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Antagonism Between Osmophilic Lactic Acid Bacteria and Yeasts in Brine Fermentation of Soy Sauce

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Brine fermentation by osmophilic lactic acid bacteria and yeasts for long periods of time is essential to produce a good quality of shoyu (Japanese fermented soy sauce). It is well known that lactic acid fermentation by osmophilic lactic acid bacteria results in the depression of alcoholic fermentation by osmophilic yeasts, but the nature of the interaction between osmophilic lactic acid bacteria and yeasts in brine fermentation of shoyu has not been revealed. The inhibitory effect of osmophilic lactic acid bacteria on the growth of osmophilic yeasts was investigated. It was recognized that osmophilic shoyu yeasts such as *Saccharomyces rouxii* and *Torulopsis versatilis* were inhibited by a metabolite produced by osmophilic lactic acid bacteria (belonging to *Pediococcus halophilus*) in brine fermentation of shoyu. The primary inhibitor was considered to be acetic acid, although lactic acid was slightly inhibitory.

Japanese fermented soy sauce, shoyu, is a dark-brown liquid with a salty taste and sharp flavor, which is made by fermenting soybeans, wheat, and salt. It is an all-purpose seasoning agent used in the preparation of foods, as well as a table condiment in Oriental and many other countries. The fermentation of shoyu is at present a two-step batch process involving the biochemical activities of three types of microorganisms: mold, lactic acid bacteria, and yeasts. The first step is to grow koji-mold, *Aspergillus sojae* or *Aspergillus oryzae*, on a mixture of cooked soybeans and roasted cracked wheat. When the mold growth has reached the desired level, the mixture of soybeans and wheat, covered by the mold mycelia, is placed into an 18% sodium chloride solution.

The second step in the preparation of shoyu is brine fermentation by osmophilic yeasts and lactic acid bacteria. During the fermentation period for 6 to 8 months under an appropriate temperature control, the enzymes from the koji mold hydrolyze most of the proteins of the materials to amino acids and low-molecular-weight peptides. Much of the starch is converted to simple sugars, which are fermented primarily to lactic acid, alcohol, and carbon dioxide. The pH drops from an initial value of 6.5 to 7.0 down to 4.7 to 4.8.

The high salt concentration, around 18%, effectively limits the growth to a few desirable osmophilic types of microorganism. Namely, at the first stage of brine fermentation, *Pediococcus halophilus* is grown, and it produces lactic acid to decrease the pH. At the second stage, *Saccharomyces rouxii* is grown, and as a result, a vigorous alcoholic fermentation occurs. At the last stage, other kinds of osmophilic yeasts such as *Torulopsis versatilis* and *Torulopsis etchelsii* are grown successively. These strains produce phenolic compounds which are specific as aroma compounds of shoyu.

Some kinds of yeasts such as *Pichia farinosa*, *Pichia miso*, *Hansenula anomala*, *Cryptococcus diffluens*, *Candida tropicalis*, and *Trichosporon behrendii*, which are not so important in the production of shoyu, are occasionally observed during the first stage of brine fermentation. At the last stage, film-forming yeasts belonging to *Saccharomyces rouxii* var. *halomembranis*, which are harmful to shoyu during use, are sometimes observed.

There are a number of reports indicating that strong lactic acid fermentation results in depression of alcoholic fermentation during the brewing of shoyu (15). However, the cause of the depression of alcoholic fermentation by lactic acid formation during the brine fermentation of shoyu has never been revealed.

Similar findings were reported in various foods such as sausages, hams, and dairy foods (3, 4, 6, 19). The purpose of the present investigation was to study the nature of the interaction between the osmophilic lactic acid bacteria and yeasts in brine fermentation of shoyu.

