

## Acid Tolerance of *Leuconostoc mesenteroides* and *Lactobacillus plantarum*†

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In this study, we determined the internal cellular pH response of *Leuconostoc mesenteroides* and *Lactobacillus plantarum* to the external pH created by the microorganisms themselves or by lactic or acetic acids and their salts added to the growth medium. Growth of *Leuconostoc mesenteroides* stopped when its internal pH reached 5.4 to 5.7, and growth of *L. plantarum* stopped when its internal pH reached 4.6 to 4.8. Variation in growth medium composition or pH did not alter the growth-limiting internal pH reached by these microorganisms. *L. plantarum* maintained its pH gradient in the presence of either 160 mM sodium acetate or sodium lactate down to an external pH of 3.0 with either acid. In contrast, the  $\Delta\text{pH}$  of *Leuconostoc mesenteroides* was zero at pH 4.0 with acetate and 5.0 with lactate. No differences were found between D-(−)- and L-(+)-lactic acid for the limiting internal pH for growth of either microorganism. The comparatively low growth-limiting internal pH and ability to maintain a pH gradient at high organic acid concentration may contribute to the ability of *L. plantarum* to terminate vegetable fermentations.

Lactic acid bacteria (LAB) are fermentative microorganisms which are capable of growth over a wide pH range in the presence of organic acids. The mechanism for acid tolerance by this group of microorganisms is not fully understood. In the natural fermentation of vegetables, a predictable sequence of microorganisms is observed, with the growth of one organism giving way to a more acid-tolerant organism (19). Typically, *Leuconostoc mesenteroides* grows early in the fermentation and *Lactobacillus plantarum* terminates the fermentation (18). The production of organic acids by LAB decreases the pH and makes the environment selective against less-acid-tolerant microorganisms.

Organic acids inhibit microorganisms by entering the cell in the undissociated form and then dissociating within the cell (14). This causes acidification of the cytoplasm and collapse of the proton motive force, resulting in inhibition of nutrient transport (4, 8). The primary mechanism of pH maintenance in microorganisms is dependent upon expulsion of protons from the cytoplasm by H<sup>+</sup>-ATPases at the expense of ATP (3, 12, 13, 20). Therefore, organic acids in the medium may contribute to growth inhibition because of increased energy consumption to maintain pH homeostasis (6). Fermentative microorganisms exhibit a greater range of internal pH values than respiratory microorganisms (3). Goodwin and Zeikus (5) attribute the tolerance of *Sarcina ventriculi* to low internal pH to physiological adaptation by the organism. They suggest that anaerobic microorganisms, in general, tolerate low internal pH, thereby decreasing dependence on energy-consuming proton pumps.

The objectives of this study were to characterize *Leuconostoc mesenteroides* and *L. plantarum* as to the limiting lower internal and external cellular pHs for growth and to determine effects of growth media on these limits. We show that growth of *Leuconostoc mesenteroides* and *L. plantarum*

stops when internal pH values of 5.4 to 5.7 and 4.6 to 4.8, respectively, are reached, independent of the growth medium. In contrast, the limiting external cellular pH is greatly influenced by the growth medium.

### MATERIALS AND METHODS

**Abbreviations.** The following parameters were measured and are abbreviated in this paper: (i) internal cell pH (pH<sub>in</sub>), (ii) extracellular pH (pH<sub>out</sub>), (iii) gradient between internal and external pH ( $\Delta\text{pH}$ ), (iv) the limiting lower external pH at which a bacterium initiates growth [pH<sub>(lei)</sub>], (v) the limiting lower external pH at which an organism terminates growth [pH<sub>(let)</sub>], and (vi) the limiting lower internal pH at which an organism terminates growth [pH<sub>(lit)</sub>]. Both pH<sub>(let)</sub> and pH<sub>(lit)</sub> were measured when sugar remained in the medium but was no longer being used. There are both upper and lower limits at which bacteria can grow. This study was concerned with only the lower limit.

