Influence of Starter Cultures Consisting of Lactic Acid Bacteria and Yeasts on the Performance of a Continuous Sourdough Fermenter

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ABSTRACT

The performance of a newly developed, continuously operating sourdough fermentation system is dependent on the attainable metabolic activity of the microorganisms under the processing conditions. Of eight pure cultures of lactic acid bacteria, one with *Lactobacillus brevis* ssp. Lindneri produced the best results, that is, a high, uniform formation of acids and a balanced proportion of acetic acid (23-33%) to total acid. Similarly good results were obtained with *L. fructivorans*. The performance of the fermenter can be improved and stabilized by adding starting cultures of yeasts isolated from sourdoughs. In the given fermentation time, fermenting sourdoughs with added yeast showed a significantly higher acid formation than did those without ($P > 95\%$) added yeast. The accelerated acid formation was due almost entirely to additional production of acetic acid. This effect was independent of the strain of yeast. Yeast additions above a certain level had no further influence.

The task of further developing the Ankerbrot-Reimelt system for the continuous production of sourdough (Vereinigte Nahrungsmittel AG 1979, Foramitti 1982) made it necessary to use microbiological possibilities for the propagation of lactic acid bacteria to be able to optimize both the process parameters of the newly developed fermentation system and the quality criteria of the sourdough that are dependent on the formation of organic acids.

For a better understanding of the problems involved, a short description is given of the fermentation system that can fulfill the requirements of German bread manufacturers. They demand a rapid, successive production of sourdoughs from various flour types and continuous removal of variable quantities of ripe doughs.

The system consists essentially of three fermenters in series connected by two mixers, pumps, and pipes. A heat exchanger is situated behind the first fermenter (Fig. 1) (Meuser et al 1990, *unpublished*). This fermenter is a very slender cylindrical tank through which dough flows continuously. The second fermenter is also cylindrical but has a considerably larger diameter. It is filled from above and emptied from below. It functions essentially as a buffer tank for the cooled dough resulting from the first fermenter. The third fermenter is constructed as a segmented tank, the segments of which are also filled from above and emptied from below.

As to the fermentation process, the operation of the first fermenter is of particular importance with respect to production capacity and the quality characteristics of the sourdoughs. Therefore, they were the aims of the microbiological investigations.

The specific characteristic of fermentation in the first fermentation stage is that the dough is split on leaving the fermenter. One part, after being mixed with flour and water, is fed back into the fermenter through its inlet. The other part represents the yield of the first fermentation stage and is fed into the rest of the system.

For the continuous operation of the first fermenter, it is necessary for the sourdough characteristics that a constant gradient remains between the fermenter inlet and outlet. Thereby, the gradient for the germ count of lactic acid bacteria plays an especially important role. Its value depends on the residence time of the sourdough, the distribution of the mass components, and the generation time of the microorganisms. To maintain a gradient, the residence time of the sourdough must be in harmony with the generation time of the lactic acid bacteria. This is the most important precondition for the continuous operation of the fermenter (Meuser et al 1990). Additionally, it is desirable for the dough to have a higher acidity at the fermenter outlet. This depends on the germ count as well as on the metabolic performance of the lactic acid bacteria. The problems to be solved were associated mainly with the characterization of the functional dependence of the process parameters and the quality characteristics of the sourdoughs on the microorganism flora.

For this purpose, various pure cultures of lactic acid bacteria were characterized with respect to their propagation and acid formation in sourdoughs. This was carried out under laboratory conditions similar to those in the technical plant for the particular parameter under investigation. After characterization, an attempt was made to optimize, both quantitatively and qualitatively, the acid formation of the pure culture found most suitable for a particular purpose. Thus the influence of the sourdough yeasts on the acid formation in particular was examined.

MATERIALS AND METHODS

With regard to the choice of materials and methods for these investigations, special reference is made to procedures published previously (Meuser et al 1990). They form the basis for the present work. Knowledge and experience gained in previous investigations were used in designing the arrangement and extent of this work and are not specifically justified here.

Lactic Acid Bacteria

Eight pure cultures of lactic acid bacteria, subdivided into two groups, were used. Those in group A were isolated from three different continuously operating sourdoughs studied in a previous research project (Table I) (AIF 1988). The species of the pure cultures in group A were determined twice. The first determination was made immediately after isolation and the second, two years

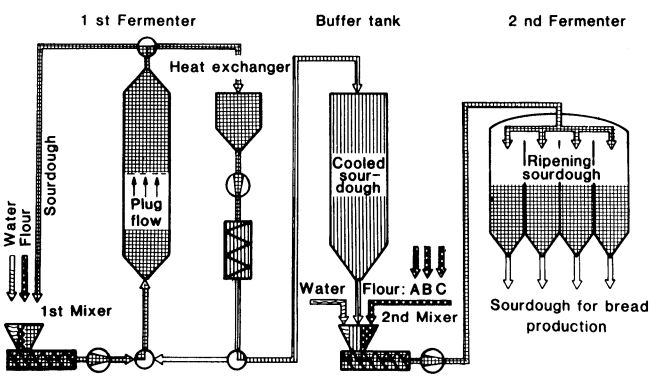

Fig. 1. Fermentation system installed in the Paech bread factory in Berlin.

