Increased Phenotypic Switching in Strains of *Candida albicans* Associated with Invasive Infections

S. JONES, G. WHITE, AND P. R. HUNTER

**Public Health Laboratory, Countess of Chester Health Park, Chester,**
**and Laboratory of**
**Hospital Infection, Central Public Health Laboratory, London,**

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This study reports the rates of phenotypic switching in strains of *Candida albicans* isolated from superficial and invasive infections. Of 19 invasive strains, 68% showed switching activity, often at very high rates, compared with only 28% of 40 strains isolated from superficial sites (*P* = 0.004).

Although colony variation in *Candida albicans* was described as early as 1935 (7), there has been renewed interest in the phenomenon since Slutsky, Bufco, and Soll (9) described high-frequency phenotypic switching in strains of *C. albicans*. Since his first paper, Soll and colleagues as well as other workers have described a variety of switching systems in strains of *C. albicans* isolated from a variety of clinical sources in different geographical areas (10). Ever since this recent paper, there has been speculation that this phenotypic switching activity may be related to virulence (10, 12). Studies of switching in cases of vaginal candidiasis have supported the role of switching in virulence (11). Despite this interest, there have not yet been any investigations into whether strains from invasive fungal disease do indeed exhibit increased rates of phenotypic switching. This paper reports such a study.

Twenty strains of *C. albicans* were isolated from vaginal swabs submitted to Chester Public Health Laboratory for the investigation of vaginitis, and an additional 20 strains were isolated from fecal samples submitted for the identification of bacterial pathogens. The 19 invasive strains had been submitted to the Mycology Reference Laboratory, Central Public Health Laboratory, London, United Kingdom, for antifungal sensitivity testing or to the Division of Hospital Infection for typing. Each invasive strain had been isolated from blood culture or a deep tissue specimen by a third laboratory. They were subsequently transferred to Chester on Sabouraud agar slopes.

The identity of each strain was confirmed by the production of germ tubes in horse serum and by the production of chlamydospores on cornmeal agar.

The method used was that described by Odds and Merson-Davies (8). Prior to examination for switching behavior, each strain was subcultured twice from single, well-isolated colonies. Finally, a suspension was made in sterile water. The concentration of yeast cells in this suspension was counted microscopically with a Fuchs Rosenthal counting chamber. The suspension was then diluted to give a concentration of 2 × 10^6 cells ml^-1. One hundred microliters of this final suspension was then inoculated onto the surface of each of four Phloxine B agar plates. This gave between 200 and 400 colonies per plate. Total counts, combining all four plates, ranged from 620 to 1,476 colonies.

Phloxine B agar plates were made according to Anderson and Soll's (1) modification of the synthetic medium of Lee et al. (6).

Inoculated plates were incubated in the dark at 25°C for 9 days. Plates were inspected on days 5 and 9 for the presence of more than one colony color or morphology. When all colonies were identical, it was assumed that phenotypic switching had not occurred. When more than one form was seen, the most frequent form was assumed to be the native type and all others were presumptive switched phenotypes. The numbers of native and presumptive switched colonies were counted.

Selected native and switched forms were reincoculated onto Phloxine B agar plates as described above to check the stability of each switch. Only if subsequent cultures of presumptive switched types were still distinct from cultures of the native forms was the original strain recorded as showing phenotypic switching.

Table 1 indicates the number of strains showing phenotypic switching behavior in each of the three groups. It can be seen that strains from the invasive group were more than twice as likely as the superficial isolates to show switching (*P* = 0.004, Fisher's exact test). In those strains that showed phenotypic switching, the rate at which invasive strains switched was also much higher than that of superficial strains (*P* = 0.0004, two-tailed Mann-Whitney U test corrected for ties). The five fecal isolates that switched had switching rates (per 1,000 colonies) of 3.5, 1.1, 1.1, 4.4, and 6.5. The six vaginal isolates that switched had rates of 4.6, 1.3, 1.1, 3.2, 3.2, and 2.1. The 13 invasive isolates that switched had rates of 25.5, 10.5, 10.5, 6.0, 17.6, 4.5, 44.1, 40.0, 7.4, 1.4, 374.1, 339.8, and 319.4.

As has already been mentioned, there has been considerable interest in whether phenotypic switching in *C. albicans* is important in the virulence of the organism. The difference in phenotypic switching activity between invasive and superficial strains in this study was dramatic. Indeed, the switching rates observed for some of the invasive strains are among the highest rates described in the literature. Although not conclusive, this association of phenotypic switching with virulence is a strong indicator that this phenomenon is indeed an important virulence factor in invasive candidiasis.

However, the invasive strains included in this study were not isolated in the same laboratory as the superficial strains and would have been subcultured more often before analysis. It is possible, therefore, that the difference in switching rates is an artifact of the handling after isolation. However, despite a considerable number of investigations into the phenomenon of phenotypic switching, no one has noted any effect of the number of subcultures on the switching rate (10).

If phenotypic switching is indeed associated with strains
from invasive infections, there are a variety of possible ways in which such behavior could contribute to virulence. Several studies have demonstrated that different phenotypes of the same strain show different levels of virulence in animal models or different levels of other potential virulence factors. For example, different switched phenotypes of the same strains of *C. albicans* isolated from patients with human immunodeficiency virus infection can show different levels of resistance to azole antifungal agents (3). Similarly, different phenotypes of the same strain can show different abilities to adhere to epithelia (5) and to produce acid protease (2).

However, the demonstration that strains with different switch phenotypes demonstrate different virulence indicators does not show that switching is an important virulence factor. It would be simpler for the organism to continue to exist in its high-virulence phenotype. What this study suggests is that the phenomenon of phenotypic switching itself is important for virulence. Hunter and colleagues previously demonstrated a very high degree of association between discontinuous fringe formation during morphotyping and fatal candidiasis (4). They made the suggestion that discontinuous fringe formation was a marker for increased phenotypic switching. The suggestion was made that phenotypic switching enabled the strain to assume the optimal phenotype for different stages of a multistage process of invasion. We still see this as the most likely explanation for the role of phenotypic switching in the differential virulence of different strains of *C. albicans*.

Clearly, an understanding of the mechanism and role of phenotypic switching in *C. albicans* will be important in the understanding of invasive candidiasis.

### REFERENCES