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Fluorescence Staining of Yeast Cells Permeabilized by Killer Toxin K1: Determination of Optimum Conditions

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Optimal assay conditions were established for the previously described method used to determine the activity of *Saccharomyces cerevisiae* pore-forming killer toxin K1. The method is based on cell staining with bromocresol purple. Sensitive cells of *S. cerevisiae* from the early exponential phase under nongrowth conditions and in the presence of glucose were the most convenient for determining the killer toxin activity. Maximum killing was reached when the suspension was buffered with 10 m*M* citrate–phosphate at pH 4.6.

KEY WORDS: Killer toxin K1; bromocresol purple staining; *Saccharomyces cerevisiae.*

INTRODUCTION

Yeast strains often achieve competitive advantage by producing toxic proteins, called killer toxins or zymocines, which kill off competing sensitive cells [1]. The genetically very well-characterized killer toxin K1 is produced by some strains of the yeast *Saccharomyces cerevisiae* [2]. Despite the advances in toxin genetics, relatively very little is known about the actual mechanism of its action on the target cell surface [3] except that it involves the formation of channels or pores in the plasma membrane. The study of the mechanism of its interaction with sensitive yeast cells requires a rapid and reliable assay of its killing action. The commonly used plating test or well test is laborious and time-consuming. We developed a new fluorescence assay for rapid estimation of killer toxin activity [4] and optimized the assay conditions [5].

MATERIALS AND METHODS

Partially purified killer toxin was obtained from cultures of *S. cerevisiae* strain T158C; *S. cerevisiae* strain S6 was used as the sensitive strain. The killer toxin activity was assayed using the bromocresol purple fluorescence (BCP) test [4].

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RESULTS AND DISCUSSION

The method is based on staining of killer toxin-exposed cells with the acid–base indicator BCP. On entering the permeabilized cells buffered at an acid pH, the dye intensifies its fluorescence and the emission maximum is shifted to higher wavelengths, probably due to its interaction with cell proteins [4]. Killer-treated cells viewed at pH 4.6, i.e., the pH optimum for the K1 toxin, in a fluorescence microscope gradually develop an intensive yellow fluorescence, while cells with intact plasma membrane remain unstained (Fig. 1). The time course of staining of killer-exposed cells demonstrates, in accordance with the previous finding that the killing is completed within 2–3 h [6], that the number of stained cells reaches its upper limit after a 150-min exposure to the toxin, when BCP is present in the suspension from the beginning (Fig. 2). The same maximum ultimate value of staining is reached within 60 min after adding the dye to already damaged cells (2–3 h after the toxin),

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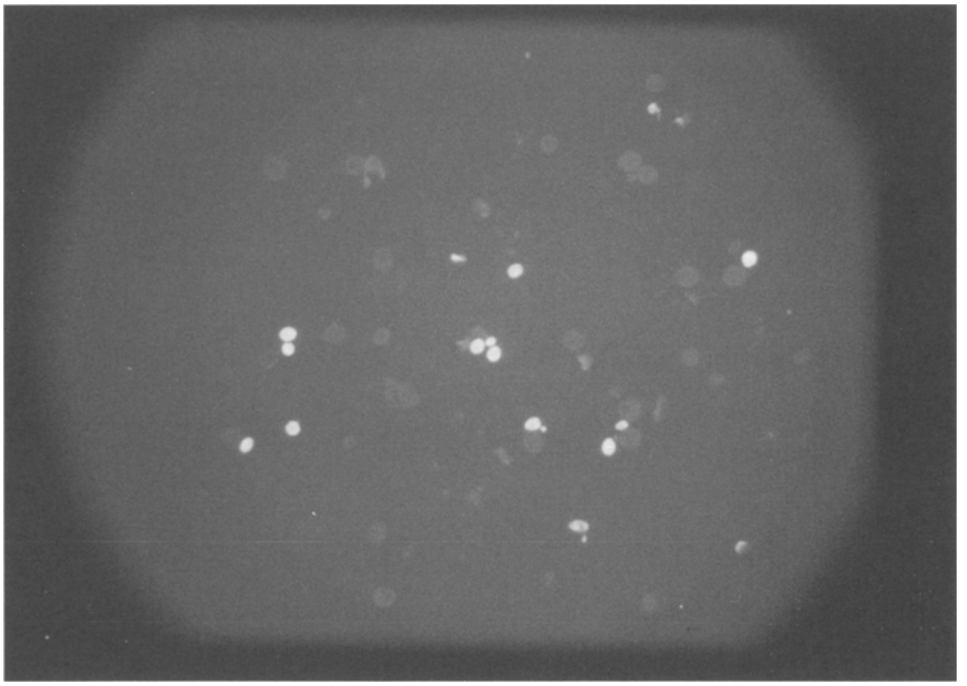

