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Microorganisms of the San Francisco Sour Dough Bread Process

I. Yeasts Responsible for the Leavening Action

T. F. SUGIHARA, LEO KLINE, AND M. W. MILLER

Western Regional Research Laboratory, Western Utilization R and D Division, U.S. Department of Agriculture, Albany, California 94710, and Department of Food Science and Technology, University of California, Davis, California 95616

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Two hundred isolates from San Francisco sour dough French bread fermentations (40 from each of five different bakeries) were screened by fermentation tests and for their ability to grow in the presence of cycloheximide (Actidione). All of the isolates from four of the bakeries and 70% of those from the fifth were unable to utilize maltose but grew well on other sugars, even in the presence of cycloheximide. The remaining few isolates from the fifth bakery utilized maltose but not galactose and were inhibited by cycloheximide. No bakers' yeast types were found. Sixteen of the maltose-negative and five of the galactose-negative isolates were subjected to more rigorous taxonomic procedures. All of the maltose-negative isolates were identified as asporogenous strains of *Saccharomyces exiguus* (*Torulopsis holmii*) and the galactose-negative ones, as *S. inusitatus*. The predominance of *S. exiguus*, its vigor in the particular acidic environment of the sour dough, and the correlation of its numbers with the leavening function constitute strong evidence on the role of this organism in the sour dough system.

This and the subsequent study on the isolation and characterization of the microorganisms involved in the San Francisco sour dough bread process (3) were prompted by the absence, to our knowledge, of any prior reports on this subject although the process has been carried out in the San Francisco bay area for over 100 years. In the bakery, the microbiological activities responsible for the leavening (rising) and souring actions in the dough are perpetuated by rebuilding a special piece of dough known in the trade as the "starter" or "mother sponge" approximately every 8 hr with fresh flour and water. This starter sponge, when fully developed, serves as the "inoculum" for each batch of bread dough.

In recent reports (4, 7), some of us have described the mechanics of the sour dough process and the nature of the acidity developed (2) and have presented preliminary information on the two principal types of microorganisms involved. One, a readily isolatable yeast, is responsible for the leavening function. The other, an unidentified bacterial type, isolated with some difficulty and apparently for the first time, has been correlated with the souring activity.

The present report supplies detailed information on the isolation, occurrence, and characterization of the sour dough yeast isolates. Characterization of the sour dough bacteria is presented in a subsequent publication (3).

MATERIALS AND METHODS

Isolation of yeasts. Sour dough starter sponges were obtained from five different sources (bakeries) in the San Francisco bay area and maintained as needed by rebuilding with flour and water as described elsewhere (4). For isolation and enumeration of the yeasts, 11 g of sour dough was aseptically blended for 90 sec at reduced speed with 99 ml of sterile 0.1% aqueous peptone and a few drops of antifoam (tributyl citrate) in a sterilized 8 oz (ca. 226.8 g) Osterizer jar. After further serial dilution, also in the sterile 0.1% peptone, samples were plated out on selected agar media by either pour or spread plate techniques and incubated 2 days at 30 C. Total yeast counts were determined by using either Mycophil agar (BBL), adjusted to *p*H 4 after autoclaving with 20% lactic acid, as the plating medium or by using yeast extract (0.5%)-glucose (1.0%)-Trypticase (1.0%) agar. APT (BBL) agar, containing 100 μg of cycloheximide (Actidione) per g added before autoclaving, was used to test the ability of yeasts to grow in the presence of cycloheximide (5, 8). Studies showed that cycloheximide added in this manner is effective in inhibiting the growth of those yeasts susceptible to it if autoclaving is kept to a minimum (15 min at 120 C) and

TABLE 1. *Carbohydrate utilization patterns indicating two types of sour dough yeasts*[a]

Yeast	Assimilation[b] of						Growth in presence of cycloheximide
	Glucose	Sucrose	Lactose	Galactose	Maltose	Raffinose	
Sour dough type 1	+	+	−	+	−	+	+
Sour dough type 2	+	+	−	−	+	+	−
Bakers'	+	+	−	+	+	+	−

[a] Liquid media were prepared by the method of Wickerham (9). Two hundred isolates were tested (40 from each of 5 sources).

[b] +, Growth, acid and gas; −, no growth.

the media are used within 2 months of preparation. The sour dough bacteria described in a subsequent publication (3) did not grow on any of the above media and hence did not pose a problem in the yeast isolation and enumeration.

Identification of yeast isolates. Approximately 40 colonies for each of the five sources were picked from the Mycophil plates, transferred to Mycophil (*p*H 4) slants, and incubated 24 to 48 hr at 30 C. A loopful was then transferred to wort broth (BBL) which, after being incubated 18 to 24 hr at 30 C, was used as the inoculum in preliminary screening tests for carbohydrate assimilation or fermentation, or both, according to the procedure of Wickerham (9). Approximately 200 isolates were tested in this manner on glucose, sucrose, galactose, lactose, maltose, and raffinose. As will be shown, the yeast isolates appeared to fall into two types. Sixteen of the predominant type from four different sources and five of the other type, all from a single source, were then subjected to the more rigorous identification procedures described by Lodder and Kreger-van Rij (6) and the supplementary procedures of Lodder (5). Ability to assimilate various carbon compounds in these tests was measured by inoculating the yeasts on solid carbon assimilation medium composed of 0.7% yeast nitrogen base (Difco), 0.5% of the carbon compound to be tested, and 2% agar. Inoculation was done with a multipoint inoculating device as modified from Beech et al. (1). Where necessary, the liquid medium technique of Wickerham (9) was used to confirm assimilations obtained on the solid medium. Sporulation was tested by incubating 14 days on Kleyn's acetate agar and V-8 agar.