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later, at the time of the fermentation trials. Differences found between the two sets of results were partly due to inadequacies in the identification method. These differences are especially noticeable when the biochemical recognition characteristics of microorganisms are changed (e.g., as a result of a long storage phase). Three pure cultures were identified as *Lactobacillus brevis* and one as *L. hilgardii.*

The lactic acid bacteria in group B also belong to the sourdough flora. They were selected from the collection at the Federal Research Centre for Cereal, Potato, and Lipid Processing (BAGKF), Detmold U., Münster (Table II). They had never been subjected to continuous fermentation conditions. Among the selected strains was one homofermentative culture (*L. plantarum*) since group A contained only heterofermentative cultures. The heterofermentative cultures in group B were *L. brevis, L. fermentum,* and *L. brevis* ssp. Lindneri. The latter requires special nutrients in the culture medium. It is necessary to use deMan, Ragosa, and Sharpe broth (Merck No. 10661) with additional fresh yeast extract, maltose, and fructose (Table II).

Yeasts from Sourdoughs

To determine the effect of the yeasts on the acid formation of the lactic acid bacteria, those present in the sourdough were isolated. The sourdoughs were prepared using the pure cultures listed in Table III and a rye flour (ash content, 1% dry subtance). The yeasts found in the dough had been present as contaminants of the flour and had propagated themselves during the repeated fermentations (compare number of sourdough sets [SDS]).

Propagation of the Microorganisms and Their Extraction for Use as Starter Cultures

The pure cultures of the lactic acid bacteria were propagated over two incubation periods in a 1,000-ml nutritive medium. In the first stage 10 ml and in the second stage 1,000 ml of nutrient was inoculated with a 1% inoculate and left to incubate at 30°C for 48 hr.

The nutrient medium for the pure cultures (except Li and C1) were prepared as follows: maltose, 20 g/L; peptone, 10 g/L; yeast extract, 10 g/L; gelatine, 10 g/L; Tween 80, 1 g/L; magnesium, 0.1 g/L, and manganese, 0.1 g/L. In all trials, the propagation of the pure cultures Li and C1 were carried out in an MRS broth with fresh yeast extract (approximately 15 g/L dry matter), maltose (7 g/L), and fructose (7 g/L). The other pure cultures that were used in the trials to optimize the acid formation were propagated in an MRS broth (Merck No. 10661).

To obtain the lactic acid bacteria as starter cultures for the sourdough preparation, 600 ml of the 1,000-ml nutrient medium (final incubation stage) was centrifuged (3,000 × *g*). The sediment was homogenized with a solution of common salt (NaCl, 8.5 g/L; peptone from casein, 1.0 g/L). The slurry acted as starter culture for an SDS of 2 kg (1,000 g of rye flour and 1,000 ml of water).

Processing Conditions

To prepare a sourdough with a pure culture, the starter culture was mixed with the necessary amount of water and rye flour (ash content, 1.0% dry substance) by the first SDS. This and the subsequent sourdoughs were fermented under the conditions outlined in Table IV. From the second SDS on, the sourdoughs were processed under conditions corresponding to those of the continuous sourdough fermentation (Meuser et al 1987).

TABLE I
Pure Cultures of Lactic Acid Bacteria Isolated from Sourdoughs (Group A)

Sourdough	Pure Culture	*Lactobacillus* Species: After First Determination	*Lactobacillus* Species: After Second Determination
A	A 1	? (Thermoph.)	*L. brevis*
C	C 1	*L. fructivorans*	*L. hilgardii*
	C 3	*L. delbrückii*	*L. brevis*
D	D 4	*L. cellobiosus*	*L. brevis*

TABLE II
Pure Cultures of Lactic Acid Bacteria Taken from a Culture Collection (Group B)

Pure Culture	Species	Culture Collection	Type of Lactic Fermentation
Br	*Lactobacillus brevis*	397	Heterofermentative
Fe	*L. fermentum*	17	Heterofermentative
Li	*L. brevis* ssp. Lindneri	242	Heterofermentative
Pl	*L. plantarum*	107	Homofermentative

TABLE III
Pure Cultures of Yeasts Isolated from Sourdoughs

Pure Culture	Sourdough Sets	Species
Fe 5/2	5 with *Lactobacillus fermentum*	*Saccharomyces cerevisiae*
Fe 20/1	20 with *L. fermentum*	*S. cerevisiae*
SF 20	20 with *L. sanfrancisco*	*S. cerevisiae* and *Pichia membranefaciens*
D4 17a	17 with D4	*S. cerevisiae* (*S. uvarum*)
Li 20b	20 with *L. brevis* ssp. Lindneri	*S. cerevisiae*
A1 20b	20 with A1	*S. cerevisiae (S. uvarum)*
C1 20b	20 with C1	*S. cerevisiae* (*S. uvarum*)

TABLE IV
Procedure for the Fermentation of Sourdoughs with Pure Cultures

Parameter	Sourdough Sets: First	Second	Third	Fourth	Fifth	Sixth
Inoculum	Bacterial sediment of the culture medium	Sourdough from the preceding set				
Sourdough-dough ratio	About 1 g (dry substance) of bacterial sediment	1:9	1:1	1:1	1:1	1:1
Dough mass, kg	2	2	2	2	2	2
Rye flour ash, % dry substance	1	1	1	1	1	1
Dough dry solids, %	43	43	43	43	43	43
Temperature, °C	30	30	30, 10, 25	30	30	30, 10, 25
Time, hr	16	8	1, 14, 1	3	3	1, 14, 1

Experimental Plan for Determining the Influence of Yeasts on the Acid-Forming Properties of Lactic Acid Bacteria

The starter cultures for the trials were obtained by propagating the required pure strains of lactic acid bacteria in their respective culture media under the specified conditions discussed earlier. Since it had been found that only 200 ml of broth was sufficient to use with the pure strains of lactic acid bacteria to obtain the starter cultures for the production of sourdoughs, these conditions were applied in these trials for the production of three sourdoughs running in parallel, to have a better direct comparison among the different sourdoughs. At the same time, the doughs were processed under standard conditions (sourdough-dough ratio, 1:1; fermentation time, 3 hr; dough dry solids, 43%; fermentation temperature, 30°C) from the second SDS. The sourdough yeasts (SYs) were multiplied in an incubation step of 48 hr at 30°C in a wort broth. Table V gives the details of how many milliliters of wort broth, with the corresponding SY, were added to the first SDS (2 kg) along with the lactic acid bacteria.