MATERIALS AND METHODS

Microorganisms. *Pediococcus halophilus* strains 7116 and 7117 were isolated from shoyu mash as lactic acid bacteria of shoyu in our laboratories (21). *Saccharomyces rouxii* IAM 4028 and IAM 4118, *Saccharomyces rouxii* var. *halomembranis* IAM 4558, *Sac-*

charomyces acidofaciens IAM 4752, *Torulopsis nodaensis* IAM 4768, *Pichia farinosa* IAM 4303, *Pichia miso* IAM 4526, *Hansenula anomala* IAM 4253, and *Cryptococcus diffluens* IAM 4875 were obtained from The Institute of Applied Microbiology, Tokyo University, Japan. *Torulopsis versatilis* IFO 0652, *Torulopsis etchelsii* IFO 1229, *Candida tropicalis* IFO 1070, and *Trichosporon behrendii* IFO 0844 were supplied from The Institute for Fermentation, Osaka, Japan. These yeasts had been originally isolated from shoyu mash and are called shoyu yeasts.

Interaction assays. Preliminary assays were conducted in digested liquid mixture of a shoyu-koji which was composed of precooked soybeans and roasted cracked wheat cultured with *A. sojae* KS.

Pediococcus halophilus strains 7116 and 7117 were incubated in the digested liquid mixture, containing 18% sodium chloride and 10% glucose, whose composition was almost equal to that at the beginning of lactic acid fermentation in shoyu production. After adequate incubation at 30°C for 7 days, the cells were removed by centrifugation (4,000 × *g* at 2°C) from the spent medium. Various shoyu yeasts as described above were incubated at 10^6 cells per ml in the spent medium, which was readjusted to 18% sodium chloride and 10% glucose at pH 5.0, and cultured statically at 30°C for 7 days. Various shoyu yeasts were incubated in the digested liquid mixture of shoyu-koji (the unspent medium) as controls. The growth rate was determined by measuring absorbancy at 660 nm, and the difference in absorbancy between the spent medium and the unspent medium indicated possible interaction.

Fractionation of medium and analyses of carboxylic acids. The components contained in the cultured media were fractionated into four fractions as shown in Fig. 1, and each fraction was added to a synthetic medium which was inoculated with shoyu yeasts and incubated at 30°C for 7 days. The medium used consisted of glucose, 100 g; vitamin-free Casamino Acids, 9 g; NaCl, 180 g; KH_2PO_4, 0.6 g; KCl, 0.4 g; $CaCl_2 \cdot 2H_2O$, 0.15 g; $MgSO_4 \cdot 7H_2O$, 0.15 g; $MnSO_4$, 2.5 mg; $FeCl_2$, 2.5 mg; thiamine hydrochloride, 0.25 mg; riboflavin, 0.1 mg; pyridoxine hydrochloride, 1 mg; niacin, 5 mg; *p*-aminobenzoic acid, 0.5 mg; biotin, 10 mg; inositol, 25 mg; calcium pantothenate, 0.5 mg; and tap water, 1 liter (pH 5.0). The effect of the four fractions on the growth of shoyu yeasts was measured in terms of absorbancy difference at 660 nm of cultured media.

The organic acids in the various media were analyzed by a carboxylic acid analyzer (Seishin Pharmaceutical Co., Ltd., Japan).

Cultural test of shoyu yeasts in synthetic medium containing carboxylic acids. *S. rouxii* IAM 4303, *Torulopsis versatilis* IFO 0652, *Torulopsis etchelsii* IFO 1229, *Pichia farinosa* IAM 4303, and *Hansenula anomala* IAM 4253 were incubated at 10^6 cells per ml of the synthetic medium; each culture contained 0 to 0.5% lactic acid, acetic acid, citric acid, malic acid, succinic acid, or formic acid, which are usual components in shoyu. The effect of these carboxylic acids on the growth of shoyu yeasts was determined by measuring absorbancy after cultivation for 7 days at 30°C.

