NOTES

Induction of Protoplasts of *Schizosaccharomyces octosporus* in a Defined Medium

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Protoplasts of *Schizosaccharomyces octosporus* were formed in a defined medium consisting of yeast nitrogen base, adenine, glucose, MgSO₄, and 2-deoxy-D-glucose.

Yeast protoplasts have been induced by either of two methods (3). The more common method utilizes the undefined enzyme complex in snail gut juice, whereas the other involves wall degradation mediated by MgSO₄ and 2-deoxy-D-glucose (2DG) (1). When protoplasts of *Schizosaccharomyces octosporus* are produced by the latter method, the MgSO₄ and 2DG are added to Sabouraud broth (1). The use of undefined media such as Sabouraud broth hampers critical biochemical studies of wall synthesis. Thus, there is a need for a defined protoplast induction medium, and such a medium has not previously been reported (3).

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**FIG. 1-4. Schizosaccharomyces octosporus in defined protoplast induction medium (DGM + 1.8 mM MgSO₄ + 5.0 mg of 2DG/100 ml) after 48 hr at 30 C. Phase contrast photomicrographs ×1,200.**

1. NRRLY 854. Haploid vegetative cells partially emerged from eroding cell walls.
2. NRRLY 855. Free protoplast from haploid cell. Wall fragment remains at lower left.
3. NRRLY 855. Protoplast formation in conjugating cells (upper pair) and in haploid vegetative cells (lower pair).
4. NRRLY 854. Large vacuolated protoplasts just prior to bursting in distilled water.
Stock strains NRRLY 854 and 855 of the fission yeast *S. octosporus* were maintained on Mycophil agar (BBL), pH 7.0, for these experiments. They were grown at 30 C in stationary culture in a liquid defined growth medium (DGM) consisting of 6.7 g of yeast nitrogen base (Difco) plus 18.9 mg of adenine HCl (Nutritional Biochemical Co.), plus 20 g of glucose (Difco)/liter of distilled water. Adenine was added because it has been reported to enhance the growth of this species (2). The yeasts were subcultured twice at 48-hr intervals. The cell concentration of the third subculture was adjusted to 10⁶ cells/ml. One milliliter of cell suspension was then added to 50 ml of either of two protoplast induction media: DGM plus 5.0 mg of 2DG/100 ml (Mann) plus anhydrous MgSO₄ to raise the molarity to 1.8M(DGM ++ high) or DGM plus 2.5 mg of 2DG/100 ml plus anhydrous MgSO₄ to raise the molarity to 1.5M(DGM ++ low). These cultures were incubated at 30 C either as unshaken cultures or on a reciprocal shaker.

Good growth of mostly haploid cells was obtained in DGM. Diploid cells were few, and ascospores were rarely seen. Significant protoplast yields (80 ± 10%) were obtained in 4 days in the DGM ++ high medium (Fig. 1-4), and only a few protoplasts (10–20%) formed in DGM ++ low medium. There were no differences in protoplast yields between shaken and unshaken cultures. Protoplasts tended to float at the top of the medium or adhered to the walls of the flasks. The protoplasts were osmotically fragile and burst in distilled water. Protoplast formation occurred by either peeling of the wall layers (Fig. 1, 3) or by erosion of the division scars as previously reported (1). The wall material dissolved shortly after protoplast release (1).

Protoplasts of *S. octosporus* have been induced previously in 48 hr in Sabouraud broth plus 1.5 M MgSO₄ plus 2.5 mg of 2DG/100 ml (1), whereas protoplast formation in DGM ++ high took twice as long and required twice the concentration of 2DG. The Sabouraud broth previously used for protoplast formation in *S. octosporus* (1) contains the same concentration of glucose as DGM ++ high. Thus, if there is a critical 2DG-glucose ratio for *S. octosporus*, it varies with the medium.

**LITERATURE CITED**