Yeast Killer Toxin K1 and Its Exploitation in Genetic Manipulations

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ABSTRACT. Principles of new techniques for selecting and/or accumulating various yeast mutants, hybrids, cybrids and transformants, and the construction of industrial strains based on features of the yeast killer system are introduced using the application of the killer system K1 as examples.

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1 KILLER SYSTEM K1

Killer yeasts secrete toxins (killer toxins, killer factors or zymocins), proteins lethal to certain sensitive yeast strains (Makower and Bevan 1963). The killing ability (K⁺) and the sensitivity to various killers (R⁻) can be routinely screened by the formation of clear zones found when a killer strain is heavily streaked on the background lawn of a sensitive strain (Fig. 1) (Woods and Bevan 1968).

Fig. 1. Screening of the killer phenotype (left) and killer toxin activity (right); left: The inhibition zone around the streak of S. cerevisiae X3 on the background lawn of S. cerevisiae S6/A; right: a well-assay based on measuring the width of the inhibition zone around the well which was filled with a killer toxin solution.
Extensive investigation of the killer character (K\(^+\), R\(^-\)) in the laboratory, industrial and natural yeast isolates using this method, provided evidence that killer strains are quite common among many species of yeasts and yeast-like organisms, e.g. Candida, Debaryomyces, Hansenula, Kluyveromyces and Pichia sp. (Phillipskirk and Young 1975), Cryptococcus sp. (Stumm et al. 1977), Rhodotorula sp. (Golubev 1989), Saccharomyces sp. (Makower and Bevan 1963, Maule and Thomas 1973, Naumov and Naumova 1973), Sporidiobolus sp. (Golubev et al. 1988), Schwannomyces sp., Torulopsis sp. (Bussey and Skipper 1975) and Ustilago sp. (Puhalla 1968). Many classes of killer strains exist, differing not only in the spectrum of their activity against sensitive strains but also by their cross reactivity, molecular features and mechanisms of action for production of their toxins and the genetic determination of killer characteristics, including immunity against their own toxins (review: Young 1987). The killer phenomenon was treated from various aspects in a number of reviews (e.g. Tipper and Bostian 1984; Wickner 1986, 1993; Bendova 1986; Young 1987; Vondrejs 1987; Bussey et al. 1990; Vondrejs et al. 1991; Tipper and Schmitt 1991).

The best understood killer system K1 of *S. cerevisiae* (reviews: Bussey et al. 1990; Tipper and Schmitt 1991; Wickner 1993) is determined by two types of dsRNA (L – large, M – medium) located in the cytoplasm as latent, noninfective, mycovirus particles. M-dsRNA is killer-toxin-specific codes for the killer toxin and also determines the host’s toxin immunity. Cloning and sequencing of the preprotoxin-coding region of the M1-dsRNA was completed in 1984 by Skipper et al. (1984). L-dsRNA which is present also in many nonkiller strains, specifies capsids of virus particles and the viral RNA-dependent RNA polymerase. The maintenance of M-dsRNA depends on the presence of L-dsRNA. In addition, it was shown that many chromosomal genes are involved in the maintenance and expression of the K1 phenotype. Recessive mutations in MAK (maintenance of killer) genes result in loss of both L and M-dsRNA (mak3, mak10, pet18) or only M1-dsRNA (more than 30 MAK genes) while recessive mutations in any one of the six SKI (superkiller) genes increase the production of toxin and suppress a major class of mak. Curing of the killer phenotype is usually due to the loss of M1-dsRNA occurring when, killer cells are cultivated at high temperatures (38–39 °C) or in the presence of subinhibitory concentrations of cycloheximide or 5-fluorouracil (Fink and Styles 1972; Wickner 1974a).

Recombinant plasmids containing M1-cDNA located next to a strong yeast promoter were constructed in order to show that M1-cDNA expression confers on the transformed cells both the ability to produce the toxin and an immunity independent of L-dsRNA (Bostian et al. 1984; Hanes et al. 1986; Lolle et al. 1984; Vernet et al. 1987). K1‘R1‘ phenotype can be artificially transmitted to spheroplasts of *S. cerevisiae* containing L-AdsRNA also by transfection with mycovirus ScV-M1, electroporation with M1-dsRNA or by using induced protoplast fusion, mating or rare mating of a killer strain with a sensitive strain (Vondrejs et al. 1982; El-Sherbeini and Bostian 1987; Salek et al. 1992). The mating, followed by sporulation, is very probably the way of killer phenotype transmission in nature. It should be noted that the acquisition of the killer phenotype (K\(^+\) R\(^-\)) provides positive autoselection for killers, because the cured cells (K\(^-\) R\(^+\)) are usually eliminated by a toxin produced by their nontreated neighbors.

Killer toxin K1 is produced by processing the N-glycosylated precursor which requires both KEX2 and KEX1 functions (for reviews see Bussey 1988; Bussey et al. 1990). This precursor is composed of several domains δ, α, γ and β (Fig. 2). The N-terminal leader sequence (δ) directs the precursor to the classic yeast secretory pathway (Bussey et al. 1983; Lolle and Bussey 1986), where signal peptidase cleavage occurs after Ala-26 (Zhu et al. 1992). N-glycosylated γ-domain is released by Kex2 cleavage next to ArgArg (RR) and LysArg (KR). Cleavage at ProArg (PR), which is important for the maturation of toxin containing α and β polypeptide chains is also catalyzed by Kex2 (Zhu et al. 1992). Carboxypeptidase Kex1 activity is responsible for trimming the dibasic C-terminus of the α-subunit (Zhu et al. 1987) and probably also the β-subunit of the mature toxin.

Sected killer toxin K1 is a heterodimer containing two polypeptide chains (α, β) interconnected by three -S-S- bonds. The maturated toxin is stable at temperatures below 23 °C, however, the more stable “mutants” (e.g. the killer toxin K1 produced by superkiller strain *S. cerevisiae* T 158 a) were also described. The optimum pH for killing of sensitive strains has a very narrow range (4.3–4.7) and the lethal effect of the toxin depends on the concentration of various ions, namely K\(^+\), Ca\(^{2+}\) and Mg\(^{2+}\) (Kurzweilová and Sigler 1993a).

The first step in the K1 killer toxin action involves a rapid binding to cell wall receptors containing 1,6-β-D-glucan as an essential component (Hutchins and Bussey 1983). This step does not require energy, and it is not followed by a killing effect when sensitive cells are cultivated in a medium without a source of energy (see below). In addition, it was shown that only specific interactions of the toxin with the cell wall are important for killing (Bussey et al. 1979; Kurzweilová and Sigler 1993b). This event is not always required for the lethal effect in the next step, because protoplasts and vesicles derived from cells of sensitive strains are also susceptible to treatment with the killer toxin K1. In addition, it was observed that spheroplasts of