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Modeling the specific growth rate of *Lactobacillus plantarum* in cucumber extract*

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Abstract. Extensive empirical research has been published on the fermentation of vegetables, but little predictive modeling of the process is available. The objectives of this study were to assess the effects of key variables involved in cucumber fermentation and to develop models for predicting the growth of *Lactobacillus plantarum* in pure and mixed culture fermentations. The growth medium for the studies was cucumber juice. The effects of various concentrations of lactic, acetic, and hydochloric acids and sodium chloride on growth at 30° C were determined in batch culture. Limiting conditions for growth were pH 3.37 (lower limit), 69 mM undissociated lactic acid, 150 mM undissociated acetic acid, or 11.8% NaCl. Acetic acid was stimulatory to growth at low concentrations (up to 40 mM) but inhibitory at higher concentrations. Lactic acid was more inhibitory than acetic acid, whether total or undissociated concentrations were used as the basis of comparison. A predictive equation for specific growth rate was developed, tested, and shown to predict growth of *L. plantarum* in batch processes reasonably well.

Introduction

Approximately 40% of the pickling cucumber crop in the United States is preserved by brine fermentation. Preservation is achieved from the conversion of the fruit sugars into lactic acid and other compounds with a resultant lowering of pH. Cucumber nutrients available for the fermentation must diffuse through the tissue and skin of the fruit into the surrounding brine. Also, sodium chloride and acetic acid added at the beginning of the process diffuse into the fruit. These components exert selective effects on growth of the natural microflora and/or on the starter culture during fermentation.

Although use of starter cultures in vegetable fermentation has been studied since the beginning of this century, indigenous lactic acid bacteria still carry out most commercial cucumber fermentations. Several circumstances are responsible for this situation: current fermentation practices are not compatible with the use of pure cultures, cultures with attributes sufficiently valuable to justify their use are lacking, and other factors (Fleming et al. 1985). *Lactobacillus plantarum*, an acid-tolerant, homofermentative (to hexoses) bacterium, has been the choice as an inoculum in controlled fermentation of cucumbers (Etchells et al. 1973). This species predominates in the final stages of cucumber fermentation, apparently because of its relative acid tolerance.

Unfortunately, fermentation by this organism alone results in concentrations of lactic acid that are too high for direct consumption of the fermented product. A minimum of 0.6% (67 mM) of lactic acid has been recommended (Etchells and Hontz 1972), but high levels of acidity may adversely influence texture retention in cucumber (Fleming and McFeeters 1981) and flavor. Some of the acid must be leached from the product before final processing, which results in a loss of other flavor and nutrient compounds. The leachate must be biodegraded, which places additional demands on waste disposal facilities. In addition, sugar may remain after the primary fermentation by lactic acid bacteria, resulting in subsequent fermentation and bloater damage by acid-tolerant fermentative yeast.

Mixed culture fermentation of cucumber juice by *L. plantarum* and fermentative yeasts was shown to divert a portion of the fermentable sugars to non-acidic endproducts (Daeschel et al. 1988). Acetic acid and NaCl added in the cover brine, together with lactic acid produced by lactic acid bacteria, influence the specific growth rate (μ) of lactic acid bacteria in cucumber fer-

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mentation. The antimicrobial activity of organic acids is due to the inhibition by free H^+, by undissociated acid, and by specific anion effects (Ingram et al. 1956).

The objectives of this reseach were to identify key variables that influence the growth rate of *L. plantarum* in cucumber juice and to develop a mathematical model for predicting μ under varying conditions. The key variables considered were concentrations of H^+, lactic acid (produced by the cells), acetic acid, and NaCl. Subsequent equations to be determined for growth of selected yeasts and for diffusion of soluble components in/out of the fruit, when combined with data for *L. plantarum* in a set of ordinary differential equations, will provide a dynamic model for mixed culture fermentation of whole cucumbers.

Materials and methods

Culture. The bacterium used in the study was *L. plantarum* MOP-3 (provided by this laboratory), and was stored at −70° C in MRS broth (Difco Laboratories, Detroit, MI) containing 16% glycerol. Isolated colonies were picked from MRS agar streak plates of the frozen culture and grown twice in cucumber juice for 12–15 h at 30° C. The inoculum growth medium was the same composition as that used for growth rate determination, but was supplemented with acetic acid (40 mM) or NaCl (6%) when these two compounds were added. The inoculum culture was diluted to an optical density at 630 nm (OD_{630}) of 0.4–0.5, and 1.0% (by volume) was added to the growth medium to give initial cell levels approximating 1×10^6 ml^{-1}.

