Isolation of Yeast Ascospores Free of Vegetative Cell Contamination

By AMITAVA CHATTERJEE, SHYAMALI GHOSH, SAMPA DAS
AND S. K. SEN*

Programme in Genetical Research, Botany Department, Bose Institute, Calcutta 700 009, India

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A method has been devised for isolating yeast ascospores and asci free from vegetative cell contamination. By careful manipulation of the size and surface properties of the spheroplasted vegetative cells and asci, a pure preparation of ascospores could be recovered by sucrose gradient centrifugation.

INTRODUCTION

The advantages of sporulating diploid yeast (*Saccharomyces cerevisiae*) for meiotic studies have been realized by a number of workers (Roth & Fogel, 1971; Simchen *et al.*, 1972; Pinon *et al.*, 1974; Esposito & Esposito, 1974; Shilo *et al.*, 1978). Unfortunately, yeast cells respond asynchronously to the sporulation stimulus in acetate-containing growth medium. Consequently, it has been difficult to analyse the meiotic developmental programme. However, characterization of developmental stages, such as readiness and commitment to meiosis has been achieved (Simchen *et al.*, 1972) by monitoring the first batch of meiotic products (asci) formed. To extend this approach, it is necessary to use the first batch of asci to monitor the features of the developmental phases of meiosis. However, suitable techniques for isolating the first batch of asci from the remaining cells were not available. Thus, it was necessary to devise a technique for isolating the first asci formed, which are quite small in size, free from any contamination by vegetative cells. Previously described techniques for spore isolation (Resnick *et al.*, 1967; Tingle *et al.*, 1974; Dawes & Hardie, 1974; Padilla *et al.*, 1975; Betz & Weiser, 1976; Nishi *et al.*, 1978; Dawes *et al.*, 1980) fell short of this requirement. The separation technique of Dawes *et al.* (1980) was considered; however, it was felt that the technique did not yield fractionation of exclusively asci/ascospores. By exploiting differences in surface properties and size of spheroplasted vegetative cells and asci of yeast a simple but effective technique has been devised.

METHODS

The diploid strain 131 (obtained from Professor G. Simchen, Jerusalem) was used, growth and sporulation conditions were those of Simchen *et al.* (1972) in which at 30 °C the onset of ascus formation took place at the 11th hour of sporulation. Harvesting of cells for the present study was carried out after 12 h.

Preparation of yeast spheroplasts. Spheroplasting of the yeast cells was by a similar method to that of Klein & Byers (1978). Cells were incubated for 30 min in 0.1 M-mercaptoethanol containing 0.2 M-Tris and 0.02 M-EDTA. They were then transferred to 1 M-sorbitol, 0.02 M-sodium citrate/phosphate buffer (pH 5.9), containing 0.01 M-EDTA with 8 mg Helicase ml⁻¹ (Pharmindustrie, Clichy). Incubation of the yeast cells was carried out for 2 h at 37 °C. All operations were carried out aseptically. Spheroplasts and other fractions were examined by phase contrast microscopy.

Viability test. Samples containing asci/ascospores were counted using a haemocytometer. After suitable dilution, they were plated on YEPD medium (1%, w/v yeast extract; 2%, w/v peptone; 2%, w/v glucose, and 2%, w/v agar) and incubated for 3 d at 30 °C. Colony counts were made to determine the viability.

RESULTS AND DISCUSSION

Our efforts have been directed towards isolating asci and ascospores intact and free from vegetative cell contamination from a heterogeneous mixture containing about 5% asci. The

Fig. 1. Phase contrast micrographs of yeast cells. (a) Spheroplasted yeast cells forming the starting material for centrifugation. The presence of few asci can be seen. (b) The pellet after 60% (w/v) sucrose centrifugation containing asci and vegetative cells in about 4 : 1 proportion. (c) The third fraction from the top, after step gradient centrifugation, containing mainly asci. (d) The bottom layer after 60% (w/v) sucrose centrifugation when treated with 5% (w/v) Sarkosyl, liberating the ascospores from the spheroplasted asci. The spheroplasted vegetative cells were selectively ruptured in the process. The photograph shows the ascospores obtained. The bar markers represent 20 μ m.

