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The K1 Toxin of *Saccharomyces cerevisiae* Kills Spheroplasts of Many Yeast Species

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The *Saccharomyces cerevisiae* K1 toxin killed spheroplasts from the genera *Candida*, *Kluyveromyces*, and *Schwanniomyces*. Cells of these organisms were toxin insensitive. The toxin bound poorly to *Kluyveromyces lactis* cells. In contrast, *Candida albicans* bound the toxin to an extent similar to that seen with *S. cerevisiae*. Thus, wall receptors can define toxin specificity and are necessary but not sufficient for toxin action on intact cells.

Yeast cells of many genera secrete protein toxins to which they are immune but which are lethal to sensitive yeast cells. Classification of killer yeast cells by cross-killing reactions established at least 11 distinct killer activities, which probably represent 11 biochemically different toxins (12). Among these toxins, the best understood are the *Saccharomyces cerevisiae* K1 and K2 toxins (3, 11).

The K1 killer toxin is a secreted, 20-kilodalton heterodimeric protein consisting of α and β subunits linked by disulfide bonds (2, 13). The action of killer toxin on sensitive yeast cells, although not fully understood, involves a set of specific cell-surface interactions. These include binding to a glucan cell wall receptor (9) and subsequent interactions at the level of the plasma membrane, causing ion leakage and cell death (3, 6, 10).

K1 and K2 killer toxins have a narrow host range; they are reported to kill only *S. cerevisiae* and *Torulopsis glabrata* (5, 12). Studies of K1 toxin action suggest that binding to the cell wall receptor, and killing of spheroplasts which involves interactions with the plasma membrane and causes ion leakage, are independent steps (3). The killing spectra of the K1 and K2 toxins at the spheroplast level have not been examined. Here, we report that spheroplasts from several yeast genera are sensitive to K1 and K2 killer toxins.

Spheroplasts were made by digestion of yeast cells with 40 μg of zymolyase-60,000 per ml (4) at 30°C for 1 to 3 h, depending on the yeast strain used. Spheroplasts were regenerated by the method of Hinnen et al. (8) in YEPD agar (1 g of yeast extract, 1 g of peptone, and 2 g of glucose per 100 ml) containing 1.2 M sorbitol at pH 4.7. Survival of cells following the spheroplast procedure was not greater than 10^{-5}, measured by plating on YEPD agar without sorbitol as an osmotic support.

The sensitivity of yeast cells and spheroplasts to the killer toxin was determined by killing zone assay in a plate test (3). An agar plate seeded with a tester strain (either whole cells or spheroplasts) was patched with killer cells or spotted with 10 μl of concentrated killer yeast culture filtrates. The inhibition of cell or spheroplast growth was shown by formation of a clear zone around the killer cells or culture filtrate (Fig. 1). Controls showed that spotting of 10 μl of buffer or water in place of killer filtrates caused no zone of growth inhibition.

The binding of the killer toxin to cell wall receptors was performed by the toxin activity removal method of Al-Aidroos and Bussey (1). The isolation of yeast alkali-insoluble cell wall glucans was as described by Hutchins and Bussey (9).

Cells and spheroplasts of nine yeast strains (Table 1) were tested for sensitivity of K1 and K2 toxins. A K1 killer strain, T158C/S14a, and a K2 killer strain, M471, were used for

