

Effects of Temperature, pH, and Potassium Lactate on Growth of *Listeria monocytogenes* in Broth

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Abstract A total of 60 growth curves were generated with combinations of temperature, pH, and potassium lactate (PL) (60% (v/v) commercial solution) to determine the lag time (LT) and specific growth rate (SGR) of *L. monocytogenes* in broth. LT and SGR were significantly ($P < 0.05$) affected by temperature, pH, concentration of PL, or the combined interaction of these factors. LT was extended and SGR was reduced significantly ($P < 0.05$) by increased concentration of PL at lower temperature and pH. Listericidal effect was observed in the broth containing 2, 3, and 4% PL at pH 5.0 and 4°C. The antimicrobial activity of PL against *L. monocytogenes* increased when the pH of the medium was decreased at all temperatures tested. The results suggest that PL has antimicrobial properties to suppress the growth of *L. monocytogenes*. Potassium lactate has many potential applications as an antimicrobial additive in variety of refrigerated ready-to-eat foods.

Keywords: *L. monocytogenes*; potassium lactate; specific growth rate; lag time; pH; temperature

Introduction

Listeria monocytogenes is a gram-positive, non-spore forming, facultative anaerobic and psychrotrophic bacterium that can cause foodborne disease in humans and animal (1-4). *L. monocytogenes* can be easily spread into the environment by water and birds, bedding, and animal feed. Because of its wide and ubiquitous distribution in nature, animal exposure to this pathogen is unavoidable (5). Listeriosis is one of the most severe food-borne diseases. It can cause meningitis, septicemia, and abortion. Though the morbidity of this disease is low (annual incidence rate ranging from 2 to 10 cases per million populations), it has a high fatality rate (20-30%) for pregnant women, newborn infants, the elderly, and other individuals with impaired T-cell immunity and compromised immune systems (6, 7). Outbreaks of human listeriosis have been reported as a result of the consumption of meat and meat products contaminated with this pathogen (8-11). This bacteria has been intensively studied due to its ability to survive and even multiply at refrigeration temperatures (12, 13). It is also known that contaminated handling equipment can serve as a source of contamination in processed foods (5, 14). In addition, there are various intrinsic and extrinsic factors in raw and processed products that play an important role in the growth kinetics of *L. monocytogenes*. Although *L. monocytogenes* and other species have been isolated from many different types of raw and processed foods, the main source and routes of contamination are still not fully understood (15-21).

Lactic acid and its potassium and sodium salts, commonly known as lactates, are generally recognized as safe (GRAS) compounds. The USDA and the FDA have

already approved its use as a flavoring agent and flavor enhancer in various meat and poultry products at a level of 2% of actual potassium lactate, which is 3.3% of the 60% solution commercially available (22). Sodium lactate and potassium lactate have been used in meat products at levels varying from 2% to 4% without adversely affecting the sensory quality of the food (23). In addition, they have been widely used as antimicrobial agents to extend the shelf life and to increase the safety of meat and poultry products by controlling bacterial outgrowth (23-26). When considering the antimicrobial effects of lactates, the impact of the lactic acid bacteria was noted because their growth inhibits the growth of other pathogenic flora (27-30). A benefit of using potassium lactate instead of sodium lactate is that the sodium content in products is not affected.

Potassium lactate (PL) is produced by the fermentation of sugar. PL has shown bacteriostatic activity against many types of flora, especially *L. monocytogenes* and lactic acid bacteria (29, 31-33). However, few studies (34-36) have been conducted on its effect on the growth response of *L. monocytogenes* in broth. Therefore, the objective of this study was to investigate the effect of potassium lactate on the growth behavior of *L. monocytogenes* in broth as a function of temperature and pH. Hopefully this data will help to determine the best conditions for controlling the growth of *L. monocytogenes*.

Materials and Methods

Test organism Cocktails of three-strains of *L. monocytogenes* (Scott A, ATCC 19111, ATCC 19116) were used throughout the study. Stock cultures were maintained biannually on tryptic soy agar (Difco, Becton Dickinson Co., U.S.A) supplemented with 20% glycerol stored at -70°C. Sub-culturing was done in tryptic soy broth supplemented with 0.6% yeast extract (TSBYE: Difco, Becton Dickinson Co., U.S.A). Subcultures were

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activated by transferring 100 μ L of each stock culture into 10 mL of TSB to be incubated for 24 hr at 35°C. Following incubation, 10 mL of each culture suspension was harvested by centrifugation at 1500 rpm for 10 minutes twice. It was then washed with 0.1M phosphate buffer solution. Serial dilutions were carried out with 0.1% sterile peptone water to get the desired concentration for inoculum preparation. A mixture containing equal numbers of cells from each strain was used as inoculum.

Preparation of antimicrobial agents PL (PURASAL P HiPure 60, Purac America Inc., Lincolnshire, U.S.A) was added to tryptic soy broth (TSB) at a total volume of 100 mL for PL solutions at the following concentrations: 1%, 2%, 3%, and 4%. To make a 1% solution of potassium lactate, 1.6 mL of PL was added to 98.4 mL TSB. A 2% solution was made by adding 3.2 mL PL to 96.8 mL TSB, and 4.8 mL PL was mixed with 95.2 mL TSB for a 3% PL solution. The 4% solution was a mixture of 6.6 mL PL with 93.4 mL TSB. Broth media without PL was prepared as a control

Determination of pH The pH of the broth media containing PL was determined using an Orion pH meter (model 420 A, Orion research Inc., Cummings Center, Beverly, USA). The media was adjusted to pH 5.0, 5.5, 6.0, 6.5, or 7.0 with 0.1N HCl or 0.1N NaOH, autoclaved for 15 min at 121°C, and cooled down to room temperature before inoculation.

