# Screening of a *Saccharomyces cerevisiae* nonessential gene deletion collection for altered susceptibility to a killer peptide

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**SUMMARY**

~4,800 *Saccharomyces cerevisiae* mutants deleted for nonessential genes were screened for alterations in susceptibility to a synthetic killer peptide (KP). None of the tested strains, including mutants resistant to conventional antifungal drugs, showed increased or decreased susceptibility to KP in comparison with the parental strain. The results may reflect the peculiar mechanism of action of KP and claim the possible avoidance of vital resistant mutants.

*KEY WORDS: Saccharomyces cerevisiae*, Deletion mutants, Killer peptide

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KP is an engineered decapeptide derived from the sequence of the variable region of the light chain of a recombinant antiidiotypic antibody representing the internal image of a *Pichia anomala* killer toxin (Polonelli *et al.*, 2003). This yeast killer toxin is characterized by a wide spectrum of antimicrobial activity mediated by the interaction with a β-glucan receptor on target microbial cells (Magliani *et al.*, 1997). KP has shown *in vitro* a microbicidal activity against different eukaryotic and prokaryotic pathogenic microorganisms, which proved to be neutralized by laminarin (a soluble β 1,3-glucan), and *in vivo* a therapeutic effect in experimental models of vaginal and systemic candidiasis, disseminated cryptococcosis and paracoccidioidomycosis (Polonelli *et al.*, 2003; Cenci *et al.*, 2004; Travassos *et al.*, 2004; Manfredi *et al.*, 2005; Fiori *et al.*, 2006; Savoia *et al.*, 2006).

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Owing to its sequence homology with critical segments of gp120 precursor, KP also proved to inhibit HIV-1 replication *ex vivo* and *in vitro* by a different mechanism of action, i.e. down-regulation of CCR5 co-receptor, and/or physical block of the gp120-receptor interaction (Casoli *et al.*, 2006).

The aim of this study was to screen a *S. cerevisiae* gene deletion strain collection to identify fungal genes responsible for alterations in susceptibility at KP concentrations exhibiting 100% and 0% killing activity against the parental yeast strain. The collection of nonessential deletion mutants constructed in the BY4741 background (*MAT*a *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*), including strains resistant to conventional antifungal drugs, was purchased from Euroscarf (Frankfurt, Germany) (Brachmann *et al.*, 1998). In a preliminary CFU assay, carried out as previously described, the activity of KP (sequence AKVTMTCSAS) at scalar concentrations against the parental strain BY4741 was evaluated to establish the peptide concentration corresponding to the 50% inhibitory concentration ($IC_{50}$) (Polonelli *et al.*, 2003). The inactive scramble peptide SP (sequence MSTAVSKCAT) was used as a growth

control. $IC_{50}$ value resulted to be $8.95\times10^{-7}$ mol/L. KP concentrations of 20 µg/ml, giving a 100% killing activity, and 0.2 µg/ml, giving no killing activity, were selected for the screening of altered susceptibility of the complete deletion strain set. For this purpose, yeast mutant strains, and the parental wild type strain BY4741, were spotted onto the surface of YEPD agar plates (1% yeast extract, 2% peptone, 2% dextrose, 2% agar, Difco) and grown for 24-48 h at 28°C. Each inoculum was prepared by touching each spot with a pointed tool and making a suspension in 100 µl sterile distilled water. The cells were then further diluted 1:10 and 1 µl of the last suspension added to wells of a microtiter plate containing 0.2 or 20 µg/ml of KP or SP in a final volume of 100 µl (5-$7\times10^2$ cells/well). Each assay was performed in duplicate. After 6 h incubation at 28°C, 100 µl of YEPD broth were added to each well, the microdilution plates were then re-incubated at 28°C and observed after 24-48 h for the presence (turbidity) or absence (optically clear) of visible growth. The growth in each well containing KP was compared with that of the SP-containing wells used as a control. None of the tested strains showed a behavior different than that of the parental wild type strain BY4741 which was used as a standard control throughout the experiments.

Based on the reputed involvement of β 1,3-glucan as a target of KP on fungal cells, further analyses were performed with some mutants deleted of genes implicated in β 1,3-glucan synthesis (Klis *et al.*, 2002). In particular, *FKS1* and *GSC2* (*FSK2*), genes well conserved among fungi coding for proteins essential for the synthesis of β 1,3-glucan, and *GAS1*, an endotransglycosylase that may be involved in extending and rearranging β 1,3-glucan chains, were selected. Notably, *GSC2* (*FSK2*) deletion mutant showed enhanced resistance to caspofungin (Lesage *et al.*, 2004; Markovich *et al.*, 2004). Since some moderately sensitive mutants may have been missed in the screening assay, KP's activity was evaluated also at the intermediate concentration of 2 µg/ml in the standardized CFU assay. Again, none of the tested strains significantly differed from the parental one (Table 1).

Based on the reported results it seems unlikely that KP susceptibility is dependent on the activities of specific gene products. It could not be ruled out whether this feature is a result of polygenic traits or dependent on essential gene functions not encompassed in the haploid yeast deletion strain collection due to lethality.

KP proved to be fungicidal to all the defective mutants including the ones reported to be resistant to conventional antifungal drugs such as

TABLE 1 - *Evaluation of killer peptide (KP) activity against Saccharomyces cerevisiae BY4741 wild type strain and 3 selected mutant strains.*

| | | | *KP activity*[a] | | |
|---|---|---|---|---|---|
| *ORF* | *Gene* | *Description* | *20 µg/ml* | *2 µg/ml* | *0.2 µg/ml* |
| Wild type | | | 100% | 91.72% | none |
| *YLR342W* | *FKS1* | Catalytic subunit of 1,3-β-glucan synthase, functionally redundant with alternate catalytic subunit Gsc2p; binds to regulatory subunit Rho1p; involved in cell wall synthesis and maintenance; localizes to sites of cell wall remodeling | 100% | 92.82%[b] | none |
| *YGR032W* | *GSC2* | Catalytic subunit of 1,3-β-glucan synthase, has similarity to an alternate catalytic subunit, Fks1p (Gsc1p); Rho1p encodes the regulatory subunit; involved in cell wall synthesis and maintenance | 100% | 73.83%[b] | none |
| *YMR307W* | *GAS1* | β 1,3-glucanosyltransferase, required for cell wall assembly | 100% | 96.73%[b] | none |

[a]Percent inhibition in comparison to the scramble peptide SP; [b]No significant difference vs wild type strain. Two-way ANOVA was performed using GraphPad Prism version 4.00 for Windows.

caspofungin and fluconazole (Anderson *et al*., 2003; Lesage *et al*., 2004; Markovich *et al*., 2004). Overall, our results show that KP is characterized by a novel mechanism of fungicidal action mirroring the biological effect of competition exerted among microorganisms in their natural habitats and exploiting an essential microbial target which might claim the avoidance of vital resistant mutants.

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