Fermentation Characteristics of Heterolactic Acid Bacteria in Green Bean Juice

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ABSTRACT

Green bean juice was fermented with 10 species (14 strains) of heterofermentative and two homofermentative lactic acid bacteria to select organisms which might be used to carry out a complete fermentation. *Lactobacillus cellobiosus* was the only organism to remove all fermentable sugars from bean juice with or without 2.5% NaCl. Nine other cultures used from 75–95% of the sugars. *Lactobacillus cellobiosus* also produced the lowest final pH among the 14 strains. A complete analysis of the major fermentation substrates and products was done for each of the organisms. Fermentation balance calculations showed a range from 74–132% carbon recovery. These bacteria showed considerable variation in the ability to degrade malic acid and to form mannitol and acetic acid.

INTRODUCTION

RECENT WORK in this laboratory has shown that *Lactobacillus plantarum* can be used to make several vegetables microbiologically stable by removing all fermentable sugars (Fleming et al., 1983). One potential problem with these fermentations is that the lactic acid flavor developed by a homofermentative organism may be too strong to be desirable for many people.

Heterofermentative lactic acid bacteria are important in many natural lactic acid fermentations (Pederson, 1979). The fact that these bacteria produce acetic acid, ethanol, mannitol, and CO_2 in addition to lactic acid (Wood, 1961) means that less acid will be produced from a given amount of sugar than occurs in homolactic acid fermentations. However, there is little information available on the quantitative substrate and the product changes caused by heterolactic acid bacteria in complex media-like foods. Thiel (1940) investigated the fermentation of milk with a number of heterofermentors. This has been the only systematic attempt to investigate the characteristics of this group of bacteria in a food. Analytical difficulties at that time resulted in an incomplete analysis of chemical changes caused by fermentation.

The objective of this investigation was to determine the detailed fermentation characteristics of a group of heterolactic acid bacteria in green bean juice. The fermentation characteristics measured were the total amount of sugar fermented, the final pH and the changes in all major substrates, i.e., glucose, fructose, sucrose, and malic acid, and products except CO_2, i.e., lactic acid, acetic acid, ethanol, and mannitol. The effect of medium on these fermentation parameters was evaluated by doing fermentations in fresh and heated bean juice with and without addition of 2.5% NaCl. The intent of this detailed analysis was to select an organism(s) with desirable characteristics for fermentation of green beans.

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MATERIALS & METHODS

Preparation of green bean juice

Fresh green beans (Bush Blue Lake, XPB 3069) were snapped and frozen at −15°C without blanching. They were partially thawed and blended with an equal weight of water. Juice was obtained by filtration through cheesecloth and centrifugation of the filtrate at 23,000 × *g* for 10 min. Juice was heated to 77°C in a boiling water bath, held at that temperature for 3 min, cooled in ice water, and centrifuged at 23,000 × *g* for 10 min. For fermentations with NaCl, dry salt was added to the supernatant juice to give a 2.5% (w/w) solution. The bean extract was filtered through a nonsterile, 0.22 μm Millipore filter. Then, 9.0 ml aliquots were sterile-filtered through a 0.22 μm Millex filter into 16 × 150 mm screw cap test tubes. Noninoculated control tubes of juice prepared in this way showed no growth during incubation.

Cultures

The cultures used to ferment green bean juice are listed in Table 1. Bacteria were grown in MRS medium (DeMan et al., 1960) at 30°C. Twelve-hour cultures were centrifuged and cells resuspended in sterile saline. Each tube was inoculated to give an initial inoculum of 10^6 CFU/ml and incubated for 4 days at 30°C in an anaerobic jar. An anaerobic hydrogen atmosphere was generated with a BBL gas pack. An indicator strip was used to assure that anaerobic conditions were maintained.

Each organism was inoculated into duplicate tubes of fresh bean juice or heated juice with and without 2.5% NaCl. After fermentation, duplicate tubes were analyzed separately.

Measurement of pH value

The pH was measured by an Orion 901 pH meter equipped with a glass electrode.

