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Microorganisms of the San Francisco Sour Dough Bread Process

II. Isolation and Characterization of Undescribed Bacterial Species Responsible for the Souring Activity

LEO KLINE AND T. F. SUGIHARA

Western Regional Research Laboratory, Western Utilization R and D Division, U.S. Department of Agriculture, Albany, California 94710

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A medium was developed which permitted isolation, apparently for the first time, of the bacteria responsible for the acid production in the 100-year-old San Francisco sour dough French bread process. Some of the essential ingredients of this medium included a specific requirement for maltose at a high level, Tween 80, freshly prepared yeast extractives, and an initial *p*H of not over 6.0. The bacteria were gram-positive, nonmotile, catalase-negative, short to medium slender rods, indifferent to oxygen, and producers of lactic and acetic acids with the latter varying from 3 to 26% of the total. Carbon dioxide was also produced. Their requirement for maltose for rapid and heavy growth and a proclivity for forming involuted, filamentous, and pleomorphic forms raises a question as to whether they should be properly grouped with the heterofermentative lactobacilli.

Previous reports (3, 8, 9) describe the nature of the San Francisco sour dough process and also identify the naturally occurring yeast moiety of the system, *Saccharomyces exiguus*, as responsible for the leavening action in the dough. The present report deals with the isolation, apparently for the first time, of undescribed, closely related lactobacilli-type strains of bacteria from various sources of sour dough and some of their properties in pure culture. Evidence supporting the role of these bacteria in the souring action in these doughs is also described elsewhere (3, 8).

MATERIALS AND METHODS

The medium developed for isolation of the sour dough bacteria from the doughs and used for pure culture broth studies is shown below. For convenience it is referred to as SDB (sour dough bacteria) agar or broth and was autoclaved before use. It contained the following: maltose, 2.0%; yeast extract (Difco), 0.3%; fresh yeast extractives (FYE), 0.5 to 1.5%; Tween 80, 0.03% (3 ml of 10% solution/liter); and Trypticase (BBL), 0.6%. The *p*H was adjusted to 5.6 with 20% lactic acid or 1 N to 6 N HCl. The FYE were usually prepared by autoclaving a 20% suspension of commercial compressed bakers' yeast in distilled water for 30 min at 15 psi, allowing the suspension to settle overnight at 2 to 8 C, decanting, and further clarifying the supernatant by centrifugation. The extract prepared in this manner contained 1.5% solids and, if not to be used within a few days, was frozen or freeze-dried immediately. Alternately, the FYE could be prepared by autolyzing a 1:1 suspension of compressed yeast in distilled water under Toluene for 3 days at 50 to 55 C, heating to boiling, and clarifying. For maximum cell yields in broth culture, a level of 1.5% FYE was used in the medium; otherwise, 0.5% sufficed.

For isolation and enumeration of the bacteria from and in the doughs, 11 g of dough was blended with 99 ml of sterile 0.1% peptone as described in the previous paper for the sour dough yeasts (9). These yeasts did not interfere with enumeration of the bacteria on SDB agar as they occurred in numbers 1/50 to 1/100 those of the bacteria, and the yeast colonies, generally one to two at the most per plate, were easily distinguishable. Spread plating of 0.1 ml portions of the appropriate serial dilution was used in preference to pour plates as it was found to provide better access to the stimulatory effects of gaseous CO_2. Incubation of SDB agar plates was generally for 2 days at 31 C in an atmosphere containing 25 to >90% CO_2.

Pure culture isolates were maintained on slants of SDB agar, transferred approximately once a month, and held at 13 C between transfers. Viability was retained better at this temperature than at 2 to 4 C. Isolates were made from each of five sources (bakeries) and were designated according to the source as strains L, P, C, B, or T.

SDB broth cultures were inoculated with 0.1 to 1.0% of a fresh 18- to 36-hr broth culture, flushed with CO_2, closed off, and shaken slowly during incubation at 30 to 31 C for a period of 1 to 2 days. Growth was estimated by turbidity or by cell volume determined on

a 10-ml sample subjected to centrifugation at 1,500 × g in a tapered tube calibrated in 0.1-ml divisions. This centrifugal force seemed to suffice due to the tendency of the cells to assume various degrees of asymmetry and to clump in broth cultures not neutralized during growth. Plate counts gave inconsistent results, apparently due to this tendency to clump.