**Organisms and culture conditions.** Microorganisms used in this study were *L. plantarum* WSO (U.S. Food Fermentation Laboratory) and *Leuconostoc mesenteroides* C33 (from J. R. Stamer, Cornell University, Geneva, N.Y.). They were maintained by weekly transfers into Lactobacilli MRS broth (Difco Laboratories, Detroit, Mich.) at 22°C. MHHD medium, a modification of HHD medium (15), was composed of Trypticase peptone (10 g/liter; BBL Microbiology Systems, Cockeysville, Md.), Casamino Acids (3 g/liter; Difco), Phytonone peptone (1.5 g/liter; BBL), yeast extract (1 g/liter; BBL), and Tween 80 (1 g/liter; Fisher Scientific Co., Fairlawn, N.J.). The medium was adjusted to pH 7.0 with HCl and autoclaved for 15 min. Glucose (Sigma Chemical Company, St. Louis, Mo.) was autoclaved separately and added to MHHD after autoclaving. The final glucose concentration in the medium was 2% (111 mM). MnSO<sub>4</sub> · H<sub>2</sub>O (Fisher) was autoclaved separately and added to the medium so that the final concentration of manganese was 10 mM.

Growth temperature was 30°C unless otherwise indicated. Cells used in all experiments were prepared by growth in

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MRS broth followed by two 18-h transfers of 1% inocula in MHHD medium.

**Limiting pH for initiating growth.** Media for  $\text{pH}_{(\text{lei})}$  studies were MHHD containing 0 to 160 mM sodium acetate (Fisher) and MHHD containing 0 to 160 mM sodium lactate (0 to 160 mM NaOH plus 0 to 160 mM L-(+)-lactic acid; Sigma). All media were adjusted to pH values between 3.0 and 7.0 with HCl or  $\text{NH}_4\text{OH}$ . Sterile acetate, lactate, and glucose solutions were added to sterile MHHD medium. All tubes were inoculated with 1% *L. plantarum* or *Leuconostoc mesenteroides*. The initial optical density at 650 nm was measured on a Lumetron colorimeter (Photovolt Corp., Indianapolis, Ind.). Tubes were incubated and examined daily for a visual change in turbidity. After 3 weeks,  $\text{pH}_{(\text{lei})}$  was measured.

**Continuous culture.** The continuous culture system consisted of a reservoir containing 8 liters of MHHD, a Gilson Minipuls 2 pump (Gilson Medical Electronics, Middleton, Wis.), a Multigen growth vessel (New Brunswick Scientific Co., Inc., Edison, N.J.) containing 600 ml of MHHD, and an overflow flask. Mixing of cells and incoming fresh medium was achieved by agitation at  $140 \pm 20$  rpm. An air vent with a  $0.45\text{-}\mu\text{m}$ -pore-size filter was left open at the top of the growth vessel. A 1% inoculum from an 18-h culture grown in MHHD was added to the growth vessel and allowed to grow as a batch culture until late log phase before fresh medium was pumped into the growth vessel. The dilution rate was adjusted to a rate less than the growth rate of the culture in order to avoid washout (9). The optical density at 650 nm was monitored. When steady state was established (i.e., stable optical density at 650 nm for 8 to 10 generations), samples were taken for internal pH measurements. The justification for use of continuous culture for pH studies was to eliminate differences in the  $\text{pH}_{\text{in}}$  response which might be a result of differences in the growth phase (i.e., log-phase cells versus stationary-phase cells) (unpublished data). Cells grown in continuous culture were used for all studies except for  $\text{pH}_{(\text{lei})}$  studies (Tables 1 to 4).