Fig. 1. Killer toxin (5×10^6 LU/ml)-treated *S. cerevisiae* S6 stained with BCP at pH 4.6. Unstained cells adsorbed only a sublethal dose of the killer toxin and consequently survived. ×630.

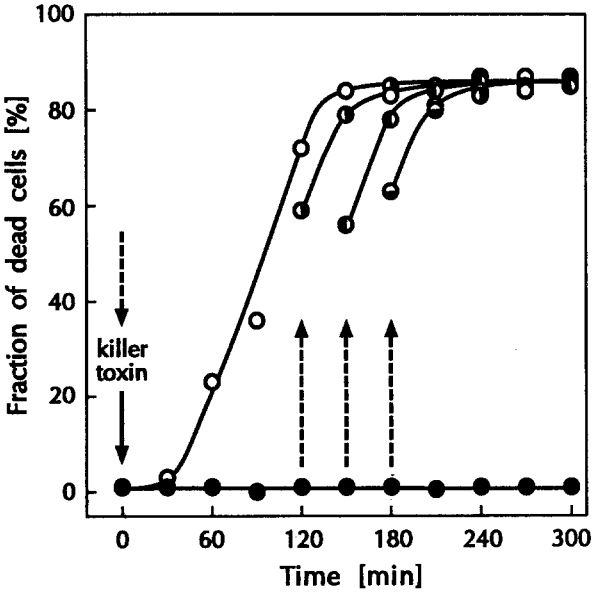

Fig. 2. The time course of staining of killer toxin-attacked cells (○). BCP was added to the suspension (dashed arrows) either together with the killer toxin (4×10^7 LU/ml; solid arrow) or 2–3 h (◑, ◐, ○) later. In the control (●) the killer toxin was replaced by the same volume of C-P buffer.

although a substantial part of the damaged cells is stained immediately. The presence of BCP has no apparent influence on killing, but considering the interaction between the dye and cell proteins, it seems advisable to stain the cells only after the killing has been completed.

It was reported that sensitive cells are most susceptible to killing in the early exponential phase when they grow most intensively [7]. To explore this assumption, we measured the effect of the toxin on exponential cells transferred into three media: nutrient-free 10 m*M* citrate–phosphate (C-P) buffer, Halvorson minimal medium supplemented with adenine (20 mg/L), and a complex medium (1% yeast extract, 1% peptone). The pH value was kept at 4.6 in all cases.

The killer activity was the highest when recipient cells were maintained under nongrowth conditions in C-P buffer in which the number of cells changed only negligibly during the test. The killer activity determined in a complex medium or under conditions of nutrient limitation in a minimal medium supplemented with adenine was lower because a fraction of sensitive cells, which adsorbed a sublethal dose of killer toxin, could continue their growth and division, thus reducing the apparent proportion of dead cells. Accordingly, the number of cells increased 1.4- to 1.6-fold in minimal medium and

Table I. Killer Activity Determined Under Different Nutrient Conditions[a]

Medium	Activity (LU/ml) × 10^8
C-P buffer	7.7 ± 0.7
Minimal with adenine	5.0 ± 0.5
Complex	3.3 ± 0.3

[a]Activities (mean ± SE) in the BCP test were measured in seven independent experiments.