Examination of the morphology of bacteria from agar plate colonies and broth cultures was routinely made on wet mounts by using a Zeiss phase microscope at 1,200× magnification.

Lactic and acetic acid were determined essentially by the column chromatography method of Wiseman and Irvin (11) with sucrose-Celite as the adsorbent and alphamine red-R as internal indicator to follow elution of the separated acid bands which were then titrated to a cresol red end point with 0.01 N NaOH. The column was capped with a mixture of sodium sulfate, Celite, and ammonium sulfate which permitted direct addition of culture filtrates after acidification with sulfuric acid without further purification. Doughs were analyzed by preparing alkaline aqueous extracts similar to the procedure of Cole et al. (1). These extracts, after centrifugation and acidification, also could be added directly to the column. In both cases, the sample being analyzed was mixed vigorously with the capping material directly on the column before elution procedures were begun.

RESULTS

Nutritional (general). Microscopically, the sour dough bacteria are readily observed in slurries made from the doughs as slender rods, short to medium length, in numbers roughly 30 to 100 times those of the sour dough yeast cells. The latter were easily counted on conventional media (8, 9) and numbered approximately 2×10^7 g of fully developed dough so that the bacteria appeared to be present in numbers of the order of magnitude of about 10^9. However, all attempts to isolate or grow these bacteria on conventional media of numerous sorts were unsuccessful. These included various plate count, milk, tomato, and orange juice, anaerobic, wort, soy, thioglycolate, coliform, and acetic acid bacteria agars as well as media used specifically for enumeration of lactic acid or other bacteria in flours and doughs (2).

The development of the medium for successful isolation of the sour dough bacteria was based on simulating some of the conditions of the natural environment in the sour dough. Thus, maltose, which is essential, was provided as the carbohydrate source since the yeast *S. exiguus*, with which these bacteria coexist, does not utilize maltose. Maltose then becomes the principal carbohydrate available in the sour dough system which is formulated without addition of sugars. (Maltose is produced after the dough is formed due to the action of amylase on free starch.) Similarly, the need for FYE and stimulation of growth by CO_2 was predicated on the fact that the system contains an active yeast. The use of Tween 80 is related to the dual consideration that this compound, or other unsaturated fatty acids, are stimulatory for many lactic acid bacteria and that flour contains about 1 to 1.5% of lipid, and two-thirds of the fatty acids in this lipid are unsaturated (5). The adjustment of the *p*H of the medium to below 6 stems from the observation that the *p*H range encountered in the starter sponge, the natural vehicle for carrying these bacteria in the bakery, is limited to the narrow range of 3.8 to 4.5 (3). Other considerations in the development of this medium include the deletion of sorbic acid from some media commonly used for lactics (2), as this compound completely inhibits the growth of the sour dough bacteria at a concentration of 0.1% (at *p*H 5.6).

The combination of nutritional factors necessary for successful isolation of the sour dough bacteria will thus be shown to include maltose, Tween 80, FYE, and an initial *p*H below 6; accordingly, the lack of success in isolating them on known media becomes understandable.

The nutritional effects of some of these factors are described in more detail.

Requirement for maltose. By using the SDB agar and the B source of sour dough, i.e., from bakery B, no bacterial growth was obtained when the following carbohydrates or substrates were substituted for maltose: xylose, arabinose, glucose, galactose, lactose, sucrose, raffinose, rhamnose, lactate, and ethanol. In the case of xylose and glucose, this was also true even when they were filter-sterilized. The same pattern was observed on three other sources of the bacteria or strains (Table 1), thus confirming the need by all strains for maltose, at least as far as initial isolation from

TABLE 1. *Evaluation of various carbohydrates for isolation of sour dough bacteria (SDB)*[a]

Strain	Growth on carbohydrates tested							
	Xylose	Arabinose	Glucose	Galactose	Sucrose	Maltose[b]	Rhamnose	Raffinose
B	−	−	−	−	−	+	−	−
P	NT[c]	−	−	−	−	+	NT	NT
L	−	−	−	−	−	+	NT	NT
T	−	NT	−	−	NT	+	−	−

[a] Sour doughs (starter sponges) from different sources plated out on SDB agar prepared with 1% of the various carbohydrates.

