Mixed Culture Fermentation of Cucumber Juice with *Lactobacillus plantarum* and Yeasts

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ABSTRACT

Saccharomyces cerevisiae and *Saccharomyces rosei* were tested for use in mixed culture fermentation of cucumber juice with *Lactobacillus plantarum*. Extent of sugar utilization and the ratio of products formed (lactic acid:ethanol) were influenced by time of inoculation and cell numbers of each microorganism. Sugar fermentation was complete within 6 days at 28°C when similar numbers of bacteria and yeasts were added simultaneously, or when yeasts were added before the bacteria. Sugar remained when only *L. plantarum* was added, or when yeasts were added in low numbers. Glycerol was produced when yeasts were present, the concentration being directly related to NaCl concentration. Results suggest advantageous uses of yeasts to complete fermentation and to modify acidity.

INTRODUCTION

LACTIC ACID BACTERIA are mainly responsible for the primary fermentation of brined vegetables such as cucumbers. Fermentative yeasts may also grow during the primary fermentation stage, and may grow exclusively during the secondary stage if fermentable sugars remain after growth by lactic acid bacteria ceases (Fleming, 1982). Fermentative yeasts traditionally have been viewed as undesirable in cucumber fermentations because of their CO_2 production and implication in bloater damage (hollowness) of the cucumbers (Etchells and Bell, 1950).

Acetate buffers have been added to cucumber brines to assure complete sugar utilization during primary fermentation (Etchells et al., 1973, 1975). Neutralization of acids during fermentation with a pH controller has also been used to assure complete sugar utilization (Fleming et al., 1983). However, only with cucumbers, green beans and peppers was complete sugar utilization accomplished.

Purging of CO_2 from fermenting cucumbers by bubbling air or N_2 through the brine is an effective means for preventing bloater damage to the cucumbers (Fleming et al., 1973a, 1973b; Costilow et al., 1977). Thus, the intentional use of fermentative yeasts in cucumber fermentations may be advantageous with the advent of purging. Selected yeasts might remove a portion of the fermentable sugars during primary fermentation, thereby assuring more rapid and complete utilization of fermentable sugars. By preventing residual sugar after primary fermentation, the time necessary to continue purging could be shortened and purging costs reduced. Also, by converting a portion of the sugars to ethanol by yeast fermentation, undesirably high lactic acid concentrations from exclusively lactic acid fermentations could be prevented. Mild acidity and other flavor characteristics imparted by the yeasts could be highly desirable. In this investigation, cucumber juice was used as a model fermentation system to represent cucumber fermentations.

The objectives of this investigation were: (1) to screen selected species of yeasts for their ability to ferment cucumber juice at varying concentrations of salt and acids, (2) to determine conditions necessary to complete cucumber juice fermentation using a mixed culture of a selected yeast with a lactic acid bacterium, and (3) to determine the effect of mixed culture fermentations on end product concentrations.

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MATERIALS & METHODS

Microbial strains

Saccharomyces cerevisiae Y-635, *Torulopsis etchellsii* Y-6651, *Saccharomyces bailli* Y-2227, *Saccharomyces rouxii* Y-2547, and *Candida utilis* Y-900, which were used in preliminary screening experiments, were obtained from the USDA-ARS, Northern Regional Research Laboratory (Peoria, IL). An additional 13 strains, including *Saccharomyces rosei,* were isolated from fermenting cucumbers and red pepper mash in our laboratory. Identification of natural isolates was done using the physiological tests suggested by Barnett and Parkhurst (1974). The lactic acid bacterium, *Lactobacillus plantarum*, WSO, is from the Food Fermentation Laboratory (author's laboratory).

Media

Yeasts were maintained in YM (Difco) broth and lactic acid bacteria in MRS (Difco) broth. Cucumber juice for growth studies was prepared by freezing fresh pickling cucumbers at −20°C overnight and then partially thawing and blending them to a homogenous slurry. The slurry was brought to boiling and rapidly cooled to approximately 25°C. Successive filtrations were done through cheesecloth, Whatman no. 1 filter paper, and sterile, 0.2μ Millipore filters. Juices were diluted twofold with sterile distilled water prior to use. Varying concentrations and combinations of NaCl (0, 3, and 5% w/v), acetic acid (0 and 0.16% w/v) and lactic acid (0, 0.1, 0.25, and 0.50% w/v) were added to cucumber juice to simulate different cucumber brine conditions. Inoculated media were incubated in static culture in 16 mm disposable test tubes with Kim Kap enclosures. In testing for glycerol production, anaerobic conditions were achieved with BBL Gas Pak jars using a carbon dioxide-hydrogen generator.

