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Preparation and Storage of Single Spores of *Saccharomyces cerevisiae*

P. ROUSSEAU AND H. O. HALVORSON
Laboratory of Molecular Biology and Department of Bacteriology, University of Wisconsin, Madison, Wisconsin

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A method is described for large-scale isolation and storage of single spores of *Saccharomyces cerevisiae*.

Physiological studies on yeast spores have been limited by the difficulty in isolating sufficient quantities of single spores for biochemical investigations. Oil-phase separation (1), liquid paraffin (4), and stable-flow free-boundry electrophoresis (2) have all been proposed for genetic studies where small populations of isolated single spores of yeast are required. Complexity of these methods, low spore yields, and clumping of spores limit their use. This report describes a method for the preparation of yeast spores which is convenient, reproducible, avoids clumping, and gives a final preparation which is suitable for studies on germination.

A diploid *Saccharomyces cerevisiae* Y-55 (3) was used in this study. The culture was grown first on slants of YMA medium (3 g of yeast extract, 3 g of malt extract, 5 g of peptone, 10 g of glucose, 20 g of agar, and 1 liter water; *p*H 5.5) for 2 days at 30 C. The cells were washed off the slant with sterile water and inoculated at a density of 5×10^4 cells per ml into medium containing 20 g of glucose, 20 g of peptone, and 10 g of yeast extract per liter of water. After 24 hr of aeration at 30 C, the culture was harvested by centrifugation in an International 267 rotor for 15 min at $550 \times g$ at 25 C, washed once with distilled water, and inoculated at a final level of 5×10^7 cells per ml of sporulating medium into 2-liter Erlenmeyer flasks containing 800 ml of medium [0.1% glucose, 0.25% yeast extract, and 2% potassium acetate). The antibiotic tetracycline (Squibb Co.) was added at a level of 20 μg/ml to prevent contamination. The flasks were aerated on a shaker (New Brunswick Scientific Co., New Brunswick, New Jersey) at 300 rev/min at 30 C for 6 days. After 132 hr, for example, sporulation was over 97%. The asci were then harvested in a Sharples centrifuge and washed twice with cold distilled water (Fig. 1A). The yield from 10 liters of sporulation medium was 85 g of wet asci.

Large-scale preparative methods require storage of the spores at some stage of their isolation. Since experience has shown that asci are more stable than isolated spores, conditions for optimal survival of asci were explored. Lyophilization of asci led to a 50% loss in viability. Also, rapid freezing of asci with dry ice and acetone in citrate-phosphate-mercaptoethylamine hydrochloride buffer, 5% glycerol, or 5% dimethyl sulfoxide led to significant decreases in viability (Table 1). When liquid asci suspensions were placed in the freezer at −15 C (slow freezing), excellent re-

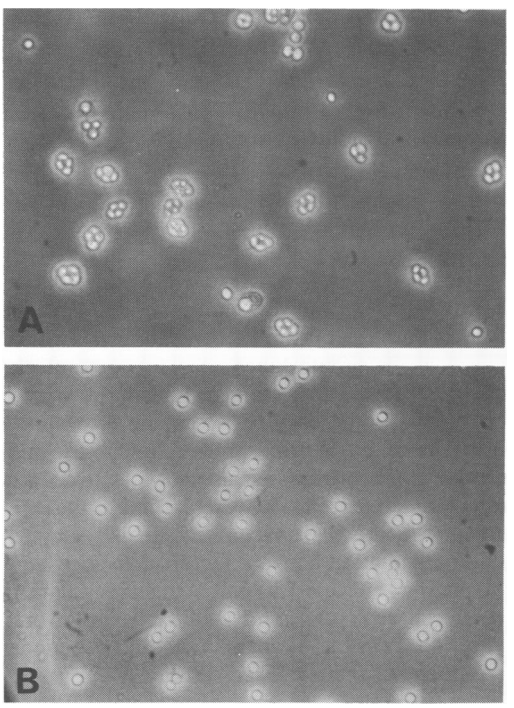

FIG. 1. *A. S. cerevisiae Y-55 asci collected after 132 hr of sporulation at 30 C. B. Purified single spores dispersed in 1% Tween 80. Both pictures were taken with a Zeiss phase contrast microscope. Primary magnification, × 720.*

TABLE I. *Effect of storage conditions on the viability of asci of S. cerevisiae*[a]

Days of storage at −15 C	Asci per ml of suspension					
	DMSO 5%		Glycerol 5%		Buffer[c]	
	Slow freeze[d]	Fast freeze[d]	Slow freeze	Fast freeze	Slow freeze	Fast freeze
0	130 × 10⁵	130 × 10⁵	130 × 10⁵	130 × 10⁵	130 × 10⁵	130 × 10⁵
3	140 × 10⁵	39 × 10⁵	130 × 10⁵	32 × 10⁵	150 × 10⁵	139 × 10⁵
41	177 × 10⁵	52 × 10⁵	122 × 10⁵	18 × 10⁵	160 × 10⁵	110 × 10⁵
70	143 × 10⁵	57 × 10⁵	165 × 10⁵	16 × 10⁵	160 × 10⁵	106 × 10⁵

[a] Viability was measured after 48 hr of growth at 30 C on YMA plates.
[b] Dimethyl sulfoxide.
[c] Medium: 0.02 M citrate, 0.02 M phosphate, 0.14 M mercaptoethylamine hydrochloride; *p*H 7.0.
[d] A 1.2-ml amount of asci suspension was placed in a 5-ml vial and either placed in a deep freezer at −15 C (slow freeze) or else emersed in a acetone-dry ice bath (fast freeze) and later transferred to −15 C.

coveries were observed even after 70 days of storage.

To prepare a suspension of single spores, a suspension of asci (about 5 to 10 OD at 600 nm) was incubated overnight at 30 C on a shaker with 5 mg per ml of Pronase (Calbiochem, Los Angeles, Calif.) in 0.02 M citrate-phosphate buffer, *p*H 7. Spores do not germinate in this medium. To disperse the spores after that incubation, Tween 80 (Nutritional Biochemicals Corp., Cleveland, Ohio) was added to a final concentration of 1%, and the suspension was passed through a French press (10 to 15 C) at 7,000 psi by using a flow rate of 20 to 25 ml per min. The suspension was then centrifuged at 25 × *g* for 20 to 25 min and the pellet was suspended in growth medium containing 1% Tween 80. Occasionally, aggregates of single spores occur which are easily removed by allowing the suspension to settle for a few minutes and discarding the sediment.

By the above procedure, suspensions of single spores can be obtained which remain dormant for several days when stored as a thick suspension at 4 C (Fig. 1B). The treatments employed (Pronase, Tween 80, or pressure to disrupt the ascus) affect neither their ability to germinate nor their viability. Higher centrifugation speeds of the single spore preparations should be avoided, since they give rise to clumps of spores which are difficult to disperse.

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