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Sporulation of Yeast Harvested During Logarithmic Growth

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Rapid and abundant sporulation of yeast can be obtained, with cells harvested during logarithmic growth, by employing potassium acetate rather than glucose as a carbon source.

Meiosis in yeast (sporulation) offers a model system for examining developmental processes in an eukaryotic organism. In meiosis in *Saccharomyces cerevisiae*, each cell normally produces four haploid ascospores. The process is synchronous (1), involves a series of morphological and physiological stages (1, 3, 7, 8), and is influenced by cultural conditions (2, 6). When a population of exponential glucose-grown cells were transferred to an acetate sporulation medium, the rate of sporulation was very poor but increased as cells entered the later stages of growth (2). Since, during this period, yeasts shift from a fermentative to an aerobic metabolism (9), sporulation in yeast, as in bacteria (5), may be dependent upon acetate metabolism. To test this possibility, sporulation of acetate-grown cultures was examined. We have obtained equally synchronous, and complete sporulation of logarithmically growing cells by cultivation of our strains on potassium acetate (KAc) medium rather than on a glucose medium.

Yeast stocks were maintained and subcultured on yeast extract-peptone-dextrose (YEPD) agar (4). The presporulation growth medium contained 6.7 g of Yeast Nitrogen Base (without amino acids; Difco), 1.0 g of Yeast Extract (Difco) per liter of 0.05 M potassium pthalate buffer (*p*H 5.0), and 1% of either glucose or potassium acetate. For each experiment, cells were transferred from YEPD agar to presporulation growth media; the cells were then maintained in continuous log phase (below 5×10^7 cells/ml), by repeated subculturing, for at least 30 hr prior to transfer to sporulation media.

Growth was observed at 600 nm with a Gilford model 2000 spectrophotometer; 1 absorbance unit in a 1-cm light path equals approximately 9×10^6 cells/ml. Cultures were maintained at 30 C ± 0.5 on a 2.54-cm stroke rotary shaker at 350 rev/min. For sporulation, cells were harvested at room temperature by rapid centrifugation from presporulation media [optical density (OD) = 1.0 to 1.5], washed once with distilled water, collected by centrifugation, suspended in 1% KAc (2×10^7 to 2.3×10^7 cells/ml, 20 to 25 ml

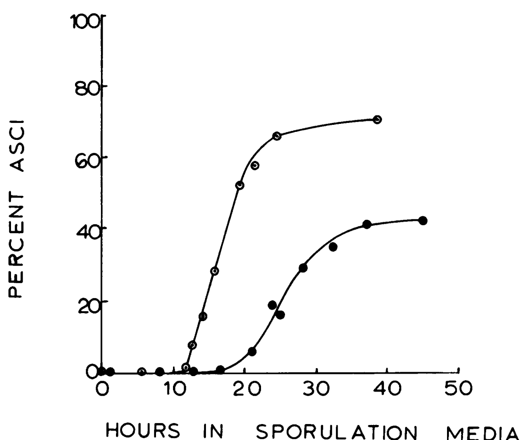

FIG. 1. *Sporulation of S. cerevisiae strain Z113. Cells were grown in either 1% glucose (●) or 1% potassium acetate (○) presporulation media. The mass doubling times were 70 and 150 min for glucose and KAc media, respectively. Cells were harvested from each medium during early log phase (OD_{600nm}, 1 to 1.5), washed, and suspended in sporulation medium (1% KAc solution) at 30 C with agitation. At intervals, samples were removed and examined for asci, cells, and buds. The per cent asci relative to the total count is plotted against the time of incubation in sporu ation medium.*

in 300-ml flask), and agitated at 30 C as above. At intervals, sporulation was examined microscopically with Zeiss phase-contrast optics (4). The percentage of asci relative to the total count of cells, buds, and asci (at least 500) were determined according to the conventions of Croes (1).

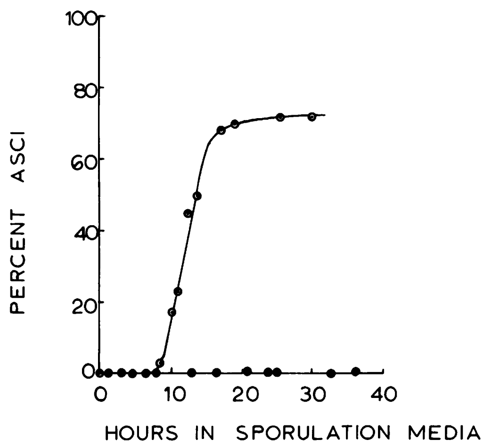

FIG. 2. *Sporulation of S. cerevisiae strain Y55. Cells were grown in either 1% glucose (●) or 1% KAc (○) presporulation media. The mass doubling times were 65 and 110 min for the glucose and KAc media, respectively. The procedure is given in the legend to Fig. 1.*

Figures 1 and 2 show the kinetics of sporulation of two strains of *S. cerevisiae* harvested during logarithmic growth on either glucose or KAc presporulation media. Strain Z113, a heterothallic diploid, sporulates more rapidly and abundantly after growth on KAc than on glucose (Fig. 1). Strain Y55 (Fig. 2), a homothallic diploid, sporulates well after growth on KAc but poorly or not at all after growth on glucose. (After 4 days, 1 to 2% asci were occasionally found.)

Growth on glucose-presporulation medium is not necessary for subsequent sporulation and may be inhibitory for some strains, possibly due to a severe glucose repression of the tricarboxylic acid cycle enzymes in some strains of yeast (3). Thus, the oxidative capacity of the cells at the time they are placed into acetate sporulation medium may play a role in the kinetics and extent of sporulation in a population of *S. cerevisiae* (1). The use of cells derived from steady-state growth on acetate eliminates the necessity for respiratory adaptation to new substrate as a condition for sporulation and facilitates investigations on those intracellular events unique to sporulation.

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