Analysis of carbohydrates, organic acids and ethanol

Liquid chromatography was done with a system which consisted of a Waters M6000A pump and model 401 refractive index detector, a Rheodyne model 7125 injector with a 10 μl loop and a Spectra-Physics 4100 computing integrator. A Bio-Rad HPX-87HM ion exchange column held at 67°C in a water bath with deionized water as the mobile phase was used for sucrose, glucose, fructose, and mannitol determinations. Dual Bio-Rad guard columns, one an anion exchange cartridge an the other a cation exchange cartridge, were connected in front of the main column and kept at room temperature to remove NaCl from the samples. For lactic acid, acetic acid, ethanol, and malic acid measurements, a Waters 5 μm, C_{18}, 8 × 100 mm Radial-Pak cartridge was used. Compounds were eluted with 0.05M phosphoric acid/NH_4OH aqueous buffer, pH 2.5. Concentrations were calculated by comparison of the peak heights with external standards for each compound.

Samples were prepared for injection into the HPLC by filtration through 0.45 μm Gelman filters. Prior to injection of samples with added NaCl into the ion exchange column, the samples were diluted with an equal amount of water to reduce the salt concentration. All samples injected into the C_{18} Radial-Pak column were first mixed with an equal volume of 2X concentrated elution buffer to assure that the organic acids would be in the protonated form.

Calculations

Carbon recoveries were calculated with the following formula:

$$\text{Carbon recovery} = \frac{[L_f - L_i] + [A_f - A_i] + [E_f - E_i] + 2[M_f - M_i]}{2[G_i - G_f] + 2[F_i - F_f] + 4[S_i - S_f] + [MA_i - MA_f]} \times 100$$

where L = lactic acid, A = acetic acid, E = ethanol, M = mannitol, G = glucose, F = fructose, S = sucrose, and MA = malic acid. The subscripts i and f indicate the initial and final molar concentrations of each compound. The determination of whether the calculated carbon recoveries were significantly different from 100% was made by use of a protected least significant difference procedure (Snedecor and Cochran, 1980).

RESULTS & DISCUSSION

SUBSTRATE DISAPPEARANCE and product formation in heated green bean juice is presented in Table 2 for each of the 17 organisms evaluated. The initial substrate concentrations in the bean juice lots used for fermentation are given in the footnote of Table 2. The qualitative product patterns did not change as a result of not heating or salt addition to the juice, but some changes in the amount of sugar degraded and final pH were observed.

Sugar utilization

Fleming et al. (1983) found that vegetables fermented by *L. plantarum* are microbiologically stable at pH 3.8 and 3.3 in hermetically sealed glass jars provided all fermentable sugars are removed. Therefore, it was of interest to identify heterofermentative organisms which could completely remove sugars during fermentation. Table 3 shows the percentage of the initial sugar removed by each organism in heated and nonheated juice with and without addition of 2.5% salt.

Lactobacillus cellobiosus was the only species tested which removed 100% of the sugars present in bean juice. This occurred in each of the test conditions. Seventy to 95% of the sugars were fermented by nine of the organisms in the absence of salt and by seven organisms with 2.5% NaCl in the medium. Conditions might be optimized so that some organisms can removal all sugars. For example, *L. plantarum*, which metabolized slightly less than 70% of the sugars in these experiments, removed all sugars from green beans in a pH-controlled fermentation (Fleming et al., 1983).

Vegetables are fermented in the presence of salt, so organisms used in these fermentations must tolerate the salt present. Table 3 shows that in most instances these bacteria metabolized less sugar with 2.5% NaCl in the bean juice. *Lactobacillus hilgardii* had the largest reduction in sugar consumption with over a 35% decline in both heated and nonheated juice. *Lactobacillus buchneri* and *L. dextranicum* were notable because they had a tendency to use more sugar in 2.5% NaCl.

Lactobacillus viridescens, *L. dextranicum*, *L. lactis*, *S. lactis* subsp. *diacetylactis*, and *S. cremoris* fermented less than 50% of the sugar in each of the four media. These organisms are usually not isolated from plant materials.

Overall, heating the juice had no effect on sugar utilization. However, *L. brevis* 70 showed a 20% decrease in sugar utilization as a result of heat treatment with salt. In a few other cases, 10–12% differences occurred.

Final pH of the fermentations

The pH of heated bean juice after 4 days' fermentation is shown in Table 4. *Lactobacillus cellobiosus*, in addition to fermenting all available sugars, also had the lowest final pH among the heterofermentative bacteria. This low pH was desirable because in a fermented product it would help prevent secondary fermentations by more acid-tolerant bacteria.

