Growth Requirements of San Francisco Sour Dough Yeasts and Bakers' Yeast

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The growth requirements of several yeasts isolated from San Francisco sour dough mother sponges were compared with those of bakers' yeast. The sour dough yeasts studied were one strain of *Saccharomyces uvarum*, one strain of *S. inusitatus*, and four strains of *S. exiguus*. *S. inusitatus* was the only yeast found to have an amino acid requirement, namely, methionine. All of the yeasts had an absolute requirement for pantothenic acid and a partial requirement for biotin. Inositol was stimulatory to all except bakers' yeast. All strains of *S. exiguus* required niacin and thiamine. Interestingly, *S. inusitatus*, the only yeast that required methionine, also needed folic acid. For optimal growth of *S. exiguus* in a molasses medium, supplementation with thiamine was required.

The microorganisms used in the San Francisco sour dough bread process have been isolated by workers in this Laboratory. The souring activity is attributed to a previously undescribed species of lactic acid bacterium, now named *Lactobacillus sanfrancisco* (1). The leavening function is performed mainly by a yeast found in the starters of several bakeries in the area. Although identified as *Torulopsis holmii*, because ascospores are not produced, terminology of the perfect form, *Saccharomyces exiguus*, is preferred (4). This yeast differs from bakers' yeast, *S. cerevisiae*, in several important aspects. It is (i) resistant to cycloheximide, (ii) unable to ferment maltose, and (iii) more tolerant to acetic acid. It is precisely the latter two properties that render this yeast a suitable partner for this association. In addition to *S. exiguus*, two other yeasts of lesser significance have also been found in starters of two other bakeries. They have been identified as *S. inusitatus* and *S. uvarum*. Both, like bakers' yeast, are maltose positive and cycloheximide susceptible; however, the former differs from bakers' yeast in being unable to ferment galactose, whereas the latter differs by fermenting raffinose to completion.

Since the discovery of *S. exiguus* in the sour dough system, efforts have been made to grow this yeast in large quantities with the ultimate aim of combining it with pure culture starters of the sour dough bacterium *L. sanfrancisco* to make sour dough bread. This would eliminate the necessity of rebuilding daily the mother sponge by the bakers as is now practiced. To produce this yeast on a scale similar to that

now used for commercial production of bakers' yeast, information relating to its nutrition and conditions of growth is needed. The purpose of the present study is to provide such information.

MATERIALS AND METHODS

Organisms. The sour dough yeasts isolated and described by Sugihara et al. (4) included four strains of *S. exiguus* (98, M58, M86, and M116) and one strain of *S. inusitatus* (M95). Additionally, *S. cerevisiae* PBV was isolated from a cake of commercial, compressed bakers' yeast, and *S. uvarum* CY2 was isolated from a sour dough starter obtained from a local bakery by Frank Sugihara. *S. uvarum* was identified by Martin W. Miller of the University of California at Davis.

Media. The media used in these studies were the following: (i) APT (BBL); (ii) GTY medium composed of 1% glucose, 1% Trypticase (BBL), and 0.5% yeast extract (BBL); (iii) yeast nitrogen base (YNB) with and without amino acids (Difco) and supplemented with 1% glucose; (iv) simulated YNB, also supplemented with 1% glucose, prepared from reagent grade ingredients in amounts stated by Difco, and from which each ingredient was omitted to test for its requirements; and (v) molasses medium prepared by adding 0.5% $(\text{NH}_4)_2\text{HPO}_4$ (wt/vol) to molasses that had been diluted to ca. 10° Brix. The molasses, heated and clarified, was obtained from Red Star Yeast Co., Oakland, Calif., in a concentrated form (40° Brix). The medium was adjusted to pH 5.0 with 1 N HCl and pasteurized under flowing steam for 20 min before use. Fresh yeast extractives (FYE) were prepared as described by Kline and Sugihara (1). The purine-pyrimidine and the salts B mixtures were described previously by Sugihara and Kline (3).

Growth measurements. Growth was measured

turbidimetrically at 675 nm on a Beckman spectrophotometer, model DB-GT. When necessary, the culture was diluted to a level at which the relationship between cell mass and optical density is linear. Dry weights were determined by centrifuging a known volume of a culture, after which the cells were washed with distilled water, dried to constant weight in a forced-air oven at 90 C, cooled in a desiccator over Drierite, and weighed.