Growth tests

Relative comparisons of growth among strains in various cucumber juice formulations were determined by measuring the specific growth rates, and generation times (Drew, 1981). Carbon recoveries were determined as described by Wood (1961).

Chemical assays

Reducing sugar concentrations were determined by the colorimetric method of Sumner and Somers (1944). Lactic acid, ethanol, and glycerol concentrations were measured using high performance liquid chromatography according to the procedures of McFeeters et al. (1984).

RESULTS & DISCUSSION

INITIALLY, five yeast species were selected for screening based upon their described properties of being salt-tolerant (>5% w/v) and their established use in food fermentations. The initial screening tests (data not shown) consisted of comparing the ability of the yeasts to grow and ferment sugars in cucumber juice containing various concentrations of NaCl and lactic acid. Under these conditions, *S. cerevisiae* Y-635 was selected for

further experiments because of its ability to grow in the presence of comparatively high concentrations of lactic acid (0.5% w/v). A second group of 13 yeasts that we originally isolated from active cucumber fermentations was screened using tests similar to that used with the first group. One isolate, identified as *S. rosei*, was chosen for further study because, like *S. cerevisiae*, it grew in the presence of a relatively high concentration of lactic acid (0.5% w/v).

Saccharomyces cerevisiae and *S. rosei*, were compared to test their ability to ferment cucumber sugars in the presence of various concentrations of lactic acid. The cucumber juice used was supplemented with acetic acid (0.16% w/v), which represents the concentration used in controlled cucumber fermentations. In the absence of lactic acid, *S. rosei* was able to ferment more than 95% of the sugar in 3 days, whereas *S. cerevisiae* needed 7 days to ferment 80% of the sugar (Fig. 1).

In the presence of 0.1% lactic acid, the same general pattern existed except for a slight inhibition of both species. At the 0.25% lactic acid concentration, *S. rosei* was severely inhibited in its ability to use the sugar, whereas *S. cerevisiae* was not. At 0.5% lactic acid, neither species grew.

Subsequently, the growth rates of the yeasts and of *L. plantarum* in cucumber juice containing 5% salt and 0.16% w/v acetic acid were determined. Under these conditions, *S. rosei* was competitive in terms of growth rate (generation time = 148 min) with the *L. plantarum* (165 min). *Saccharomyces cerevisiae* was the slower growing of the two yeasts (200 min), and lagged behind the growth of *L. plantarum*.

Both yeasts were used with *L. plantarum* in mixed culture fermentations of cucumber juice. In these experiments the inocula consisted of one concentration of *L. plantarum* and various concentrations of the yeast. In addition, the time of inoculation of each species was varied.

By varying the inoculum level of *S. cerevisiae* it was possible to manipulate the product ratios of lactic acid to ethanol and at the same time to ferment all of the sugar (Table 1). In addition, the final acidity was reduced, with a higher final pH, when *S. cerevisiae* was added with *L. plantarum*, as compared to *L. plantarum* alone. A similar pattern was observed when *S. rosei* was added with *L. plantarum* (Table 2) except that greater amounts of ethanol were produced, which probably reflects the faster growth rate of this yeast under the test conditions. Varying the time of inoculation of the yeast and *L. plantarum* had a significant effect on the end-product ratios. When inoculation with yeast preceded that of *L. plantarum*, a