RESULTS

Growth inhibition of shoyu yeasts by culture filtrates of *Pediococcus halophilus*. The growth of various shoyu yeasts incubated in the spent or unspent medium of *Pediococcus halophilus* is shown in Table 1. In all tested shoyu yeasts, the growth in the spent medium was remarkably reduced, compared with the growth in the unspent medium. From this result, the antagonistic action of *Pediococcus halophilus* toward shoyu yeasts was very evident.

The inhibitory effect of culture filtrates of *Pediococcus halophilus* varied with the species

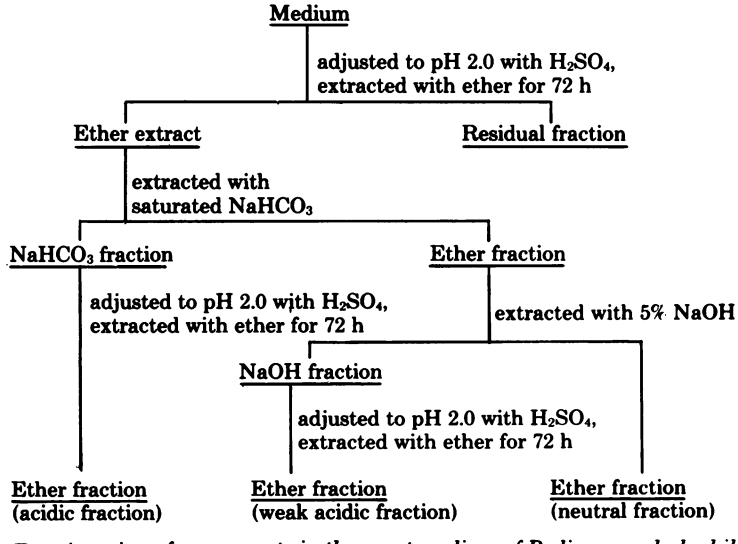

FIG. 1. *Fractionation of components in the spent medium of Pediococcus halophilus 7117.*

of shoyu yeasts: the inhibitory effect of the spent medium of *Pediococcus halophilus* on the growth of tested shoyu yeasts increased in the following order: *Trichosporon behrendii*, *Cryptococcus diffluens*, *Candida tropicalis*, *Hansenula anomala*, *Picha farinosa*, *Pichia miso*, *Saccharomyces acidofaciens*, *Saccharomyces rouxii*, *Torulopsis nodaensis*, *Torulopsis etchelsii*, *Torulopsis versatilis*, and *Saccharomyces rouxii* var. *halomembranis*. This order corresponds fairly well to the flora shift of shoyu yeasts in the brine fermentation of shoyu (1, 14).

TABLE 1. *Inhibitory effect of spent media of Pediococcus halophilus strains 7116 and 7117 on growth of various shoyu yeasts*[a]

Culture	Growth		
	Unspent medium	7116 spent medium	7117 spent medium
Trichosporon behrendii IFO 0844	0.805	0.060	0.065
Cryptococcus diffluens IAM 4875	0.850	0.105	0.117
Candida tropicalis IFO 1070	0.785	0.110	0.115
Hansenula anomala IAM 4253	0.780	0.113	0.117
Pichia farinosa IAM 4303	0.752	0.125	0.130
Pichia miso IAM 4526	0.720	0.142	0.150
Saccharomyces acidofaciens IAM 4752	0.710	0.205	0.235
Saccharomyces rouxii IAM 4028	0.700	0.220	0.245
Saccharomyces rouxii IAM 4118	0.705	0.225	0.240
Torulopsis nodaensis IAM 4768	0.710	0.255	0.250
Torulopsis etchelsii IFO 1229	0.750	0.301	0.305
Torulopsis versatilis IFO 0652	0.725	0.310	0.315
Saccharomyces rouxii var. *halomembranis* IAM 4558	0.705	0.315	0.320

[a] Shoyu yeasts were cultured statically in the unspent or spent media of *Peidococcus halophilus* strains 7116 and 7117 at 30°C for 7 days, and growth rate was determined by measuring absorbance of broth diluted 10-fold at 660 nm.

