Metabolism of lactic acid in fermented cucumbers by *Lactobacillus buchneri* and related species, potential spoilage organisms in reduced salt fermentations☆

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ABSTRACT

Recent evidence suggests that *Lactobacillus buchneri* may play an important role in spoilage-associated secondary fermentation of cucumbers. Lactic acid degradation during fermented cucumber spoilage is influenced by sodium chloride (NaCl) concentration, pH, and presence of oxygen. Objectives were to evaluate these factors on lactic acid utilization by *L. buchneri*, and to compare the biochemical changes to those which occur during fermented cucumber spoilage. Effects of NaCl (0, 2, 4, and 6% w/w), pH (3.8 vs 5.0), and aerobic environment were investigated using fermented cucumber media (FC) inoculated with spoilage microorganisms. At pH 3.8, *L. buchneri* degraded lactic acid in all NaCl concentrations. The highest rate of lactic acid utilization occurred in FC with 2% NaCl ($P < 0.05$). Lactic acid utilization was nearly identical under aerobic and anaerobic conditions, indicating that oxygen does not influence lactate metabolism by *L. buchneri*. Lactic acid utilization was accompanied by increases in acetic acid and 1,2-propanediol, and *Lactobacillus rapi* was able to convert 1,2-propanediol to propionic acid and propanol. *L. buchneri* initiated spoilage in a wide range of environmental conditions that may be present in commercial cucumber fermentations, and *L. rapi* may act syntrophically with *L. buchneri* to produce the commonly observed spoilage metabolites.

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1. Introduction

Fermented cucumbers are the raw material for production of many commonly consumed pickle products, such as hamburger dill chips and sweet relish. Cucumbers are typically fermented in brine solutions in large open tanks and may be held in bulk storage for many months prior to processing. Naturally occurring lactic acid bacteria, primarily *Lactobacillus plantarum*, carry out the fermentation converting cucumber sugars into lactic acid and lowering the pH. Spoilage-associated secondary fermentation may occur in the bulk storage phase, especially when the salt concentration is too low.

Recent evidence in the study of fermented cucumber spoilage indicates the potential involvement of *Lactobacillus buchneri*. The chemical changes that have been observed during fermented cucumber spoilage (Fleming et al., 1989, 2002; Kim and Breidt, 2007)

were similar to those demonstrated in silage inoculated with *L. buchneri* as a fermentation adjunct (Driehuis et al., 1999). Furthermore, *L. buchneri* has been isolated repeatedly from fermented cucumbers that had undergone spoilage, characterized by decreased concentrations of lactic acid, increased pH, and increased concentrations of acetic and propionic acids (Franco et al., 2012; Johanningsmeier et al., 2012). Several other lactic acid bacteria (LAB) have been isolated from fermented cucumber spoilage, but only *L. buchneri* was found to initiate lactic acid utilization in fermented cucumber media (Johanningsmeier et al., 2012). Therefore, it is hypothesized that *L. buchneri* may play an important role in the initiation of secondary fermentations that lead to fermented cucumber spoilage.

In silage fermentation, lactic acid degradation by *L. buchneri* has been demonstrated to be a useful metabolic pathway that results in increased aerobic stability of the silage. Inoculation of silage with *L. buchneri* resulted in decreased lactic acid content and increased concentrations of acetic acid, propionic acid, and propanol (Driehuis et al., 1999). The anaerobic degradation of lactic acid by *L. buchneri* in pure cultures in laboratory medium required induction by acidic conditions and did not require an external electron acceptor. Products of the degradation of lactic acid were acetic acid and 1,2-propanediol (Oude Elferink et al., 2001). In contrast to other

studies on lactate utilization by LAB (Bobillo and Marshall, 1991; Lindgren et al., 1990; Murphy et al., 1985; Viega-da-Cunha and Foster, 1992), this particular metabolism in *L. buchneri* was not found to support an increase in cell numbers (Oude Elferink et al., 2001). Another lactobacillus species, *Lactobacillus dioliverans*, that was able to degrade 1,2-propanediol to propionic acid and propanol, was isolated from aerobically stable silages that had been inoculated with *L. buchneri* (Krooneman et al., 2002). Accordingly, the use of *L. buchneri* to enhance silage preservation and the proposed metabolic pathway for lactic acid degradation has been reviewed (Holzer et al., 2003). Subsequently, a combination of *L. buchneri* and *L. dioliverans* was shown to increase propionic acid concentrations during sourdough fermentation, resulting in increased antifungal activity in the bread products (Zhang et al., 2010). It is not yet known if similar syntrophic organisms are working in concert with *L. buchneri* during fermented cucumber spoilage.