To determine the influence of the size of the SY addition on acid formation, three sourdoughs were processed in parallel, whereby two doughs were given different concentrations of SY at the first SDS, while the third was given none. The doughs were processed to at least 10 SDS. *L. fermentum* was used as lactic acid bacteria since, during the increase in the yeast cell count from the 11th to the 17th SDS, this species led to a large increase in both acidity and acid ratio.

TABLE V
Experimental Plan for the Investigation of the Influence of Yeasts on Acid Formation of Lactic Acid Bacteria

	Pure Culture of Lactic Acid Bacteria				
Yeasts	Fe	D2	Li	D4	C1
Fe 5/2	1 ml	...	...	...	...
Fe 20/1	1 ml	...	...	...	...
SF 14	...	1 ml	...	...	...
D4 17 a	...	1 ml	1 ml	1 ml	...
Li 20b	...	...	1 ml	...	...
A1 20	...	...	...	1 ml	1 ml
C1 20b	...	...	...	...	1 ml
Baker's yeast	...	...	1 g	...	...
Yeast extract	...	...	0.5/g/SDS[a]	...	...
Control[b]	X[b]	X	X	X	X

[a]Sourdough set.
[b]X = No yeast added.

The SY strain Fe 20/1 was added in amounts of 1 and 9 ml. Afterwards, the influence of different SYs on the acid formation of various pure cultures of lactic acid bacteria was examined. The individual trial compositions are summarized in Table IV.

For comparison, a common baker's yeast and a yeast extract (Merck No. 3753) were also included in the investigations. The baker's yeast was given only to the first SDS, as was the case for the SY, whereas 1 g of the yeast extract was added to every SDS.

Characterization of the Sourdoughs

The sourdoughs were characterized by measuring the following criteria using standard methods: acidity, pH, lactic and acetic acid content, and germ counts for lactic acid bacteria and yeasts (Meuser et al 1990). The analysis samples were extracted at the times given in Tables VI and VII.

Determination of the Species of Lactic Acid Bacteria

To determine the degree of contamination of the sourdoughs by other lactic acid bacteria and the resultant possible changes to the microflora during the continuous processing, the lactic acid bacteria in the sourdoughs was taxonomically determined. Colonies were isolated from cultures (so-called "cultures in higher level") that had been prepared to determine the germ count of the individual SDS. The colonies were chosen according to their morphologies in the cultures in higher levels. Since the sourdoughs had been set with pure cultures, the colonies in the cultures in higher levels (from which those containing a maximum of 100 colonies were chosen) generally looked very similar. Experience indicated that to make a quantitative assessment of any changes to the microorganism culture, only two colonies from each set needed to be examined. The isolated colonies as well as the pure cultures were examined taxonomically using the API-50 test (API 1986) at the BAGKF.

Statistical Evaluation of the Trials

The influence of the process parameters on acidity and on the rise in acidity were determined by examining the differences between the measured values with the help of the *t* test. The values used were taken from samples of sourdoughs that had been processed for 3 hr. The sourdoughs were set three times a day. Each time, the second SDS was used to produce an SDS that was cooled after 1 hr of fermentation at 10°C. This SDS was used as starter material for the first SDS on the following day. The experiments were carried out on four consecutive days

TABLE VI
Influence of the Amount of Sourdough Sets on Growth and Acid Formation of the Lactic Acid Bacteria of Group A

	Sourdough Characteristics[a]						
					Lactic Acid Bacteria		
Sourdough	Sourdough Set	Rise in Acidity (°A/3 hr)	Acidity After 3 hr (°A)	Acid Ratio (%)	Counts (CFU/g)	Germination Time (min)	Yeast Counts (CFU/g)
A1	5	2.6	8.1	10.1	12.2×10^8	156	...
A1	14	4.4	11.3	21.2	21.3×10^8	255	460.0×10^5
A1	20	4.6	11.9	23.8	16.2×10^8	136	480.0×10^5
C3	5	2.4	8.2	10.0	12.0×10^8	154	...
C3	11	3.0	9.2	14.5	20.1×10^8	218	50.0×10^5
C3	17	3.9	10.4	24.2	17.9×10^8	180	330.0×10^5
C3	20	3.1	9.5	26.1	18.2×10^8	190	470.0×10^5
D4	5	3.0	9.5	12.9	19.9×10^8	135	0.1×10^5
D4	11	2.8	9.3	13.9	18.3×10^8	144	2.8×10^5
D4	17	4.6	11.1	18.7	19.0×10^8	191	290.0×10^5
D4	20	5.0	11.8	...	...	...	...
D4	26	5.4	12.8	...	9.4×10^8	186	260.0×10^5
C1	5	3.6	10.7	23.0	20.4×10^8	199	0.1×10^5
C1	11	4.3	10.9	23.9	23.3×10^8	247	4.4×10^5
C1	14	4.2	10.7	26.0	15.8×10^8	131	46.0×10^5
C1	20	4.2	10.7	33.2	21.9×10^8	266	230.0×10^5

[a]°A = milliliters of 0.1*N* NaOH/10 g of diluted sourdough, acid ratio = acetic acid/(lactic acid + acetic acid) × 100, CFU = colony forming units.

so that the sourdoughs were set a total of 12 times. The measurements of the samples from the noncooled doughs (eight in all) were used for the *t* test calculations.

