Relationship Between Yield of Protoplasts and Growth Phase in Saccharomyces

M. M. SHAHIN

Institute of Biophysics; Free University of Berlin, 1 Berlin 33, Germany

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A number of generations must occur in the presence of glucose before daughter cells of stationary or old cultures become amenable to protoplast formation by the action of snail enzyme.

The yield of protoplast formation varied greatly with different strains of the same species. For example, Rost and Venner (5) found that protoplast formation varied from 1 to 100% depending on the strains of Saccharomyces tested. The basis of these different susceptibilities to attack by snail enzyme has not yet been adequately described.

The purpose of this communication is to show the effect of different growth phases of the preculture and main culture on the yield of protoplasts in Saccharomyces, because these factors may account for the variation in rate and yield in the preparation of protoplasts in different laboratories. Furthermore, the optimal conditions for the formation of a high yield of stable protoplasts are shown.

Three almost isogenic and homozygous strains were used: the wild-type strains 211-1b/a (haploid), 211/aα (diploid), and 415/aαaα (tetraploid); their origin has already been described (2, 3). The cells were kept on agar slants and stored at 4 C. Samples were taken from the slants, inoculated into yeast-peptone-glucose (YPG) medium (1% Difco yeast extract, 0.5% Difco peptone, 2% glucose), and incubated at 30 C on a shaker (preculture). Cell samples were taken at various times from the preculture, inoculated into fresh YPG medium, and incubated, with shaking, at 30 C (main culture).

For the preparation of protoplasts, the digestive juice of the snail Helix pomatia, in samples obtained from L'Industrie Biologique Francaise, 35-49 Quai du Moulin de Cage, Gennevilliers, Seine, France, was used. The general method for obtaining protoplasts described by Eddy and Williamson (1) was modified as follows. Cells of the main culture from various stages of the logarithmic growth phase and from the transition phase were collected by centrifugation, washed three times with distilled water, and suspended in 0.05 M citrate-phosphate buffer (pH 6). The cell suspension (10¹⁷ to 10¹⁸ cells/ml), the stabilizer MgSO₄ (1.2 M), and a solution of snail juice diluted with 1.5 volumes of citrate-phosphate buffer were mixed in the ratio 4:10:1, respectively, and incubated at 25 C for 2.5 hr, with gentle shaking at 30 min intervals. Spherical bodies which ruptured within 1 min after dilution with water were considered to be protoplasts.

The degree of ploidy was found to have no effect on the yield of protoplasts; the results for diploid strain 211 are shown in the figures. A growth curve of the preculture and a curve indicating the change of pH values of the medium during the period of growth are shown in Fig. 1, whereas Fig. 2 shows a growth curve of the main culture.

Figure 3 shows the effects of different main-culture ages as well as different preculture ages; it is apparent that the maximum yield of protoplasts always occurred when the cells were taken from a 6-hr-incubated main culture and that yield was independent of the age of the preculture. At this time the cells have spent approximately 5 hr in the logarithmic growth phase, which is equivalent to about three doubling times. A 4-hr main culture has undergone about two cell generations, whereas after 8 hr of main culture incubation the cells are no longer in the logarithmic phase but in the transition phase. As far as preculture age is concerned, 12 hr incubation time proved to be more favourable than all others. Figure 1 shows that the logarithmic phase has just ended after an incubation time of 12 hr and that the cells are entering the transition phase. Cells taken after 108 hr of preculture incubation, however, have already spent about 80 hr in the sta-
protoplast formation decreased as soon as the culture outgrew logarithmic phase and entered transition phase. This growth phase is characterized by the depletion of glucose in the medium. During this phase the cells utilize as a carbon source the ethanol which they produced during logarithmic phase (4).

To test the effect of ethanol on protoplast formation, a main-culture medium was used that contained ethanol (0.5%, v/v) as carbon source instead of 2% glucose. Under these conditions no protoplasts were formed, even after many cell generations in the logarithmic phase. Similarly, when a low concentration of glucose (0.125%) was used, no protoplast formation was observed. However, if ethanol (0.5%, v/v) and glucose (2%) were both present

tionary phase of growth and exhibit the lowest yield of protoplasts. If the duration of the logarithmic phase of the main culture is lengthened by supplying more medium or by using a smaller inoculum, or both, more cell generations are formed during logarithmic phase. Under these conditions a high yield of protoplasts can be obtained even if cells from an old preculture are used. Cells taken from an 108-hr preculture and an 18-hr main culture will yield ≥98% protoplasts (Fig. 4). Even cells that have spent 6 months at 4 C in stationary phase on agar slants were able to be transformed to protoplasts at the same high frequency after 26 hr in the main culture; this involved 16 generations of logarithmic growth in the main culture.

In all experiments the frequency of protoplast formation decreased as soon as the culture outgrew logarithmic phase and entered transition phase. This growth phase is characterized by the depletion of glucose in the medium. During this phase the cells utilize as a carbon source the ethanol which they produced during logarithmic phase (4).

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in the main culture, optimal protoplast formation was observed.

Preliminary experiments indicate that the pH of the culture has no effect on protoplast formation, although this particular observation needs more investigation to be reliable.

It is evident from the results shown in Fig. 3 and 4 that the amount of cell wall components refractory to the snail enzyme treatment increases with the duration of the stationary phase of the preculture and decreases progressively during the logarithmic phase of the main culture. It should be emphasized that the first few generations of cells formed in the main culture in the presence of glucose still contained snail enzyme-resistant wall components if the main culture was started with cells from an old preculture. Therefore, logarithmic phase cells are not always the most suitable for obtaining a high yield of protoplasts. A number of generations must occur in the presence of glucose before daughter cells of stationary or old cultures become amenable to protoplast formation by the action of snail enzyme. It is possible that this involves the dilution by growth of some inhibitory or resistant component(s) formed in the wall in stationary-phase cells.

LITERATURE CITED