Environmental factors such as sodium chloride (NaCl) concentration, terminal pH, and exclusion of oxygen have a significant impact on cucumber fermentation and microbial stability during storage in commercial tanks of up to 40,000-L capacity. It has been shown that addition of hydrochloric acid to reduce the pH to 3.5 after fermentation can substantially slow the utilization of lactic acid in fermented cucumbers where the terminal pH was between 3.6 and 3.8 and NaCl concentration was 4.8–5.5% (Fleming et al., 2002). However, at reduced NaCl concentrations, spoilage potential was demonstrated at pH 3.5 within 3 months of anaerobic incubation (Kim and Breidt, 2007). Reduced salt fermented cucumbers (0, 2, and 4% NaCl) with a terminal pH as low as 3.2 were also subject to spoilage under anaerobic conditions (Johanningsmeier et al., 2012). Kim and Breidt (2007) found that spoilage rate was increased at pH 5.0 and low NaCl (2% wt/vol) under anaerobic conditions and proposed using these conditions for studying spoilage in a model system. In contrast, Johanningsmeier et al. (2012) found that the rate of lactic acid utilization was increased at pH 3.8 as compared to pH 5.0 in fermented cucumber media (FC) inoculated with spoilage microorganisms from a reduced salt fermentation source, but exhibited similar behavior to the previously reported pH effect when inoculated with a spoilage culture from a commercial fermentation that had been subcultured in the laboratory at pH 5.0 and 2% NaCl. The difference in results between these two studies can be explained by the selection of different lactate metabolizing microorganisms based on the acid sensitivities of the varying microflora present in each sample. The individual effects of NaCl concentration and pH on anaerobic lactic acid utilization by a pure culture of *L. buchneri* in fermented cucumbers have not been studied.

Most investigations on lactic acid degradation in fermented cucumbers have been carried out in sealed fermentation jars or an anaerobic chamber due to evidence that spoilage can occur under anaerobic conditions (Fleming et al., 1989). The translation of this research to commercial fermentation tanks where air purging is commonly used has been questioned, and the role that aerobic microorganisms have in the initiation of spoilage-associated secondary fermentation has been recently investigated (Franco and Pérez-Díaz, 2012a). A few species of LAB have been shown to metabolize lactic acid under either aerobic or anaerobic conditions with a variety of co-factors and end products (Bobillo and Marshall, 1991; Franco and Pérez-Díaz, 2012b; Johanningsmeier et al., 2012; Lindgren et al., 1990; Murphy et al., 1985; Oude Elferink et al., 2001; Viega-da-Cunha and Foster, 1992). However, the influence of aerobic and anaerobic environments on the metabolism of lactic acid by *L. buchneri* in fermented cucumbers has not been reported. The objectives of this study were to evaluate the effects of NaCl concentration, pH, and aerobic environment on lactic acid utilization

by *L. buchneri*, and to relate the resulting biochemical changes to those observed during mixed culture spoilage of fermented cucumbers.

2. Materials and methods

2.1. Spoilage inocula sources

A *L. buchneri* strain (Culture Collection ID LA1147, USDA-ARS Food Science Research Unit, Raleigh, NC, USA) isolated from spoiled fermented cucumbers brined with 2% NaCl (Johanningsmeier et al., 2012) was tested for its ability to degrade lactic acid in FC under a variety of environmental conditions. Brines from a reduced NaCl cucumber fermentation and a commercial cucumber fermentation that had undergone undesirable secondary fermentation were also used as spoilage inocula. The reduced NaCl spoilage source has been previously described (Johanningsmeier and McFeeters, 2011). The source of spoilage microorganisms labeled as commercial spoilage throughout this text was obtained from a commercial brining facility after it was observed that a tank of fermented cucumbers was undergoing post-fermentation spoilage. A complete description of these spoilage sources is available elsewhere (Franco et al., 2012; Johanningsmeier et al., 2012).