[b] Counts on fully developed doughs plated out on maltose-SDB agar ranged from 70×10^7 to 300×10^7 per g of dough.

[c] NT, not tested.

TABLE 2. *Effect of maltose level on growth and pH drop in broth cultures*[a]

Maltose level (%)	Strain L (24 hr)			Strain C (41 hr)		
	pH	Cell vol (ml/10 ml of culture)	Turbidity[b]	pH	Cell vol (ml/10 ml of culture)	Turbidity
0.10	4.50	0.02	0.08	5.07	0.01	0.09
0.25	4.45	0.03	0.23	4.80	0.02	0.15
0.50	4.40	0.07	0.47	4.48	0.04	0.30
1.00	4.15	0.11	0.63	4.17	0.06	0.35
1.50	4.15	0.11	0.60	4.10	0.08	0.39
2.00	4.15	0.10	0.62	4.10	0.07	0.39

[a] Containing 1.5% fresh yeast extractives in sour dough bacteria (SDB) broth.
[b] Optical density measured at 525 nm in Bausch and Lomb Spectronic 20 after first diluting culture with distilled water 1:5. Turbidity figures corrected for turbidity in uninoculated SDB broth as a function of pH.

the sour doughs was concerned. No difference in growth response with one strain was observed with maltose from six different commercial sources, minimizing the possibility that the growth requirement was contributed by an impurity in the maltose.

The concentration of maltose required for heavy growth appeared to be unusually high, suggesting that a permeability or other effect might be involved. On agar plates, growth rate and colony size for one strain tested (B) were noticeably greater at 2.4% maltose than at 1.0%. In broth culture (Table 2), growth was slight at 0.10 to 0.25% maltose and increased sharply up to 1.0%. In other studies, additional slight increases in growth and acid production have been observed with increasing maltose levels up to 2.0%, the level adopted for the SDB medium.

Studies in progress indicate, however, that some of the strains subcultured in pure broth culture may adapt slowly to glucose, but, again, a high concentration (>1.0%) is required for good growth and there is considerable variation among the strains in this respect.

FYE. Without the use of any FYE solids, growth was very slow, requiring 4 to 6 days for colonies to achieve sufficient size to be enumerated, and irregular, both in rate of growth and shape of colonies. Increasing the level of other ingredients such as commercial dried yeast extract or casein hydrolysate (Trypticase) did not substitute for the FYE nor did addition of sodium acetate (0.05 to 0.30%) or vitamin B12 (2.5 μg/ml). A level of 0.5% FYE gave adequate growth for most purposes, such as enumeration of colonies by plate counts after 2 days of incubation, but growth was markedly further stimulated by increasing the level of FYE to at least 1.5%. This is illustrated in Table 3 for two strains grown in broth where the cell volumes are 2 to 3 times greater at 1.5% FYE than at 0.5%. The components in the FYE essential for vigorous growth remain to be determined. Freeze drying and storage of the solids in air at refrigerator temperatures was a satisfactory way of preserving the growth-stimulating properties for at least several months.

Tween 80. As tested on the B and L strains, no growth was observed on SDB plate agar after 2 days of incubation when the Tween 80 was omitted from the medium. With strain B, a level of 0.02% (2 ml of 10% Tween 80/liter) was necessary for good growth on SDB plate agar and 0.035 to 0.05% was necessary for heavy growth. Accordingly, the SDB medium was formulated with 0.03% Tween 80. No other unsaturated fatty acids or their esters were tested.