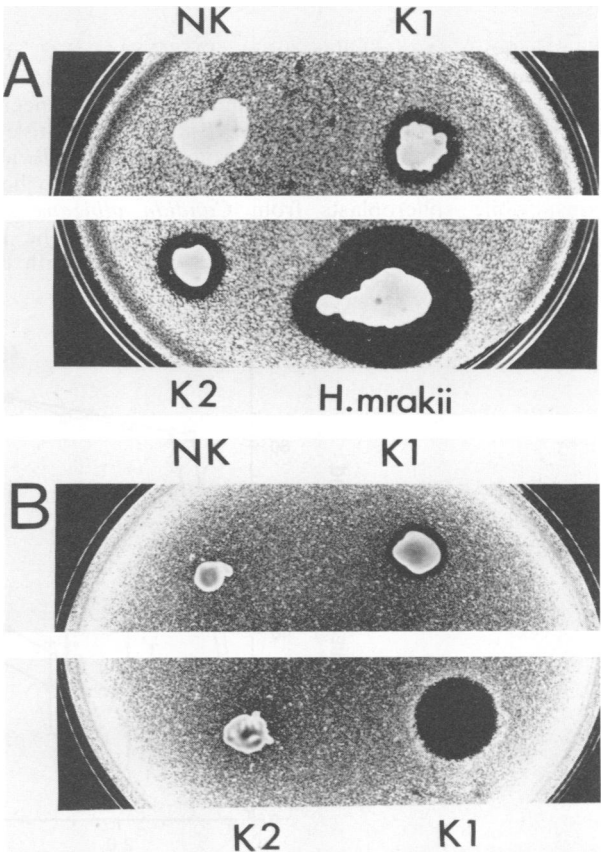

FIG. 1. Effects of killer toxins on yeast spheroplasts. YEPD agar plates with 1.2 M sorbitol at pH 4.7 were seeded with spheroplasts of *K. lactis* (A) and *C. albicans* (B). The seeded plates were patched with 10 μl of the indicated killer or nonkiller (NK) cells or culture filtrates and incubated at 18°C for 2 to 3 days. See Table 1 for strains used.

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TABLE 1. Sensitivities of yeast spheroplasts to K1 and K2 killer strains

Strains tested for spheroplast sensitivity[a]	Killing activity of strain[b]:		
	T158c/S14a (K1 toxin)	M471 (K2 toxin)	T158c/ S14a.H.C.[c]
Saccharomyces cerevisiae S6	+	+	−
Saccharomyces cerevisiae NCYC 1324	+	+	−
Candida albicans ATCC 10261	+	−	−
Candida utilis NCYC 707	+	+	−
Candida buinensis ATCC 58432	−	−	−
Hansenula mrakii NCYC 500	−	−	−
Kluyveromyces lactis 2105-1D	+	+	−
Pichia kluyveri ATCC 24241	−	−	−
Schwanniomyces alluvius ATCC 26075	+	−	−

[a] NCYC, National Collection of Yeast Cultures, England; S6, sensitive tester strain, wild type; 2105-D, killer strain from N. Gunge.

[b] +, Spheroplasts sensitive to killer strains; −, spheroplasts insensitive to killer strains.

[c] T158c/S14a.H.C., Sensitive nonkiller strain derived from T158c/S14a by heat curing of KIL-K1 double-stranded RNA.

killing activity. A nonkiller strain, T158C/S14a.H.C., derived from T158C/S14a by heat curing of the K1 double-stranded RNA, was used to determine killing effects specific to the K1 toxin. Only cells of *S. cerevisiae* were sensitive to the toxins. However, spheroplasts from *Candida utilis* and *Kluyveromyces lactis* were found to be sensitive to both toxins, while spheroplasts from *Candida albicans* and *Schwanniomyces alluvius* were sensitive only to the K1 toxin (Table 1 and Fig. 1). No killing was seen with the heat-cured nonkiller derivative of the K1 strain, indicating that killing seen with the K1 strain was K1 toxin specific. *Hansenula mrakii*, *Candida buinensis*, and *Pichia kluyveri* spheroplasts were insensitive to both toxins. The results indicate that both killer toxins have a far wider spheroplast-killing spectrum than that found at the whole-cell level.

Previous work with *S. cerevisiae* cells indicated a requirement for a (1→6)-β-D-glucan cell wall receptor for toxin-dependent killing to occur (9). Mutants with defects in the *KRE1* gene of *S. cerevisiae* have altered wall (1→6)-β-D-glucan, fail to bind to the toxins, and are toxin resistant (9). One reason for toxin insensitivity in non-*Saccharomyces* yeasts could be lack of such a (1→6)-β-D-glucan receptor. To test this possibility, *C. albicans* and *K. lactis* cells were assayed for toxin-binding ability. A sensitive *S. cerevisiae* strain, 463-1C, and an isogenic receptor-defective *kre1* mutant, of *S. cerevisiae* 463-1B, were used for comparative binding studies (Fig. 2), in which toxins could be seen to bind with far less avidity to the resistant mutant than to the sensitive parent cells. *K. lactis* cells showed reduced binding of toxin compared with that of the sensitive *S. cerevisiae* strain, 463-1C, and binding similar to that of the receptor-defective resistant mutant, 463-1B. This result suggests that *K. lactis* lacks an effective wall receptor, and this lack may be sufficient to prevent toxin action.

Results with a toxin from *H. mrakii* are consistent with this explanation. The *H. mrakii* toxin kills spheroplasts (Fig. 1) but not whole cells of *K. lactis*. This toxin kills *S. cerevisiae* cells and also appears to use a (1→6)-β-D-glucan wall receptor; we have found that *S. cerevisiae kre1* mutants are resistant to it. Lack of such a glucan receptor on *K. lactis* cells could explain the insensitivity of these cells to the *H. mrakii* toxin.