**Internal pH measurement.** The  $\text{pH}_{\text{in}}$  and cell volume were determined by using radioactive probes (all isotopes were purchased from Du Pont Co., Wilmington, Del.), as described by Rottenberg (21). Cells were centrifuged at  $22^\circ\text{C}$  (Sorvall RC-5B; Du Pont) at  $9,000 \times g$  for 10 min, suspended in MHHD, and centrifuged again. For determination of internal pH, washed cells were suspended to an optical density at 650 nm of 1.00 in the test medium. A 1-ml aliquot was placed into each of four microcentrifuge tubes. To each tube,  $^3\text{H}_2\text{O}$  (5  $\mu\text{Ci}$ ) was added to determine the total volume. For extracellular volume determination, [ $^{14}\text{C}$ ]sorbitol (3  $\mu\text{Ci}$ ) was added to two of the tubes. For  $\text{pH}_{\text{in}}$  determination, [ $^{14}\text{C}$ ]salicylic acid (3  $\mu\text{Ci}$ ) was added to the remaining two tubes. Following a 15-min incubation, 0.5 ml of silicone fluid (Dow Corning Corp., Midland, Mich.) (1) was added to each tube. The tubes were centrifuged in an Eppendorf 5414 microcentrifuge (Brinkmann Instruments, Inc., Westbury, N.Y.) at  $22^\circ\text{C}$  at  $9,800 \times g$  for 6 min. A 100- $\mu\text{l}$  sample of supernatant was pipetted into a scintillation vial containing 5 ml of Scintiverse (Fisher) and 0.5 ml of 0.6 N perchloric acid. The remaining fluid was aspirated from the tube, and the entire pellet was transferred to a scintillation vial containing 5 ml of Scintiverse and 0.5 ml of 0.6 N perchloric acid. The vials were placed into a LS501 scintillation counter (Beckman Instruments, Inc., Fullerton, Calif.) for determining counts per minute. Both  $\text{pH}_{\text{in}}$  and the intracellular volume were calculated on the basis of the counts per minute in the pellet and the supernatant (21). All pH values presented in

tables and figures are means. Means of  $\text{pH}$ s were obtained by converting pH to  $\text{H}^+$  concentration, averaging, and then taking  $-\log_{10}$  of the mean of the  $\text{H}^+$  concentration.

**$\text{pH}_{(\text{lei})}$  studies.** Media used for  $\text{pH}_{(\text{lei})}$  studies were MHHD (3% glucose) containing 0, 100, or 200 mM sodium acetate with an initial pH of 7.0 (adjusted with  $\text{NH}_4\text{OH}$ ); MHHD (3% glucose) containing 0, 100, or 200 mM sodium lactate [using either D(-)- or L(+)-lactic acid] adjusted with  $\text{NH}_4\text{OH}$  to an initial pH of 7.0; MRS broth with an additional 1% sterile glucose (55 mM) added after autoclaving; and cucumber juice. Cucumber juice was prepared as described by McFeeters et al. (16). Microorganisms were transferred from MRS broth into either MRS broth, MHHD, or cucumber juice. After 18 h, a 1% inoculum was transferred into the same medium (e.g., cucumber juice into cucumber juice). The cultures were incubated for 18 h, and from this a 1% inoculum was transferred to 200 ml of the same medium and incubated. Daily, 0.5-ml aliquots were removed for pH and glucose determinations. Glucose was measured by using the colorimetric assay of Sumner and Sisler (22). The  $\text{pH}_{(\text{lei})}$  was determined to occur within 1 week after inoculation (sugar remained in the medium but was no longer being used by microorganisms). One-milliliter aliquots were taken at this point, and the  $\text{pH}_{\text{in}}$  was determined as described above.

**Determination of end products.** The concentrations of lactic and acetic acids were determined by using high-performance liquid chromatography (17). Acids were analyzed with an Aminex HPX-87H column (Bio-Rad Laboratories, Richmond, Calif.) with a cation guard column. A refractive index detector was used for quantitation of the acids.

## RESULTS

**Effect of  $\text{pH}_{\text{out}}$  on  $\text{pH}_{\text{in}}$ .** The minimum pH used in this study was 3.0, since the internal pH of microorganisms grown below pH 3.0 could not be determined because the  $\text{pK}_a$  of the probe is too high. Neither *L. plantarum* nor *Leuconostoc mesenteroides* maintained a constant  $\text{pH}_{\text{in}}$  over the range of  $\text{pH}_{\text{out}}$  studied (Fig. 1). As the  $\text{pH}_{\text{out}}$  was decreased, the  $\text{pH}_{\text{in}}$  decreased. The  $\text{pH}_{\text{in}}$  of each microorganism approached 4.5 when the  $\text{pH}_{\text{out}}$  neared 3.0 (HCl was the acidulant).