Experimental procedure and assessment of growth One hundred mL of sterile TSB containing different concentrations of PL was transferred into sterile 500-mL Erlenmeyer flasks. From the working culture, 1 mL of inoculum was added into each of these flasks to give a final inoculum of approximately to 10^2 - 10^3 CFU/mL. The flasks were incubated at various temperatures. Sampling was done at regular intervals to determine inhibitory effects on bacterial growth under all conditions tested. The number of viable bacteria was determined by spreading 100 μ L of suspension onto the surface of duplicate plates of modified oxford medium base agar (Difco, Becton Dickinson Co., U.S.A). The plates were then incubated at 35 for 24 hrs. Colonies were counted by using standard plate count methods.

Statistical analysis Each experiment featured two replications per treatment. Growth curves were generated using GraphPad PRISM 4 (Graphic Pad Software San Diego, USA). From GraphPad PRISM figures, specific growth rate (SGR) and lag time (LT) were determined. Results were analyzed by ANOVA and means were separated using Duncan's multiple range test ($P < 0.05$) using the Statistical Analysis Systems SAS® version 8.2 (SAS Institute, Cary, NC, U.S.A).

Results

Effect of potassium lactate at 4°C Fig. 1 shows the effect of the PL on the growth pattern of *L. monocytogenes* in TSB stored at 4°C. The values for LT of *L. monocytogenes* as function of temperature, pH, and PL

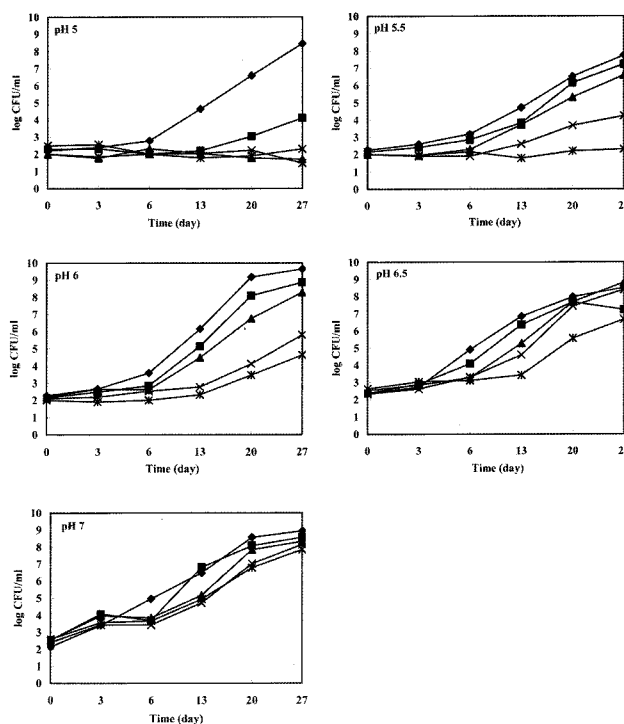


Fig. 1. Growth of *L. monocytogenes* in TSB containing different concentrations of potassium lactate as a function of pH at 4°C (Control \blacklozenge , Potassium lactate 1% \blacksquare , 2% \blacktriangle , 3% \times , 4% $*$).

concentration are shown in the Table 1. The data on the effects of temperature, pH, and PL concentration on SGR is shown in Table 2. At pH 5, growth of *L. monocytogenes* was seen in the control after 6 days at a specific growth rate of 0.31 \log_{10} CFU/day. Addition of 1% PL in the broth only extended LT by 15.6 days and did not decrease the SGR of *L. monocytogenes*. No growth was observed in broth containing 2, 3, or 4% PL for the first 27 days. At pH 5.5, growth started in the untreated control broth after 4 days. Addition of 1% PL significantly ($P < 0.05$) increased the LT over that of the control, but PL concentrations of more than 2% were needed to significantly reduce SGR ($P < 0.05$). There was no significant ($P > 0.05$) difference in SGR (0.46 \log_{10} CFU/day) between controls at pH 6.0 or 6.5. However, LT was still significantly ($P < 0.05$) extended by increases in PL concentrations at both pH 6 and 6.5, while no significant differences ($P > 0.05$) in SGR were noticed among the different concentrations tested at pH over 6. At pH 7, *L. monocytogenes* started to grow within 9 hours. However, addition of PL significantly ($P < 0.05$) increased LT and reduced SGR. Overall, the inhibitory effect of PL on the growth of *L. monocytogenes* was accelerated with increases in PL concentration.

Effect of potassium lactate at 7°C Fig. 2 represents the growth pattern of *L. monocytogenes* observed at 7°C. At pH 5, significant antimicrobial effects were observed at all concentrations of PL. Addition of PL to the medium extended LT and reduced SGR. At this refrigerated temperature, addition of 1% PL significantly ($P < 0.05$) extended LT and decreased SGR compared to control up to pH 6.0. However, no significant ($P > 0.05$) difference in

Table 1. Lag time for the growth of *Listeria monocytogenes* at different temperatures, pH, and potassium lactate concentrations