Trypticase and commercial yeast extract. Trypticase at a 1.0% level and commercial dried yeast extract at a level of 0.3% were empirically included in early attempts to devise a medium for isolation of the bacteria and were present when the first weak growth was observed. Several commercial dried yeast extracts and autolysates were tested; they varied in their growth-stimulation properties although none of them produced good growth. However, since a rather high level of

TABLE 3. *Effect of level of FYE on growth in broth cultures*[a]

FYE (%)	Cell vol (ml)	
	B strain (24 hr)	L strain (24 hr)
0.25	0.03	0.04
0.50	0.04	0.08
1.00	0.07	0.12
1.25	0.10	0.13
1.50	0.13	0.18

[a] FYE, freshly prepared yeast extractives.

TABLE 4. *Effect of Trypticase level in SDB agar on isolation of bacteria from sour dough (B strain)*

Trypticase (%)	Colony appearance and size[a]	Count[b] (per g of dough)
0	Small, faint	110
0.2	Medium	115
0.6	Large, heavy	160
1.0	Large, heavy	177

[a] After 2 days at 31 C.
[b] Values to be multiplied by 10^7.

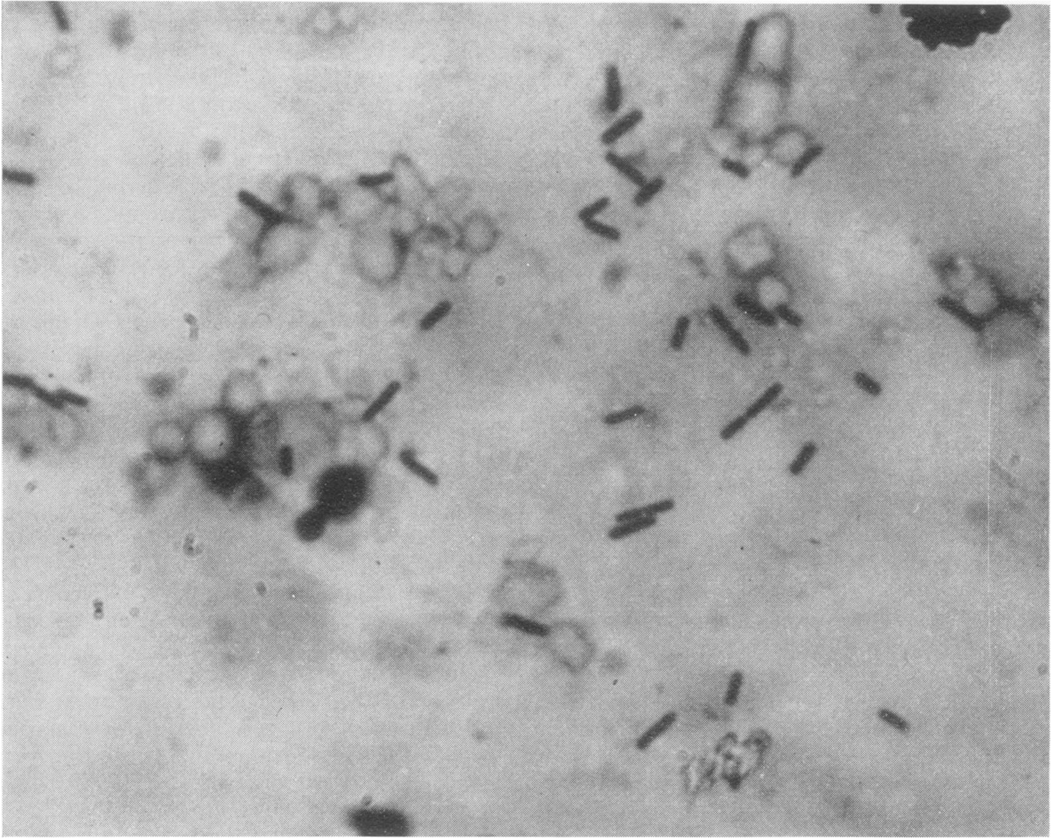

FIG 1. *Photomicrograph of aqueous slurry prepared from sour dough (starter sponge source C) showing numerous bacteria and a few yeast cells.* × *1,000. Crystal violet.*

FYE solids is necessary for heavy growth, the commercial yeast extract was arbitrarily left in the medium for its minor contribution. Trypticase appeared to be more significantly stimulatory for growth, at least for the one strain tested (Table 4). Both the colony size and bacterial count increased up to a 0.6% Trypticase level which was then adopted for use in the SDB medium.