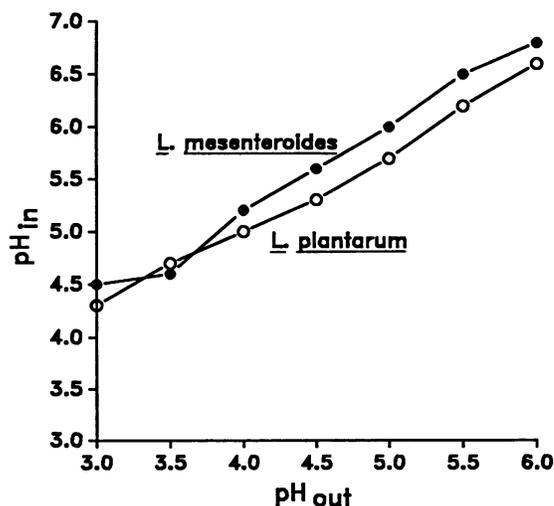


FIG. 1. Effect of  $\text{pH}_{\text{out}}$  on  $\text{pH}_{\text{in}}$  of washed cells of *L. plantarum* (○) and *Leuconostoc mesenteroides* (●) suspended in MHHD acidified with HCl.

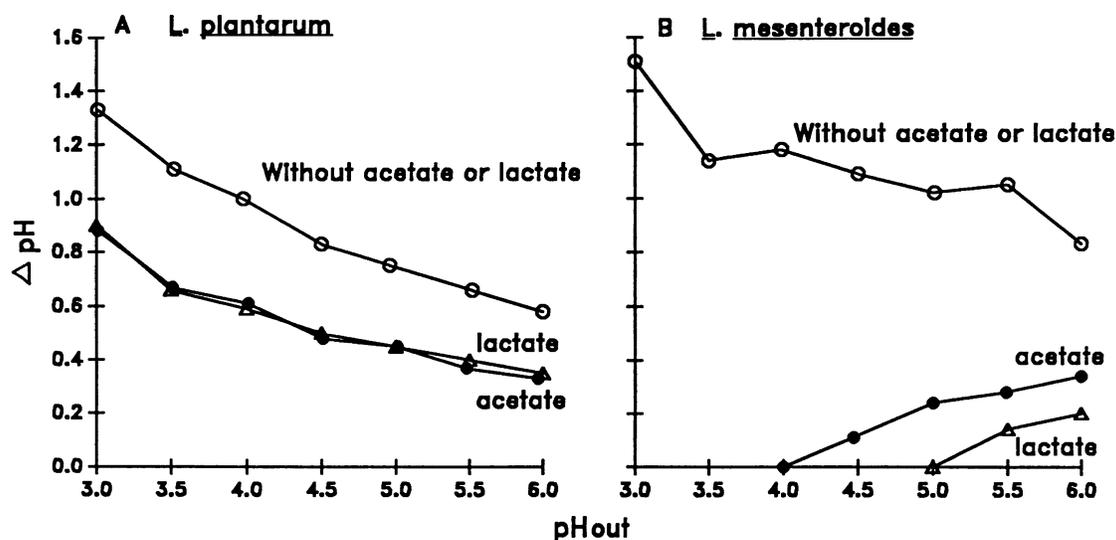


FIG. 2. Effect of  $\text{pH}_{\text{out}}$  and sodium acetate or sodium lactate on  $\Delta\text{pH}$  of *L. plantarum* (A) and *Leuconostoc mesenteroides* (B). Cells were washed and suspended in MHHD. Symbols:  $\circ$ , without buffer;  $\bullet$ , 160 mM sodium acetate;  $\triangle$ , 160 mM sodium lactate.

