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Effect of Potassium on *Saccharomyces cerevisiae* Resistance to Fluconazole

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Susceptibility of strain S288c of *Saccharomyces cerevisiae* to fluconazole was assayed in the presence and absence of KCl. Addition of 150 mM KCl renders the strain more sensitive to the antifungal agent. The effect is caused by the K^+ ion rather than the anion or the osmolarity of the medium. The increase in sensitivity does not modify the values of intracellular and extracellular pH established in the presence of KCl.

Fluconazole is a widely used antifungal agent, especially in the treatment of invasive mycosis. The generation of resistant mutants operates against the effectiveness of fluconazole treatment (1, 5, 7). Consequently, it is important to study the conditions under which resistance to the antifungal emerges impaired or diminished.

The potassium ion is normally found in *Saccharomyces cerevisiae* growth media. However, in concentrations of 150 mM or higher, it depolarizes the cellular membrane of this organism (3, 4). Bearing this fact in mind, we investigated the effect of potassium ions on the susceptibility of *S. cerevisiae* strain S288c to fluconazole.

Number of colonies. *S. cerevisiae* strain S288c was grown to 1.5×10^8 cells/ml on SD synthetic medium (Difco Laboratories, Detroit, Mich.) containing 2% (wt/vol) D-glucose. To study the effect of KCl using solid SD, an aliquot from a preculture grown for 24 h at 30°C was diluted and used to inoculate plates to obtain 300 to 500 colonies per plate. Plates were incubated at 30°C, and at 72 h the number of colonies was recorded. With 10 μg of fluconazole per ml and 150 mM KCl, fluconazole had only a slight effect on the number of colonies. However, the inhibition increased at a concentration of 20 μg/ml (Fig. 1).

Halo formation. To determine whether the difference observed with KCl is due to an osmotic effect or to the presence of chloride ions, parallel assays were carried out using sorbitol and $MgCl_2$. From a suspension of 1.0×10^6 cells/ml, 200-μl portions were dispersed onto plates of SD medium supplemented with 300 mM sorbitol, 150 mM KCl, or 100 mM $MgCl_2$. Glass-fiber filters (Schleicher & Schuell, Inc., catalog no. 3362), 8 mm in diameter, were impregnated with 25-μg portions of fluconazole and placed in the center of the seeded plates. After incubation at 30°C for 48 h, the diameters of the inhibition halos were measured and compared (Fig. 2). The largest halo was formed on the plate containing KCl. In the medium with $MgCl_2$ the halo diameter was similar to that of the control. However, in this medium roughly 200 to 250 small colonies appeared inside the halo; they are not visible in the photograph. Similar results were obtained using lower KCl concentrations, ranging from 50 to 100 mM.

Internal and external pHs. The potassium ion is able to produce intracellular alkalinization concomitantly with extracellular acidification (2, 6). A possible explanation for the increase in susceptibility observed with KCl is that fluconazole may interfere with processes that release protons from the intracellular space.

To exclude this possibility, cells were incubated with 150 mM KCl, with and without the addition of fluconazole, and intracellular pH was assayed with fluorescein diacetate (FDA) (8). By assuming a medium volume of 1.0 ml below the halo (Fig. 2), the fluconazole concentration may be estimated to be 25 μg/ml. This concentration was used for the FDA assay. A suspension of 3.0 mg (dry weight) of yeast per ml in 4 mM potassium phthalate buffer (pH 4.5) was incubated with 150 mM KCl and 0.13 mM FDA with and without 25 μg of fluconazole per ml for 30 min at 30°C. Following incubation, the mixture was centrifuged and the precipitate was suspended in 1.0 ml of the buffer. After measuring the fluorescence using 50 μl of this suspension, the pH value was interpolated using a calibration curve. The curve was determined by adding an aliquot from hydrolyzed FDA to 1.8-ml portions of solutions with different pH values. The extracellular pH of the supernatant

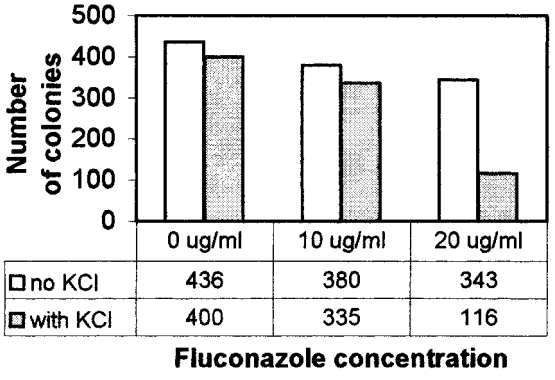

FIG. 1. Fluconazole inhibition of strain S288c on solid SD medium containing 150 mM KCl. Values are from one representative experiment.

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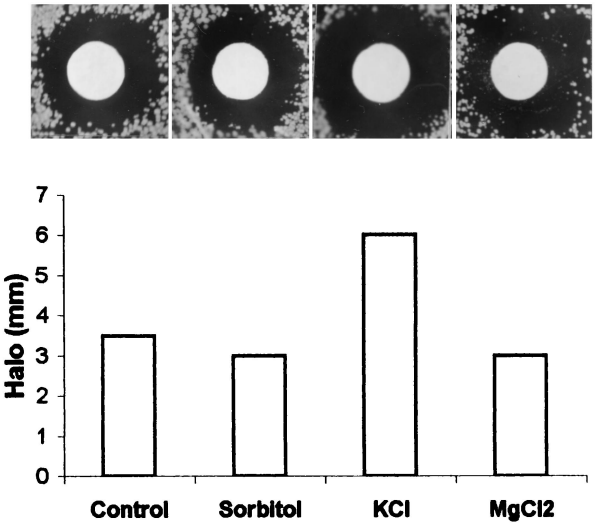

FIG. 2. (Top) From left to right, halos from control and 300 mM sorbitol-, 150 mM KCl-, and 100 mM $MgCl_2$-supplemented media. Photographs were taken after 48 h of incubation at 30°C. Filters contained 25 μg of fluconazole. (Bottom) Sensitivity reported as halo radius.

tant of a suspension slightly buffered with 4 mM potassium phthalate (pH = 4.5) was determined by using a pH electrode.

For this purpose, 13.3 mg (dry weight) of yeast/ml was incubated with 150 mM KCl, with and without the addition of 100 μg of fluconazole per ml. The fluconazole concentration was increased to maintain the same ratio of fluconazole/mg of yeast as that used for internal pH measurement. After centrifugation, the pH value of the supernatant fluid was determined. The pH values obtained did not show differences, regardless of whether the cells were incubated with or without fluconazole (Table 1). Therefore, it can be excluded that fluconazole alters the alkalinization and acidification processes elicited by the ion.

TABLE 1. Intracellular and extracellular pH values for strain S288c cells

Treatment[a]	Intracellular pH	Extracellular pH
None (control)	6.12	4.60
FLC	6.08	4.57
KCl	6.63	4.43
FLC and KCl	6.60	4.42

[a] FLC, fluconazole.

The results show that the potassium ion increases the susceptibility of *S. cerevisiae* strain S288c to fluconazole. The elucidation of the mechanism by which the ion exercises this effect needs to be established through future investigations.

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