In instances where the amount of sugar fermented was not greatly affected by the addition of NaCl, the final pH was about 0.3 units lower in 2.5% NaCl compared to fermentations without added NaCl. Examples are *L. cellobiosus*, *L. fermentum* and *L. plantarum*. This is probably caused by

Table 1—Cultures used for inoculation of green bean juice

Species	Culture no.	Hetero-fermentative	Homo-fermentative
Lactobacilli			
L. brevis	50	X	
L. brevis	70	X	
L. brevis	ATCC 14869	X	
L. buchneri	ATCC 4005	X	
L. cellobiosus	ATCC 11379	X	
L. fermentum	ATCC 14931	X	
L. hilgardii	ATCC 8290	X	
L. plantarum	WSO		X
L. viridescens	ATCC 12706	X	
Leuconostocs			
L. dextranicum	ATCC 19255	X	
L. lactis	ATCC 19256	X	
L. mesenteroides	LC 33	X	
L. mesenteroides	42	X	
L. mesenteroides	43	X	
L. paramesenteroides	NCDO 803	X	
Streptococci			
S. cremoris	ATCC 9625		X
S. lactis diacetylactis	ATCC 13675		X

Table 2—Changes in substrates and products after fermentation of heat-treated bean juice by 17 lactic acid bacteria

		0% NaCl added								2.5% NaCl added							
		Substrate fermented (mM)				Product formed (mM)				Substrate fermented (mM)				Product formed (mM)			
Species	Batch no.[a]	Sucrose	Glucose	Fructose	Malic acid	Lactic acid	Acetic acid	Ethanol	Mannitol	Sucrose	Glucose	Fructose	Malic acid	Lactic acid	Acetic acid	Ethanol	Mannitol
L. brevis 50	I	0	18.1	25.1	9.3	36.2	8.0	38.0	13.6	0	19.4	24.2	9.3	34.8	9.3	27.3	16.2
L. brevis 70	I	0	17.3	20.6	9.3	27.3	7.8	29.5	12.8	0	13.5	18.5	9.3	17.4	7.5	19.0	17.3
L. brevis ATCC 14869	II	0	18.3	18.8	10.1	29.9	10.7	14.5	12.3	0	10.7	7.2	8.2	14.7	7.4	5.2	10.6
L. buchnerii ATCC 4005	II	1.9	21.2	26.3	3.8	27.6	15.9	14.4	26.1	3.2	21.0	26.3	2.7	23.7	20.0	10.7	31.8
L. cellobiosus ATCC 11739	II	3.7	24.7	26.3	8.7	45.1	20.3	21.7	26.5	3.7	24.7	26.3	8.5	41.5	21.1	20.9	35.5
L. fermentum ATCC 14931	I	1.6	13.3	25.7	5.7	21.5	12.7	21.9	22.2	2.0	11.2	26.4	7.8	20.2	8.4	22.4	27.9
L. hilgardii ATCC 8290	II	0	20.3	21.6	3.9	33.6	8.1	33.1	6.7	0	12.0	11.1	1.5	12.8	8.3	10.3	13.3
L. plantarum WSO	I	0	17.2	20.6	9.3	90.5	0	0	0	0	18.1	16.7	9.3	87.2	0	0	0
L. viridescens ATCC 12706	II	0	14.9	2.4	8.5	29.7	5.3	21.7	0	0	14.6	0	7.5	22.0	2.8	18.0	0
L. dextranicum ATCC 19255	II	0	8.3	0	0.6	8.1	0	10.8	0	1.4	7.9	0.5	0.7	4.9	0	8.2	0
L. lactis ATCC 19256	II	2.0	17.8	4.8	8.3	27.6	3.9	18.9	2.8	0.7	11.4	7.3	2.6	7.4	0	7.4	9.8
L. mesenteroides LC 33	I	2.0	16.4	26.3	0.4	23.2	15.3	27.6	24.5	2.6	13.6	22.0	0.5	15.4	12.8	19.3	14.5
L. mesenteroides 42	I	1.8	15.6	27.8	1.5	20.5	13.2	22.7	25.6	1.1	12.2	25.4	0.6	14.4	12.5	18.2	21.3
L. mesenteroides 43	I	1.9	14.8	27.8	0.7	25.2	15.7	25.5	28.3	0.8	12.1	25.5	0	17.6	15.3	16.5	21.6
L. paramesenteroides NCDO 803	I	0	16.7	27.8	9.3	30.1	10.3	23.7	19.1	0	10.8	22.1	2.1	12.6	7.1	18.9	23.0
S. cremoris ATCC 9625	I	0	10.2	6.0	4.1	20.3	0	16.4	0	0.4	1.6	4.7	1.6	4.2	0	13.1	0
S. lactic diacetylactis 13675	I	0	5.2	8.7	2.9	13.9	0	15.9	0	0	1.8	7.9	1.0	6.0	0	13.2	0