**Effect of sodium acetate or sodium lactate on the  $\Delta\text{pH}$ s of *L. plantarum* and *Leuconostoc mesenteroides*.** The  $\Delta\text{pH}$ s of both *L. plantarum* and *Leuconostoc mesenteroides* increased with decreasing external pH with HCl as the acidulant (Fig. 2). Addition of either 160 mM sodium acetate or 160 mM sodium lactate decreased the  $\Delta\text{pH}$  but did not collapse the pH gradient of *L. plantarum* (Fig. 2A). However, addition of either 160 mM sodium acetate or 160 mM sodium lactate collapsed the pH gradient of *Leuconostoc mesenteroides* (Fig. 2B). Sodium lactate was more effective than sodium acetate at decreasing the  $\Delta\text{pH}$  of *Leuconostoc mesenteroides*.

Also, the  $\text{pH}_{\text{in}}$  responses of *L. plantarum* and *Leuconostoc mesenteroides* were measured at levels of sodium acetate or sodium lactate ranging from 0 to 160 mM, with a constant

external pH of 5.0 (adjusted with HCl) (Fig. 3). Increasing the concentration of either sodium acetate or sodium lactate decreased the  $\Delta\text{pH}$  of *L. plantarum* and *Leuconostoc mesenteroides* (Fig. 3). No difference was observed between the effect of sodium acetate or sodium lactate on the  $\Delta\text{pH}$  of *L. plantarum* (Fig. 3A). However, a striking difference was observed between the two acids with respect to *Leuconostoc mesenteroides* (Fig. 3B). Addition of sodium lactate resulted in a dramatic decrease and eventual collapse of the pH gradient of *Leuconostoc mesenteroides*. At external pH 5.0, sodium acetate decreased but did not collapse the pH gradient of *Leuconostoc mesenteroides*.

**Effect of growth medium on  $\text{pH}_{(\text{let})}$ ,  $\text{pH}_{(\text{let})}$ ,  $\text{pH}_{(\text{lit})}$ , and terminal acid concentration.** *L. plantarum* initiated growth at a lower pH (3.0) than did *Leuconostoc mesenteroides* (3.5)

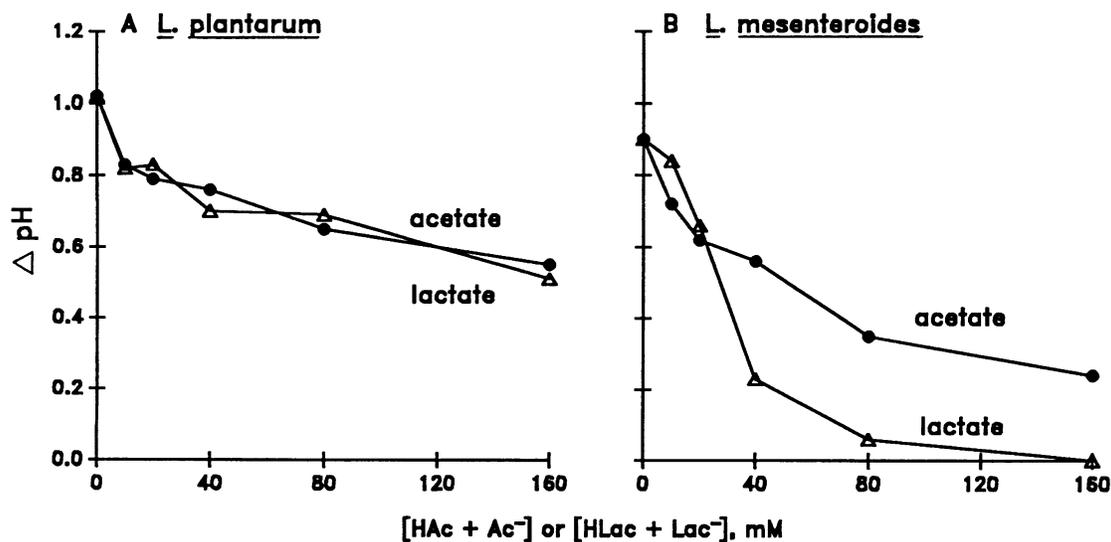


FIG. 3. Effect of sodium acetate or sodium lactate concentration on  $\Delta\text{pH}$  of *L. plantarum* (A) and *Leuconostoc mesenteroides* (B) at an external pH of 5.0. Cells were washed and suspended in MHHD. Symbols:  $\bullet$ , sodium acetate;  $\triangle$ , sodium lactate.

