A New Killer Factor Produced by a Killer/Sensitive Yeast Strain

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SUMMARY

The isolation of a new killer/sensitive phenotype of the yeast, *Saccharomyces cerevisiae*, is described. Killer/sensitive yeast cells are killed by the killer factor (KF₁) secreted by killer yeast cells. The killer/sensitive cells also secrete a new killer factor (KF₂) which kills sensitive cells. The production of KF₂ by killer/sensitive cells renders them less sensitive to KF₁ than sensitive cells. Sensitive cells are most susceptible to the action of KF₂ in log phase. KF₂ is a thermostable protein-containing killer factor.

INTRODUCTION

Killer, sensitive and neutral strains of the yeast *Saccharomyces cerevisiae* were discovered by Bevan & Makower (1963). Killer cells kill sensitive cells by secreting into the medium a toxic protein-containing 'killer factor' (KF₁) (Woods & Bevan, 1968; Bussey, 1972) to which they themselves are immune. Neutral cells are resistant to KF₁ and do not kill sensitive cells. The killer and neutral phenotypes are under the control of at least one nuclear gene and two types of cytoplasmic determinant which confer the killer and neutral phenotypes respectively (Bevan & Somers, 1969; Somers & Bevan, 1969). A new species of double-stranded RNA has been detected in all killer and neutral strains and is lacking in some sensitive strains (Berry & Bevan, 1972).

We report the discovery of a fourth yeast phenotype termed 'killer/sensitive' which is sensitive to KF₁ but produces a new 'killer factor' (KF₂) which kills sensitive cells.

METHODS

Strains. Wild-type killer, neutral and killer/sensitive yeast strains and an adenine mutant sensitive strain were used. All the strains originated from the wild-type haploid strains WT₄ and WT₅ of the Oxford stock collection (Woods & Bevan, 1966). The killer/sensitive strain originated as a spontaneous mutant in a sensitive strain.

Seeded-agar phenotype test. The phenotypes of the strains derived from single cells were determined by the seeded-agar phenotype test described by Woods & Bevan (1968). Killer colonies were identified by zones of inhibition on agar plates seeded with sensitive yeast (10⁴ cells/ml). Since all the agar medium contained methylene blue which is a specific stain for dead yeast cells (Lindgren, 1949), the sensitive colonies were identified by a dark zone of dead cells on agar plates seeded with killer yeast (10⁵ cells/ml). Neutral colonies showed no reaction on either killer or sensitive agar plates.

Killer solutions. Killer solutions containing either KF₁ or KF₂ were obtained by incubating killer or killer/sensitive yeast in buffered (pH 4.8) yeast complete medium supplemented with 0.05 % (w/v) gelatin at 22 °C for 3 days (Bevan, 1955; Woods & Bevan, 1968). The

cells were removed by centrifugation and the solutions sterilized by the addition of chloroform (10 %, v/v).

Well test. Killer solutions were assayed for the presence of KF_1 or KF_2 by the well-test assay technique of Woods & Bevan (1968). Zones of inhibition were obtained on plates seeded with sensitive yeast (5×10^3 cells/ml).

Killer activity. The killer activities of KF_1 and KF_2 solutions were studied by adding 6 ml of the respective killer solutions to 3 ml of threefold concentrated complete medium containing log-phase sensitive or killer/sensitive yeasts (10^3 or 10^5 cells/ml). Viable counts were then determined at different time intervals and compared with control cultures. Controls consisted of adding 6 ml of sensitive solution (medium in which sensitive yeast had grown) to 3 ml of threefold concentrated complete medium containing log-phase sensitive cells.

Effects of physical and chemical agents. The effects of temperature, surface inactivation, pH, papain (Koch Light), pronase (Miles-Seravac), DNase (Miles-Seravac), RNase (Miles-Seravac), sodium dodecyl sulphate and KCl were studied on standard KF_2 solutions or KF_2 solutions concentrated fourfold by freeze drying. Inactivation of KF_2 was determined by the well test.