The statistical calculations were made on an Apple Macintosh Plus personal computer using Systat software (Demonstration Version 3.1) (Wilkinson 1987).

DISCUSSION

It is known that lactic acid bacteria, which are suitable as sourdough starters for application in the fermentation system, must multiply sufficiently during the residence time in the system and also produce enough acids (Meuser et al 1987, AIF 1988, Meuser et al 1990). Under the process conditions used until now, which are to be regarded as standard conditions for the continuous operation of the first stage (sourdough-dough, ratio 1:1; dough dry solids, 43%; fermentation time, 3 hr; rye flour ash, 1% d.s.; fermentation temperature, 30° C), the sourdough, leaving the first stage of the fermenter, has an acidity of approximately 9–12° A, representing a rise in acidity of about 3–4° A/3 hr (° A = milliliters of 0.1*N* NaOH/10 g of diluted sourdough). This acidification is sufficient to bake rye bread with the sourdough. It was found that the acid ratio of 1, which expresses the ratio of acetic acid to lactic and acetic acid, is 20–30%, so that the usually desired bread taste is maintained. During the residence time in stage 1, the microorganisms of the starter culture double in number. As a result, under standard conditions the generation time is the same as the residence time. All the following reported trials with pure cultures are based on these standard conditions, whereby the expression of the chosen characteristics represent the criteria for the selection of a suitable starter culture.

Comparison of the Acid Formation and Propagation of the Lactic Acid Bacteria

Initially, the pure cultures isolated from sourdoughs were examined for lactic acid bacteria. Afterwards, the pure cultures chosen from the collection at the BAGKF were investigated. Because of the different origins of the pure cultures, and to present the findings more clearly, the individual results are reported separately.

Propagation and Acid Formation of the Lactic Acid Bacteria Isolated from the Sourdoughs

The most important analysis results of the propagation and acid formation of the pure cultures of lactic acid bacteria (group A) isolated from sourdoughs are presented in Table VI. To characterize the growth of the lactic acid bacteria, the germ count at the beginning of each fresh SDS is given with the generation time, calculated from their propagation during the fermentation time. In addition, for the characterization of the acid formation, the acidity as well as its rise during the fermentation time of 3 hr and the acid ratio are given. As far as technically possible, pure cultures were examined after the same SDS. Thus, the results in Table VI refer to the number of SDS. Additionally, a yeast count was made to determine whether, during the course of the fermentation, the pure cultures had been contaminated by yeasts from the flour.

For all pure cultures of group A, the acidity and the acid ratio increased during the course of fermentation up to the 26th SDS. In comparison with the standard conditions, the pure culture C1 was found to be especially suitable. Sourdoughs inoculated with this culture had a high acidity level, which remained stable throughout the trials. The same was true for the rise in acidity. The acid ratio, with values of 23–33%, was in the desirable range for production of tasty sourdough breads. Except for a longer adaptation phase of 14 or 17 SDS, the pure cultures A1 and D4 were similarly suitable.

During the trials, the germ count of the lactic acid bacteria in the individual sourdoughs varied widely, as did their generation times (Table VI). It was especially notable that with the pure culture D4, despite an initial generation time of less than 3 hr, the germ count reduced considerably from the 17th to the 26th SDS. In contrast, no reduction in germ count was measured for the pure cultures A1, C1, and C3. However, the germ count as well as the generation time showed large fluctuations, whereby the generation time tended to be longer for larger initial germ counts than it was for the others.

During the experiments, the sourdoughs inoculated with pure cultures of group A were contaminated with yeasts via the flour. The yeasts multiplied in the sourdoughs, and their germ count increased with the number of SDS. For the 20th SDS, the germ count of yeasts for all starter cultures examined was about 10^7 colony forming units per gram. For a few sourdoughs, the yeast multiplication was associated with an increase in acid formation. This result is of interest since it is known that sourdough yeasts can influence the acid formation of lactic acid bacteria (Pelshenke and Schulz 1942, Spicher and Schröder 1978, Spicher et al 1981, Spicher et al 1982).

TABLE VII
Influence of the Amount of Sourdough Sets on Growth and Acid Formation of the Lactic Acid Bacteria of Group B

	Sourdough Characteristics[a]						
					Lactic Acid Bacteria		
Sourdough	Sourdough Set	Rise in Acidity (°A/3 hr)	Acidity After 3 hr (°A)	Acid Ratio (%)	Counts (CFU/g)	Germination Time (min)	Yeast Counts (CFU/g)
Li	5	4.0	10.8	11.6	...*	...*	1.5×10^5
Li	11	3.8	10.6	20.0	0.3×10^8	180	210.0×10^5
Li	17	4.0	11.5	24.3	1.5×10^8	122	320.0×10^5
Li	20	4.7	12.1	23.9	3.6×10^8	209	340.0×10^5
Fe	5	2.0	7.8	8.1	5.4×10^8	178	0.9×10^5
Fe	11	3.2	8.9	8.2	7.9×10^8	183	24.0×10^5
Fe	17	5.2	11.8	24.5	8.1×10^8	182	460.0×10^5
Fe	20	4.9	12.3	23.8	10.9×10^8	348	320.0×10^5
Pl	5	2.8	8.7	1.4	7.0×10^8	142	38.0×10^5
Pl	14	2.9	8.2	1.0	7.8×10^8	265	570.0×10^5
Pl	17	1.9	7.2	1.2	6.8×10^8	184	760.0×10^5
Pl	20	2.6	8.2	1.8	6.5×10^8	203	850.0×10^5
Br	5	3.0	9.0	15.2	6.2×10^8	199	0.5×10^5
Br	11	3.4	9.3	20.2	5.8×10^8	210	6.2×10^5
Br	17	2.9	8.2	22.8	3.9×10^8	165	63.0×10^5
Br	20	2.9	8.7	28.5	2.8×10^8	184	340.0×10^5

[a]° A = milliliters of 0.1*N* NaOH/10 g of diluted sourdough, acid ratio = acetic acid/(lactic acid + acetic acid) × 100, CFU = colony forming units, * = $< 10^7$.