Growth medium. Cucumber juice for growth studies was prepared by freezing fresh pickling cucumbers at −20° C overnight and then partially thawing and blending to a homogeneous slurry (Daeschel et al. 1988). The slurry was distributed into jars of convenient sizes and stored at −20° C. Before use the slurry was thawed, heated to 80° C, rapidly cooled to room temperature, and centrifuged at 10000 *g* for 20 min. The juice was diluted twofold with distilled water, and 200-ml portions were added to each fermentor. Each portion was individually sterilized by filtration through a 0.22-μm filter (Costar, Cambridge, Mass., USA). The chemicals used in the study were hydrochloric acid, DL-lactic acid, acetic acid, and NaCl (Aldrich, Milwaukee, Wis., USA).

Fermentors. Water-jacketed jars from Wheaton (Millville, N.J., USA), of 200-ml working volume, were used as uncontrolled-pH batch-growth systems. The growth medium was agitated by a magnetic stirrer and maintained at 30° C. Compressed N_2 was humidified, sterilized (0.22-μm Millex-FG_{50} filter, Millipore, Bedford, Mass., USA), and released into the headspace of the fermentor at a rate of 2.5 l h^{-1} to assure anaerobic conditions in all the experiments. During batch growth, 3-ml samples were removed aseptically by syringe from the 200-ml initial broth volume at intervals of 1–6 h (depending on the fermentation rate) until growth ceased, used for OD and pH measurement, and then frozen for future HPLC analysis.

Analytical methods. Cell growth was followed by measuring the OD_{630} of the medium in a 1.5-ml glass cuvette using a Novaspec II spectrophotometer (Pharmacia, Piscataway, N.J., USA). The linear range extended to $OD_{630} = 0.45$. During growth, if the OD_{630} was higher than 0.30, the sample was diluted to within a range of 0.1–0.3 using distilled water. Standard curves were used to relate OD_{630} to cell concentration [colony-forming units (cfu ml^{-1}]. Viable cells were enumerated in MRS agar (Difco, Detroit, Mich., USA), incubated at 30° C. One unit of OD_{630} was found to be eqivalent to 2.5×10^8 cfu/ml. NaCl was determined by titration with standard $AgNO_3$ using dichlorofluorescein as an indicator (Fleming et al. 1992). All other components were determined using HPLC; each sample was diluted twofold using distilled water, and 1.5-ml aliquots were centrifuged at 12000 *g* (Eppendorf centrifuge model 5415, Westburg, N.Y., USA). Glucose, lactic acid, and acetic acid were analyzed with an Aminex HPX-87H column (Bio-Rad Laboratories, Richmond, Calif., USA) at 65° C, a Waters Differential Refractometer (Waters Associates, Milford, Mass., USA) and a Shimadzu integrator (Shimadzu, Columbia, Md., USA). The column was eluted with 0.01 M H_2SO_4 at a flow rate of 0.7 ml min^{-1} (Lazaro et al. 1989).

The concentration of the undissociated lactic acid [HLa] was calculated from the equation:

$$[\mathrm{HLa}] = \frac{[\mathrm{La_t}]}{(1+10^{\mathrm{pH}-\mathrm{p}K'})} \quad (1)$$

where

$$\mathrm{p}K' = \mathrm{p}K - \frac{Z^2 \cdot (0.51)\sqrt{\Gamma/2}}{1+1.6\sqrt{\Gamma/2}} \quad (2)$$

and $[La_t]$ is the total lactic acid concentration, pK is the −log dissociation constant, equal to 3.86, Z is the ion charge, and $\Gamma/2$ is the ionic strength (Westcott 1978). For undissociated acetic acid [HAc], the same equations were used with the pK of 4.75 (see nomenclature for subsequent definitions).

Model development. To develop a mathematical representation of the μ of *L. plantarum* as a function of the dynamic chemical variables during cucumber fermentation (pH, lactic acid, acetic acid, and NaCl concentrations), the effect of each variable was studied individually. For each set of fermentations, medium pH was adjusted from about 5.95 (initial cucumber juice pH) to 3.6 by hydrochloric, lactic, or acetic acid addition. The medium adjusted with hydrochloric acid was used to establish the effect of hydrogen ion concentration, $[H^+]$ on the μ of the bacteria without influence of lactic or acetic acid. To determine the individual effect of [HLa], the [HLa] for each fermentation was calculated as described above and the corresponding effect on the overall μ was estimated after eliminating the $[H^+]$ effect, using Eq. 1. A similar procedure was used to ascertain the [HAc] effect. The influence of NaCl was obtained with concentrations in the range 1–12%.

The μ were calculated from linear regression analysis of the initial exponential portion of the growth curves. Since the inoculum was grown in the same medium as the growth medium, no lag in growth was observed.