Potassium Lactate (%)	Lag time (day)																													
	pH 5			pH 5.5			pH 6			pH 6.5			pH 7																	
	Temperature (°C)																													
	4	7	10	13	16	25	4	7	10	13	16	25	4	7	10	13	16	25												
0	4.75 ^a	4.23 ^a	4.63 ^a	4.49 ^a	0.02 ^a	0.04 ^a	3.91 ^a	2.14 ^a	2.09 ^a	0.36 ^a	0.05 ^a	0.02 ^a	3.96 ^a	2.24 ^a	1.32 ^a	0.41 ^a	0.01 ^a	0.00 ^a	0.6 ^a	0.51 ^a	1.63 ^a	0.40 ^a	0.05 ^a	0.01 ^a	0.34 ^a	0.51 ^a	1.07 ^a	0.26 ^b	0.07 ^a	0.01 ^a
1	15.6 ^b	6.87 ^b	5.07 ^a	1.49 ^b	0.18 ^c	0.08 ^b	6.75 ^b	4.15 ^b	2.27 ^a	0.59 ^b	0.06 ^a	0.04 ^b	4.88 ^b	3.57 ^b	1.74 ^b	0.47 ^b	0.06 ^b	0.01 ^b	2.05 ^b	1.77 ^b	1.64 ^a	0.44 ^a	0.08 ^b	0.01 ^a	0.41 ^a	1.77 ^b	1.00 ^a	0.19 ^a	0.09 ^b	0.02 ^b
2	ND	8.52 ^b	8.40 ^d	2.22 ^c	0.10 ^b	0.08 ^b	6.84 ^b	6.73 ^c	7.15 ^c	1.41 ^d	0.10 ^a	0.04 ^b	5.49 ^b	4.94 ^c	1.90 ^b	0.5 ^{bc}	0.07 ^b	0.04 ^c	4.90 ^c	2.28 ^c	1.68 ^a	0.43 ^a	0.15 ^c	0.01 ^a	2.65 ^b	2.28 ^c	1.19 ^a	0.53 ^d	0.17 ^c	0.03 ^c
3	ND	11.8 ^c	7.50 ^b	2.49 ^d	0.61 ^d	0.26 ^c	9.43 ^c	7.00 ^c	6.51 ^b	1.21 ^c	0.11 ^a	0.08 ^c	11.9 ^c	5.18 ^c	2.83 ^c	0.65 ^c	0.28 ^c	0.04 ^c	5.56 ^d	3.19 ^d	1.79 ^a	0.52 ^b	0.33 ^d	0.01 ^a	6.95 ^d	3.19 ^d	1.49 ^b	0.58 ^c	0.33 ^d	0.05 ^d
4	ND	38.3 ^d	8.0 ^{bc}	2.68 ^e	1.09 ^e	0.49 ^d	ND	9.05 ^d	7.72 ^d	2.67 ^e	1.05 ^b	0.10 ^d	11.9 ^c	6.44 ^d	2.93 ^c	0.69 ^c	0.33 ^d	0.10 ^d	10.7 ^e	5.78 ^e	2.50 ^b	0.61 ^c	0.37 ^c	0.03 ^b	5.21 ^c	5.78 ^e	2.37 ^c	0.45 ^c	0.40 ^e	0.05 ^e

ND denotes not detected. Means within a column with different superscripts are significantly different (p<0.05) representing three separate experiments.

Table 2. Specific growth rate for the growth of *Listeria monocytogenes* at different temperatures, pH, and potassium lactate concentrations

Potassium Lactate (%)	Specific growth rate (Log CFU/ml/day)																													
	pH 5			pH 5.5			pH 6			pH 6.5			pH 7																	
	Temperature (°C)																													
	4	7	10	13	16	25	4	7	10	13	16	25	4	7	10	13	16	25												
0	0.31 ^a	0.54 ^a	1.29 ^a	2.47 ^a	4.55 ^a	17.0 ^a	0.28 ^a	0.55 ^a	1.37 ^a	2.53 ^a	5.43 ^a	18.8 ^a	0.46 ^a	0.71 ^a	1.20 ^a	2.43 ^a	4.80 ^a	20.7 ^a	0.46 ^a	0.61 ^a	1.34 ^a	2.81 ^a	5.12 ^a	20.6 ^a	0.56 ^a	0.61 ^a	1.39 ^a	2.32 ^a	5.50 ^a	23.9 ^b
1	0.61 ^b	0.34 ^b	1.06 ^b	1.74 ^b	4.10 ^b	13.8 ^b	0.28 ^a	0.39 ^b	1.15 ^b	1.77 ^b	5.33 ^a	12.5 ^b	0.37 ^b	0.57 ^b	1.19 ^a	2.29 ^a	4.5 ^{ab}	17.3 ^b	0.44 ^a	0.62 ^a	1.28 ^a	2.74 ^a	4.8 ^{ab}	19.1 ^{ab}	0.39 ^b	0.62 ^a	1.3 ^{ab}	2.26 ^a	4.95 ^b	22 ^{ab}
2	ND	0.17 ^c	0.65 ^c	1.25 ^c	3.14 ^c	7.72 ^c	0.26 ^b	0.23 ^c	0.78 ^c	1.74 ^b	3.17 ^b	10.3 ^c	0.40 ^c	1.1 ^{ab}	1.81 ^b	4.27 ^b	13.5 ^c	0.35 ^b	0.54 ^b	1.26 ^b	2.13 ^b	4.6 ^{bc}	18.4 ^b	0.40 ^b	0.54 ^b	1.3 ^{ab}	2.54 ^b	4.41 ^c	21 ^{bc}	
3	ND	0.16 ^c	0.50 ^d	1.1 ^{cd}	3.12 ^c	7.4 ^{cd}	0.17 ^c	0.16 ^d	0.64 ^d	1.29 ^c	3.15 ^b	7.16 ^d	0.24 ^d	1.07 ^b	1.20 ^c	2.82 ^c	10.6 ^d	0.33 ^b	0.44 ^c	1.01 ^b	2.03 ^b	4.2 ^{cd}	14.6 ^c	0.38 ^b	0.44 ^c	1.26 ^b	2.54 ^b	3.45 ^d	20.2 ^c	
4	ND	0.11 ^d	0.33 ^c	1.08 ^d	2.44 ^d	6.46 ^d	ND	0.12 ^c	0.60 ^d	1.19 ^c	3.30 ^b	6.05 ^c	0.19 ^c	0.87 ^c	0.71 ^d	2.65 ^c	9.96 ^d	0.33 ^b	0.38 ^d	0.84 ^c	1.92 ^b	4.04 ^d	12.5 ^d	0.30 ^c	0.38 ^d	1.22 ^b	1.89 ^c	4.36 ^c	14.5 ^d	

ND denotes not detected. Means within a column with different superscripts are significantly different (p<0.05) representing three separate experiments.