To examine the inhibitory effect of the spent medium of *Pediococcus halophilus* 7116, *Hansenula anomala* IAM 4253, *Pichia falinosa* IAM 4303, *Saccharomyces rouxii* IAM 4028, and *Torulopsis versatilis* IFO 0652 were incubated in the unspent medium, the spent medium containing 0.1% yeast extract, the spent medium containing 0.1% vitamin-free Casamino Acids, and the spent medium containing 0.1% yeast extract and 0.1% vitamin-free Casamino Acids at 30°C for 7 days (Table 2). Even if vitamin-free Casamino Acids and yeast extract were added to the spent medium, recovery of the growth of shoyu yeasts could not be observed. This result suggested that the inhibitory effect of *Pediococcus halophilus* on the growth of shoyu yeasts was not caused by the consumption of vitamins or amino acids or both in the medium, but caused by the accumulation of some inhibitory substance produced by *Pediococcus halophilus*.

Characterization of inhibitory substances. The metabolite of *Pediococcus halophilus* was considered to inhibit the growth of shoyu yeasts. Therefore, the spent medium of *Pediococcus halophilus* 7117 was fractionated into four fractions: acidic fraction, weak acidic fraction, neutral fraction, and residual fraction as shown in Fig. 1; these were added separately to the synthetic medium. *Pichia miso* IAM 4526, *Saccharomyces rouxii* IAM 4118, and *Torulopsis etchelsii* IFO 1229 were then incubated in these synthetic media at 30°C for 7 days, and the inhibitory effect of the four fractions on the growth of the tested yeasts was examined in terms of optical density of cultured broths at 660 nm (Table 3). The acidic fraction exhibited the strongest inhibitory effect on the growth of the tested yeasts among the four fractions.

TABLE 2. *Effect of Casamino Acids and yeast extract on growth of shoyu yeasts incubated in spent medium of Pediococcus halophilus 7116*[a]

Medium	Growth[b]			
	Hansenula anomala IAM 4253	*Pichia farinosa* IAM 4303	*Saccharomyces rouxii* IAM 4028	*Torulopsis versatilis* IFO 0652
Unspent medium	0.790	0.510	0.820	0.835
Spent medium + 0.1% Casamino Acids	0.130	0.115	0.230	0.295
Spent medium + 0.1% yeast extract	0.125	0.120	0.235	0.305
Spent medium + 0.1% Casamino Acids + 0.1% yeast extract	0.135	0.115	0.235	0.300

[a] Shoyu yeasts were cultured statically in the various media at 30°C for 7 days.
[b] The growth rate was determined by measuring absorbance of broth diluted 10-fold at 660 nm.

The component contained in the acid fraction was recognized to be similar to carboxylic acids (16). Therefore, the carboxylic acids contained in the unspent medium and the spent medium of *Pediococcus halophilus* strains 7116 and 7117 were analyzed (Table 4). The carboxylic acid contained in the unspent medium was recognized to be similar to citric acid. On the other hand, the carboxylic acids contained in the spent medium resembled lactic and acetic acids.

Next, we examined the effect on the growth of shoyu yeasts of the carboxylic acids that are contained in common shoyu (8) and are recognized to be metabolites of *Pediococcus halophilus* (7, 24). The growth rate of *Hansenula anomala* IAM 4253, *Pichia farinosa* IAM 4303, *Saccharomyces rouxii* IAM 4028, and *Torulopsis versatilis* IFO 0652, cultured in synthetic media each containing 0 to 0.5% lactic, acetic, citric, malic, succinic, or formic acid, is shown in Table 5. The growth of the tested yeasts was remarkably inhibited by formic acid or acetic acid. Formic acid was not found in the spent medium of *Pediococcus halophilus*, as shown in Table 4, and the growth rate of the tested yeasts in synthetic medium containing 0.25% acetic acid corresponded fairly well to that in the spent medium that contained about 0.2% acetic acid. Lactic and acetic acid contents in common shoyu were recognized to be about 1% and 0.2%, respectively. From these facts as described above, acetic acid which was produced by *Pediococcus halophilus* from citric acid (abundant in soybeans) (7, 24) was considered as the cause of the microbial antagonism in shoyu fermentation.