2.2. Growth media preparation

Size 2B pickling cucumbers were obtained from a local processor, blended into slurry, and frozen at −10 °C until needed. Fresh cucumber slurry was thawed, pressed through cheesecloth and centrifuged in 250-mL bottles at 23,400 × g for 15 min to remove particulate matter. After centrifugation, NaCl, yeast extract, peptone and water were added to yield final concentrations of 67% fresh cucumber, 4% NaCl, 1% yeast extract, and 1% peptone. The resulting modified cucumber slurry (mCS) growth media was sterile-filtered with a 0.2 μm bottle top filter (Nalgene FAST PES, 0.2 μm pore size, 90-mm-diameter membrane, Daigger, Vernon Hills, IL, USA) and stored at 4 °C until use.

2.3. Fermented cucumber media preparation

Size 2B cucumbers (32–38 mm in diameter) were washed, packed into 3.84-L glass jars, and covered with brine (55:45 cucumber:brine ratio) to equilibrate at 0.25% (w/w) calcium chloride (CaCl$_2$) and 4% (w/w) NaCl. Brined cucumbers were inoculated with 10^6 CFU/g *Lactobacillus plantarum* MOP3 starter culture (Culture Collection ID LA0219, USDA-ARS Food Science Research Unit, Raleigh, NC, USA). Jars were closed with lids that were heated in boiling water to soften the plastisol liner, and a rubber septum was inserted into the lid of each jar to allow sampling of the brine with a syringe. Three replicate jars were packed and stored at ambient temperature (21–25 °C) for 11 months. Fermentation was monitored by measuring pH and changes in organic acids. Fermented cucumbers were cut into pieces and blended into slurry. The slurry was pressed through cheesecloth and centrifuged in 250-mL bottles at 23,400 × g for 15 min to remove particulate matter. The pH of the clarified slurry was raised from 3.2 to 3.8 by addition of 6 N sodium hydroxide to increase the rate that spoilage would occur (Fleming et al., 2002; Kim and Breidt, 2007). The pH-adjusted, clarified slurry was sterile-filtered with a Nalgene FAST PES 0.2 μm pore size, 90-mm-diameter membrane, bottle-top filter apparatus (Daigger, Vernon Hills, IL, USA) to produce a sterile fermented cucumber slurry medium (FC) for inoculation with potential spoilage microorganisms.

2.4. Effect of pH on lactate metabolism by L. buchneri

FC media was adjusted to 6% NaCl and pH 3.8 or 5.0 prior to sterile filtration. Twelve mL of FC medium was then aseptically transferred into sterile 15-mL conical tubes. The loosely capped tubes were placed into an anaerobic chamber for 3 d prior to inoculation with 10^6 CFU/mL L. buchneri strain LA1147. Samples were aseptically withdrawn at several times during anaerobic incubation at ambient temperature (∼25 °C). Lactic acid concentration was measured by HPLC.

2.5. Effect of aerobic environment on lactate metabolism by L. buchneri

Reduced NaCl and commercial spoilage brines (1 mL each) were inoculated into 9 mL mCS and incubated anaerobically at 30 °C for 2 weeks. L. buchneri strain LA1147 was streaked onto de Man, Rogosa, Sharpe (MRS) agar (Becton, Dickinson, and Company, Sparks, MD, USA) and incubated anaerobically at 30 °C for 4 days. Three isolated colonies were transferred to 9 mL mCS and incubated anaerobically at 30 °C for 2 weeks. One milliliter of each of the three resulting spoilage cultures was transferred to 25 mL mCS and incubated anaerobically at 30 °C for 6 days to prepare inocula for reproduction of spoilage in FC. Spoilage inocula were centrifuged to pellet cells and the spent growth media was discarded. Cells were washed with 5 mL FC and resuspended in 15 mL FC. Conical centrifuge tubes containing 5 mL FC were inoculated in triplicate with 0.5 mL of each spoilage culture and incubated anaerobically at ambient temperature (25 °C) along with triplicate non inoculated FC controls. A duplicate set of treatments was incubated under static aerobic conditions at 25 °C. Samples were aseptically withdrawn at 9, 22, 51, 77, 96, and 146 days of incubation and stored at −80 °C until analysis.