RESULTS

Seeded phenotype test

Killer/sensitive colonies produced zones of inhibition on agar plates seeded with sensitive yeast. The zones of inhibition were approximately half the width of those formed by killer colonies on the same sensitive background. Woods & Bevan (1968) reported zones of inhibition by killer colonies on a sensitive background containing 10^5 cells/ml, but killer/sensitive colonies produced detectable zones only at 10^4 cells/ml. Killer/sensitive yeast streaked on agar plates seeded with killer yeast were stained with methylene blue, indicating that they had been killed. Killer yeast also produced a zone of inhibition on a killer/sensitive background. No reaction was observed between killer/sensitive and neutral cells.

Killer activity

The killer activities of KF_1 and KF_2 solutions obtained after 3 days' growth at 22 °C are shown in Fig. 1. Killer/sensitive cells were markedly more resistant to KF_1 than sensitive cells. Although KF_1 and KF_2 solutions were produced under the same conditions, the killer activity of the KF_1 solution against sensitive cells was considerably greater than that of KF_2 solutions.

The susceptibility of log- and resting-phase sensitive cells to KF_2 was determined by comparing the percentage of killed cells in log- and resting-phase sensitive cultures. Sensitive cells were most susceptible to KF_2 while growing logarithmically. Similar results were obtained by Woods & Bevan (1968) and Bussey (1972) regarding the susceptibility of sensitive cells to KF_1 .

The stability of the KF_2 killer-cell complex was determined by comparing the percentage of killing between cells exposed to KF_2 which were either plated directly or washed and shaken twice in 0.85 % (w/v) saline before plating. Since the two treatments showed the same percentage of killing, the KF_2 killer-cell complex was not readily dissociable. In this respect it resembles the KF_1 killer-cell complex (Woods & Bevan, 1968; Bussey, 1972). All the strains were regularly cloned to ensure that mixed populations were not used.

Fig. 1. Killer activities of KF₁ and KF₂ solutions. Log-phase sensitive cells were added to KF₁ and KF₂ solutions. Controls (broken lines) consisted of adding the sensitive or killer/sensitive cultures to sensitive solutions (medium in which sensitive cells had grown). ●—●, Sensitive cells in KF₁ solution; ○---○, sensitive control. ■—■, Killer/sensitive cells in KF₁ solution; □---□, killer/sensitive control. ▲—▲, Sensitive cells in KF₂ solution; △---△, sensitive control.

The results of the following genetic crosses (see Table 1) indicate that the genetic control of the 'new' killer is completely different from that of the 'orthodox' killer and that we have isolated a 'new' killer strain with a different genetic control from the 'orthodox' killer. The interesting result from the crosses (Table 1) is the phenotype of the diploids, which were all sensitive after crossing the killer/sensitive (K/S) with a sensitive (S) (cross a). The normal situation of an equivalent cross with an 'orthodox' killer is shown in cross b where all the diploids were killer. The sensitive phenotype was dominant in the diploid resulting from a killer/sensitive $\times$ sensitive cross but the killer/sensitive character was not lost since, on tetrad analysis, it segregated 2 K/S:2 S. In the crosses between the killer/sensitive and killer (K) strains the orthodox killer character dominated the killer/sensitive character. The segregation of 4 K:0 K/S is to be expected since our killer/sensitive mutant came from a *M(o)* sensitive. The results of the killer/sensitive $\times$ neutral crosses were also different from killer $\times$ neutral crosses. Again the neutral characteristic dominated, whereas with orthodox killer $\times$ neutral crosses the killer characteristic was dominant.

Table 1. *Results of genetic crosses and tetrad analyses*

These took place between killer/sensitive (K/S) $\times$ sensitive (S) strains, killer (K) $\times$ sensitive strains, killer/sensitive $\times$ killer strains, and killer/sensitive $\times$ neutral (N) strains.