The inhibitory effect of acetic acid on the growth of the tested yeasts increased in the order: *Hansenula anomala*, *Pichia farinosa*, *Saccharomyces rouxii*, and *Torulopsis versatilis*, as in the previous test of the inhibitory effect of the spent medium of *Pediococcus halophilus* on the growth of shoyu yeasts. This order was recognized to correspond as well to the flora shift of shoyu yeasts in shoyu fermentation.

TABLE 3. *Inhibitory effect on the growth of shoyu yeasts of four fractions from spent medium of Pediococcus halophilus 7117*[a]

Added fraction	Growth[b]		
	Pichia miso IAM 4526	*Saccharomyces rouxii* IAM 4118	*Torulopsis etchelsii* IFO 1229
None	0.805	0.745	0.730
Acidic	0.170	0.305	0.335
Weak acidic	0.735	0.625	0.688
Neutral	0.810	0.735	0.720
Residual	0.785	0.705	0.725
All four	0.155	0.285	0.297

[a] Various shoyu yeasts were cultured statically at 30°C for 7 days in the synthetic medium or in the synthetic media containing fractions from the spent medium of *Pediococcus halophilus* 7117.

[b] The growth rate was determined by measuring absorbance of broth diluted 10-fold at 660 nm.

DISCUSSION

In general, several kinds of microorganisms are involved in the fermentation or the putrefaction of various foods. When different kinds of microorganisms exist in foods, a phenomenon such as competition or antagonism is observed among these microorganisms.

Most investigation on microbial interactions focuses on the lactic acid bacteria. Some lactic streptococci produce the well-characterized antibiotics nisin and diplococcin (2, 13, 17). Antibiotics produced by lactobacilli also have been reported, including acidophilin and lactocidin from *Lactobacillus acidophilus* (26; J. R. Vakil and K. M. Shahani, Bacteriol. Proc., p. 9, 1965), lactolin from *Lactobacillus plantarum* (10, 11), and lactobacillin, which was later identified as hydrogen peroxide, from *Lactobacillus lactis* (27).

Other workers, however, have claimed that lactic acid (9, 25), hydrogen peroxide (3), or unidentified heat-labile substances (20) are responsible for the inhibitory effects associated with lactobacilli. The leuconostocs, which are component strains of certain mixed-strain starter cultures, have been shown to be inhibitory to *Salmonella gallinarum* and certain

TABLE 4. *Carboxylic acid content in unspent or spent media of Pediococcus halophilus strains 7116 and 7117*[a]

Sample	Carboxylic acid content (%)							
	Pyroglutamic acid	Lactic acid	Acetic acid	Pyruvic acid	Formic acid	Malic acid	Citric acid	Succinic acid
Unspent medium	0.031	0.001	0.010	Trace	Trace	0.011	0.925	0.006
Spent medium (strain 7116)	0.061	0.690	0.215	Trace	Trace	0.020	Trace	0.008
Spent medium (strain 7117)	0.053	0.710	0.195	Trace	Trace	0.021	Trace	0.009

[a] Carboxylic acids in the samples were analyzed by a carboxylic acid analyzer.