[a] Bean juice batch I contained 2.6 mM sucrose, 22.0 mM glucose, 27.8 mM fructose, and 9.3 mM malic acid. Batch II had 3.7 mM sucrose, 24.7 mM glucose, 26.3 mM fructose, and 10.1 mM malic acid.

the increase of ionization constants which occurs as the ionic strength is increased (Perrin and Dempsey, 1974).

Fermentation patterns

The hexose monophosphate pathway for heterolactic acid fermentation requires the production of 1 mole lactic acid and 1 mole of ethanol per mole of hexose metabolized (Wood, 1961) (Fig. 1). In most cases, a mixture of acetic acid and ethanol were formed rather than just ethanol. This resulted when fructose was reduced to mannitol.

Data in Fig. 2 show some product/substrate ratios for selected organisms calculated from Table 2. *Lactobacillus plantarum* gives a typical homofermentative product pattern. Two moles of lactic acid were formed per mole of hexose fermented. In contrast, the other organisms produce about 1 mole of lactic acid per mole of sugar metabolized for energy. Ethanol, acetic acid and considerable amounts of mannitol were formed.

Streptococcus lactis subsp. *diacetylactis* and *S. cremoris* have an unexpected fermentation pattern in bean juice. These species, which are used commercially in cheese fermentations, are classified as homofermentative organisms. However, they have been shown to produce heterolactic products (Platt and Foster, 1958; Shahani and Vakil, 1962). Thomas et al. (1979) found that when grown anaerobically at low dilution rates in a chemostat, *S. lactis* and *S. cremoris* converted as little as 1% of the glucose to lactic acid, while formate, acetate and ethanol became the major products. Their data were consistent with the operation of the glycolytic pathway to produce pyruvate and a "phosphoroclastic" cleavage of pyruvate to give the observed products.

The present data show a heterofermentative product pattern, but only lactic acid and ethanol were found (Fig. 2). Moreover, the carbon recovery was near 100% (Table 5). This heterofermentative pattern occurred in batch culture with glucose and fructose as the primary carbon sources. Neither Thomas et al. (1979) nor Brown and Collins (1977) were able to produce a heterolactic fermentation with high glucose levels in the medium. The reason for production of a heterolactic compound in bean juice is not known.

Table 3—Effect of heat treatment of the juice and salt addition on sugar utilization by different lactic acid bacteria in green bean juice fermentations

Species	Sugar utilization (%)[a] Heated no salt	Unheated no salt	Heated 2.5% NaCl	Unheated 2.5% NaCl
L. brevis 50	78.6	80.9	79.4	86.0
L. brevis 70	75.6	85.9	58.2	78.0
L. brevis ATCC 14869	63.6	61.8	42.2	30.6
L. buchneri ATCC 4005	87.6	88.4	94.7	91.8
L. cellobiosus ATCC 11739	100.0	100.0	100.0	100.0
L. fermentum ATCC 14931	76.8	87.4	75.7	70.0
L. hilgardii ATCC 8290	71.8	84.2	36.4	45.8
L. plantarum WSO	68.8	68.9	63.3	69.0
L. viridescens ATCC 12706	29.5	32.3	30.2	21.0
L. dextranicum ATCC 19255	11.1	15.5	22.6	19.2
L. lactis ATCC 19256	45.7	50.7	36.4	34.4
L. mesenteroides LC 33	85.0	82.4	74.1	83.0
L. mesenteroides 42	85.6	84.4	72.4	76.7
L. mesenteroides 43	84.2	83.4	71.5	77.0
L. paramesenteroides NCDO 803	80.9	82.7	59.8	54.4
S. cremoris ATCC 9625	29.4	33.8	12.8	25.6
S. lactic diacetylactis 13675	25.2	28.8	17.7	16.7

[a] Sugar utilization was calculated as the ratio of the molar concentration of sugars metabolized to the initial sugar concentrations. The sucrose concentration was multiplied by 2, since it is a disaccharide.