Gaseous atmosphere. The bacteria appeared more or less indifferent to oxygen as they grew out just as well under anaerobic conditions, providing CO_2 was present. Early studies, particularly before formulation of the SDB medium was completed, showed marked beneficial effects of CO_2. Colonies developed on plate agars much more rapidly in the presence of 25 to >90% CO_2 as compared to air and even the latter was more beneficial than a nitrogen atmosphere. However, with the completed medium the stimulatory effects of CO_2 were found to be variable both from time to time for a single strain and to vary between strains. When CO_2 was beneficial, a level of at least 25% was frequently found to give maximum growth stimulation. Accordingly, for most studies, either a flush with CO_2 or a level of 25 to >90% CO_2 in the atmosphere was employed. Addition of bicarbonate as a source of CO_2 was not considered due to the requirement of the medium for a low *p*H (<6).

General properties. The sour dough bacteria, in the stage of active early growth in either the dough or pure culture and before the total acidity is developed, appear as short to medium slender rods or very short chains with only a minor tendency to form bent or filamentous forms (Fig. 1, 2). However, they showed an unusual proclivity for assuming involuted, filamentous, and occasionally pleomorphic forms even in relatively young cultures (24 to 48 hr). On plate agars, when vigorous, they grow out as smooth, round, translucent colonies of about 1 mm or less diameter. They are gram-positive in the early stages of growth, nonmotile, and catalase-negative, as indicated by lack of gas evolution when a 10% solution of H_2O_2 is poured on the colonies on a spread agar plate. In the doughs, approximately 70 to 80% of the