2.6. Effect of NaCl

Cucumbers were fermented without NaCl in the presence of 50 mM acetic acid, 25 mM $CaCl_2$, and 10^6 CFU/mL L. plantarum strain LA0219 starter culture (Fleming et al., 1995). Cucumbers were washed but not blanched prior to packing. HPLC analysis of acids and sugars was conducted to verify fermentation was complete. Fermented cucumbers were blended with equal parts brine and stored at −10 °C until needed. Slurry was processed as previously described. After centrifugation, NaCl was added to individual aliquots of clarified fermented cucumber slurry to yield NaCl concentrations of 0, 2, 4, and 6% (w/w). The pH was then adjusted to pH 3.8 and the solutions were sterile-filtered. The resulting media were inoculated in the anaerobic chamber at ambient temperature with 10^6 CFU/mL L. buchneri and spoilage cultures as described above. Three biological replicates were conducted on three separate days. Samples were aseptically withdrawn after 2, 4, 6, 8, 12, and 22 weeks anaerobic incubation and stored at −80 °C for chemical analysis.

2.7. Utilization of 1,2-propanediol by spoilage organisms

FC was prepared as previously described, adjusted to 6% NaCl and pH 3.8, and supplemented with 30 mM 1,2-propanediol (Sigma–Aldrich, St. Louis, MO, USA) prior to sterile-filtration. Sterile 1,2-propanediol supplemented FC was dispensed into sterile conical centrifuge tubes and placed in the anaerobic chamber for 3 days prior to inoculation with 10^6 CFU/mL spoilage organisms. Spoilage organisms from reduced NaCl and commercial sources were cultured as described above. LAB spoilage isolates (Johanningsmeier et al., 2012), L. buchneri strain LA1147, Pediococcus ethanolidurans strain LA1139, Pediococcus parvulus strain LA1140, Lactobacillus rapi strains LA1165 and LA1169, Lactobacillus parafarraginis strain LA1153, and Lactobacillus harbinensi/perolens strain LA1162, (Culture Collection of the USDA-ARS Food Science Research Unit, Raleigh, NC, USA) were grown anaerobically on MRS agar at 30 °C. Three isolated colonies were transferred to MRS broth and incubated anaerobically at 30 °C for 5 days. An aliquot of each culture was transferred 1:10 into mCS broth and incubated anaerobically at 30 °C for 5 days. Cells were harvested by centrifugation (6800 × g for 10 min), washed with 1,2-propanediol-FC, and resuspended in 1,2-propanediol-FC. Tubes of 1,2-propanediol-FC were inoculated in triplicate in the anaerobic chamber and incubated at ambient temperature (∼25 °C). Samples were aseptically withdrawn after 8, 24, 50, and 93 days and stored at −80 °C for chemical analysis.

2.8. Quantification of metabolites by HPLC

HPLC quantification of glucose, fructose, glycerol, ethanol, propanol, 1,2-propanediol, malic, succinic, D/L-lactic, acetic, propionic, and butyric acids was used to measure anaerobic lactic acid degradation and formation of spoilage metabolites. HPLC analysis was conducted with minor modification of the method published by McFeeters and Barish (2003). Briefly, components of samples were separated on an Aminex HPX-87H resin column (300 × 7.8 mm, Bio-Rad Laboratories, Hercules, CA, USA) with 0.03 N sulfuric acid eluant at a flow rate of 0.6 mL/min. The column temperature was held at 37 °C to separate propionic acid, a potential spoilage metabolite, from an unknown component that frequently occurs in fermented cucumbers. Sugars and alcohols were quantified in the same analysis using a refractive index detector connected in series.

2.9. Quantification of D- and L-lactic acid

In addition to HPLC analysis of D/L-lactic acid, D- and L-lactic acid isomers were individually quantified for selected experiments using an enzymatic assay based on conversion of lactate to pyruvate by D-lactate dehydrogenase and L-lactate dehydrogenase, respectively (D-/L-lactic acid kit Catalog# K-DLATE, Megazyme International Ireland Limited, Bray, Co. Wicklow, Ireland). The NADH produced by the reaction was measured spectrophotometrically at 340 nm with a Cary 300 Bio UV–Visible Spectrophotometer (Agilent Technologies (formerly Varian), Santa Clara, CA, USA).

2.10. pH measurements

Measurement of pH was done at ambient temperature with an Accumet AR25 pH meter (Catalog #13-636-AR25A, Fisher Scientific, Pittsburgh, PA, USA) equipped with a gel-filled combination pH electrode (Catalog #13-620-290, Fisher Scientific) that was calibrated with certified standards of pH 2.00, 4.00, and 7.00 (Fisher Scientific).