The sourdoughs inoculated with pure cultures were contaminated from the flour not only by yeasts but also by associated lactic acid bacteria. It had to be assumed that these also propagate in the sourdoughs under the conditions applied and that they, too, can influence the fermentation results.

From the taxonomic analysis of the extracted isolates for lactic acid bacteria, species other than those added were found in the four different sourdoughs during the course of the experiments, which lasted for 20 SDS except for sourdough D4, which was run for 26 SDS (Table VIII). For example, the sourdoughs inoculated with the pure culture A1, which had been determined to be *L. brevis,* the two colonies from the fifth SDS were identified as *L. cellobiosus.* At the 11th SDS, one colony was identified as *L. casei* and the other as *L. brevis.* At the 20th SDS, both colonies were found to be *L. brevis.* The pure culture C1 had been identified in the first examination (1989) as *L. fructivorans* (Table I). However, at the second examination it was identified as *L. hilgardii,* although this determination was not definite. In these sourdoughs, initially inoculated with this culture, all samples were found to be *L. fructivorans* except for the two colonies of the 14th SDS. The sourdoughs produced with pure culture C3 were examined at the fifth and the 11th SDS. One colony at the fifth SDS was *L. casei alactosus,* while the others were the same as the inoculated species, *L. brevis.* In the sourdoughs from pure culture D4, the majority of the colonies were *L. brevis.* One colony each of the 11th and 17th SDS were identified as *L. cellobiosus.*

The results demonstrate clearly that the individual pure cultures, although they were classified as the same species in three cases, adapted to different extents to the prevailing fermentation conditions or were contaminated to varying degrees by the microflora present in the flour. The contamination with lactic acid bacteria was of less significance. The selection pressure originating from each pure culture was evidently sufficient over the 20 or 26 SDS to maintain its influence on the sourdough characteristics.

Propagation and Acid Formation of the Lactic Acid Bacteria Originating from the BAGKF Collection

The results for group B were similar to those for group A. This was especially true for the propagation of SY present through contamination of the sourdoughs via the flour and for the propagation of the lactic acid bacteria. Thus, throughout the 10 days during which the experiment was running at a fermentation time of 3 hr, no washing out of the lactic acid bacteria was observed. This is clearly indicated by the values for the germ count of the lactic acid bacteria and their generation times (Table VII).

In contrast to the difficulties encountered with the propagation of the species *L. brevis* ssp. Lindneri in a culture broth, none were encountered with the continuous fermentation of the sourdoughs. The sourdoughs that were inoculated with these species displayed high, stable values for acidity and its increase during 20 SDS, similar to those of the pure culture C1 (group A). Except for the fifth SDS, the acid ratio was also relatively stable at 20–24%. On the other hand, when the pure culture Fe was used, an adaptation phase of 17 SDS had to be taken into account until a similar acid formation could be achieved. The two other pure cultures, Pl and Br, are less suitable due to their low acid formation under standard conditions.

The sourdoughs inoculated with these pure cultures were also examined for possible changes in the microflora during the continuous processing. No changes in the microflora were detected in any of the four sourdoughs (Pl, Br, Fe, and Li) throughout the 20 SDS. However, the sourdough inoculated with *L. brevis* was found to contain *L. hilgardii* since it was not able to ferment melibiose.

TABLE VIII
Determination of the Microflora of the Sourdough Sets Inoculated with Pure Cultures (Group A) at the First Sourdough Set

Sour-dough Set	Pure *Lactobacillus* Culture[a]			
	L. brevis	*L. hilgardii* ?	*L. brevis*	*L. brevis*
5a	*L. cellobisus*	*L. fructivorans*	*L. casei ala.*	L. brevis
5b	*L. cellobisus*	*L. fructivorans*	*L. brevis*	L. brevis
11a	*L. casei ala.*	*L. fructivorans*	*L. brevis*	L. cellobiosus
11b	*L. brevis*	*L. fructivorans*	*L. brevis*	L. brevis
14a	ND[a]	*L. brevis*	ND	ND
14b	ND	*L. brevis*	ND	ND
17a	ND	ND	ND	*L. cellobiosus* ?
17b	ND	ND	ND	*L. brevis*
20a	*L. brevis*	*L. fructivorans*	ND	*L. brevis*
20b	*L. brevis*	NG	ND	*L. brevis*
26a	E	E	E	*L. brevis*
26b	E	E	E	*L. brevis*

[a]ND = not determined, NG = no growth, E = end of investigation, ? = not definitely identified.

Optimization of the Starter Cultures for the Fermentation System

The results of the examination of the propagation and acid-forming properties of the applied lactic acid bacteria showed that the sourdoughs were contaminated with yeasts. In addition, acidity increased with processing time. Therefore, we investigated whether the increasing acid formation was due to adaptation of the lactic acid bacteria on the culture medium or was caused by a synergistic effect of the multiplying yeasts on the metabolic activity of the lactic acid bacteria.

Influence of the Yeasts on the Acid Formation of Lactic Acid Bacteria

It is known that certain yeasts can influence to different degrees the acid formation of particular lactic acid bacteria (Pelshenke and Schulz 1942, Spicher and Schröder 1978, Spicher et al 1981, Spicher et al 1982). For this reason we investigated whether the addition of SY, isolated from sourdoughs, could affect the acid-forming properties of the lactic acid bacteria in the sourdoughs. First we investigated the effect on the acid formation by adding varying quantities of yeast at the first SDS.

