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"Killer Character" of *Saccharomyces cerevisiae*: Curing by Growth at Elevated Temperature

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Normal "killer" strains of *Saccharomyces cerevisiae*, when grown at 37 to 40 C, produce almost exclusively nonkiller cells due to loss or mutation of at least part of the non-chromosomal killer genome.

The killer character of *Saccharomyces cerevisiae* is a non-Mendelian genetic element which gives strains carrying it (i) the ability to kill sensitive strains by secreting a killer substance, and (ii) immunity to the action of the killer substance (3, 9, 11). Evidence has been presented suggesting that the cytoplasmic genetic element may be double-stranded ribonucleic acid (1, 2, 11). Fink and Styles (5) have found that growth of killer strains in the presence of low concentrations of cycloheximide converts them to sensitive, nonkiller strains. This report concerns the conversion of killers to nonkiller, sensitive strains induced by growth at elevated temperatures.

Methods used for assay of killing ability and resistance were those of Somers and Bevan (9) and Fink and Styles (4), and have been previously described (R. Wickner, Genetics, in press). The phenotypes of strains, with regard to their killing ability (K) and resistance to killing (R), are denoted K^+R^+ (normal killer), K^-R^+ (neutral phenotype), or K^-R^- (sensitive nonkiller). The strains used here are listed in Table 1. Genetic analysis was carried out by the usual methods (6). Cells were grown on 2% agar containing 1% yeast extract, 2% peptone, and 2% dextrose (YPAD agar).

Various strains were plated for single colonies on YPAD plates at 23, 30, 37, and 40 C. The number of colonies formed was constant up to and including 37 C, but was reduced by about fivefold at 40 C for most strains. The colonies were replica plated to a lawn of the sensitive strain 5×47 on YPAD plates containing methylene blue and buffered at pH 4.7 to test for killing at 23 C (4, 9). Most of the colonies formed at 37 or 40 C showed no evidence of killing or were a mixture of killer and sensitive cells, whereas those formed at 23 or 30 C from killer strains generally showed normal killing (Table 2). The temperature required to "cure" varied from one strain to another, but all strains showed increasing frequency of nonkiller colonies with increasing temperature. This "curing" of the plasmid is distinct from the thermolabil-

TABLE 1. *Strains of S. cerevisiae*

Strain designation	Killer phenotype	Other markers	Reference or source
D585-11C	K^+R^+	*α lys1*	F. Sherman
D587-4B	K^+R^+	*α his1*	F. Sherman
A364A	K^+R^+	*a ade1 ade2 ura1 his7 tyr1 lys2 gal1*	(5)
A5969A	K^+R^+	*α trp1 his4-331 leu2-3 thr4 MAL2*	(7)
5X47	K^-R^-	*a his1 trp1 ura3* / *α + + +*	
D585-11CXD587-4B	K^+K^+	*a his1 lys1* / *α + +*	
53	K^-R^-	*a ade1*	S. Henry
D587-4B K68	K^-R^+	*α his1*	Mutant derived from D587-4B (R. Wickner, unpublished data)

TABLE 2. *Killer phenotype at 23 C after growth at various temperatures*

Strains	Killer Phenotype[a]	No. of Colonies[b]											
		23 C			30 C			37 C			40 C		
		K^+	Mixed	K^-	K^+	Mixed	K^-	K^+	Mixed	K^-	K^+	Mixed	K^-
D587-4B	K^+R^+	130			120			4	1	26			150
D585-11C	K^+R^+	107	1		105			55	56	64	3	5	59
A364A	K^+R^+	30			49	1		30	2		4	22	75
A5969A	K^+R^+	52		2	29	10	2		15	39			25
D585-11C XD587-4B	K^+R^+	105	1	1	21	1	2		55	72	1		16
5X47	K^-R^-			65			65			133			40
		R^+		R^-				R^+		R^-			
D587-4B K68	K^-R^+	25								25			

[a] The killer phenotype of all colonies was determined at 23 C.

[b] The total colonies for a given strain varies with temperature here, because different numbers of colonies were tested for killing.

ity of the killer substance observed by Woods and Bevan (11), because the ability of the grown cells to kill has been assayed at 23 C, a temperature at which the killer substance is fully active.

"Cured" colonies of each strain breed true at 23 C (over 100 colonies checked for each of five K^- derivatives of each of the first five strains in Table 2), and 28 of 29 heat-cured K^- colonies of D587-4B were also R^-. A mutant of D587-4B (called K68) which had lost the ability to kill, but retained a plasmid that imparts resistance to killing (R. Wickner, unpublished data), could be "cured" of its resistance by growth at 37 C.

Diploids formed from each of six heat-cured K^-R^- derivatives of D587-4B and strain 53 (K^-R^-) were K^-R^- (over 200 of each tested), and sporulation yielded 4 K^-R^-:0 segregation (six or more tetrads examined for each of the six K^-R^- derivatives checked). Diploids formed from each of six heat-cured D587-4B (K^-R^-) and either strain A364A (K^+R^+) or strain D585-11C (K^+R^+) were all K^+R^+ and yielded 4 K^+R^+:0 segregation on sporulation. Thus, as was found for cycloheximide curing (4), growth at elevated temperatures resulted in partial or complete loss of the killer cytoplasmic genetic element. Mating of heat-cured strains with cycloheximide-cured strains yields only K^-R^- diploids.

Sherman showed that heat induces cytoplasmic petite (ρ^-) mutations (8). Among colonies of D587-4B and D585-11C grown at 37 C, I have found $\rho^+K^-R^-$, $\rho^-K^-R^-$, $\rho^+K^+R^+$, and $\rho^-K^+R^+$ colonies. This again reflects the nonidentity of the killer and ρ cytoplasmic determinants (4).

The curing effect of growth at elevated temperatures appears to be a property of all normal killer strains rather than a temperature-sensitive mutation. Efforts are currently under way to further define this phenomenon by isolating "incurable" strains.

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