3. Results and discussion

Lactic acid utilization by L. buchneri in FC media occurred under a variety of environmental conditions. At an initial pH of 3.8, L. buchneri was able to degrade lactic acid in FC with all four NaCl concentrations tested (0, 2, 4, and 6%) and under both aerobic and anaerobic atmospheres. In all cases, decreases in lactic acid concentration were accompanied by increases in acetic acid and 1,2-propanediol as the primary end products with small increases in ethanol (Table 1). Across multiple independent replications and

Table 1
Chemical changes in fermented cucumber media with varying NaCl concentrations after 84 days anaerobic incubation with *L. buchneri*.

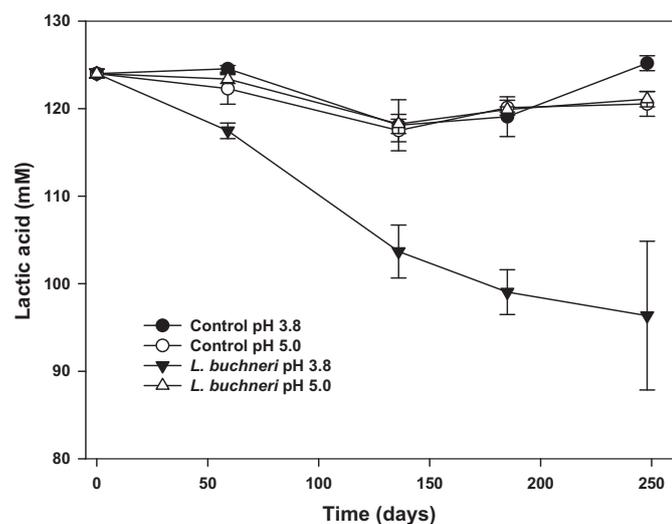| Treatment	NaCl (%)	pH	D/L-lactic acid (mM)	Acetic acid (mM)	1,2-propanediol (mM)	Ethanol (mM)	Carbon Balance[a] (%)
Control	0	3.86 ± 0.01	112.1 ± 3.3	72.4 ± 1.8	ND[b]	18.7 ± 2.9	NA[c]
L. buchneri	0	4.32 ± 0.07	67.1 ± 7.6	96.6 ± 4.0	17.5 ± 2.7	23.7 ± 2.5	104.2 ± 8.8
Control	2	3.85 ± 0.02	112.8 ± 1.1	72.9 ± 0.5	ND	19.2 ± 4.0	NA
L. buchneri	2	4.60 ± 0.14	39.5 ± 9.5	106.0 ± 5.2	24.7 ± 3.4	31.1 ± 3.1	94. 6 ± 6.1
Control	4	3.86 ± 0.02	110.5 ± 1.4	72.2 ± 0.4	ND	18.2 ± 4.1	NA
L. buchneri	4	4.35 ± 0.05	60.7 ± 3.3	97.1 ± 2.7	18.4 ± 1.5	25.3 ± 2.9	100.6 ± 10.6
Control	6	3.86 ± 0.01	110.9 ± 0.8	72.2 ± 0.5	ND	18.7 ± 3.5	NA
L. buchneri	6	4.14 ± 0.07	83.2 ± 7.5	86.6 ± 3.8	10.8 ± 2.7	19.5 ± 2.4	92.4 ± 11.5

[a] Carbon balance was calculated for each replicate as the molar sum of the products (increases in acetic acid, 1,2-propanediol, and ethanol) divided by the decrease in molar concentration of lactic acid substrate and multiplied by 100 to be expressed as a percent. It was assumed that for each mole of lactic acid converted to acetic acid, 1 mol of carbon dioxide was formed (CO_2 not quantified).
[b] None detected.
[c] Not applicable.