TABLE 1. Effect of sodium acetate or sodium lactate on pH<sub>(lei)</sub> and pH<sub>(let)</sub> of *L. plantarum* and *Leuconostoc mesenteroides*<sup>a</sup>

Condition (mM)	<i>L. plantarum</i>		<i>Leuconostoc mesenteroides</i>	
	pH <sub>(lei)</sub>	pH <sub>(let)</sub>	pH <sub>(lei)</sub>	pH <sub>(let)</sub>
<b>Sodium acetate</b>				
0	<3.00	2.80	3.50	3.33
10	<3.00	2.88	3.50	3.41
20	<3.00	2.91	4.00	3.72
40	3.50	3.18	4.50	3.91
80	3.50	3.40	4.50	4.28
160	4.00	3.61	4.50	4.33
<b>Sodium lactate</b>				
0	<3.00	2.82	3.50	3.39
10	3.50	3.07	4.00	3.76
20	3.50	3.15	4.00	3.88
40	3.50	3.26	4.50	4.11
80	4.00	3.65	5.00	4.63
160	4.50	3.90	5.50	4.75

<sup>a</sup> Growth was defined as an increase in turbidity and a decrease in pH. pH<sub>(lei)</sub> values <3.0 were not tested. Therefore, pH<sub>(lei)</sub> may be <3.00 for *L. plantarum* at 0 to 20 mM sodium acetate or 0 mM sodium lactate.

(Table 1). The presence of sodium lactate in the growth medium increased pH<sub>(lei)</sub> and pH<sub>(let)</sub> for both microorganisms. Higher concentrations of sodium acetate were required to yield results similar to those found with sodium lactate. The pH<sub>(let)</sub> of both microorganisms varied greatly (Tables 1 to 4).

For *L. plantarum* and *Leuconostoc mesenteroides*, lactic acid and acetic acid production and pH<sub>(let)</sub> were dependent upon the growth medium (Tables 2 and 3). However, the pH<sub>(lit)</sub> was relatively constant. The pH<sub>(lit)</sub> of *L. plantarum* varied 0.2 pH unit between the highest and lowest pH<sub>in</sub> (Table 2). With *L. plantarum*, the lowest pH<sub>(let)</sub> (3.24) was reached in MHHD without buffer. The highest pH<sub>(let)</sub> (3.92) was reached in MHHD buffered with 200 mM sodium acetate. Despite the variation among acid levels and pH<sub>(let)</sub>, the pH<sub>(lit)</sub> of *L. plantarum* had a narrow range from 4.8 (MHHD buffered with 200 mM sodium lactate) to 4.6 (unbuffered MHHD). With *Leuconostoc mesenteroides*, pH<sub>(lit)</sub> varied by 0.3 pH unit from 5.4 to 5.7, and pH<sub>(let)</sub> varied by 2.3 units from 5.18 to 3.8 (Table 3).

Both microorganisms produced more lactic acid in media buffered with sodium acetate (MHHD with two levels of acetate and MRS broth) than in unbuffered medium, media buffered with sodium lactate, or cucumber juice. In addition, *Leuconostoc mesenteroides* grown in MHHD with 200 mM sodium lactate yielded no measurable lactic acid.