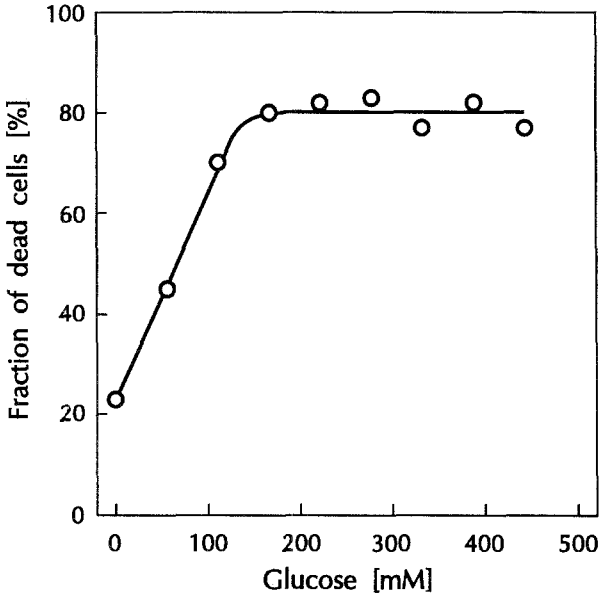

Fig. 3. Effect of increasing concentrations of glucose on the extent of killing, expressed as the percentage of the maximal activity attained (in this experiment 8 × 10^7 LU/ml).

2.3- to 2.6-fold in complex medium compared with the sample containing buffer only. When these values were used to correct the corresponding killer toxin activities in Table I for possible cell growth, the killing efficiency was about the same in the buffer and in the two media.

We also tested the dependence of killing on the energy status of the cells by determining the activity of a killer toxin preparation in the presence of different concentrations of glucose as an energy source. The toxin activity increased with increasing glucose concentration, reaching a limiting value at about 200 m*M* glucose (Fig. 3). A similar "saturation effect" was described in various *S. cerevisiae* strains for the dependence of the total proton extrusion on glucose concentration [8]. Because this process reflects the ability of the yeast to build up

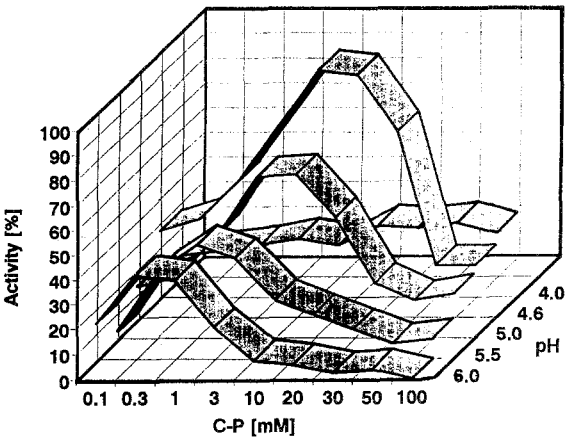

Fig. 4. Dependence of toxin activity on pH and on the concentration of C-P buffer, expressed as the percentage of the maximal activity attained (in this experiment 8 × 10^7 LU/ml).

a proton-motive force, this finding implies that the extent of killing is connected with the magnitude of the proton-motive force maintained across the plasma membrane of sensitive cells.

To sum up, the killing depends on the energy state of sensitive cells [6]; the cells should be in the early exponential phase, but further intensive growth is unnecessary for the action of the toxin (Table I).

In the presence of high concentrations of glucose (200 m*M*), yeast cells strongly acidify the outer medium [8] and change its buffer power, which may affect toxin action. We therefore measured killer toxin activity at different concentrations of C-P buffer, in the pH range 4.0–6.0 (Fig. 4). Maximum killing was reached in 10 m*M* C-P buffer at pH 4.6 (optimum pH for killer action). With increasing buffer pH, the maximum activity decreased and was shifted to lower buffer concentrations. These data document the importance of keeping the correct pH value and corresponding buffering capacity during experiments with metabolizing sensitive cells and killer toxin K1.

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