Table 4—Final pH of green bean juice fermented by different lactic acid bacteria

Species	pH Value Heated no salt	Heated 2.5% NaCl
L. brevis 50	4.12	4.01
L. brevis 70	4.12	4.15
L. brevis ATCC 14869	4.29	4.48
L. buchneri ATCC 4005	4.01	3.68
L. cellobiosus ATCC 11739	3.92	3.56
L. fermentum ATCC 14931	4.31	4.03
L. hilgardii ATCC 8290	3.86	3.88
L. plantarum WSO	3.45	3.16
L. viridescens ATCC 12706	4.44	4.29
L. dextranicum ATCC 19255	4.64	4.57
L. lactis ATCC 19256	4.29	4.44
L. mesenteroides LC 33	4.08	4.09
L. mesenteroides 42	4.10	4.05
L. mesenteroides 43	4.06	4.07
L. paramesenteroides NCDO 803	4.15	4.19
S. cremoris ATCC 9625	4.22	5.10
S. lactis diacetylactis 13675	4.47	4.96

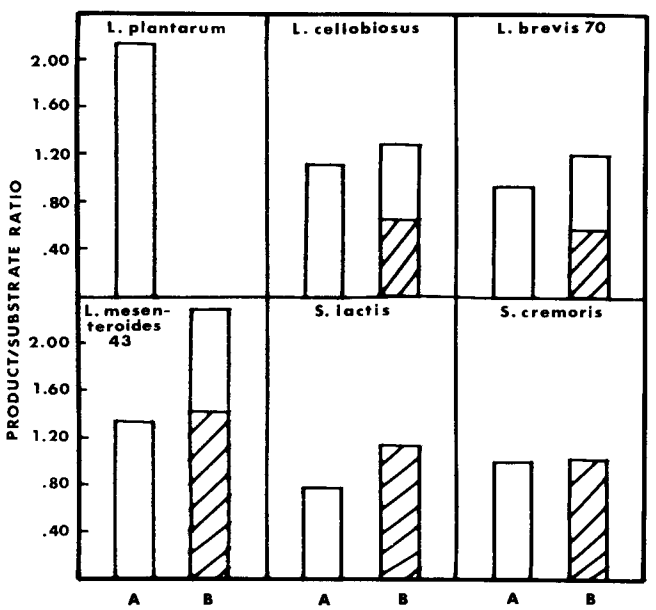

Fig. 2—Relationships between the moles of product formed and substrate fermented for selected lactic acid bacteria. For each organism bar "A" shows the ratio of lactic acid formed/hexose fermented. Bar "B" shows the ratio of 2-carbon products/hexose fermented. The hatched part of B is the ethanol produced/hexose fermented; the open bar is the acetic acid produced/hexose fermented.

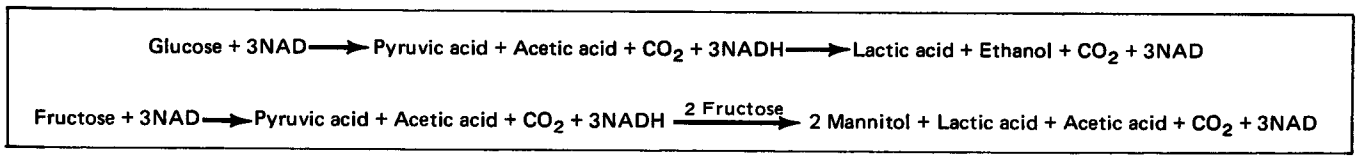

Fig. 1—Degradation of glucose and fructose by the hexose monophosphate heterofermentative pathway.