TABLE 2. Effect of growth medium on lactic acid and acetic acid concentrations and limiting pHs of *L. plantarum*

Growth medium <sup>a</sup>		Lactic acid (mM)		Acetic acid (mM)		pH <sub>(let)</sub>	pH <sub>(lit)</sub>
Basal	Addition	Made	Total	Made	Total		
MRS	55 mM glucose	224	224	8	74	3.81	4.79
CJ	None	110	110	3	3	3.36	4.73
MHHD	None	147	147	5	5	3.24	4.60
MHHD	100 mM NaOAc	227	227	0	98	3.65	4.80
MHHD	200 mM NaOAc	272	272	0	195	3.92	4.67
MHHD	100 mM NaOLac	169	269	7	7	3.64	4.67
MHHD	200 mM NaOLac	83	278	8	8	3.85	4.83

<sup>a</sup> CJ, Cucumber juice; NaOAc, sodium acetate; NaOLac, sodium lactate. Minimum residual sugar concentration was 30 mM. Initial pH was 7.00.

TABLE 3. Effect of growth medium on lactic acid and acetic acid concentrations and limiting pHs of *Leuconostoc mesenteroides*

Growth medium <sup>a</sup>	Lactic acid (mM)		Acetic acid (mM)		pH <sub>(let)</sub>	pH <sub>(lit)</sub>	
	Made	Total	Made	Total			
MRS	55 mM glucose	104	104	7	73	4.38	5.57
CJ	None	29	29	24	24	3.80	5.49
MHHD	None	34	34	5	0	4.02	5.41
MHHD	100 mM NaOAc	67	67	2	100	4.60	5.66
MHHD	200 mM NaOAc	69	69	0	192	4.94	5.67
MHHD	100 mM NaOLac	21	121	7	25	4.62	5.69
MHHD	200 mM NaOLac	0	195	6	6	5.18	5.54

<sup>a</sup> CJ, Cucumber juice; NaOAc, sodium acetate; NaOLac, sodium lactate. Minimum residual sugar concentration was 100 mM. Initial pH was 7.00.

**Effect of D(-) and L(+) isomers of lactic acid on pH<sub>(let)</sub> and pH<sub>(lit)</sub> of *L. plantarum* and *Leuconostoc mesenteroides*.** Since *L. plantarum* produces both D(-) and L(+) isomers of lactic acid and *Leuconostoc mesenteroides* produces only D(-)-lactic acid, we studied the effect of each isomer on the pH<sub>(let)</sub> and pH<sub>(lit)</sub> of the microorganisms. The responses of both *Leuconostoc mesenteroides* and *L. plantarum* were not dependent upon the isomer of lactic acid used (Table 4).

DISCUSSION

This study shows that, regardless of the growth medium, growth of *L. plantarum* and *Leuconostoc mesenteroides* ceases when a specific pH<sub>in</sub> is reached. The pH<sub>(let)</sub> data (Tables 1 to 4) indicate that the medium pH alone cannot define the conditions which will prevent growth of microorganisms. The composition of the growth medium, including initial pH and the presence and type of organic acid, determines pH<sub>(let)</sub>. Similar results showing that there is a minimum pH<sub>in</sub> which is compatible with growth have been reported for *Clostridium thermoaceticum* (1). Similarly, oral streptococci stop growth at a pH<sub>in</sub> of 5.7 (11). Furthermore, Kashket (10) found that growth of *Lactobacillus acidophilus*, *Lactobacillus delbrueckii*, and *Lactobacillus bulgaricus* stops when a pH<sub>in</sub> near 4.4 is reached. In our study, growth of *Leuconostoc mesenteroides* stopped when a pH<sub>in</sub> of 5.4 to 5.7 was reached, while growth of *L. plantarum* stopped when a pH<sub>in</sub> of 4.6 to 4.8 was reached. Our data indicated that *L. plantarum* and *Leuconostoc mesenteroides*

TABLE 4. Comparison of D(-)- and L(+)-lactic acid on pH<sub>(let)</sub> and pH<sub>(lit)</sub> of *L. plantarum* and *Leuconostoc mesenteroides*

Microorganism	Growth medium lactic acid addition <sup>a</sup>	pH <sub>(let)</sub>	pH <sub>(lit)</sub>
<i>L. plantarum</i>	None	3.23	4.62
	100 mM L-(+)	3.65	4.70
	100 mM D(-)	3.64	4.72
	200 mM L-(+)	3.86	4.82
	200 mM D(-)	3.88	4.82
<i>Leuconostoc mesenteroides</i>	None	3.99	5.47
	100 mM L-(+)	4.61	5.63
	100 mM D(-)	4.58	5.60
	200 mM L-(+)	5.08	5.50
	200 mM D(-)	5.11	5.52