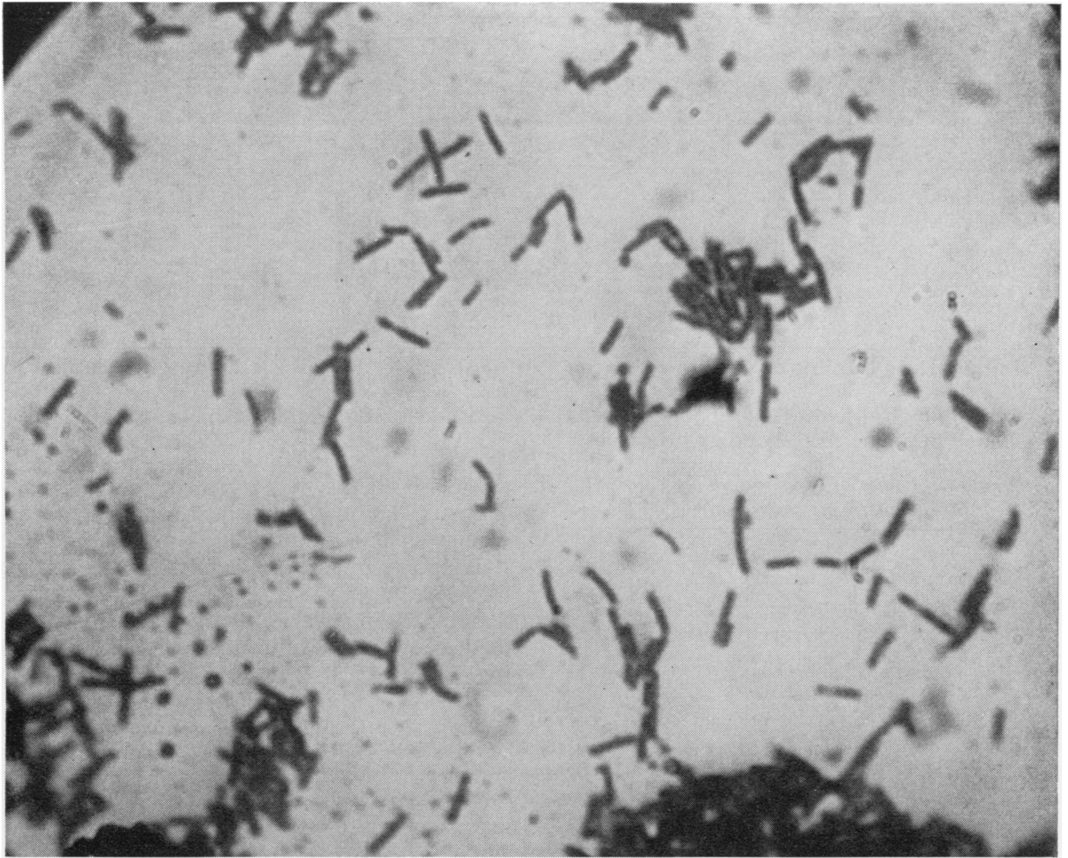

FIG 2. *Photomicrograph of pure 24-hr broth culture of sour dough bacteria.* × *1,000. Strain C, crystal violet.*

total organic acidity produced in the natural system is lactic; the remainder is acetic (3). The proportion of acetic acid is somewhat lower in pure cultures and appears to vary between strains. Gas is produced by all strains in pure culture and is virtually all absorbed by saturated $Ba(OH)_2$, indicating that it is probably CO_2. These bacteria are mesophilic and do not exhibit any unusual heat or salt tolerance. A detailed description of some of these properties follows.

Heat-resistance and salt-tolerance. SDB broth cultures (12 and 24 hr) of three different strains (B, L, and T) were subjected to submersion in a bath at 60 C for 15 min in a manner which included a 3- to 4-min come-up time. The 12-hr cultures had counts varying from 19×10^7 to 34×10^7 cells per ml and the 24-hr cultures, 150×10^7 to 160×10^7 per ml. No viable survivors were found in any instance at the greatest dilution tested ($<10^6$), suggesting that these bacteria do not possess any unusual thermal tolerance.

Four strains (B, L, T, and C) were tested for growth in SDB broth containing 4 or 6.5% NaCl. No development of turbidity was evident even after 4 to 6 days of incubation, suggesting no unusual salt tolerance. Salt at a much lower con-

TABLE 5. *Effect of temperature on growth*[a]

Temp (C)	Growth on slants[b] at day			Growth in SDB broth (0.5% FYE)[c]							
				Cell vol (ml of cells/10 ml of culture) at day				*p*H at day			
	1	2	3	1	2	3	6	1	2	3	6
13	−	±	+1	0	0.03	0.04	0.07	5.2	4.5	4.2	3.9
24	+2	+3	+4								
31	+3	+4	+4	0.09	0.14	0.15	0.14	4.2	3.9	3.8	3.7
37	−	+1	+2								
45				0	0	0	0	5.1	5.2	5.2	5.3

[a] Data shown are for L strain. Similar results obtained with B and T strains with possible exception of slightly slower growth at 13 C.

[b] Inoculated from fresh slants.

[c] SDB, sour dough bacteria; FYE, fresh yeast extractives.

TABLE 6. *Effect of initial pH on growth*[a]

Initial *p*H	T strain				L strain			
	Cell vol[b] (ml)		*p*H		Cell vol[b] (ml)		*p*H	
	24 hr	48 hr	24 hr	48 hr	24 hr	44 hr	24 hr	44 hr
6.5 (unadjusted)	0.01	0.04	5.6	4.2	0.00	0.10	5.9	4.1
6.0	0.02	0.05	4.3	3.8	0.07	0.12	4.3	3.8
5.5	0.03	0.08	4.1	3.8	0.15	0.13	4.2	3.8
5.0	0.07	0.19	4.1	3.7	0.19	0.17	4.1	3.7
4.5	0.02	0.08	3.9	3.6	0.04	0.09	4.1	3.7
4.0	0.00	0.08	4.0	3.6	0.00	0.00	4.0	3.9

[a] Initial *p*H adjustment of sour dough bacteria broth (0.5% fresh yeast extractives) was made with HCl.

[b] Cell volumes for cultures dropping below *p*H 4.5 were corrected for slight sediment forming in uninoculated media at corresponding *p*H values.