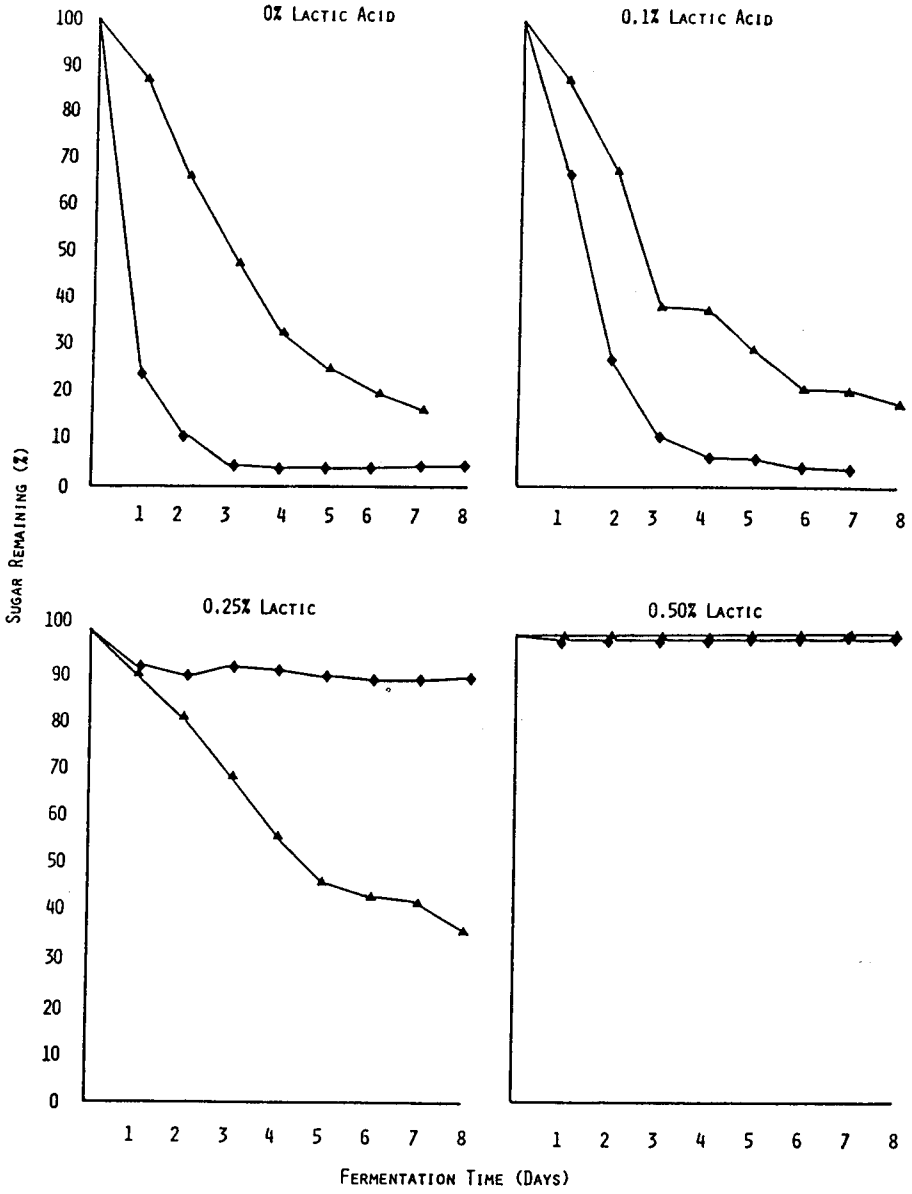

Fig. 1—Sugar fermentation by yeasts in cucumber juice at 30°C containing 5% w/v NaCl, 0.16 w/v acetic acid and various concentrations of lactic acid. ▲ = S. cerevisiae; ♦ = S. rosei.

Table 1—Fermentation of cucumber juice with L. plantarum and *S. cerevisiae* incubated at 30°C for 6 days

S. cerevisiae inoculum added (log CFU/mL)	Final pH	Fermentation products (mM) Lactic acid	Ethanol	Residual sugar (mM)	% Carbon recovery
24 hr before	2.7	3.23	76	45	4	90.3
L. plantarum[a]	3.7	3.57	42	70	<1	78.9
	4.7	3.93	25	80	<1	73.9
	5.7	3.99	23	92	<1	81.0
Same time as	2.7	3.18	77	28	17	97.2
L. plantarum	3.7	3.10	95	26	15	108.0
	4.7	3.24	72	49	<1	85.2
	5.7	3.60	40	69	<1	76.8
24 hr after	2.7	3.16	74	32	20	103.9
L. plantarum	3.7	3.09	84	29	14	99.1
	4.7	3.12	85	28	14	99.1
	5.7	3.09	80	24	12	91.2
L. plantarum only		3.13	91	0	31	113.8
S. cerevisiae only		4.02	4	86	<1	63.4
Uninoculated control		4.05	2	3	71	

[a] *L. plantarum* was added at a level of 6.2 log CFU/mL.

Table 2—Fermentation of cucumber juice with L. plantarum and *S. rosei* incubated at 30°C for 6 days

S. rosei inoculum added (log CFU/mL)	Final pH	Fermentation products (mM) Lactic acid	Ethanol	Residual sugar (mM)	% Carbon recovery
24 hr before	2.5	3.47	33	105	<1	100.0
L. plantarum[a]	3.5	3.64	25	114	<1	100.7
	4.5	3.91	14	109	<1	89.1
	5.5	4.02	11	117	<1	92.7
Same time as	2.5	3.03	82	27	14	99.1
L. plantarum	3.5	3.09	69	65	<1	97.1
	4.5	3.29	44	88	<1	95.7
	5.5	3.35	42	93	<1	97.8
24 hr after	2.5	3.06	78	17	28	115.9
L. plantarum	3.5	3.06	79	14	25	105.7
	4.5	3.13	68	69	<1	99.3
	5.5	3.06	77	49	6	100.0
L. plantarum only		3.07	76	0	32	102.7
S. rosei only		3.92	0	124	<2	89.9
Uninoculated control		3.94	0	0	69	---

[a] *L. plantarum* was added at a level of 6.2 log CFU/mL.