<sup>a</sup> Minimum residual glucose concentration was 80 mM, and the basal growth medium was MHHD. Initial pH was 7.00.

do not maintain  $\text{pH}_{\text{in}}$  values near neutral. Neither microorganism maintained a constant  $\text{pH}_{\text{in}}$  and, as the  $\text{pH}_{\text{out}}$  decreased, the  $\text{pH}_{\text{in}}$  decreased (Fig. 1). Both microorganisms initiated growth under conditions where their  $\text{pH}_{\text{in}}$  was below neutral. This may give these microorganisms a competitive advantage over less-acid-tolerant microorganisms which are eliminated early in a fermentation. Other fermentative microorganisms including *C. thermoaceticum* (1), *Clostridium acetobutylicum* (7), *S. ventriculi* (5), and other LAB (10) do not maintain internal pHs near neutral. Goodwin and Zeikus (5) concluded that since fermentative microorganisms obtain less energy from substrates than do respiratory microorganisms, fermentative microorganisms adapt to low pH through mechanisms which are not energy consuming. In our study, the  $\text{pH}_{\text{in}}$  of *Leuconostoc mesenteroides* and *L. plantarum* was near 4.5 when the  $\text{pH}_{\text{out}}$  was 3.0 (acidified with HCl). Similarly, the  $\text{pH}_{\text{in}}$  of *S. ventriculi* (5) and LAB (10), including *L. acidophilus*, *L. bulgaricus*, and *L. delbrueckii*, approached 4.5 as the  $\text{pH}_{\text{out}}$  decreased. Kashket (10) observed that the addition of buffer prolongs growth of LAB because these microorganisms maintain  $\text{pH}_{\text{in}} > 4.4$  for a greater length of time than microorganisms grown without buffer. This might explain the higher acid production in media buffered with sodium acetate as compared with that in unbuffered medium or media containing sodium lactate (Tables 2 and 3). In contrast, lactic acid may decrease the growth of *Leuconostoc mesenteroides* by accelerating the rate at which its  $\text{pH}_{\text{(lit)}}$  is reached.

The undissociated form of an organic acid is assumed to be the most toxic form for microorganisms (4, 8, 14). At all pH levels studied, lactic acid was more effective than acetic acid in decreasing the  $\text{pH}_{\text{in}}$  of *Leuconostoc mesenteroides*, even though the undissociated acid concentration of acetic acid (136 mM) was higher than that of lactic acid (9 mM). Therefore, lactic acid might affect pH homeostasis through a mechanism which is not solely dependent upon the undissociated acid molecule or the  $\text{H}^+$  concentration, thus supporting a role for anion inhibition or for a specific target which lactic acid may attack. Thus, during vegetable fermentation, lactic acid produced by the microorganisms may serve to eliminate *Leuconostoc mesenteroides* early in the fermentation.

Despite low pH and high lactic or acetic acid concentration, *L. plantarum* maintained a pH gradient (Fig. 2A and 3A). The ability of *L. plantarum* to maintain its pH gradient under such conditions may be a contributing factor to the ability of the organism to terminate most vegetable fermentations. Conversely, the inability of *Leuconostoc mesenteroides* to maintain its pH gradient in the presence of acetic or, especially, lactic acid may contribute to its early elimination in vegetable fermentations. Also, the  $\text{pH}_{\text{(lit)}}$  of *L. plantarum* is lower than that of *L. mesenteroides*, which may contribute to its greater acid tolerance. Bender and Marquis (2) attribute acid tolerance of bacteria to the amount and activity of  $\text{H}^+$ -ATPases in the cell membrane. Further research into the mechanisms of acid tolerance, including  $\text{H}^+$ -ATPases, of LAB will lead to a greater understanding of the selective pressures involved in determining the ecology of vegetable fermentations.

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