Cultural conditions. Cultures were grown in 250-ml, cotton-plugged Erlenmeyer flasks containing 100 ml of media and incubated at 30 C on a rotary shaker (New Brunswick) operated at ca. 100 rpm.

RESULTS AND DISCUSSION

Amino acid requirements. Growth of all yeasts except *S. inusitatus* M95 was not affected by omitting either histidine, methionine, or tryptophan from a simulated YNB medium. *S. inusitatus*, however, grew only 4 to 12% as well when methionine was omitted as in the complete medium.

Vitamin requirements. Vitamins were omitted systematically from the simulated YNB medium (Table 1). All yeasts, including bakers' yeast (strain PBY), had an absolute requirement for pantothenic acid and a partial requirement for biotin. Growth of all yeasts, except strain PBY, was only slightly affected by the absence of inositol. *S. inusitatus* M95 was the only yeast showing a requirement for folic acid. The requirement for folic acid in this yeast may be related to its requirement for methionine, since this vitamin is known to be involved in the transfer of methyl groups (2). In addition to these vitamins, all strains of *S. exiguus* had an absolute requirement for niacin and thiamine. Thus, *S. exiguus* is nutritionally more fastidious than bakers' yeast.

Trace-metal requirements. The trace-element requirements of these yeasts in simulated

YNB were determined by comparing their growth in simulated YNB medium with and without added metals. The metals were added as the following salts in amounts stated for the commercially prepared YNB by Difco: H_3BO_3 , $CuSO_4$, KI, $FeCl_3$, $MnSO_4$, $NaMoO_4$, and $ZnSO_4$. No significant effect of trace metal was noted. A slight decrease in growth of strains 98 and M116 of *S. exiguus* occurred when manganese was not added. Since no attempt was made to free the medium of trace metals, these results imply that the trace metals are not required in amounts greater than normally present in commercial YNB medium.

Effect of pH on growth in complex media. Since the sour dough yeasts are, in general, nutritionally more fastidious than bakers' yeast, their growth was compared to that of baker's yeast in the complete APT medium. This was tested at the normal pH of the medium (6.7) and when acidified to 4.5. No significant difference could be observed in growth yield in the two media. However, at any given pH, comparison of cell yields after 27 h of incubation showed that *S. uvarum* grew as well as *S. cerevisiae* PBY and *S. inusitatus* grew almost as well, but that the four strains of *S. exiguus* grew less than half as well.

Growth in molasses with various supplements. Since cane and beet molasses are relatively inexpensive but nutritious sources of carbohydrates, large-scale production of the sour dough yeasts will probably be on these substrates. Growth of these yeasts on molasses media is shown in Table 2. Of the two mixtures of molasses available, 60% beet-40% cane supported more growth than 80% beet-20% cane, probably because cane molasses generally contains five times as much thiamine as beet molasses. The yeasts can be ranked in the follow-

TABLE 1. Vitamin requirements of various yeasts

Medium	Turbidity at 675 nm after 48 h at 30 C						
	PBY	CY2	M95	98	M58	M86	M116
Complete	100 ^a (0.90)	100 (0.95)	100 (0.84)	100 (0.85)	100 (0.90)	100 (0.94)	100 (0.80)
No biotin	57	65	67	23	48	47	35
No pantothenic acid	9	9	21	2	3	4	12
No folic acid	106	100	10	95	101	101	102
No inositol	106	81	90	88	97	90	90
No niacin	100	93	98	18	8	13	19
No <i>p</i> -aminobenzoic acid	103	100	108	94	100	98	81
No pyridoxine	106	100	99	92	93	82	85
No riboflavin	103	100	98	85	92	94	75
No thiamine	100	87	106	2	1	1	1

^a Turbidity in complete medium is taken as 100%, and the actual value is shown in parenthesis.

ing order with respect to growth yields: bakers' yeast (strain PBY), taken as 100%; *S. uvarum* CY2, 75%; *S. exiguus* M58, 68%; *S. exiguus* 98, 60%; *S. exiguus* M86 and M116, 38%; and *S. inusitatus* M95, 32%. The reason for the low yield of *S. inusitatus* is very likely that molasses is limiting in methionine and/or folic acid. Increasing the concentration of $(\text{NH}_4)_2\text{HPO}_4$ to 10 g/liter had no effect on cell yields (Table 3). Although doubling the molasses concentration

TABLE 2. Growth yields of various yeasts on molasses

Strain	Dry wt (mg/100 ml) in 27 h	
	80/20 ^a	60/40 ^b
PBY	833	953
CY2	622	690
M95	307	299
98	499	576
M58	572	616
M86	311	364
M116	302	378

^a 80% beet molasses-20% cane molasses.