RESULTS

The 200 yeast isolates from five sources were found to fit into one of two types (Table 1). The predominant type (sour dough no. 1) was unable to assimilate maltose but utilized other sugars, even in the presence of cycloheximide. The other type (sour dough no. 2) was unable to utilize galactose, and its growth on other sugars was inhibited by cycloheximide. A strain of bakers' yeast, isolated from commercial compressed yeast, was also tested for comparative purposes and, as expected, was able to utilize both maltose and galactose, thus being readily distinguished from the sour dough yeasts.

The distribution of the two types of yeast isolates recovered from the five sources was determined in two ways. First, the 40 isolates from each source were grouped by their fermentation characteristics and cycloheximide-resistance (Table 2). The maltose-negative type which grew well in the presence of cycloheximide was found to be the only yeast present in four of the five sources

TABLE 2. *Distribution of two types of sour dough yeasts by source (Bakery)*

Source	No. which were		
	Maltose-negative[a]	Galactose-negative[b]	Bakers'
T	40	0	0
C	40	0	0
P	40	0	0
L	40	0	0
B	28	12	0

[a] Sour dough yeast type 1 (Table 1).

[b] Sour dough yeast type 2 (Table 1).

TABLE 3. *Characteristics used in identifying 16 yeast isolates as Saccharomyces exiguus and five as S. inusitatus*[a]

Assimilation or reaction on	*S. exiguus*[b] (imperfect form)	*S. inusitatus*
Glucose	F	F
Galactose	F	−
Maltose	−	F
Sucrose	F	F
Trehalose	+	+
Melibiose	−	F*
Melezitose	−	+
α-Methyl-D-glucoside	−	+
Glucono-δ-lactone	W	−
DL-Lactic acid	−	+
Ethyl amine	−	−
Growth at 30 C	+	+
Growth at 37 C	−	+
Growth on 100 μg of cycloheximide per g	+	−
Production of acid	W	W

[a] Identification procedures established by Lodder (5).

[b] Symbols: F, fermentation and assimilation positive; +, positive assimilation or growth reaction; W, positive but weak; −, no assimilation or growth; and *, ferments only half of the disaccharide.

and the predominant type in the fifth. Differential counts in the sour doughs were also run on various agars as follows. The maltose-negative type was specifically isolated and enumerated on the APT agar (BBL) containing 100 μg of cycloheximide per g. The galactose-negative type was determined either by difference between the counts on Mycophil and APT-cycloheximide agars or specifically on a medium containing yeast extract (0.25%), Trypticase (0.5%), and maltose (0.25%). The level of maltose was kept low in this medium so that its impurities would not promote the growth of the other yeast type. These differential counts over a period of time confirmed the distribution of the two types of yeast in the five sources as established by the fermentation tests. Thus, for the four sources containing only the maltose-negative cycloheximide-resistant yeast, counts determined on the APT-cyclohexim ide agar were always as high as or higher than those determined on Mycophil agar. The proportions of the galactose-negative strain in the fifth source averaged out about 30% as determined either by the difference between the counts on Mycophil and APT-cycloheximide agars or specifically on the yeast extract-Trypticase-maltose agar.

Sixteen of the maltose-negative isolates selected from four different sources and five of the galactose-negative isolates from the one source were subjected to the more rigorous taxonomic testing illustrated in Table 3. Based on these characteristics, the maltose-negative isolates were all identified as the imperfect form of *Saccharomyces exiguus* (*Torulopsis holmii*). Ascospore formation was not observed. The galactose-negative isolates were all identified as *Saccharomyces inusitatus*. Representative cultures of each type have been deposited in the culture collection at the USDA Northern Regional Research Laboratory, Peoria, Ill.

DISCUSSION

The presence of *S. exiguus* in sour doughs to the virtual exclusion of other yeasts and the correlation of its presence and numbers with the leavening function in San Francisco sour dough as described in an earlier report (7) appear to establish its role in this system.

Its presence after so many years of being subcultured in the sour dough can probably be attributed, in part, to its vigor in this particular acidic environment which contains a substantial proportion of acetic acid in the *p*H range of 3.8 to 4.5. However, in view of the extremely unusual ability of this sour dough system to resist invasion by other microorganisms, the possibility is also suggested that *S. exiguus* may coexist with the sour dough bacteria because of its resistance to an antibiotic substance produced by the latter.

Also contributing to the successful coexistence of *S. exiguus* with the sour dough bacteria is the observation that the latter require or greatly prefer maltose which *S. exiguus* does not utilize. Thus, they are not competitive for the same carbohydrate source in the dough. No carbohydrates are added in the formulation of this sour dough and the maltose, utilized by the bacteria, is formed in the dough by amylase action on free starch. The *S. exiguus* utilizes the approximately 2% of non-maltose carbohydrates contained in the flour.

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