approach was based on two facts. First, spheroplasted vegetative cells and asci differ in size and surface properties and secondly, it was found it was possible to destroy vegetative cells in preference to the spores by lysis. The technique that was devised to fulfil our requirements is described as follows. Spheroplasts produced from cells harvested from the sporulation medium were pelleted free of enzyme and resuspended in a small volume of 1 M-sorbitol, 1.5 ml of this suspension was layered on to 2.5 ml 60% (w/v) sucrose and centrifuged at 3500 g_{av} in a swing-out rotor for 5 min. The upper and lower layers were carefully separated. The upper layer was free of spores while the lower layer contained 75–80% asci (Fig. 1 *b*) and was pelleted. The pellet was suspended in a small volume of 1 M-sorbitol and charged on to a sucrose step gradient made by layering 1.5 ml each of 65%, 60%, 55% and 50% (all w/v) sucrose solution. Gradient centrifugation was carried out at 30 °C in a swing-out rotor at 4750 g_{av} for 30 min. Four clear bands were formed, and each was then carefully removed using a Pasteur pipette. The quality of asci (Fig. 1 *c*) obtained from the third fraction from the top was by far the best and was fairly free from contamination. The mixture for step gradient centrifugation contained 77% of asci and 23% of vegetative cells. These asci distributed after centrifugation amongst the four bands from the top in the following proportion 0.2 : 28 : 39 : 32.4, each band containing varying amounts of vegetative cells. The third band containing 39% of the total asci was the purest fraction having only 1.1% vegetative cell contamination (Table 1). Evaluation of this fraction containing asci

Table 1. *Distribution and viability of cell types after spheroplasted yeast cells were centrifuged on sucrose solution*

	Vegetative cells (%)	Asci or ascospores* (%)	Approximate recovery of asci/ascospores* (%)	Viability of ascospores (%)
1. Starting material	95.1	4.9		
2. Pellet from centrifugation through 60% (w/v) sucrose	22.8	77.2	100	98.8
3. Fractions separated on the step gradient of sucrose (from top downwards):				
Fraction I	99.2	0.8	0.2	97.4
Fraction II	39.0	61.0	28	98.49
Fraction III	1.1	98.9	39	98.23
Fraction IV	19.7	80.3	32.4	96.89

* Observations have been tabulated on the basis that an ascus contains four ascospores.

indicated that it was completely free from any other cellular contamination. The ascospores liberated from the asci retained complete viability (Table 1). The viability of the ascospores on storage was well maintained through the use of a citrate/phosphate buffer (Rousseau & Halvorson, 1969). These preparations were found to be suitable for use in biochemical and genetic studies. Although there was some quantitative loss of asci in the course of the procedure, the high order of purity of the preparation made up for this loss. The method was found to be reproducible and the fraction of total asci recovered in the third fraction of the step gradient was fairly consistent from one separation to another. The present technique is simple, rapid and more effective when dealing with low percentages of sporulated yeast cells than to that described by Betz & Weiser (1976).

In cases where the loss of asci affected the suitability of the technique, an alternative protocol was adopted. The pellet, after centrifugation through 60% (w/v) sucrose, containing chiefly asci and some vegetative cells could alternatively be suspended in 3 ml 1 M-sorbitol containing 5% (w/v) Sarkosyl NL 97 (Geigy) which was then incubated at 65 °C for 15 min. This treatment preferentially lysed the vegetative cells, and ascospores were liberated from the asci (Fig. 1d). This method was found to be suitable for some biochemical uses, although it was not suitable for genetic studies, since Sarkosyl treatment affected the viability of the ascospores adversely. Moreover, the preparation obtained by this technique was not as free from cellular contamination as could be obtained with the step gradient procedure.

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