^b 60% beet molasses-40% cane molasses.

TABLE 3. Effect of doubling ammonium phosphate concentration and molasses concentration on growth yield of various yeasts

Strain	Dry wt ^a		
	10° Brix molasses, 5 g of $(\text{NH}_4)_2\text{HPO}_4$ /liter	10° Brix molasses, 10 g of $(\text{NH}_4)_2\text{HPO}_4$ /liter	20° Brix molasses, 5 g of $(\text{NH}_4)_2\text{HPO}_4$ /liter
PBY	100.0 (964.0)	97	108
CY2	100.0 (678.0)	93	85
M95	100.0 (327.8)	97	127
98	100.0 (520.0)	99	53
M58	100.0 (562.5)	92	35
M86	100.0 (360.8)	98	49
M116	100.0 (369.0)	88	68

^a Dry weight in 10° Brix molasses and 5 g of $(\text{NH}_4)_2\text{HPO}_4$ per liter is taken as 100%, and the actual value, expressed as milligrams per 100 ml of medium, is shown in parenthesis.

to 20° Brix was without effect on the cell yield of strain PBY, it increased slightly the yield of *S. inusitatus* M95 and curtailed drastically the yields of all strains of *S. exiguus* and *S. uvarum* CY2.

Since all strains of *S. exiguus* require pantothenate, thiamine, niacin, and biotin, these four vitamins were added to molasses medium singly and in combination to determine the effect on cell yield. Only the addition of thiamine increased cell yields; the other three vitamins were without effect. Thus for these strains of *S. exiguus*, molasses appears to be limiting only with respect to thiamine. Addition of thiamine in amounts greater than 400 $\mu\text{g/ml}$ did not result in additional growth of *S. exiguus*. Supplementation of molasses with the purine-pyrimidine mixture and/or with the salts B mixture had no effect on growth.

Supplementation of molasses with FYE increased growth of all sour dough yeasts except strain M58 (Table 4), but the increment of stimulation was approximately equivalent to that obtained by the addition of thiamine alone. Therefore, it is very likely that FYE is merely furnishing this vitamin, since no additional growth of *S. exiguus* occurred when FYE and thiamine were both added to molasses. Addition of FYE to molasses doubled the yield of *S. inusitatus* M95 probably by satisfying its need for folic acid or methionine. FYE addition to molasses did not stimulate growth of strain PBY or M58, indicating that unsupplemented molasses is providing all the growth factors

TABLE 4. Effect of various supplementation on the growth yield of yeasts in a molasses medium

Strain	Relative growth yield ^a in 27 h					
	Thiamine	Pantothenate	Niacin	Biotin	Vitamin mixture	FYE
PBY	96	98	99	97	98	103
CY2	103	102	101	101	NT ^c	126
M95	101	97	97	100	NT ^c	203
98	134	99	99	102	139	127
M58	112	101	99	102	121	87
M86	168	98	100	99	172	152
M116	138	103	101	102	154	154

^a The table is a composite of results from several experiments. The values shown are the percent growth yields obtained, with unsupplemented molasses medium control taken as 100%. The actual values of these never varied by more than 10% from experiment to experiment.

^b Vitamin mixture consisted of a mixture of the vitamins listed in the preceding four columns, at the same concentrations.

^c Culture not tested.

needed by this yeast. Curiously it was consistently noted during these experiments that upon addition of thiamine to either YNB or molasses the final pH's of the cultures of *S. exiguus* were always higher by about half of a pH unit. Whether this increase in pH reflects the greater amount of growth or a shift to a different mode of metabolism is, at present, not known.

Using the molasses medium described here, it has been possible to grow sour dough yeast (*S. exiguus* 98) in a 14-liter fermentor with pH controlled at 5.0 to give cell yields of approximately 750 mg/100 ml on a dry weight basis. Yeasts grown in this manner have been successfully used as starter for making sour dough French bread.

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