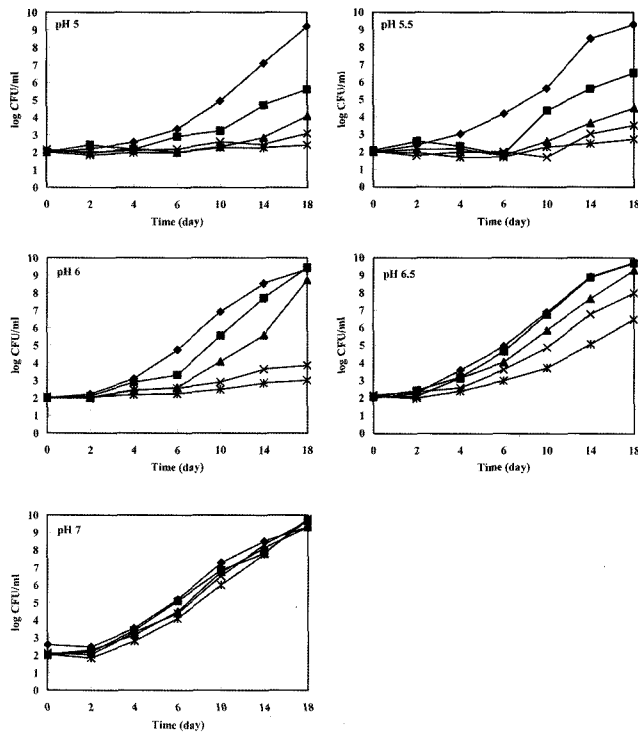


Fig. 2. Growth of *L. monocytogenes* in TSB containing different concentrations of potassium lactate as a function of pH at 7°C (Control ◆, Potassium lactate 1% ■, 2% ▲, 3%×, 4% *).

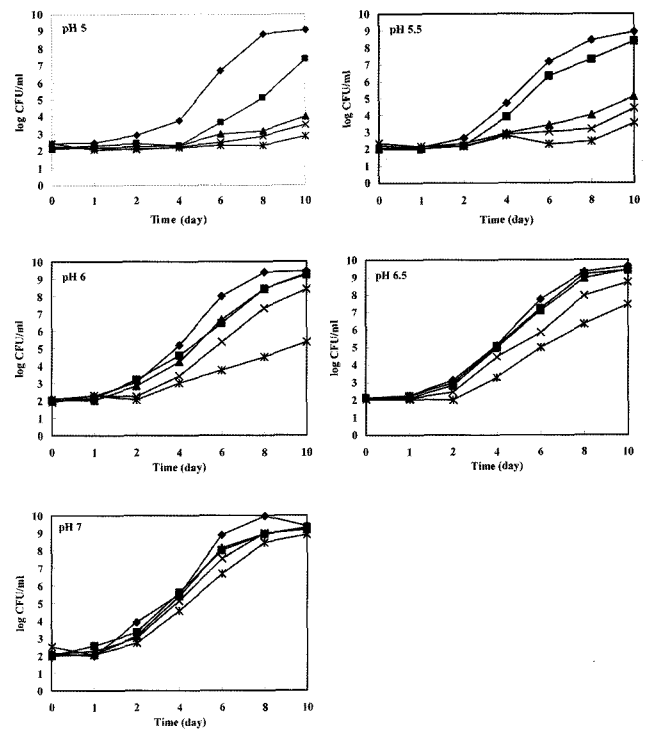


Fig. 3. Growth of *L. monocytogenes* in TSB containing different concentrations of potassium lactate as a function of pH at 10°C (Control ◆, Potassium lactate 1% ■, 2% ▲, 3%×, 4% *).

the growth rate of *L. monocytogenes* was noticed at pH 6.5 and 7. In addition, the growth rate was not significantly ($P > 0.05$) different from control, but LT was still significantly ($P < 0.05$) extended, even with PL concentrations of 1% at pH 6.5 or 7 (Table 1). At pH 7, the respective values for LT and SGR were significantly different ($P < 0.05$) among the different concentrations of PL. LT was significantly increased with PL concentration, while SGR decreased as PL levels increased.

Effect of potassium lactate at 10°C The effect of PL on the growth pattern of *L. monocytogenes* at 10°C is shown in Fig. 3. At pH 5, bacterial growth was observed in broth without PL after 4 days at an SGR of 1.29 log₁₀CFU/day. The LT was extended by the addition of PL in the medium. The SGR was significantly ($P < 0.05$) decreased with higher PL concentration. At pH 5.5, growth was observed after 2 days. However, only SGR was significantly ($P < 0.05$) affected by addition of 1% PL. No significant difference ($P > 0.05$) in SGR values between 3% or 4% PL was observed at pH 5.5. When pH was increased to 6, the growth of *L. monocytogenes* was significantly inhibited ($P < 0.05$) by PL at concentrations of 3% and 4%. When the pH of the control medium was increased to 6.5, addition of PL up to 3% did not extend LT. However, the growth rate was delayed by 3% or 4% PL. At pH 7.0, the growth was significantly ($P < 0.05$) delayed only by 4% PL.