TABLE 5. *Inhibitory effect of carboxylic acids on growth of shoyu yeasts*[a]

Acid added	Concn (%)	Growth[b]			
		Hansenula anomala IAM 4253	*Pichia farinosa* IAM 4303	*Saccharomyces rouxii* IAM 4028	*Torulopsis versatilis* IFO 0652
None		0.855	0.840	0.820	0.830
Pyroglutamic acid	0.25	0.860	0.835	0.820	0.820
	0.50	0.855	0.830	0.815	0.825
Lactic acid	0.25	0.840	0.780	0.805	0.800
	0.50	0.800	0.745	0.770	0.785
Acetic acid	0.25	0.115	0.130	0.230	0.240
	0.50	0.060	0.075	0.115	0.130
Formic acid	0.25	0.085	0.095	0.120	0.135
	0.50	0.030	0.050	0.080	0.105
Malic acid	0.25	0.855	0.840	0.815	0.825
	0.50	0.850	0.842	0.810	0.818
Citric acid	0.25	0.715	0.750	0.795	0.800
	0.50	0.695	0.708	0.752	0.770
Succinic acid	0.25	0.680	0.685	0.745	0.765
	0.50	0.645	0.595	0.655	0.704

[a] Various shoyu yeasts were cultured statically at 30°C for 7 days in synthetic medium (pH 5.0) containing 0.25% or 0.5% of various carboxylic acids.

[b] The growth rate was determined by measuring absorbance of broth diluted 10-fold at 660 nm.

other gram-negative bacteria. The main inhibitor produced was identified as acetic acid, which was much more antagonistic than lactic or mineral acids at comparable pH value (22). Pinheiro et al. (18) have shown that acetic acid is responsible for some of the inhibition caused by *Lactobacillus citrovorum*.

In the case of traditional fermentation method of Japanese rice wine, sake, it was reported that sake yeasts (belonging to *Saccharomyces cerevisiae*) are inhibited by lactic acid produced by lactic acid bacteria (*Lactobacillus sake, Leuconostoc mesenteroides*) (23).

In the brine fermentation of shoyu, microbial antagonism has been observed between osmophilic lactic acid bacteria and yeasts; however, this is the first report to examine this microbial antagonism. As a result of this investigation, the inhibitory effect of osmophilic lactic acid bacteria on the growth of osmophilic shoyu yeasts is considered to be due to metabolites produced by the osmophilic lactic acid bacterium *Pediococcus halophilus*. The primary inhibitor contained in the metabolite is acetic acid, although lactic acid is also slightly inhibitory.

Hentges (5) has noted that lower pH causes greater inhibition of *Shigella* by formic and acetic acids. The toxicity of undissociated acetic acid has been recognized for yeasts (12). Therefore, the influence of pH on the inhibitory activity of acetic and lactic acids for shoyu yeasts is now under investigation.

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LITERATURE CITED

1. **Asao, Y., T. Sakasai, and T. Yokotsuka.** 1967. Flavorous components produced by yeast fermentation. J. Agric. Chem. Soc. Japan **41**:434–441.
2. **Berridge, N. J.** 1949. Preparation of the antibiotic nisin. Biochem. J. **45**:486–493.
3. **Dahiya, R. S., and M. L. Speck.** 1968. Hydrogen peroxide formation by lactobacilli and its effect on *Staphylococcus aureus*. J. Dairy Sci. **51**:1568–1572.
4. **Gilliland, S. E., and M. L. Speck.** 1972. Interactions of food starter cultures and food-borne pathogens: lactic streptococci versus *Staphylococci* and *Salmonellae*. J. Milk Food Technol. **35**:307–310.
5. **Hentges, D. J.** 1967. Influence of pH on the inhibitory activity of formic and acetic acids for *Shigella*. J. Bacteriol. **93**:2029–2030.
6. **Itou, H.** 1968. Putrefaction of miso-paste. J. Brew. Soc. Japan **63**:405–409.
7. **Kanbe, T., T. Iwasa, and T. Sakasai.** 1978. Production of organic acids by *Pediococcus halophilus* in soy sauce fermentation. J. Jpn. Soy Sauce Res. Inst. **5**:15–20.