Table 5—Carbon recovery of different lactic acid bacteria in green bean juice fermentations

Species	Carbon recovery (%)	
	Heated no salt	Unheated no salt
L. brevis 50	114.0	124.6
L. brevis 70	97.8	98.0
L. brevis ATCC 14869	86.4	113.6
L. buchneri ATCC 4005	103.7	94.5
L. cellobiosus ATCC 11739	111.7	107.4
L. fermentum ATCC 14931	135.5	126.3
L. hilgardii ATCC 8290	102.4	74.1
L. plantarum WSO	106.8	107.7
L. viridescens ATCC 12706	116.1	104.3
L. dextranicum ATCC 19255	101.0	108.3
L. lactis ATCC 19256	85.2	79.4
L. mesenteroides LC 33	123.6	119.3
L. mesenteroides 42	113.1	115.5
L. mesenteroides 43	132.1	130.4
L. paramesenteroides NCDO 803	104.1	110.2
S. cremoris ATCC 9625	101.4	91.5
S. lactis diacetylactis 13675	97.7	99.0

Production of mannitol and acetic acid

Conversion of fructose to mannitol is a major reason for low acid production relative to sugar consumption by heterolactic acid bacteria. When the $NADH_2$ produced during sugar degradation is used to reduce fructose rather than acetic acid, mannitol and acetic acid will appear as fermentation products instead of ethanol (Martinez et al., 1963). Data in Table 6 show the ratio of mannitol production to fructose disappearance in heat-treated bean juice. Six strains converted from 81–96% of the available fructose to mannitol. For these organisms, glucose was the main source of energy, with the fructose acting primarily as an electron acceptor. *Lactobacillus brevis, L. lactis* and *L. paramesenteroides* produced about 1 mole of mannitol for every 2 moles of fructose fermented. So, on an overall basis, fructose can be considered to be split between energy production and oxidation of $NADH_2$. Finally, two of the heterolactic organisms and both of the streptococci were unable to produce mannitol.

On a molar basis, 50% as much acetic acid as mannitol should be formed, since it requires 2 moles of $NADH_2$ to reduce acetic acid to ethanol, but only 1 mole to reduce fructose. The bacteria that did not form mannitol, as would be expected, did not produce acetic acid. When mannitol accumulated, 52–143% as much acetic acid as mannitol was found. The fact that more than 50% as much acetic acid was observed in several cases suggested that the organisms could produce some acetic acid from alternative sources.

High mannitol production would not be a primary reason for choosing a heterofermentative organism for vegetable fermentations. However, it would be a desirable characteristic since it would result in relatively low acid production.

Malic acid degradation

Degradation of malic acid to lactic acid and CO_2 by a malolactic enzyme is common in lactic acid bacteria. Since malic acid is the major organic acid in green beans (Bakowski et al., 1964), disappearance of malic acid is likely to occur during fermentation. Data in Table 7 show the percentage of malic acid degraded in the heat-treated juice. Seven organisms degraded over 80%, five species metabolized from 30–60% of the initial malic acid and four cultures, including all three *L. mesenteroides* strains, used 15% or less of the malic acid. The small changes observed in the four strains suggest that they may not produce a malolactic enzyme. Data in Table 8 show a comparison of the malic acid degradation between strains of different species used in this survey and in a study by Winter and Kandler (1977). The table shows strain differences in four of the seven species common to both surveys. In addition, Winter and

Table 6—Relationship among fructose, mannitol and acetic acid in green bean juice[a] after fermentation with different lactic acid bacteria

Species	Mannitol formed, moles	Acetic acid formed, moles
	Fructose used, moles	Mannitol formed, moles
L. brevis 50	0.51	0.57
L. brevis 70	0.48	0.61
L. brevis ATCC 14869	0.55	0.83
L. buchneri ATCC 4005	0.93	0.61
L. cellobiosus ATCC 11739	0.88	0.77
L. fermentum ATCC 14931	0.81	0.57
L. hilgardii ATCC 8290	0.27	1.21
L. plantarum WSO	0	–
L. viridescens ATCC 12706	0	–
L. dextranicum ATCC 19255	0	–
L. lactis ATCC 19256	0.40	1.43
L. mesenteroides LC 33	0.87	0.62
L. mesenteroides 42	0.87	0.52
L. mesenteroides 43	0.96	0.55
L. paramesenteroides NCDO 803	0.58	0.54
S. cremoris ATCC 9625	0	0
S. lactis diacetylactis 13675	0	0

[a] The bean juice was heated with no NaCl added.