To examine the acid formation from the three differently started sourdoughs, each further SDS was analyzed for pH and acidity at the time of setting as well as after 3 hr or after the cold storage. In addition, the third, sixth, and 12th SDS were examined after 3 hr for lactic and acetic acid content. The acidity measurements were averaged and presented graphically for evaluation of the results. Figure 2 shows the average rise in acidity over 12 SDS and the average acidity for the three different doughs.

The results clearly indicate that after a fermentation time of 3 hr, the acidity in the two doughs to which the sourdough yeast Fe 20/1 had been added at the first SDS was higher than that in the doughs without added yeast. Thus, the average acidity value of the sourdoughs with added yeast was 9.9°A, compared with 8.6°A for the other without added yeast. On average, the rise in acidity was 3.3°A/3 hr in the sourdoughs with yeast and only 2.6°A/3 hr in those without.

Interestingly, the higher acidity was due to increased production

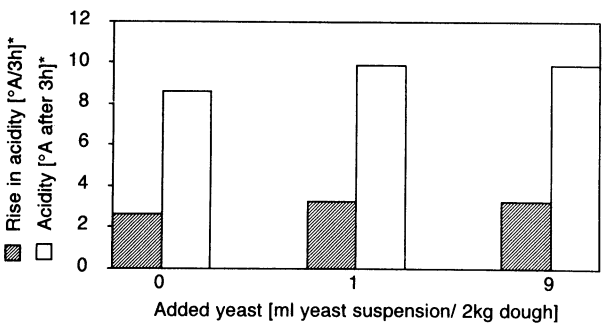

Fig. 2. Influence of the amount of added sourdough yeast Fe 20/1 to the first sourdough set (SDS) on acid formation of *L. fermentum* during the fermentation experiment. °A = ml of 0.1*N* NaOH/10 g of diluted sourdough, * = mean value of the experiment.

of acetic rather than lactic acid. The lactic acid contents of the sourdoughs without added yeast were even higher than of those with yeast (Fig. 3). In the same experimental series, we then examined whether acid formation would also increase in other lactic acid bacteria on addition of SY. The experiments were carried out according to the plan given in Table VI.

The values in Tables IX and X demonstrate that in all experiments, the addition of SY to the doughs led to an increase in acidity as well as an increased rise in acidity. Although the differences in acidity between the sourdoughs with and without SY was statistically significant for all 10 cases ($P \geq 95\%$), this was true in only four cases for the rise in acidity. However, since the statistical probability for three further results was $\geq 90\%$, it can be assumed that, within the given working area, the increased rise in acidity is dependent on the SY.

The highest acidity was obtained by the sourdough produced with the pure culture Li. The difference between the sourdoughs with and without SY addition amounted to about 1° A. The highest calculated value of 1.25° A was found for the sourdoughs with the pure culture D4 and the SY A1 20b. The mean increase in rise in acidity was about 0.5° A. The largest value of 0.74° A was obtained for the same sourdoughs.

The increase in rise in acidity was the lowest for the sourdoughs inoculated with the pure culture C1 and the SYs A1 20b and C1 20b as well as for the pure culture Fe and the SY Fe 5/2. This is indicated by the increasing rise in acidity and the values for the statistical probability for the differences between the sourdoughs with and without SY.

As for the previous experiment (influence of the amount of yeast on acid formation), SY had the greatest influence on the formation of acetic acid. Thus, all sourdoughs to which SY had been added at the first SDS were found to have a higher acetic acid content than those without SY.

This was also true even for the pure culture C1, for which only a small difference in acidity could be determined for the sourdoughs with and without SY addition (Fig. 4). For example, the acetic acid content in the sourdough without SY addition, at the sixth SDS after 3 hr of fermentation was 0.086 g/100 g and 0.130 or 0.139 g/100 g for doughs with added SY.

This result, which is important for the compilation of a starter culture especially suitable for the fermentation system, leads to the question as to whether the observed influence on acetic acid formation can result only from SY or whether it can also be caused by other yeasts and yeast extracts. To answer this question, four doughs inoculated with *L. brevis* ssp. Lindneri were set up

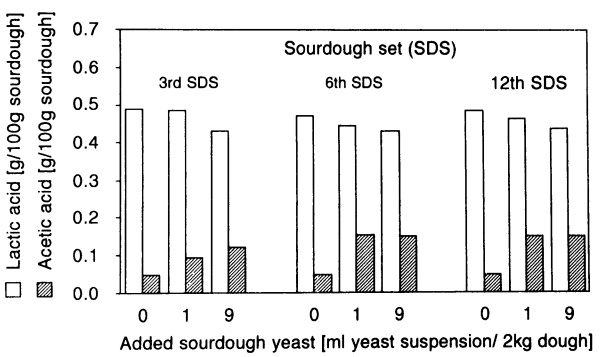

Fig. 3. Influence of the amount of added sourdough yeast Fe 20/1 to the first sourdough set (SDS) on acetic and lactic acid content of the sourdoughs inoculated with *Lactobacillus fermentum*.

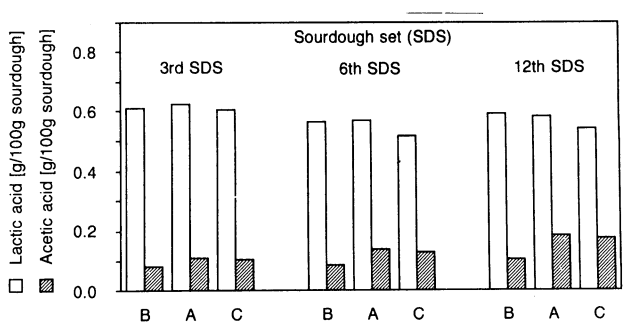

Fig. 4. Influence of the sourdough yeasts A1-20 (A) and C1-20 (C) on lactic and acetic acid content of the sourdoughs inoculated with the pure culture C1 (B). SDS = sourdough set.