To define the mathematical relationship between μ and component concentrations, several models were fitted to the data using non-linear regression and goodness-of-fit criteria. The resultant data indicated the best model for establishing relevant coefficients. Analysis was performed with SAS software (NLIN, SAS Institute, Cary, N.C., USA), using the iterative modified Gauss-Newton method (SAS 1988). Table 1 presents the more common equations tested to describe cell growth under product inhibition conditions.

Results

For growth of *L. plantarum* in cucumber juice at 30° C, the four independent variables studied were acetic acid, lactic acid, H^+, and NaCl concentrations. Both acetic acid and NaCl typically are added to cover brines of cucumbers prior to fermentation. Lactic acid is produced during the fermentation. The pH may or may not be adjusted to a specific value, but under com-

Table 1. Model equations for cell growth under product-inhibited conditions

Kientic model, f(C)[a]	Form	Reference
$\mu=\mu_0\left\{1-\left(\frac{[C]}{[C]_{max}}\right)\right\}$	Linear	Ghose and Tyagi (1979)
$\mu=\mu_0\left[1-\left(\frac{[C]}{[C]_{max}}\right)\right]^{\alpha}$	Non-linear	Levenspiel (1980)
$\mu=\mu_0\left\{1-\left(\frac{[C]}{[C]_{max}}\right)^{a}\right\}$	Non-linear	Luong (1985)
$\mu=\mu_0\exp(-K_i[C])$	Exponential	Aiba et al. (1968)
$\mu=\mu_0\left(\frac{K_i}{K_i+[C]}\right)$	Hyperbolic	Aiba and Shocla (1969)
$\mu=\mu_0\left(\frac{K_{i1}}{K_{i1}+[C]}-K_{i2}[C]\right)$	Non-linear	Kuhn (1991)

[a] Where [C] = inhibitory product concentration and $[C]_{max}$, K_i, α, a, K_{i1}, and K_{i2} are adjusted coefficients

mercial conditions it normally is not controlled. The above four variables were shown in preliminary experiments to have significant effects on cucumber juice fermentation. Temperature, another important factor, was not varied in order to contain the study within manageable limits.

pH effect

The variation of *L. plantarum* μ with pH in the presence of hydrochloric, lactic, or acetic acid is shown in Fig. 1. When lactic or hydrochloric acids were added to establish pH values above 3.8, the specific growth rates were influenced primarily by $[H^+]$ and not by acid

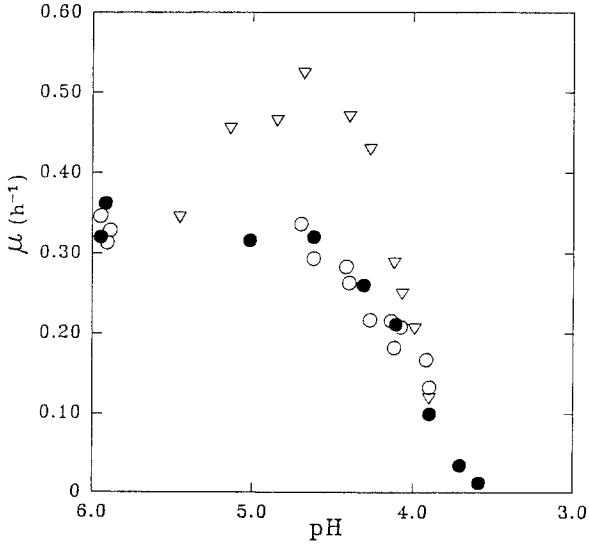

Fig. 1. Specific growth rate (μ) of *Lactobacillus plantarum* at different pHs, using hydrochloric (○), acetic (▽), or lactic (●) acid

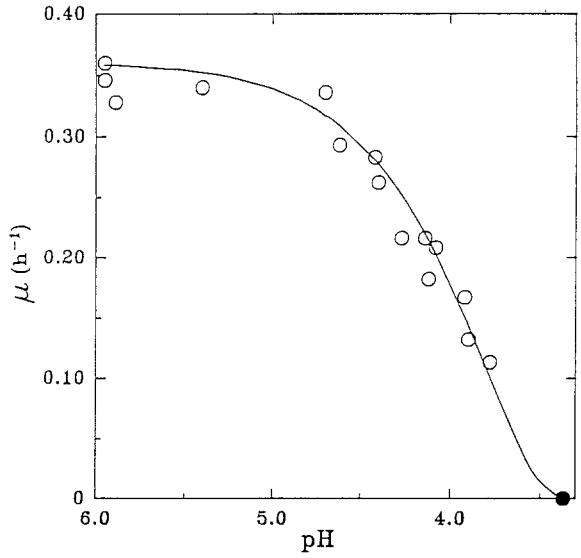

Fig. 2. Effect of pH on the μ of *L. plantarum*: HCl was added (○) to adjust pH. The *solid curve* represents fitted values (Eq. 3). The *filled circle* represents the terminal growth-limiting pH in a batch process

type. Acetic acid addition resulted in an increase in μ within the range of pH 4.0–5.5, compared to the μ at pH 6.0. This stimulatory effect of acetic acid was not observed at pH values below 4.0. Growth was not observed at initial medium pH values below 3.7 with any of the acids. However, in media where *L. plantarum* had already initiated substantial growth, cell growth continued until the pH decreased to 3.37. Data for growth at pH values lower than 3.7 thus represent μ measured during the latter phase of growth.