Effect of potassium lactate at 13°C Fig. 4 demonstrates the effect of potassium lactate on the growth pattern of *L. monocytogenes* at 13°C as a function of pH and PL concentration. Overall, the growth of *L. monocytogenes*

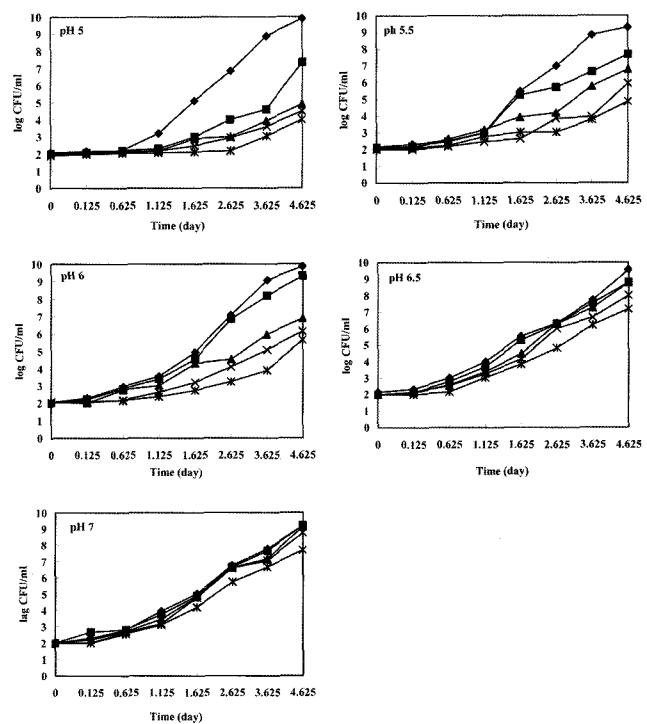


Fig. 4. Growth of *L. monocytogenes* in TSB containing different concentrations of potassium lactate as a function of pH at 13°C (Control ◆, Potassium lactate 1% ■, 2% ▲, 3%×, 4% *).

was much faster than at 10°C. At pH 5, a standard growth rate of 2.47 log₁₀CFU/day was observed after 27 hr in control media. Addition of PL suppressed the growth of *L.*

monocytogenes. However, at least 3 % concentrations of PL were required to cause significant change ($P < 0.05$) in growth rates. At pH 5.5, antimicrobial ability in PL concentrations of 1% and 2% was observed to be significantly different ($P < 0.05$) from that in 3% and 4% PL. However, there was no significant difference ($P > 0.05$) between 1% PL and 2% PL or between 3% and 4% PL. At pH 6 and pH 6.5, bacterial growth was observed in control broth after 3 hr of incubation. At these pH values, at least 2% PL was required to control the growth of *L. monocytogenes*. At pH higher than 6.0, no significant ($P > 0.05$) antimicrobial effect was observed with PL at concentrations between 3 and 4%. At pH 7.0, LT was 3 hr and SGR was $2.32 \log_{10} \text{CFU/day}$ in untreated broth. LT was significantly ($P < 0.05$) affected by 3% PL and standard growth rate was similarly altered at 4% PL.

Effect of potassium lactate at 16°C The effect of PL on the growth pattern of *L. monocytogenes* at 16°C is shown in Fig. 5. Overall, the growth was accelerated at 16°C, regardless of pH. At pH 5, the growth of in control was twice as fast as that at 13°C. Bacterial growth was observed in control after 4 hr of incubation. At pH 5.5, addition of 4 % PL caused significant ($P < 0.05$) extension of LT, while PL concentrations of more than 2 % had no significant ($P < 0.05$) effect on the growth rate. Above pH 6.0, addition of PL significantly ($P < 0.05$) extended the LT of *L. monocytogenes*, but did not significantly affect its SGR (Table 2)

Effect of potassium lactate at 25°C Fig. 6 shows the effect of PL on the growth pattern of *L. monocytogenes* in

broth stored at 25°C. Almost all treatments showed listerial growth before 1 hour. LT was highly suppressed. On the other hand, SGR was highly increased. At pH 5 and 5.5, LT and SGR were still significantly affected by addition of PL, regardless of PL concentration. As the pH was increased above 6.0, at least 4% PL was required to suppress the growth of *L. monocytogenes*. At pH 6, SGR was significantly different ($P < 0.05$) under all conditions tested. As the pH of the medium was increased to 6.5, growth was seen within 0.125 days. Only a 4% concentration of PL could significantly ($P > 0.05$) increase LT, whereas anything more than 2% PL was enough to significantly reduce SGR ($P < 0.05$). At pH 7, SGR was highest in control ($23.9 \log_{10} \text{CFU/day}$) and lowest in 4% PL (14.5).

Discussion

These results confirmed the antimicrobial efficacy of PL against the growth of *L. monocytogenes* in TSB medium. From this data, it can be inferred that the listeristatic effect of PL was due to synergistic effects of low temperature and pH. The inhibitory effect of PL on the growth of *L. monocytogenes* was greater at lower pH than that at higher pH. In the present study, we observed that the values of LT and SGR were significantly affected ($P < 0.05$) by pH and temperature. We found a progressive increase in LT with decreases in pH values. This result is in agreement with the findings of previous researchers (37-39). The observed increase in antimicrobial effects at lower pH is well documented in the literature (29, 40, 41), where it has been explained as being due to decreased

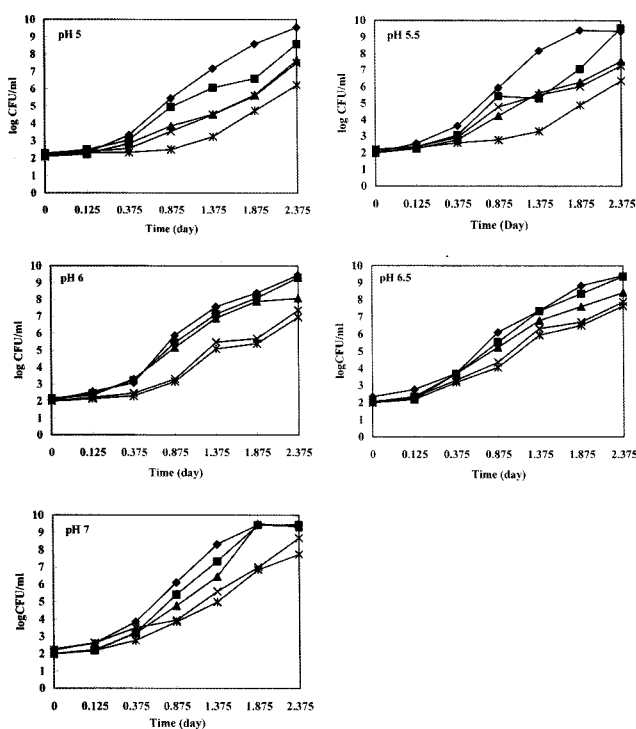


Fig. 5. Growth of *L. monocytogenes* in TSB containing different concentrations of potassium lactate as a function of pH at 16°C (Control \blacklozenge , Potassium lactate 1% \blacksquare , 2% \blacktriangle , 3% \times , 4% $*$).