	Cross			
	a	b	c	d
Phenotypes	K/S $\times$ S	K $\times$ S	K/S $\times$ K	K/S $\times$ N
Genotypes	?M(o) $\times$ m(o)	M(k) $\times$ m(o)	?M(o) $\times$ M(k)	?M(o) $\times$ M(n)
No. of diploids	25	20	5	4
Phenotypes of diploids	S	K	K	N
No. of tetrads	18	10	5	4
Segregation	2 K/S : 2 S	2 K : 2 S	4 K : 0 K/S	4 N : 0 K/S

Effects of enzymes

The inactivation of fourfold concentrated KF₂ solutions by 1 mg/ml concentrations of papain, pronase, DNase and RNase was determined. KF₂ was inactivated by papain and pronase but was not affected by DNase and RNase. Similar results were obtained with KF₁ (Woods & Bevan, 1968; Bussey, 1972) indicating the proteinaceous nature of both KF₁ and KF₂. Bussey (1972) reported that KF₁ was rapidly inactivated by 0.001 % (w/v) sodium dodecyl sulphate but was stable in 0.5 M-KCl. Although KF₂ was rapidly inactivated by 0.001 % (w/v) sodium dodecyl sulphate it was also inactivated by 0.5 M-KCl after 2 h.

Effect of temperature and surface inactivation

KF₁ is characterized by being thermolabile. Woods & Bevan (1968) showed that KF₁ was totally inactivated after 90 min at 28 °C ($t_{\frac{1}{2}}$ = 45 min at 28 °C) and Bussey (1972) reported for KF₁ that $t_{\frac{1}{2}}$ = 15 min at 32 °C. In contrast, KF₂ was markedly more thermostable ($t_{\frac{1}{2}}$ = 4 h at 60 °C).

Woods & Bevan (1968) reported that KF₁ in complete medium was sensitive to surface inactivation and was completely inactivated after 10 min shaking on a mechanical shaker. This surface inactivation could be prevented by the addition of gelatin. Complete medium solutions of KF₂ were shaken on the mechanical shaker and only 33.3 % inactivation occurred after 10 min.

Effect of pH

The optimum pH for the production and stability of KF₁ lies within the narrow range pH 4.6 to 4.8 and no active KF₁ solutions were produced in complete medium above pH 5.0 (Woods & Bevan, 1968). Killer/sensitive cells were incubated at 22 °C for 3 days in complete medium buffered at pH values 3.6, 4.0, 4.4, 4.8, 5.4 and 5.8 with 0.077 M-citrate/phosphate buffer, and after removal of the cells the killer activity was determined by the well test. The optimum pH for the production of KF₂ was between pH 4.0 and 4.8 but considerable KF₂ activity (60 % of optimum activity) was observed at pH 5.8.

DISCUSSION

We describe a killer/sensitive yeast phenotype which produces a new killer factor (KF₂). The strain originated as a spontaneous mutant from a sensitive strain. Bussey (1972) reported a sensitive strain obtained as a spontaneous clone from a killer strain which makes some killer factor (KF₁); this sensitive strain grows at the same rate as the killer so there is

probably not much self killing. The production of KF_2 by killer/sensitive cells renders them less sensitive to KF_1 than sensitive cells.

The killer/sensitive yeast strain secreted a thermostable protein-containing killer factor (KF_2) which was different from the thermolabile killer factor produced by the killer cells studied by Woods & Bevan (1968) and Bussey (1972). Solutions of KF_2 were markedly less active against sensitive cells than KF_1 solutions. Zones of inhibition produced by killer/sensitive cells on a sensitive background were detected only at a tenfold lower concentration of background sensitive cells than that required to show zones with killer cells. This may be because of limited production of KF_2 by killer/sensitive cells or because KF_2 is less active than KF_1 . At present only low-titre KF_2 solutions have been obtained and attempts to concentrate the factor by techniques other than freeze drying have failed. The highest concentration of KF_2 obtained has been insufficient for analysis by the well test after sucrose density gradient centrifugation and gel filtration. Disintegration of killer/sensitive cells did not increase the titre of KF_2 solutions. The isolation of mutants capable of producing high-titre KF_2 solutions and the genetic control of KF_2 are being investigated.

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