To propose a more realistic mathematical relationship than that posed by the pH of 3.7 for arrestment of growth initiation, the terminal growth-limiting pH of 3.37 was added in the set of data (filled circle, Fig. 2). The equation that provided a best fit of the data among those tried (Table 1) was a non-linear equation proposed by Levenspiel (1980).

$$\mu=\mu_0 f_1([H^+])=\mu_0\left(1-\frac{[H^+]}{[H^+]_{max}}\right)^{\alpha_1} \quad (3)$$

When Eq. 3 was fitted with the hydrochloric acid data (solid line, Fig. 2) the best-fit values were: $\mu_0=0.35\ h^{-1}$, $[H^+]_{max}=0.427$ mM (pH = 3.37), and the coefficient $\alpha_1=2.6$.

Total lactic acid effect

Figure 3 shows the effect of $[La_t]$ on the μ of *L. plantarum*. The non-linear model (solid line, Fig. 3) proposed by Levenspiel (1980) again provided the best fit:

$$\mu([La_t])=\mu_0\left(1-\frac{[La_t]}{[La_t]_{max}}\right)^{\alpha_2} \quad (4)$$

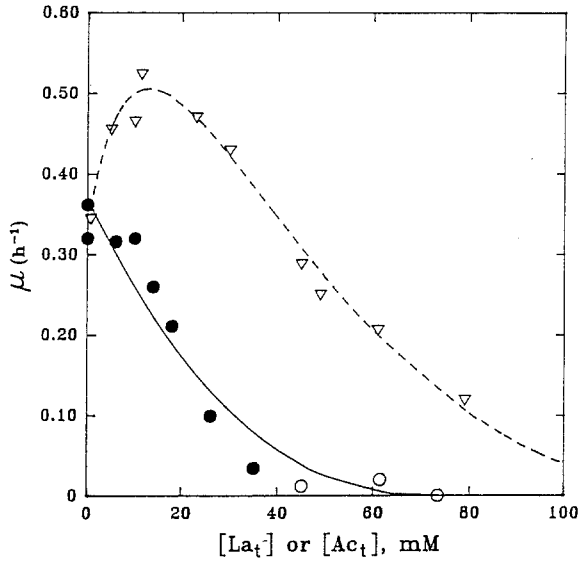

Fig. 3. Effect of total lactic [La_t] and total acetic acid [Ac_t] concentrations on the μ of *L. plantarum*. The *solid* and *dashed curves* represent fitted values (Eq. 4 and 5): ●, La_t added; ○, La_t produced; ▽, Ac_t added

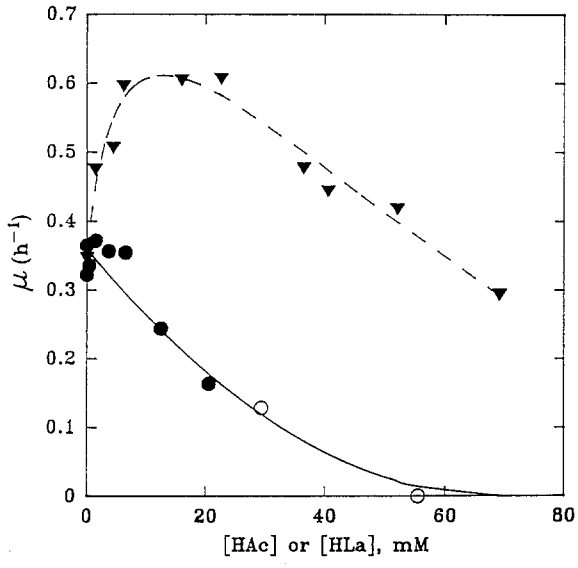

Fig. 4. Effect of undissociated lactic [HLa] and acetic acid [HAc] concentrations on the μ of *L. plantarum*. The *solid* and *dashed curves* represent fitted values (Eqs. 6 and 7): ●, HLa added; ○, HLa produced; ▼, HAc added

The best-fit parameters were $\mu_0 = 0.37\ h^{-1}$, $([La_t]_{max}) = 73.5$ mM and the coefficient $\alpha_2 = 2.4$. The difference between this estimate of the μ_0 ($0.37\ h^{-1}$) and the previous estimate ($0.35\ h^{-1}$) reflects experimental error.