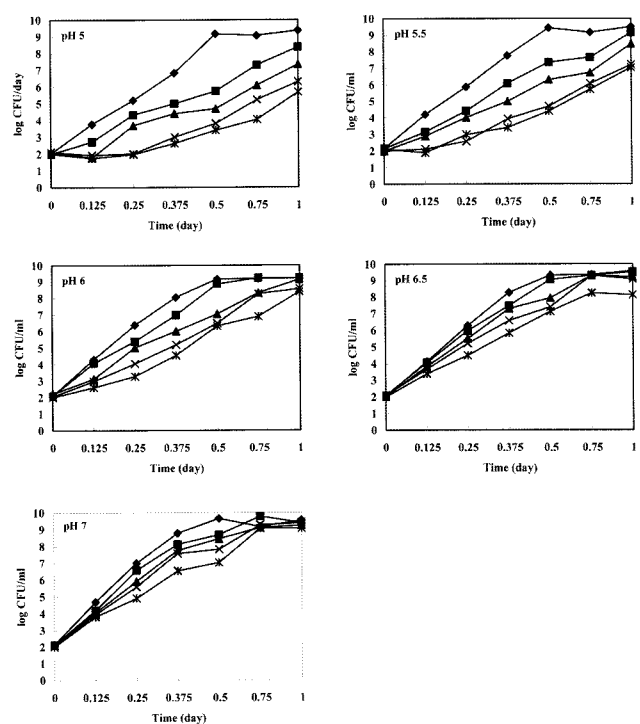


Fig. 6. Growth of *L. monocytogenes* in TSB containing different concentrations of potassium lactate as a function of pH at 25°C (Control \blacklozenge , Potassium lactate 1% \blacksquare , 2% \blacktriangle , 3% \times , 4% $*$).

interfacial tension of the bacterial lipid membrane. Similarly, PL was observed to have higher antimicrobial activity at lower temperatures. It has been suggested by researchers that a higher rate of ion diffusion due to increased permeability in the microbial cell membrane is the reason for increased antimicrobial activity (40, 42). The increased growth and extended survival of the pathogen in most cases is consistent with the previous results observed at higher temperatures. It is widely reported in the literature that decreases in temperature reduce the growth kinetics of *L. monocytogenes* (43) and increases LT (39). These findings were confirmed in the present study, where LT was extended and SGR was significantly reduced ($P < 0.05$) at lower temperatures. On the other hand, at higher temperature values, the growth of the *L. monocytogenes* was highly increased and LT was highly suppressed.

The bacteriostatic and bactericidal effects of lactates, either alone or in combination with diacetate, have been well documented (11, 23, 29, 44-48). The effectiveness of the antimicrobial activity of lactates against microorganism growth is primarily due to their ability to depress water activity, as sodium lactate is a hygroscopic salt. It binds with water and lowers water activity. Another mechanism of microbial inhibition by sodium lactate is due to its weak lipophilic acidic nature. After it passes through the cell membranes in an undissociated form, it acidifies the cell cytoplasm and interferes with the bacteria's life functions, resulting in a longer dormant lag phase phase of the microorganism's growth (23).

Additionally, considering the antimicrobial effect of PL, various theories have been suggested regarding the mechanism or contributing factors of lactate for the control of microbial growth. Studies suggest that effective microbial growth inhibition by an organic acid occurs only when an appropriate amount of undissociated molecules are present (49-53) and intracellular pH is lowered. Lowering of intracellular pH is mainly due to the penetration of undissociated lactic acid into the cell. Undissociated weak acid molecules rapidly diffuse into the plasma membrane, liberating protons, causing acidification of cytoplasm, and finally, preventing microbial growth (41). Additionally, disturbances in the activity of enzymes and nucleic acids interrupt cell metabolism and cause cell death (54). The degree of dissociation is also affected by environmental pH, as the proportion of the acid form increases with decreases in pH. In our present study, the antimicrobial effect of PL was accelerated at lower pH and temperatures values. Similarly, a listericidal effect was observed at pH 5.0 and 4°C where more than 2% PL concentration was required.

In the meantime, our simulated study on a food system (sausage) revealed that the growth of *L. monocytogenes* in TSB media was much faster than that of sausage (data not shown). Similar results, with lower maximum potential density and slower growth rate, were observed when the growth of *L. monocytogenes* was comparatively studied on iceberg lettuce and solid broth media (13). The differential growth kinetics between broth and food systems might be due to differences in availability and diffusion of nutrients within the matrix, competitive microflora in the food system (13), the possibility of pre-existing antimicrobial

constituents in the food, or other factors. Nevertheless, our data clearly demonstrated the inhibitory effect of PL on *L. monocytogenes* growth in laboratory media. However, additional work is necessary for the application of PL in processed foods with various intrinsic and extrinsic parameters that can affect microbial stability and food value: water activity, pH, temperature, atmosphere, and redox potential. In addition, the specific type and nature of the microbial flora and certain food components are variables that need to be considered. In conclusion, the growth of *L. monocytogenes* was significantly inhibited in TSB containing 1-4% PL at pH 6.0 or lower when refrigerated at temperatures below 10°C. However, at higher pH and temperatures, PL was observed to have significant antilisterial activity at concentrations of 3 to 4 % in broth media. The maximum permitted PL concentration is 2% (3.3% of the 60 percent commercially available solution) for various emulsified meat products such as frankfurters, bologna and wieners. Therefore, PL alone may not be an effective treatment in these kinds of products. Thus, it might be necessary to investigate the effects of PL in association with other natural antimicrobials on the control of *L. monocytogenes* at both refrigerated and ambient temperatures.