Table 3—Fermentation products from cucumber juice containing various amounts of salt by S. cerevisiae under anaerobic and aerobic conditions at 28°C for 7 days

Salt conc (%)	Incubation condition	ETOH (mM)	Glycerol (mM)	Residual sugars (mM)	% Carbon recovery[a]
0	Anaerobic	101.9	2.3	0.9	83.6
2	Anaerobic	99.9	7.0	1.0	85.9
4	Anaerobic	93.2	12.3	2.0	86.2
6	Anaerobic	74.4	16.0	5.6	78.5
0	Aerobic, static	94.5	3.5	0.7	78.4
2	Aerobic, static	87.9	8.9	0.9	77.7
4	Aerobic, static	79.0	14.7	1.4	75.8
6	Aerobic, static	74.2	18.2	3.5	77.4

[a] Based on the conversion of sugars to ethanol, CO_2, and glycerol. CO_2 was not measured, but it is assumed that 1 mole CO_2 is produced with each mole of ethanol produced. The initial reducing sugar in the cucumber juice was 63.2 mM.

greater proportion of ethanol to lactic acid was formed as compared to simultaneous inoculation or inoculation of the yeast 24 hr after *L. plantarum*.

Carbon recovery varied considerably in completed mixed culture fermentations employing yeasts and *L. plantarum*. Values were calculated under the assumption that the yeast quantitatively converted hexose to ethanol and CO_2. Lower recovery values were observed with *S. cerevisiae* than with *S. rosei*. *Lactobacillus plantarum*, when grown alone in the same cucumber juice medium, had carbon recovery values greater than or equal to 100%. We have found carbon recoveries to exceed 100% in the fermentation of whole cucumbers by *L. plantarum* (Fleming et al., 1988). Various explanations were offered for such high carbon recoveries, some of which may be applicable to this study. These included the possibility that not all substrates were accounted for; cell wall material, for example, could have degraded during brine storage to yield fermentable substrates. Also, malic acid, which is present in cucumbers (McFeeters et al., 1982), may have been degraded to lactic acid, but it was not included as a possible substrate.

Several explanations may exist for the low carbon recoveries with yeast: (1) Since the cultures were grown in static culture, a proportion of the hexoses may have been respired as $CO_2 + H_2O$; (2) the yeasts may have produced products that we did not measure initially such as glycerol, which some yeasts are capable of producing (Phaff et al., 1966).

Glycerol production by *S. cerevisiae* was tested in cucumber juice under anaerobic and static incubation conditions. The data show (Table 3) that greater amounts of glycerol and lower amounts of ethanol were produced as the concentration of salt was increased in the cucumber juice. This was observed for both anaerobic and static conditions of growth. Carbon recoveries were consistently higher under anaerobic conditions compared with static conditions, regardless of the salt concentration.

The production of glycerol from glucose under high NaCl conditions was demonstrated by Wei et al. (1982) with Bakers yeast grown in a gelatin matrix. A possible mechanism for glycerol production was presented by Unemoto et al. (1967), who proposed that high NaCl levels inhibited pyruvate carboxylase, which forced phosphoglyceraldehyde to be a substitute electron acceptor resulting in glycerol formation.

Glycerol, like ethanol, would be a neutral fermentation product and would not contribute acidity to a fermentation. It is not known whether glycerol would be a stable product or be used as a substrate by other microorganisms present in mixed culture fermentations.

SUMMARY

FROM THIS STUDY we can conclude that: (1) *S. cerevisiae* and *S. rosei* are suitable for use in mixed culture fermentations of cucumber juice; (2) complete fermentation of cucumber juice can be achieved by using a yeast in mixed culture with *L. plantarum* without having to add buffer; and (3) the final pH and product concentrations in such fermentations can be manipulated by varying the inoculum size and the time of inoculation of each species. When N_2 is used in purging cucumber fermentation tanks to prevent bloater damage due to CO_2 accumulation, it is possible that selected yeasts can serve useful purposes in cucumber fermentations.

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Ms received 9/8/87; revised 12/14/87; accepted 12/16/87.

Paper no. 11226 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, NC 27695-7601.

This investigation was supported in part by a research grant from Pickle Packers International, Inc., St. Charles, IL.