Total acetic acid effect

Figure 3 shows the stimulatory effect of acetic acid on *L. plantarum* growth with a positive maximum effect in the range of 10–20 mM. Beyond 40 mM, cell growth was inhibited when compared with the maximum specific growth rate (μ_{max}) observed in the absence of acetic acid. To adequately describe the observed acetic acid stimulation-inhibition effects, we propose Eq. 5. The first part of the equation, a modification of the Monod model, models growth stimulation and dominates at low acetic acid concentrations, whereas the second part accounts for [HAc] and [H^+] inhibition and drives the rate to zero at high acetic acid concentration, $[Ac_t]_{max}$ (broken line, Fig. 3):

$$\mu([Ac_t]) = \mu_0 \underbrace{\left(1 + \frac{\beta_1 [Ac_t]}{K_m^{AC} + [Ac_t]}\right)}_{\text{Stimulation}} \underbrace{\left(1 - \frac{[Ac_t]}{[Ac_t]_{max}}\right)^{\alpha_3}}_{\text{Inhibition}} \quad (5)$$

The fitted rate constants were $\mu_0 = 0.33\ h^{-1}$, $\beta_1 = 2.2$, $K_m^{Ac_1} = 15.8$ mM, $[Ac_t]_{max} = 150$ mM, and $\alpha_3 = 2.9$.

Undissociated lactic acid effect

Figure 4 shows the effect of undissociated lactic acid. The solid line represents the predicted values from Eq. 6.

$$\frac{\mu}{f_1([H^+])} = \mu_0 f_2([HLa]) = \mu_0 \left(1 - \frac{[HLa]}{[HLa]_{max}}\right)^{\alpha_4} \quad (6)$$

The fitted parameters were $\mu_0 = 0.37\ h^{-1}$, $[HLa]_{max} = 69$ mM, and $\alpha_4 = 2.0$.

Undissociated acetic acid effect

Figure 4 also presents the effect of undissociated acetic acid on the μ of *L. plantarum*. The same equation used to describe the [Ac_t] effect fits the data for [HAc] (Eq. 7; broken line, Fig. 4), with rate constants $\mu_0 = 0.35\ h^{-1}$, $\beta_2 = 1.5$, $K_m^{HAc} = 5.8$ mM, $[HAc]_{max} = 150$ mM, and $\alpha_5 = 1.7$.

$$\frac{\mu}{f_1([H^+])} = \mu_0 f_3([HAc]) = \mu_0 \left(1 + \frac{\beta_2 [HAc]}{K_m^{HAc} + [HAc]}\right)\left(1 - \frac{[HAc]}{[HAc]_{max}}\right) \quad (7)$$

NaCl effect

The effect of NaCl concentration on growth rate is shown in Fig. 5. The μ increased slightly as the NaCl concentration rose from 0 to 2%. Beyond 5%, cell growth was reduced in a nearly linear fashion, and no growth was observed at 12% NaCl. To fit the growth rate we used the same equation as for total acetic acid (Eq. 6).

$$\mu = \mu_0 f_4([NaCl]) = \mu_0 \underbrace{\left(1 + \frac{\beta_3 [NaCl]}{K_m^{NaCl} + [NaCl]}\right)}_{\text{Stimulation}} \underbrace{\left(1 - \frac{[NaCl]}{[NaCl]_{max}}\right)^{\alpha_6}}_{\text{Inhibition}} \quad (8)$$

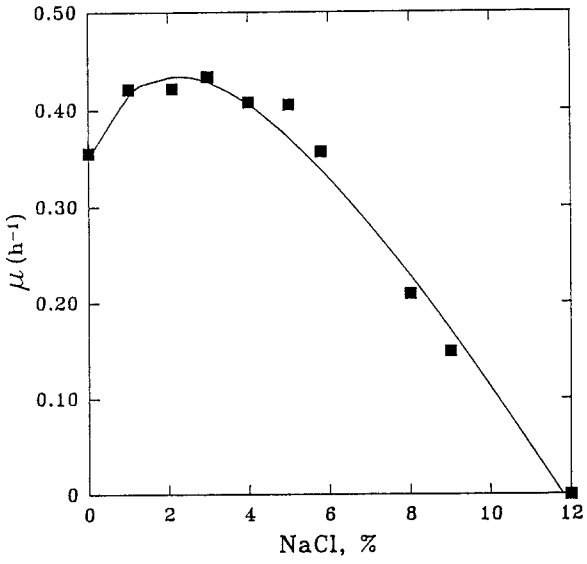

Fig. 5. Effect of NaCl concentration on the μ of *L. plantarum*. The *solid curve* represents fitted values (Eq. 8)

The best-fit values were $\mu_0 = 0.35\ h^{-1}$, $\beta_3 = 1.6$, $K_m^{NaCl} = 4.47\%$, $[NaCl]_{max} = 11.8\%$, and $\alpha_6 = 1$ (solid line, Fig. 5).