Acknowledgments

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References

- Gitter M. Listeriosis in farm animals in Great Britain. pp 191-200. In: Isolation and identification of microorganism of medical and veterinary importance. Collins CH, Grange JM (ed). Academic Press, Inc., London (1985)
- Donnelly CW, Briggs EH. Psychrotrophic growth and thermal inactivation of *Listeria monocytogenes* as a function of milk composition. J. Food Prot. 49: 994-998 (1986)
- Marth EH. Disease characteristics of *Listeria monocytogenes*. Food Technol. 42: 165-168 (1988)
- Oh DH, Marshall DL. Influence of Temperature, pH, and Glycerol Monolaurate on Growth and Survival of *Listeria monocytogenes*. J. Food Prot. 56: 744-749 (1993)
- Kim J, Marshall DL. Effect of lactic acid on *Listeria monocytogenes* and *Edwardsiella tarda* attached to catfish skin. Int. J. Food Microbiol. 18: 589-596 (2001)
- Rocourt J, BenEmbarek P, Toyofuku H, Schlundt J. Quantitative risk assessment of *Listeria monocytogenes* in ready-to-eat foods: the FAO/WHO approach. FEMS Immun. Med. Mic. 35: 263-267 (2003)
- Park HJ, Lee NK, Kim KT, Ha JU, Lee DS, Paik HD. Inhibition of *Listeria monocytogenes* in vacuum or modified atmosphere-packed ground beef by lactococcal bacteriocins. Nutraceut. Food 8: 196-199 (2003)
- McLauchlin J, Hall SM, Velani SK, Gilbert RJ. Human listeriosis and pate: a possible association. Brit. Med. J. 303: 773-775 (1991)
- Anon MLT. Update: Multistate outbreak of listeriosis United States, 1998-1999. J. Am. Med. Assoc. 281: 317-318 (1999)
- Ryser ET, Marth EH. *Listeria*, Listeriosis, and Food Safety. Marcel Dekker, New York, USA. pp 505-564 (1999)
- Mbandi E, Shelef LA. Enhanced antimicrobial effects of combination of lactate and diacetate on *Listeria monocytogenes* and *Salmonella* spp. in beef bologna. Int. J. Food Microbiol. 76: 191-198 (2002)
- Bahk GJ, Kim YS, Shin EH, Roh WS, Kim JW. Monitoring of *Listeria monocytogenes* in an ice cream manufacturing plant in