8. **Kanbe, T., Z. Ozawa, F. Noda, and T. Sakasai.** 1975. Determination of organic acids in soy sauce by a new carboxylic acid analyzer. J. Jpn. Soy Sauce Res. Inst. **1:** 142–148.
9. **Kao, C. T., and W. C. Frazier.** 1966. Effect of lactic acid bacteria on growth of *Staphylococcus aureus*. Appl. Microbiol. **14:**251–255.
10. **Kodama, R.** 1952. Studies on lactic acid bacteria. 1. Production of antibiotic substance by lactic acid bacteria. J. Antibiot. **5:**46–49.
11. **Kodama, R.** 1952. Studies on lactic acid bacteria. II. Lactolin, a new antibiotic substance, produced by lactic acid bacteria. J. Antibiot. **5:**72–74.
12. **Levine, A. S., and G. R. Fellers.** 1940. Action of acetic acid on food spoilage microorganisms. J. Bacteriol. **39:** 499–513.
13. **Mattick, A. T. R., and A. Hirsch.** 1944. A powerful inhibitory substance produced by group N streptococci. Nature (London) **154:**551.
14. **Mogi, K., and K. Mogi.** 1968. An observational method of yeast-flora in soy sauce fermentation. J. Agric. Chem. Soc. Jpn. **42:**466–470.
15. **Nagase, I., and K. Koshida.** 1969. On the effect by an addition of the fermentative microorganism in the brewing of soy sauce. Seasoning Sci. Jpn. **16:**21–33.
16. **Noda, F., T. Sakasai, and T. Yokotsuka.** 1975. Effect of ether extract of soy sauce on *Saccharomyces rouxii*. J. Food Sci. Technol. Jpn. **22:**530–535.
17. **Oxford, A. E.** 1944. Diplococcin, anti-bacterial protein elaborated by certain milk streptococci. Biochem. J. **38:** 178–182.
18. **Pinheiro, A. J. R., J. Liska, and C. E. Parmelee.** 1968. Properties of substances inhibitory to *Pseudomonas fragi* produced by *Streptococcus citrovorus* and *Streptococcus diacetilactis*. J. Dairy Sci. **51:**183–187.
19. **Price, R. J., and J. S. Lee.** 1970. Inhibition of *Pseudomonas* species by hydrogen peroxide producing lactobacilli. J. Milk Food Technol. **33:**13–18.
20. **Sabine, D. B.** 1963. An antibiotic-like effect of *Lactobacillus acidophilus*. Nature (London) **199:**811.
21. **Sakaguchi, K.** 1958. Taxonomic studies on *Pediococcus soyae* nov. sp., the soy sauce lactic acid bacteria. Bull. Agric. Chem. Soc. Jpn. **22:**353–362.
22. **Sorrells, K., and M. L. Speck.** 1970. Inhibition of *Salmonella gallinarum* by culture filtrates of *Leuconostoc vitrovorum*. J. Dairy Sci. **52:**239–241.
23. **Tani, Y., M. Shimoide, K. Sumino, and S. Fukui.** 1963. Influence of culture conditions on coagulation of *Saccharomyces sake* occurring in media containing lactic acid. J. Ferment. Technol. Jpn. **41:**445–450.
24. **Terasawa, M., K. Kadowaki, H. Fujimoto, and M. Goan.** 1979. Citric acid metabolism by lactic acid bacteria in *shoyu*-mash. J. Jpn. Soy Sauce Res. Inst. **5:**15–20.
25. **Tramer, J.** 1966. Inhibitory effect of *Lactobacillus acidophilus*. Nature (London) **211:**204–205.
26. **Vincent, J. G., R. C. Veomett, and R. F. Riley.** 1959. Antibacterial activity associated with *Lactobacillus acidophilus*. J. Bacteriol. **78:**477–484.
27. **Wheater, D. M., A. Hirsch, and A. T. R. Mattick.** 1951. Lactobacillin, an antibiotic from lactobacilli. Nature (London) **168:**659.