Combined effect

The individual effects of H^+ (Eq. 3), undissociated lactic acid (Eq. 6), undissociated acetic acid (Eq. 7), and NaCl (Eq. 8) were then combined, assuming a multiplicative overall effect, to provide a model to predict μ:

$$\mu = \mu_0\, f_1([H^+])\, f_2([HLa])\, f_3([HAc])\, f_4([NaCl]) \quad (9)$$

The values for the constants are summarized in Table 2. The reported value of μ_0 was obtained by averaging estimates from individual additive experiments.

This general procedure has been used by others (Kuhn 1991; Yabannavar and Wang 1991). To test the model, combinations of lactic acid, acetic acid, and NaCl in various concentrations were evaluated (Table 3) as to their effects on growth of *L. plantarum*. The observed growth rates were predicted well, as shown by comparison of predicted and experimental resuls (Fig. 6). The goodness-of-fit of the observed values against the predicted values was subjected to a chi-square test; the fit was significant at the 0.005 level.

Table 2. Parameter values for *Lactobacillus plantarum* specific growth rate (μ) model (Eq. 9)

Influencing parameter	Symbol	Value obtained
Cucumber juice only	μ_0	0.34 h^{-1}
$[H^+]$	$[H^+]_{max}$	0.427 mM
	α_1	2.6
[HLa]	$[HLa]_{max}$	69 mM
	α_3	2.0
[HAc]	β_1	1.5
	K_m^{HAc}	5.8 mM
	$[HAc]_{max}$	150 mM
[NaCl]	α_5	1.7
	β_3	1.6
	K_m^{NaCl}	4.5%
	$[NaCl]_{max}$	11.8%

Table 3. Combinations of acetic and lactic acids and NaCl used to test growth prediction Eq. 9[a]

Treatment	NaCl (%)	$[Ac_t]$ (mM)	$[La_t]$ (mM)
A	3	40	10
B	5	10	10
C	3	40	10
D	5	10	10
E	3	40	28
F	5	10	28
G	3	40	28
H	5	10	28
I	0	5	0
J	0	10	0
K	0	0	10
L	0	0	30
M	3	0	0
N	5	0	0
P	8	0	0
R	9	0	0
S	4	5	0
T	4	40	0
U	6	40	0

[a] See Fig. 6 for the relationship between observed and predicted μ values

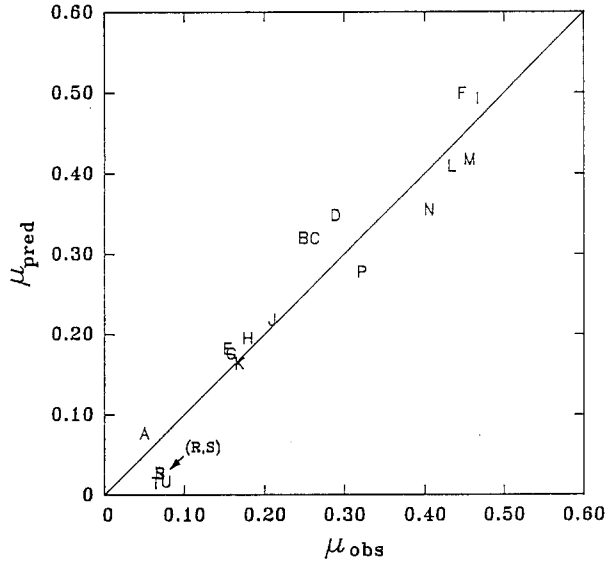

Fig. 6. The relationship of predicted μ (μ_{pred}) of *L. plantarum* using Eq. 9 and observed μ (μ_{obs}) of 19 independent fermentations (*letters*) using different combinations of initial concentrations of lactic acid, acetic acid, and NaCl (Table 3). The *solid line* represents 100% agreement between the predicted and observed values