- Korean Food Sci. Biotechnol. 12: 680-682 (2003)
13. Koseki S, Isobe S. Growth of the *Listeria monocytogenes* on iceberg lettuce and solid media. *Int. J. Food Microbiol.* 101: 217-225 (2005)
 14. Farber JM., Peterkin PI. *Listeria monocytogenes*, a foodborne pathogen. *Microbiol. Rev.* 55: 476-511 (1991)
 15. Ojienyi B, Wegener HC, Jensen NE., Bisgaard M. *Listeria monocytogenes* investigations in seven Danish abattoirs. *J. Appl. Bacteriol.* 80: 395-401 (1996)
 16. McCarthy SA. Incidence and survival of *Listeria monocytogenes* in ready-to-eat seafood products. *J. Food Prot.* 60: 372-376 (1997)
 17. Valdimarsson G, Einarsson H, Gudbjornsdottir B, Magnusson H. Microbiological quality of Icelandic cooked-peeled shrimp (*Pandalus borealis*). *Int. J. Food Microbiol.* 45:157-161 (1998)
 18. Gravini, R. Incidence and control of *Listeria* in food-processing facilities. pp 657-709. In: *Listeria, Listeriosis and Food Safety*. Ryse ET, Marth EH,(ed). Marcel Dekker, New York, USA (1999)
 19. Norrung B, Andersen JK, Schlundt J. Incidence and control of *Listeria monocytogenes* in foods in Denmark. *Int. J. Food Microbiol.* 53: 195-203 (1999)
 20. Capita R, Alonso-Calleja C, Moreno, B, Garcia-Fernandez MC. Occurrence of *Listeria species* in retail poultry meat and comparison of a cultural/immunoassay for their detection. *Int. J. Food Microbiol.* 65: 75-82 (2001)
 21. Gudbjornsdottir B, Suihko ML, Gustavsson P, Thorkelsson G, Salo S, Sjoberg AM, Niclasen O, Bredholt S. The incidence of *Listeria monocytogenes* in meat, poultry and seafood plants in the Nordic countries. *J. Food Microbiol.* 21: 217-225 (2004).
 22. De Vegt B. Salt of the earth. *Food Processing.* 10:15 (1997)
 23. Stekelenburg FK. Enhanced inhibition of *Listeria monocytogenes* in Frankfurter sausage by the addition of potassium lactate and sodium diacetate mixtures. *Food Microbiol.* 20: 133-137 (2003)
 24. Miller RK, Acuff GR. Sodium lactates affect pathogens in cooked beef. *J. Food Sci.* 59: 15-19 (1994)
 25. Qvist S, Sehersted K, Zeuthen P. Growth suppression of *Listeria monocytogenes* in a meat product. *Int. J. Food Microbiol.* 24: 283-293 (1994)
 26. Aran N. The effect of calcium and sodium lactates on growth from spores of *Bacillus cereus* and *Clostridium perfringens* in a 'sous-vide' beef goulash under temperature abuse. *Int. J. Food Microbiol.* 63: 117-123 (2001)
 27. Deumier F, Collignana A. The effects of sodium lactate and starter cultures on pH, lactic acid bacteria, *Listeria monocytogenes* and *Salmonella spp.* levels in pure chicken dry fermented sausage. *Meat Sci.* 65: 1165-1174 (2003)
 28. Gou P, Guerrero L, Gelabert U, Arnau J. Potassium chloride, potassium lactate and glycine as sodium chloride substitutes in fermented sausages and in dry-cured pork loin. *Meat Sci.* 32: 37-48 (1995)
 29. Wit JC, de Rombouts FM. Antimicrobial activity of sodium lactate. *Food Microbiol.* 7: 113-120 (1990)
 30. Stillmunkes AA, Prabhu GA, Sebranek JG, Molins RA. Microbiological safety of cooked beef roasts treated with lactate, monolaurin or gluconate. *J. Food Sci.* 58: 953-958 (1993)
 31. Williams SK, Rodrick GE, West RL. Sodium lactate affects shelf life and consumer acceptance of fresh catfish (*Ictalurus nebulosus*, *marmoratus*) fillets under simulated retail conditions. *J. Food Sci.* 60: 636-639 (1995)
 32. Duffy LL, Vanderlinde PB, Grau FU. Growth of *Listeria monocytogenes* on vacuum-packed cooked meats: effects of pH, a_w , nitrite and ascorbate. *Int. J. Food Microbiol.* 23: 377-390 (1994)
 33. Johansen C, Gram L, Meyer AS. The combined inhibitory effect of lysozyme and low pH on growth of *Listeria monocytogenes*. *J. Food Prot.* 57: 561-566 (1994)
 34. Buchanan RL, Golden MH, Whiting RC, Phillips JG, Smith JL. Non-thermal inactivation models for *Listeria monocytogenes*. *J. Food Sci.* 59(1): 179-188(1994)
 35. Buchanan RL, Golden MH. Model for the non-thermal inactivation of *Listeria monocytogenes* in a reduced oxygen environment. *Food Microbiol.* 12: 203-212 (1995)
 36. Buchanan RL, Golden MH, Phillips JG. Expanded models for the non-thermal inactivation of *Listeria monocytogenes*. *J. Appl. Microbiol.* 82: 567-577 (1997).
 37. Robinson TP, Ocio MJ, Kaloti. A, Mackey BM. The effect of the growth environment on the lag phase of *Listeria monocytogenes*. *Int. J. Food Microbiol.* 44: 83-92 (1998)
 38. El-Kest ES, Marth EH. Temperature, pH and strain of pathogen as factors affecting inactivation of *Listeria monocytogenes* by chlorine. *J. Food Prot.* 51: 622-625 (1988)
 39. Lambert RJ, Stratford M. Weak-acid preservatives: modeling microbial inhibition and response. *J. Appl. Microbiol.* 86: 157-164 (1999)
 40. Wilkins PO, Bourgeois R, Murray RGE. Psychrotrophic properties of *Listeria monocytogenes*. *Can. J. Microbiol.* 18: 543-551 (1972)
 41. Duff G, Whiting RC, Sheridan JJ. The effect of a competitive microflora, pH and temperature on the growth kinetics of *Escherichia coli* O157:H7. *Food Microbiol.* 16: 299-307 (1999)
 42. Weaver RA, Shelef LA. Antilisterial activity of sodium, potassium or calcium lactate in pork liver sausage. *J. Food Safety* 13: 133-146 (1993)
 43. Shelef LA, Addala L. Inhibition of *Listeria monocytogenes* and other bacteria by sodium diacetate. *J. Food Safety* 14: 103-115 (1994)
 44. Shelef LA, Potluri V. Behavior of foodborne pathogens in cooked liver sausage containing lactates. *Food Microbiol.* 12: 221-227 (1995)
 45. Blom H, Nerbrink E, Dainty R, Hagtveldt T, Borch E, Nissen H, Nesbakken T. Addition of 2.5% lactate and 0.25% acetate controls growth of *Listeria monocytogenes* in vacuum-packed, sensory acceptable serelat sausage and in cooked ham stored at 48°C. *Int. J. Food Microbiol.* 38: 71-76 (1997)
 46. Barmपाली MI, Koutsoumanis KP, Geomaras I, Belk KE, Scanga JA, Kendall PA, Smith GC, Sofos JN. Effect of antimicrobials as ingredients of pork bologna for *Listeria monocytogenes* control during storage at 4 or 10°C. *Food Microbiol.* 22: 205-211 (2005)
 47. Ingram M, Ottoway FJH, Coppock JBM. The preservative action of acid substances in food. *Chem. Ind.* 75: 1154-1163 (1956)
 48. Macris, BJ. Mechanisms of benzoic acid uptake by *Saccharomyces cerevesia*. *Appl. Microbiol.* 30: 503-506 (1975)
 49. Eklund T. The antimicrobial effect of dissociated and undissociated sorbic acid at different pH levels. *J. Appl. Bacteriol.* 54, 383-389 (1983)
 50. Osthold W, Shin HK, Dresel J, Leistner L. Improving the storage life of carcasses by treating their surfaces with an acid spray. *Fleischwirtsch.* 64: 828-830 (1984)
 51. Bacus J. Microbial control methods in fresh and processed meats. *Recip. Meat. Conf. Proc.* 41: 7-10 (1988)
 52. Maas MR, Glass KA, Doyle MP. Sodium lactate delays toxin production by *Clostridium botulinum* in cook-in-bag turkey products. *Appl. Environ. Microbiol.* 55L: 2226-2229 (1989)
 53. Papadopoulos LS, Miller RK, Acuff GR, Vanderzant C, Cross HR. Effect of sodium lactate on microbial and chemical composition of cooked beef during storage. *J. Food Sci.* 56: 341-347 (1991)
 54. Shelef LA. Antimicrobial effects of lactates: a review. *J. Food Prot.* 57: 445-450 (1994)