TABLE IX
Influence of Sourdough Yeasts on the Mean Acidity Value of Sourdoughs Fermented with Pure Cultures of Lactic Acid Bacteria

	Acidity (° A) and Statistical Probability (*P*)[a] for Pure Cultures of Lactic Acid Bacteria[a,b]					
Yeasts	**Fe 1**	**Fe 2**	**D2**	**Li**	**D4**	**C1**
Control	8.80					
Fe 5/2	9.77					
P, %	99.30					
Control		8.58				
Fe 20/1		9.89				
P, %		99.90				
Control			8.94			
SF 14			10.11			
P, %			99.90			
Control			8.94	10.13	8.81	
D4 17a			10.16	10.71	9.71	
P, %			99.90	99.90	99.60	
Control				10.13		
Li 20b				10.84		
P, %				99.80		
Control					8.81	9.40
A1 20b					10.06	10.41
P, %					99.70	98.90
Control						9.40
C1 20b						10.46
P, %						99.90

[a] *P* = statistical probability of a difference between the mean acidity value of the sourdoughs fermented with and without sourdough yeasts, ° A = milliliters of 0.1*N* NaOH/10 g of diluted sourdough.

TABLE X
Influence of Sourdough Yeasts on the Mean Value for the Rise in Acidity of Sourdoughs Fermented with Pure Cultures of Lactic Acid Bacteria

	Rise in Acidity (° A/3 hr) and Statistical Probability (*P*) for Pure Cultures of Lactic Acid Bacteria[a]					
Yeasts	**Fe 1**	**Fe 2**	**D2**	**Li**	**D4**	**C1**
Control	2.83					
Fe 5/2	2.86					
P, %	27.10					
Control		2.64				
Fe 20/1		3.26				
P, %		99.40				
Control			2.73			
SF 14			3.03			
P, %			94.20			
Control			2.83	3.36	2.54	
D4 17a			3.30	3.76	3.20	
P, %			99.50	92.00	99.80	
Control				3.36		
Li 20b				3.59		
P, %				94.50		
Control					2.54	2.91
A1 20b					3.28	3.06
P, %					99.90	66.80
Control						2.91
C1 20b						2.96
P, %						29.50

[a] *P* = statistical probability of a difference between the mean value for the rise in acidity of the sourdoughs fermented with and without sourdough yeasts, ° A = milliliters of 0.1*N* NaOH/10 g of diluted sourdough.

in parallel, whereby sourdough yeast D4 17a was added to one dough, a baker's yeast to a second, and a yeast extract to a third.

The results for the acetic and lactic acid concentrations in the third, sixth, ninth, and 12th SDS (Fig. 5) clearly show that only the SY and the baker's yeast affected the formation of acetic acid but not the yeast extract. The experimental results for the dough with yeast extract were practically the same as those with only *L. brevis* ssp. Lindneri.

The sourdoughs inoculated with the starter culture Li and the SY D4 17a had the highest acidity (11.24° A) and the largest rise in acidity (3.66° A/3 hr) (Table XI). The acidity achieved by the addition of SY and baker's yeast was significantly different from that of the sourdoughs with and without yeast extract, which didn't differ from one another.

Thus, one can conclude that the presence of living SY or baker's yeast is a necessary precondition for an increase in acetic acid formation within the framework of these experimental conditions.

TABLE XI
Influence of Sourdough Yeast D4 17a, Baker's Yeast, and Yeast Extract on the Mean Values for Acidity and the Rise of Acidity of Sourdoughs Inoculated with *Lactobacillus brevis* ssp. Lindneri

Mean Values[a]	Sourdough Yeast D4 17a	Baker's Yeast	Yeast Extract	Control
Acidity, °A	11.24	10.98	10.60	10.41
Rise of acidity, °A/3 hr	3.66	3.60	3.49	3.35

[a]°A = milliliters of 0.1*N* NaOH/10 g of diluted sourdough.

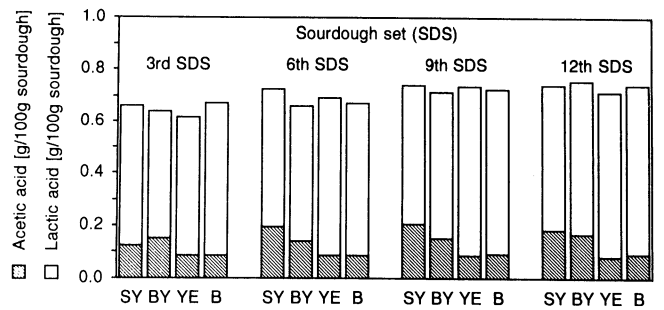

Fig. 5. Influence of the sourdough yeast (SY) D4-17a, baker's yeast (BY), and yeast extract (YE) on lactic and acetic acid content of the sourdoughs inoculated with *Lactobacillus brevis* ssp. Lindneri (B). SDS = sourdough set.