TABLE 7. *Effect of type of acid used for pH adjustment on growth*[a]

Acidified with	Cell vol (ml)							
	L strain[b]		T strain		C strain		B strain	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
HCl.....	0.19	0.17	0.07	0.19	0.07	0.08	0.14	0.14
Acetic...	0.14	0.17	0.04	0.16	0.06	0.09	0.10	0.14
Lactic...	0.08	0.16	0.04	0.12	0.06	0.08	0.12	0.12

[a] Sour dough bacteria broth containing 0.5% fresh yeast extractives adjusted to an initial *p*H of 5.0.

centration (0.5%) does, however, stimulate growth in aqueous flour suspensions.

Optimum temperature for growth. Three strains (L, B, and T) were tested for growth rate both on slants and in broths at temperatures varying from 13 to 45 C (Table 5). They all appeared mesophilic, growing out most rapidly at about 31 C, with growth being very slow at 13 and 37 C. No growth was observed at 45 C.

Optimum pH for growth. As noted earlier, these bacteria exist in their natural habitat within the narrow *p*H range of 3.8 to 4.5; in developing an artificial medium for their growth, it was found necessary to adjust the starting *p*H of the media to below 6. The data in Table 6 suggest an optimum initial *p*H in unbuffered SDB broth of about 5.0 for the strains shown (T and L). Similar results were obtained with the B and C strains. The growth observed at *p*H 6.5 was very slow (virtually none at 24 hr) and may have resulted from the slow production of acid, dropping the *p*H to the more favorable zones. When growth was attempted on plate agars at *p*H 6.5, it was not only very slow but the colonies were highly irregular. The relatively slow growth in broth at *p*H 4.0 to 4.5 may be an artifact because significant turbidity and sediment develops in the uninoculated SDB broth acidified to this region, and it is possible that essential nutrients are being removed.

Comparison of the effects of different acids used for initial *p*H adjustment on the growth of four strains (C, T, L, and B) showed HCl was in

TABLE 8. *Lactic and acetic acid production in pure sour dough bacteria broth cultures*[a]

Strain[b]	Acid production		
	Total (μmole/ml)	Per cent as lactic acid	Per cent as acetic acid
L (5 days)	146.2	96	4
B (2 days)	67.8	75	25
T (2 days)	71.2	91	9
C (5 days)	132.3	90	10

[a] Corrected for lactic and acetic acids in uninoculated medium. Fresh yeast extractives, 1.5%.

[b] Time in parentheses indicates age of culture when analyzed.

TABLE 9. *Gas*[a] *production in SDB broth cultures* (1.5% FYE)[b]

Strain	Turbidity				Per cent gas[c]			
	12 hr	1 day	2 days	3 days	12 hr	1 day	2 days	3 days
B	+2	+3	+4	+4	0	TR	50	75
C	+2	+3	+4	+4	0	0	30	50
L	TR	+2	+4	+4	0	0	30	65
T	+4	+4	+4	+4	0	15	65	75

[a] Virtually completely absorbed by saturated $Ba(OH)_2$.

[b] SDB, sour dough bacteria, FYE, fresh yeast extractives; TR, trace.

[c] Per cent of the volume of the inverted tube occupied by the gas formed.

some instances markedly preferable to lactic and generally slightly preferable to acetic acid (Table 7).

Acid and gas production. In the natural sour dough environment, as mentioned above, acetic acid quite regularly comprises 20 to 30% of the total acidity produced. We have also recently found this to be the case when the sour dough bread is made with pure cultures in place of the usual "starter." However, the proportion of acetic acid produced in pure culture may be somewhat lower and is also highly variable (Table 8). The reason for this variability is not, as yet, apparent. All strains, however, produce approximately the same amount of total acidity.

Substantial gas production, assumed to be CO_2, was observed by the inverted tube method, but its evolution was delayed somewhat even after substantial growth was observed (Table 9). Qualitatively, there does not appear to be a good correlation between the proportion of acetic acid (Table 8) and the amount of CO_2 produced.

Table 9 also illustrates the slower growth of the L strain on the SDB medium. This has been irregularly observed although occasionally difficulty is even encountered in plating this strain out from the dough, and colonies that do appear may be irregular.