C. albicans, in contrast, showed a toxin-binding capacity similar to that of the sensitive *S. cerevisiae* strain yet remained toxin insensitive. To exclude the trivial possibility that a protease or other factor inactivated the toxin, thus leading to apparent toxin binding in our assay, we isolated

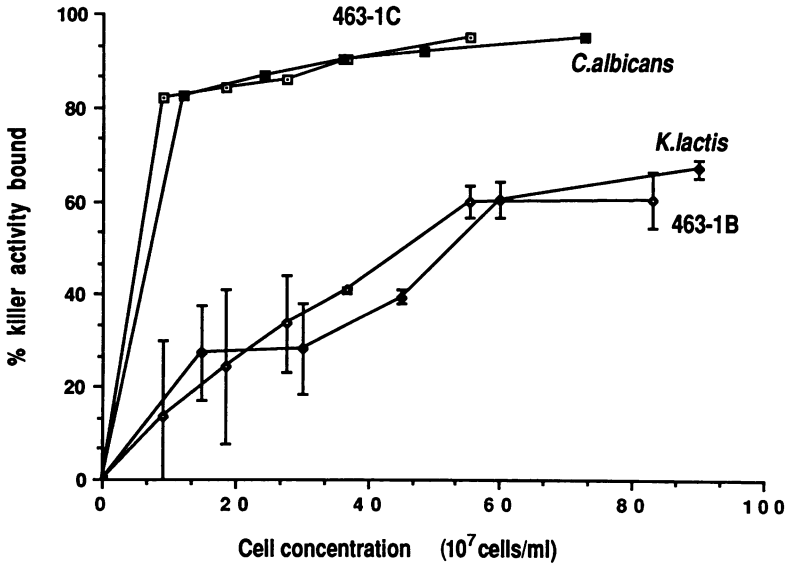

FIG. 2. Binding of killer toxin by sensitive, insensitive, and resistant yeast cells. Yeast cells at a range of concentrations were incubated in 1 ml of medium with a fixed amount of killer toxin for 30 min at 4°C. The cells were pelleted by centrifugation. Toxin activity remaining in the supernatant was assayed by a zone test (1), and bound toxin was calculated by difference. Each Error bar represents 1 standard deviation. 463-1C, Sensitive *S. cerevisiae* strain; 463-1B, isogenic wall receptor-defective *kre1* mutant.

alkali-insoluble cell wall glucan from *C. albicans* and repeated the toxin-binding assay, with results similar to those obtained with whole cells (data not shown). In addition, we showed that this toxin binding was reversible with pH; toxin activity was removed by the glucan at pH 4.7 but could be dissociated from the glucan at pH 7.6, as had been found for toxin binding to *S. cerevisiae* glucan (9). The *C. albicans* cell wall is known to contain a (1→6)-β-D-glucan (7), and binding of the toxin is likely to be to this glucan. Our findings show that for *C. albicans*, the K1 killer toxin binds to a cell wall receptor and can kill spheroplasts, yet it fails to kill yeast cells. Clearly, mere binding of the toxin to the cell wall is not sufficient here to effect action of the toxin at the plasma membrane. It is possible that there are additional unidentified wall components required for killer toxin action that are missing in the wall of *C. albicans*. Alternatively, there may be some structural differences in the wall of *C. albicans* which prevent accessibility of the glucan receptor-bound toxin to the plasma membrane.

Our results have some bearing on the possibility of a plasma membrane receptor necessary for toxin action (4, 11). If the toxin was nonspecific and free to insert into any lipid bilayer, the spheroplasts of all yeasts would be toxin sensitive. This is not the case, as spheroplasts of three of nine yeast strains tested were unaffected by toxin action. These insensitive spheroplasts could inactivate the toxin or may be lacking in some necessary receptor at the plasma membrane. If there is a plasma membrane receptor, it is not species specific, being functionally conserved among at least four yeast genera.

This work demonstrates that the yeast cell wall plays an important role in determining toxin action and can establish the apparent specificity of these toxins. A cell wall glucan receptor is necessary for the K1 and K2 toxins to function on cells, but binding to this receptor may not be sufficient. Such findings focus attention on the lack of knowledge about the structure of the yeast cell wall and how it functions in permitting protein passage. The observation that toxin resistance is conferred at the cell wall level in yeast cells with sensitive spheroplasts suggests that by modifying wall structure, sensitive cells could be constructed. For example, expression of (1→6)-β-D-glucan in *K. lactis* may make these cells toxin sensitive. Alternatively, the idea of constructing mutant or hybrid toxins that can recognize effective receptors on the walls of insensitive yeast cells while retaining the ability to kill spheroplasts could result in the construction of new toxins for these yeast genera.

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