Formula for the calculation of bacterial growth

$$N_t = \frac{N_{max}}{1 + e^{\ln\left(N_{max}/N_0 - 1\right) - t\ 1/g}}$$

Generation time = constant = 40 min

$N_{max} = 1\ 10^9$ —●—*No=3* —◇—*No=5* —○—*No=6* 10^8

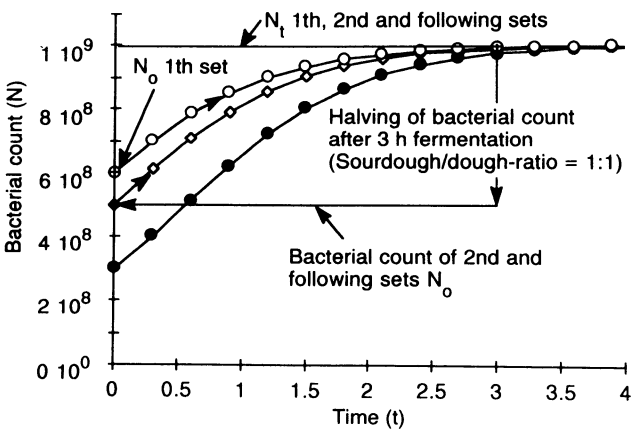

Fig. 6. Theoretical consideration of the influence of the initial bacteria count on the growth of lactic acid bacteria. N = germ count, g = generation time, N_{max} = maximum attainable germ count, t = time, ln = natural logarithm. e = base to the natural logarithm.

CONCLUSIONS

In general, the results obtained, while characterizing the propagation of lactic acid bacteria, confirm that generation time tends to increase with higher initial germ counts. This observation indicates that the propagation of the lactic acid bacteria during the transition phase between exponential and stationary growth phases is self-regulated. This transition phase is characterized by a decrease in growth rate for increasing germ counts. This can be demonstrated theoretically on the basis of a logistical growth model (Edwards and Wilke 1968).

In this model (Fig. 6), the theoretical development of the propagation of a lactic acid bacteria population is presented according to the logistical growth law for different initial germ counts in the area of the transition phase by constant values for the generation time and the maximum attainable germ count. For example, when fermentation begins with a relatively high initial germ count, the germ count can no longer double within a defined period. Since by a sourdough application with an initial sourdough-dough ratio of 1:1 the germ count is halved for each newly set dough, the new SDS has a smaller initial germ count than that of its predecessor. Due to the smaller initial germ count, which is connected to a higher growth rate, the microorganisms multiply more rapidly during the fermentation time so that the germ count is doubled.

Thus it follows that, as long as the residence time is the same as the shortest generation time that a lactic acid bacteria culture can attain in the exponential growth phase, the culture can not be washed out of the dough in the continuous operation of the first stage. One can consider the influence of a reduction of the sourdough-dough ratio on the multiplication of the microorganisms during continuous operation in a similar way. These considerations will form the basis of a further publication concerning the optimization of the performance of the fermentation system, for which not only the propagation of the lactic acid bacteria but also the acid formation must be taken into account.

The results reported here clearly indicate that the pure cultures C1 and Li will be especially suitable for this purpose. The results demonstrate further that the acid formation of these cultures is positively influenced by the presence of an SY with a germ count of about 10^7 colony forming units per gram. This leads especially to a stronger acetic acid formation. Thus, it is advantageous for higher acid production and increased acetic acid formation when the microflora of the continuously fermenting sourdough contain yeasts in addition to lactic acid bacteria. This is of great importance for the compilation of starter cultures for the fermentation system.

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LITERATURE CITED

ARBEITSGEMEINSCHAFT INDUSTRIELLER FORSCHUNGSVEREINIGUNGEN. 1988. FORSCHUNGSVORHABEN 6542, Untersuchung des Einflusses kontinuierlicher Sauerteigführungen auf die Brotqualität. Technische Universität Berlin, Institute für Lebensmitteltechnologie, Getreidetechnologie: Berlin.

APPAREIL ET PROCÉDÉ D'IDENTIFICATION. 1986. API 50 CH und API 50 CHL. API SYSTEM SA: Montalieu Vercieu, France.

EDWARDS, V. H., and WILKE, C. R. 1968. Mathematical representation of a batch culture data. Biotechnol. Bioeng. 10:205.
FORAMITTI, A. 1982. Kontinuierliche und mechanisierte Herstellung von Sauerteig. Getreide Mehl Brot 2:47.
MEUSER, F., FABER, C., and MAR, A. 1987. Continuous sour-dough fermentation with a two-stage pilot fermenter. Page 150 in: Cereals in a European Context. I. D. Morton, ed. Ellis Horwood Ltd. and VCH Verlagsgesellschaft: Chichester, England.
MEUSER, F., FABER, C., VOLLMAR, A., and SPICHER, G. 1990. Studies on acid growth of microorganisms in a continuously operating sourdough fermenter. Food Biotechnol. 4:185.
PELSHENKE, P. F., and SCHULZ, A. 1942. Untersuchungen über Sauerteighefe. Vorratspflege Lebensmittelforsch. 5:154.
SPICHER, G., and SCHRÖDER, R. 1978. Die Bedeutung der "sauerteighefen" für die Sauerteiggärung. Getreide Mehl Brot 32:295.
SPICHER, G., RABE, E., SOMMER, E., and STEPHAN, H. 1981. Die Mikroflora des Sauerteigs. XIV. . Mittl.: Über das Verhalten homofermentativer Sauerteigbakterien und Hefen bei gemeinsamer Kultur. Z. Lebensm. Unters. Forsch. 173:291.
SPICHER, G., RABE, E.; SOMMER, E., and STEPHAN, H. 1982. Die Mikroflora des Sauerteigs. XV. Mittl.: Über das Verhalten heterofermentativer Sauerteigbakterien und Hefen bei gemeinsamer Kultur. Z. Lebensm. Unters. Forsch. 174:222.
VEREINIGTE NAHRUNGSMITTEL AG 1979. Verfahren und Vorrichtung zum Reifen von Sauerteig. Österreichisches Patent 361.423: Vienna.
WILKINSON, L. 1987. Systat: The System for Statistics. Systat, Inc.: Evanston, IL.

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