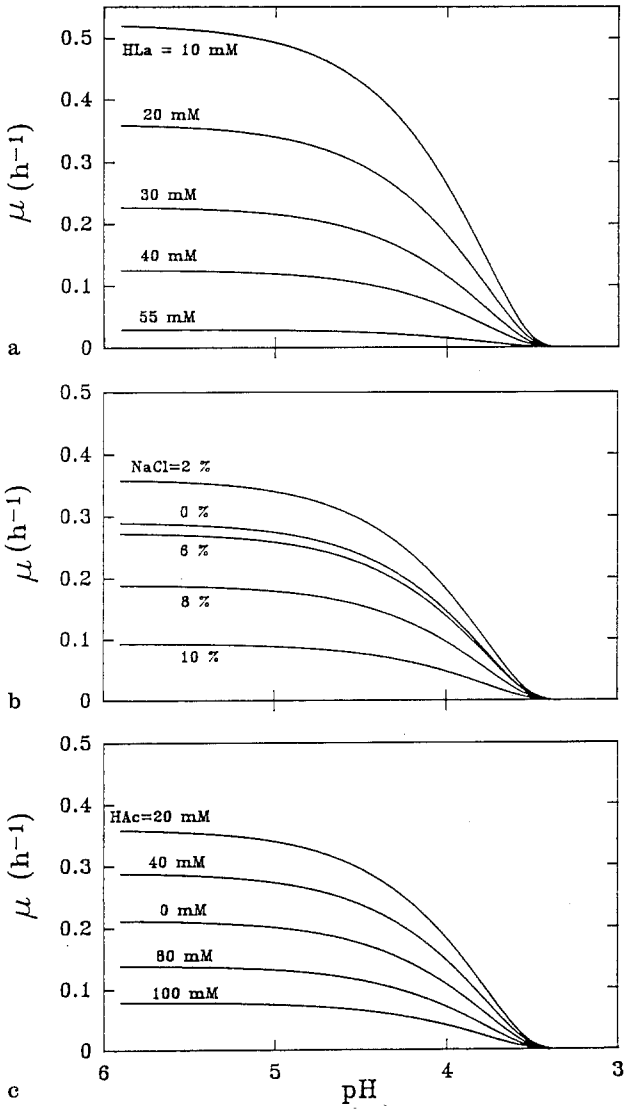

Fig. 7a–c. Predicted effect of pH on the μ of *L. plantarum*. **a** Variable concentrations of undissociated lactic acid (HLa), 20 mM undissociated acetic acid (HAc) and 2% NaCl. **b** Variable NaCl concentrations, 20 mM HLa and 20 mM HAc. **c** Variable concentrations of HAc, 20 mM HLa and 2% NaCl

Example applications of Eq. 9, illustrating predicted effects of variables on the μ of *L. plantarum*, are illustrated in Fig. 7. In Fig. 7a, [HAc] and [NaCl] were kept constant and the effect of pH was demonstrated for different [HLa]. In Fig. 7b, undissociated lactic acid and undissociated acetic acid were kept constant, and the effect of pH was demonstrated for different NaCl concentrations. In Fig. 7c, [HLa] and [NaCl] were kept constant, and the effect of pH was demonstrated for different [HAc].

Discussion

A mathematical model was developed to describe the growth of *L. plantarum* in cucumber juice at 30° C with different concentrations of acids (lactic and acetic) and NaCl, and various pHs. The model can be used to predict the growth of *L. plantarum* in the fermentation of cucumbers. It may also be useful to predict growth in other media with excess sugar.

Weak acids show antimicrobial activity primarily in the undissociated state, but $[H^+]$ itself is also inhibitory. Since the relationship between the undissociated and dissociated state of the acid depends on the pH of the medium, buffer capacity will also be important. For batch vegetable fermentation by lactic acid bacteria where the pH is not controlled and the buffer capacity of the medium is extremely low, growth limitation by achievement of low pH is likely to be very significant.

When hydrochloric acid was used to lower the pH, we assumed the effect on bacterial growth was directly attributed to $[H^+]$ in the medium. *L. plantarum* is considered an acid-tolerant bacterium compared to other lactic acid bacteria. Growth was not observed at initial medium pH values below 3.7 with any of the acids. However, in media where *L. plantarum* had already initiated substantial growth, cells continued growth until the pH decreased as low as 3.37.

Mechanisms of such acid tolerance have been proposed. Tang et al. (1989), working with *Clostridium formicoaceticum,* and Foster (1992), using *Salmonella typhimurium,* observed similar behavior to that for *L. plantarum*. Foster (1992) suggests a two-stage induction process as a system of cell protection, the first triggered at low acidity and a second stage induced at moderate acidity. Both systems must be induced before the cell can be exposed to severe acid conditions. McDonald et al. (1990) showed that the internal pH of *L. plantarum* is lowered when the cells are exposed to acid conditions, but the cells continue to grow until a limiting internal pH is reached. The authors refer to a minimal internal pH of 4.6–4.8 for growth, with a ΔpH of 0.9–1.0 units representing external pHs of 3.6–3.9.

In determining the lower pH limit for cell division, it was necessary to follow growth to the stationary phase. *L. plantarum* produced acid even after growth had ceased. Apparently, such stationary-phase acid production was due to energy generation for cell maintenance. Studies to define the maintenance coefficient of *L. plantarum* and the effect of pH on the death rate of the cells will follow this study in order to complete the model development for acidity predictions.