DISCUSSION

Slight differences were observed among the four strains (L, B, C, and T) studied in some detail. (P strain appeared the same as the L strain and was not examined further.) The differences were evident in colony appearance, degree of nutritional fastidiousness, i.e., how they grew in the SDB medium, tendency to form elongated or swollen forms, tendency to clump in broth culture, ability to adapt to glucose, and proportions of lactic and acetic acid produced. Thus, nutritionally, the T strain appeared to be the least fastidious and the L strain the most. The latter also exhibited the maximum tendency for clumping in broth and produced the lowest proportion of acetic acid. However, all had the following characteristics in common: gram-positive, nonmotile, slender rods (at times), catalase-negative, indifferent to oxygen, inhibited by sorbic acid; required low *p*H, Tween 80, and FYE for good growth; stimulated by CO_2 on occasion, and produced lactic and acetic acids and CO_2. These characteristics tend to group them with the lactobacilli, in particular with the heterofermentative type. However, their requirement for maltose and proclivity for forming involuted, filamentous, and pleomorphic forms might place them with *Bifidobacterium* or *Actinomyces* whose overlapping with lactobacilli is described by Moore and Holdeman (6). Lactobacilli also, however, can be induced to assume odd shapes (4, 7, 10).

Work is underway at another laboratory (under contract from us) to determine the genetic relationship of these sour dough bacteria to known species of lactobacilli. If our preliminary identification is confirmed, we would like to suggest these bacteria be designated officially as *Lactobacillus sanfrancisco* in honor of their unique role in this uniquely situated process.

Another interesting aspect of this sour dough system containing these bacteria and certain yeasts is its self-protective nature, i.e., its incredible resistance to contamination by other microorganisms which has been maintained for decades. No doubt the acetic acid produced in the *p*H range of 3.8 to 4.5 may contribute to this protection. However, considering the finding that the only yeasts which appear to survive significantly in this system are cycloheximide resistant, we speculate that possibly these bacteria may produce related antibiotics. This postulate is currently under investigation by us.

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LITERATURE CITED

1. Cole, E. W., W. S. Hale, and J. W. Pence. 1963. The effect of proofing and baking on concentration of organic acids, carbonyl compounds and alcohols in bread doughs prepared from pre-ferments. Cereal Chem. 40:260–265.
2. Hesseltine, C. W., R. R. Groves, R. Rogers, and H. R. Burmeister. 1969. Aerobic and facultative microflora of fresh and spoiled refrigerated dough products. Appl. Microbiol. 18:848–853.
3. Kline, L., T. F. Sugihara, and L. B. McCready. 1970. Nature of the San Francisco sour dough French bread process. I. Mechanics of the process. Bakers Dig. 44:48–50.
4. Kojima, M., S. Suda, S. Hotta, and K. Hamada. 1968. Induction of pleomorphism in *Lactobacillus bifidus*. J. Bacteriol. 95:710–711.
5. Meeham, D. K. 1964. Lipids. *In* I. Hlynka (ed.), Wheat chemistry and technology. American Association of Cereal Chemists, Inc., St. Paul.
6. Moore, W. E. C., and L. V. Holdeman. 1970. Gram-positive nonsporeforming anaerobic bacilli. *In* J. E. Blair, E. H. Lennette, and J. P. Truant (ed.), Manual of clinical microbiology. American Society for Microbiology, Bethesda, Md.
7. Nowakowska-Waszczuk, A. 1967. The effect of certain cations on the growth of *Lactobacillus lactis* and *L. delbrueckii*. Acta Microbiol. Pol. 14:145–154.
8. Sugihara, T. F., L. Kline, and L. B. McCready. 1970. Nature of the San Francisco sour dough French bread process. II. Microbiological aspects. Bakers Dig. 44:51–53, 56–57.
9. Sugihara, T. F., L. Kline, and M. W. Miller. 1971. Microorganisms of the San Francisco sour dough bread process. I. Yeasts responsible for the leavening action. Appl. Microbiol. 21: 456–458.
10. Sundman, V., and K. A. F. Björksten. 1958. The globular involution forms of the bifid bacteria. J. Gen. Microbiol. 19:491–496.
11. Wiseman, H. G., and H. M. Irvin. 1957. Determination of organic acids in silage. J. Agr. Food Chem. 5:213–215.