Frozen Concentrated Cultures Of *Kluyveromyces fragilis*

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Story in Brief

Concentrated cultures of *Kluyveromyces fragilis* Y-1156, prepared by suspending the yeast cells in 10 percent nonfat milk solids (NFMS), were frozen at −19 C and −196 C. Cells in concentrated cultures stored at −19 C survived better than those held in liquid nitrogen (−196 C). The age of the yeast culture at the time of freezing affected survival. Cells from the midexponential growth phase survived better at −19 C than those from the stationary phase. The opposite was observed for the yeasts when frozen at −196 C. The highest levels of survival appeared at the midstationary phase. The addition of glucose to the suspending medium, in amounts up to 30 percent, resulted in increased storage stability at both storage temperatures.

Introduction

Research conducted at Oklahoma State University has substantiated that the whey produced as a by-product of the cottage cheese industry can be cultured with the yeast *Kluyveromyces fragilis* and the biochemical oxygen demand (BOD) of the liquid reduced significantly (Knight *et al.*, 1972). In return, a yeast-whey protein material can be recovered which may have some value as a nutritious food supplement for humans and animals alike. Storage of the yeast culture required for this fermentation poses several problems. In the past, large volumes of the yeast-whey mixture have been held back and used as a starter for the next fermentation (Smith *et al.*, 1977). This procedure is troublesome and requires considerable time and storage space. Many times such a procedure results in reduced viability of the yeast culture.

The objective of this study was to find a means of storing yeast culture concentrates in a frozen state, at either −19 C or −196 C, in such a manner that the culture would retain its viability and activity. Storage of such concentrated cultures would require less space and simplify preparation and utilization. Positive results from this experiment could have important applications for other areas of the food industry using yeast cultures in processing.

Experimental Procedure

The medium for growing cells of *K. fragilis* for preparing concentrated cultures was pepsinized whey. It was prepared by dissolving dried Krafen sweet whey (obtained from Kraft Foods Co.) in distilled water (5 g per 100 ml). The reconstituted whey was adjusted to pH 3.0 with 30 percent citric acid and pepsin (25 mg per 100 ml; Sigma Chem. Co.) was added. The mixture was incubated 30 min at 37 C followed by adjusting the pH to 6.5 with 20 percent NaOH. The pepsinized whey was sterilized by autoclaving 15 min at 121 C.

In experiments to determine the effect of cellular age and freezing temperature on survival, four 250-ml Erlenmeyer flasks containing 50 ml of pepsinized whey were placed in a 35 C reciprocating shake water bath (82 strokes of 40 mm per min) and inoculated with 0.5 ml of a *K. fragilis* Y-1156 yeast culture. One flask was removed at 8, 11, 14 and 17 hr. The cells were harvested by centrifugation at 12062 x g for 10 min at 10 C. The cells were resuspended in a total of 5 ml of cold sterile 10 percent nonfat milk solids (NFMS). This suspension was placed in two vials for freezing at −19 C and −196 C. The cultures were plated on Sabouraud Dextrose Agar before freezing and following a three-day storage period. All plates were incubated for 48 hr at 35 C.

Additional experiments were conducted to study the possibility of using glucose as a cryoprotective agent. Ten 250-ml Erlenmeyer flasks, each containing 80 ml of pepsinized whey, were inoculated with 0.8 ml of a *K. fragilis* Y-1156 broth culture. The flasks were incubated 15 hr in a 35 C reciprocating shake water bath as described in the previous paragraph. To prepare the concentrated cultures for freezing, the cells from all 10 flasks were harvested as previously described. The cells were resuspended in a total of 20 ml of cold sterile 10 percent NFMS. Five gram aliquots of this concentrated culture were placed into each of four test tubes, containing 15 ml of 10 percent NFMS and glucose in the amounts of either 0, 2, 4 or 6 grams. This yielded concentrated cultures containing 0, 10, 20 and 30 percent glucose. Four vials (2 g) of each preparation were frozen at −19 C and four at −196 C. One vial of each sample was plated on Sabouraud Dextrose Agar before freezing and after 1, 7, 14 and 21 days storage. All plates were incubated at 35 C for 48 hr.

Results and Discussion

The survival of *K. fragilis* Y-1156 in concentrated cultures during frozen storage varied with physiological age and storage temperature (Table 1). Cultures frozen at −19 C survived much better than did those frozen at −196 C. Greatest survival of cultures frozen at −19 C was observed for those frozen after 8 hr growth. The number of cells surviving freezing after 11 hr growth was somewhat lower than observed for the 8 hr culture. The percentages of survival of the yeast cultures frozen at the 14 and 17 hr growth periods were

Table 1. Effect of cellular age and freezing temperature on survival of *K. fragilis* Y-1156

Growth period (hr)	Initial population/g	% Survival	
		−19 C	−196 C
8	2.0×10^8	80	1
11	6.2×10^8	57	11
14	2.0×10^9	65	12.5
17	1.9×10^9	65	9

Table 2. Effect of glucose concentration, storage time and freezing temperature on survival of *K. fragilis* Y-1156

Glucose concentration (%)	Storage time (days)	% Survival			
		Trial 1		Trial 2	
		−19 C	−196 C	−19 C	−196 C
0	1	90	7.5	85	12
	7	19	5	48	12
	14	49	6	39	10
	21	5	1	32	10
10	1	74	4	98	4
	7	60	5	68	6
	14	79	5	63	4
	21	37	2.5	55	4
20	1	79	8	100	6
	7	66	10	85	6
	14	80	9	85	4
	21	54	8	81	6
30	1	84	96	83	7
	7	69	94	97	8
	14	82	101	83	5
	21	83	100	79	10

slightly higher than at 11 hr but not as high as for the 8 hr cultures. This slight increase may have resulted from higher initial populations for the 14 and 17 hr cultures.

The yeast cultures frozen at −196 C responded in a contrary manner to those frozen at −19 C. There was a negligible amount of survival (1 percent) from cells frozen after an 8 hr incubation. The survival rates seemed to peak between growth times of 11 and 14 hr and decrease again at the 17 hr point.

It appeared from these results that cells of *K. fragilis* grown approximately 14 hr would be most suitable for further freezing trials at both storage temperatures. The use of cryoprotective agents in the suspending media was the next area studied in attempts to increase freezing survival. Glucose was added in various concentrations to concentrated cultures of *K. fragilis* to determine its effect on yeast survival during frozen storage.

The addition of glucose to the suspending medium had no beneficial effect on survival after a one-day storage period at −19 C (Table 2). However, during extended storage (−19 C) periods of 7 to 21 days, the number of yeast cells surviving increased as the amount of glucose in the medium increased.

The concentrated cultures frozen at −196 C again exhibited much lower survival than those frozen at −19 C. Ten and twenty percent glucose provided no protection to cells frozen at −196 C in either trial. At the 30 percent glucose level, however, a discrepancy was observed between the two trials; in trial 1 greater than 90 percent survived at −196 C, while in trial 2 the survival was 10 percent or less. Experimentation is currently being conducted to determine the cause of this difference. It may have resulted from slight differences in the physiological ages of the cultures in the two trials.

It was also observed in this study that damage to the concentrated cultures frozen at −19 C occurred over the total storage period at a slow rate. The damage to yeast cells frozen at −196 C seemed to occur almost immediately, and the viability of these cells changed little during the study period as a whole. The differences in the effect of physiological age of the cells on survival at −19 C and at −196 C suggests that there may be more than one major factor involved in producing cells that will survive freezing during extended storage periods.

Literature Cited

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