Table 7—Degradation of malic acid in bean juice by different strains of lactic acid bacteria

Species	Malic acid utilized (%)	
	Heated no salt	Heated 2.5% salt
L. brevis 50	100	100
L. brevis 70	100	100
L. brevis ATCC 14869	100	79.3
L. buchneri ATCC 4005	35.4	24.4
L. cellobiosus ATCC 11739	83.4	86.5
L. fermentum ATCC 14931	61	83.3
L. hilgardii ATCC 8290	36.1	10.9
L. plantarum WSO	100	100
L. viridescens ATCC 12706	81.3	81.4
L. dextranicum ATCC 19255	5.2	5.8
L. lactis ATCC 19256	85.5	22.5
L. mesenteroides LC 33	3.8	4.9
L. mesenteroides 42	15.8	6.8
L. mesenteroides 43	7.4	0
L. paramesenteroides NCDO 803	100	22.2
S. cremoris ATCC 9625	44.3	16.6
S. lactis subsp. *diacetylactis* B 675	31.1	11.1

Table 8—Comparison of malic acid degradation capability of lactic acid bacteria between strains used by Winter and Kandler (1977) and this study

Species	Winter and Kandler (1977)	This study
L. plantarum	+, +[a]	+
L. brevis	+	+, +, +
L. fermentum	–	+
L. dextranicum	–	–
L. lactis	–	+
L. mesenteroides	+, +[b], +[b]	–, –, ?
L. paramesenteroides	–	+

[a] A separate symbol is used for each strain reported. + indicates more than 10% of the initial malic acid degraded. – indicates less than 10% degradation.
[b] Schutz and Radler, 1974.

Kandler (1977) found that one of seven *L. casei* strains lacked the ability to degrade malic acid. These results suggest that strain differences are likely to occur for this characteristic among lactic acid bacteria.

Carbon recovery in green bean juice fermentations

Table 5 is a list of carbon recoveries calculated for each organism in nonheated and heated bean juice. Use of a protected least significant difference method gave a ±12%, 95% confidence interval for the heated juice and a ±11%, 95% interval for the nonheated case. These criteria were used to classify the carbon recovery for each organism as equal to or different than 100%. *Lactobacillus brevis* ATCC 14869 and *L. lactis* had <100% recovery in both media. This indicates that some of the substrates may have been converted to nonmeasured products. Five organisms, including all three *L. mesenteroides* strains, had carbon recoveries in excess of 100%. This suggests that some unrecognized substrates have been converted to the measured products. In the nonheated juice, eight, and in the heated juice, nine of the 17 organisms tested had a carbon recovery of 100% within the confidence limits given above. These results suggest that HPLC can give an adequate accounting of the major substrates and products of heterolactic acid fermentations in a complex medium. This type of analysis will help recognize situations in which substantial amounts of either substrates or products are missed in complex media. This information may be used to guide the search for additional reactions which lactobacilli may carry out in food fermentations.

CONCLUSIONS

LACTOBACILLUS CELLOBIOSUS appeared to be the organism most likely to carry out a complete fermentation of green beans among the 14 heterofermentative strains tested. It metabolized all sugars from sterile bean juice whether or not 2.5% NaCl was added. It decreased the pH to less than 3.6, which was the lowest final pH among these organisms. Since most of the fructose in the juice was converted to mannitol, *L. cellobiosus* had a relatively low level of acid production compared to the amount of sugars utilized. However, nine other organisms fermented 75–95% of the sugars, so it may be possible to obtain complete fermentations with some of these organisms if conditions are optimized. Further evaluation of the organisms which grew well in bean juice must be carried out to determine whether similar fermentations will occur in blanched, snapped beans.

Since a detailed analysis of fermentation substrates and products has been obtained, the data may serve to aid in the selection of suitable bacteria for other investigations. Three interesting results from these analyses have been found: (1) *S. lactis* and *S. cremoris* showed a heterofermentative product pattern in batch cultures; (2) four species of lactobacilli, in addition to the instance previously reported for *L. casei* (Winter and Kandler, 1977), show strain variability in the ability to degrade malic acid; (3) several bacteria had carbon recoveries significantly different from 100%, which suggests the possibility that unrecognized reactions may occur in a complex growth medium like bean juice.

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