Acidification of cucumber juice with lactic acid resulted in an inhibitory effect on growth beyond the H^+ effect. Growth inhibition was attributed primarily to the protonated lactic acid form. Yabannavar and Wang (1991) showed that for *L. delbrueckii* the growth inhibitory effect by lactate is small when compared with that by uncharged lactic acid. Using their growth inhibition equation, we calculate that growth of *L. delbrueckii* was reduced by 95% at either 69 mM [HLa] or 893 mM $[La^-]$. We found that growth of our *L. plantarum* culture was inhibited by 95% at 51 mM [HLa].

Acetic acid, when added in low concentration (10–20 mM, Fig. 3), stimulated the growth of *L. plantarum*, as earlier observed by McDonald (1988). A possible cause is limitation in cucumber juice by some specific

nutrient that could be substituted by acetate. The stimulatory effect of acetic acid has been shown for many strains of *Streptococcus lactis* and *S. cremoris* (Collins et al. 1950) and *S. diacetilactis* (Collins and Bruhn 1970). Collins and Bruhn (1970) found that *S. diacetilactis* growing in a partially defined, lipoic-acid-free medium had a μ_{max} when 2.0–4.0 g l^{-1} acetate was added and that DL-α-lipoic acid, reduced lipoic acid, and natural substances containing lipoic acid replaced the response to acetate. They also found that acetate was incorporated in the lipid fraction of the cells.

Beyond the stimulatory effect, a further increase in the [HAc] resulted in an inhibitory effect on the μ of *L. plantarum*, but this effect was smaller than that caused by undissociated lactic acid (Fig. 4). From the proposed model, only 20 mM undissociated lactic acid was necessary to reduce the μ of *L. plantarum* by 50%, and 69 mM caused complete growth inhibition. In comparison, 68 mM undissociated acetic acid was necessary for 50% reduction in μ and 150 mM for complete inhibition.

We conclude from the model (Eq. 9) that $[H^+]$ and [HLa] are major factors limiting growth of *L. plantarum* in cucumber fermentation. The model suggests that cells having unlimited carbohydrate will cease to grow at either pH 3.37 or an [HLa] of 69 mM.

Cells grew vigorously in NaCl concentrations up to 5%, but no growth was observed at 12%. Similary, McDonald (1988) found no difference in the μ of *L. plantarum* at 2 and 4% NaCl, but a reduction of 45% in the growth rate at 6%.

The prediction equation was developed for growth of *L. plantarum* in cucumber juice. For other media, μ must be determined in the presence and absence of acetate to confirm if acetate stimulates growth and to define μ_0. The equation also will be useful in predicting the μ of *L. plantarum* in the fermentation of whole cucumbers. To be applicable to brined cucumbers, however, the effects of diffusion must be considered. Cucumbers typically are brined at a ratio of about 60% cucumbers to 40% cover brine, by volume. Acetic acid and NaCl added to the brine diffuse into the cucumbers and nutrients of the cucumbers diffuse into the brine. To complicate matters further, cucumbers vary in diffusivity to these components, depending upon size, lot, and possibly other factors (Potts et al. 1986). Furthermore, bacteria may grow within the brine, as well as within the cucumbers (Daeschel and Fleming 1981). Further studies are underway to better understand the effects of diffusion on the growth of *L. plantarum* in pure and mixed culture fermentation when whole cucumbers are brined.

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Nomenclature. α, β, K_m, coefficients determined by model fitting; a, K_i, K_{i1}, K_{i2}, adjusted coefficients; $[Ac^-]$, dissociated acetic acid concentration (mM); $[Ac_t]$, total acetic acid ($[HAc]+[Ac^-]$) concentration (mM); [C], inhibitory component concentration (mM); f_1, f_2, f_3, f_4, functions of $[H^+]$, [HLa], [HAc], and [NaCl], respectively; [HAc], undissociated acetic acid concentration (mM); $[H^+]$, hydrogen ion concentration (mM); [HLa], undissociated lactic acid concentration (mM); K_m, that concentration (mM) of the stimulatory chemical added at which the specific growth rate is half its maximum value (equivalent to the Michaelis constant); $[La^-]$, dissociated lactic acid concentration (mM); $[La_t]$, total lactic acid ($[HLa]+[La^-]$) concentration (mM); μ, specific growth rate (h^{-1}); μ_0, specific growth rate at zero concentration of additive (h^{-1}); [NaCl], sodium chloride concentration (%, w/v); pK, negative log dissociation constant; pK', negative log apparent dissociation constant; $[\ ]_{max}$, concentration (mM) of added chemical at which the specific growth rate first became zero, determined by model fitting; Z, ion charge; $\Gamma/2$, ionic strength.

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