) were selected. Two type strains from the American Type Culture Collection (ATCC), *C. albicans* ATCC 90028 and *C. parapsilosis* ATCC 22019, were also included in the study. Stock cultures were initially maintained at  $-70^{\circ}\text{C}$ . After recovery and after ensuring the purity of cultures, yeast isolates were cultured on Sabouraud dextrose agar (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) and stored at 4 to  $6^{\circ}\text{C}$  for up to 4 days. The identity of the organisms was reconfirmed by the germ tube test and the commercially available API 32C identification kit (Analytical Profile Index; BioMérieux SA, Marcy l'Étoile, France). The type strains *Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis* ATCC 14990 were used, respectively, as positive and negative controls for coagulase expression. Coagulase production was assessed by the presence of a clot that could not be resuspended by gentle shaking after inoculation of each strain into a glass tube with

500  $\mu\text{l}$  of EDTA-rabbit plasma (Difco Laboratories, Detroit, Mich.) and incubation for 4 h at  $35^{\circ}\text{C}$ . If no clot formed, the tube was reincubated and reexamined at 24 h (5). The Pastorex Staph-Plus Kit (Bio-Rad) was used in accordance with the manufacturer's instructions. This test is based on latex particles sensitized with human fibrinogen and monoclonal antibodies directed to the simultaneous detection of clumping factor, staphylococcal protein A, and capsular polysaccharides. The ability to produce coagulase in tubes differed among species (Table 1). Although only two *C. albicans* strains induced clot formation after 4 h of incubation, most of the *C. albicans* (88.5%) and *C. tropicalis* (82.6%) strains were able to induce clot formation from EDTA-rabbit plasma at 24 h. Lower percentages were detected among *C. parapsilosis* (34.5%), *C. guilliermondii* (33.3%), and *C. glabrata* (20%), also after incubation for 24 h. None of the *C. krusei* strains tested was able to produce coagulase. Positive latex test results were obtained with 68.9% of the *C. albicans* strains, 56.5% of the *C. tropicalis* strains, and 6.9% of the *C. parapsilosis* strains. None of the *C. glabrata*, *C. guilliermondii* or *C. krusei* strains reacted positively in this test. All of the positive results obtained with the latex test corresponded to positive EDTA-rabbit plasma test results. No correlation could be established regarding the expression of or ability to produce coagulase and the site of origin of the isolate.

Hydrolytic enzyme activities that are expressed at the surface of microbial pathogens are, most probably, able to cause damage to host cells in vivo (8). However, definitive proof of the involvement of individual enzymes in pathogenesis is always hard to achieve. Extensive research has been focused on proteinases like secreted aspartyl proteinases (2, 3), phospholipases (4), and hemolysins (6, 7). However, quite a few older papers refer to enzymes such as plasma coagulase (14, 15) and invariably studied a reduced number of *Candida* strains. According to our indirect results involving a considerable number of strains, its activity is almost ubiquitous among *C. albicans*, common in *C. tropicalis*, occasional in *C. parapsilosis*, and rare or absent in other species. Plasma coagulase, an enzyme that binds plasma fibrinogen and activates a cascade of reactions that induce plasma to clot, is also used to help in identifying *S. aureus*. Different diagnostic kits like Pastorex Staph-Plus are commercially available and being increasingly used in clinical microbiology laboratories, replacing the conventional coagu-

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TABLE 1. *Candida* spp. inducing clot formation from plasma and giving positive results with the Pastorex latex agglutination test

Species	Total no. of strains	% of strains positive	
		Tube coagulase test	Pastorex latex test
<i>C. albicans</i>	70	88.5	68.9
<i>C. tropicalis</i>	23	82.6	56.5
<i>C. glabrata</i>	25	20	0
<i>C. parapsilosis</i>	29	34.5	6.9
<i>C. krusei</i>	11	0	0
<i>C. guilliermondii</i>	3	33.3	0

lase test because they are apparently more specific and considerably faster. However, the reagents used in such tests also agglutinate different *Candida* species, making the test specificity rather low. This fact might be explained by antigen similarity between staphylococci and some yeasts, mainly *C. albicans* and *C. tropicalis*, resulting in a cross-reaction. The increasing need for faster microbiological results to support therapeutic decisions demands faster identification. Increasingly, many products or samples, like central venous catheters, are cultured without previous direct microscopic examination. Thus, the growth of a few uncharacteristic white colonies following a short incubation period (e.g., 24 h) could lead to a latex coagulase test being performed (takes only a few seconds) without a previous microscopic confirmation of growth and consequently the reporting of results that, if they are positive, might be taken for *S. aureus*. A misidentification in the laboratory setting of a large university hospital, handling a considerable number of samples daily and relying mostly on automated devices for identity confirmation, could lead to a serious mis-

take. The need to reconfirm identification would increase costs, apart from delaying considerably a definitive microbiological diagnosis.

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