varying NaCl concentrations, we found that 1 mol of lactic acid was anaerobically metabolized by *L. buchneri* in fermented cucumber media to produce approximately 0.5 mol acetic acid, 0.37 mol 1,2-propanediol, and 0.13 mol ethanol. These products are consistent with the pathway proposed by Oude Elferink et al. (2001). However, the ratio of end products differed somewhat in FC from that previously reported in microbiological media. We found a slightly greater proportion of ethanol produced in FC than in the afore-mentioned study where it was reported that 1 mol of lactic acid was converted to 0.48 mol acetic acid, 0.48 mol 1,2-propanediol, and 0.04 mol ethanol.
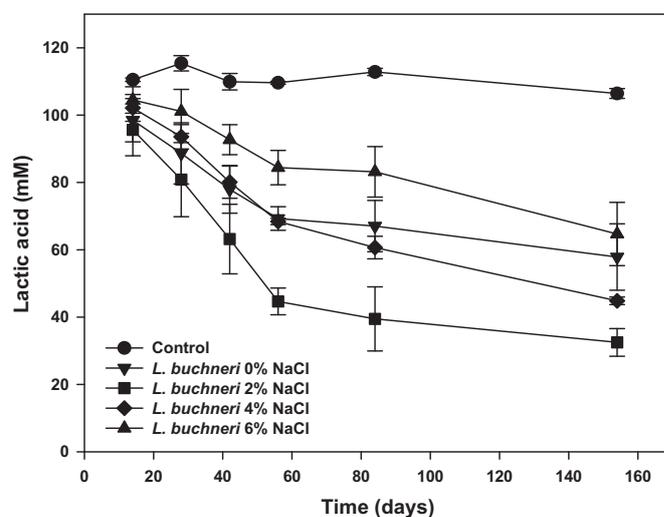
L. buchneri did not metabolize lactic acid in FC with 6% NaCl when the initial pH was 5.0 (Fig. 1). Oude Elferink et al. (2001) have shown that the metabolism of lactic acid to acetic acid and 1,2-propanediol by *L. buchneri* in laboratory media required acid in-duction. They observed that lactic acid was degraded upon incu-bation in MRS when cells were grown in chemostat cultures at pH 3.8, 4.0, and 4.3, but not at 5.8. Similar behavior observed in FC containing 6% sodium chloride suggests that the low pH that is typical of fermented cucumbers may be favorable for induction of the lactic acid degrading activity of *L. buchneri*. It has also been found that *L. buchneri* can degrade lactic acid in fermented cu-cumber media containing 1.1% calcium chloride at pH 3.2 (Franco and Pérez-Díaz, 2012b). Further research to determine the lower pH limit for lactic acid utilization by *L. buchneri* in cucumber fer-mentations is warranted.

There was a significant effect of NaCl concentration on lactic acid utilization by *L. buchneri* ($P < 0.05$). Although lactic acid was degraded by *L. buchneri* in FC with a wide range in salt concen-trations (0, 2, 4, and 6% NaCl), the rate of lactic acid utilization was slowest in 6% NaCl (Fig. 2). More lactic acid was used in 0 and 4% NaCl FC, and the highest rate and greatest extent of lactic acid utilization occurred in 2% NaCl FC. It was interesting that *L. buchneri* had greater metabolism of lactate in 2% NaCl than in salt-free FC media, suggesting the possibility that sodium dependent trans-porters may be involved. Lactic acid bacteria isolated from meat curing brines had a specific NaCl requirement of up to 2% for optimal growth (Goldman et al., 1963), which may be related to transport processes and energy transduction. Na^+ dependent transporters have been identified in lactic acid bacteria (Poolman, 1993) and are widely acknowledged in gram-positive bacteria (Reizer and Peterkofsky, 1987). However, the pathways for lactic acid utilization by lactic acid bacteria and the transport processes involved have not been fully characterized. Nonetheless, the increased metabolism of lactic acid in 2% NaCl FC by *L. buchneri* revealed in this study may explain the increased susceptibility to spoilage of cucumbers fermented with reduced NaCl concentra-tions that was observed in pilot scale (4000-L) closed tank fer-mentations (Fleming et al., 1989, 2002) and model systems (Johanningsmeier et al., 2012; Kim and Breidt, 2007).

The rate of lactic acid utilization was nearly identical in both aerobic and anaerobic atmospheres (Data not shown. Information

Fig. 1. Effect of initial pH on D/L-lactic acid utilization by *L. buchneri* in fermented cucumber medium (6% NaCl). Control samples were not inoculated.

Fig. 2. D/L-lactic acid utilization by *L. buchneri* in fermented cucumber media (pH 3.8) with four NaCl concentrations. Control samples were not inoculated.
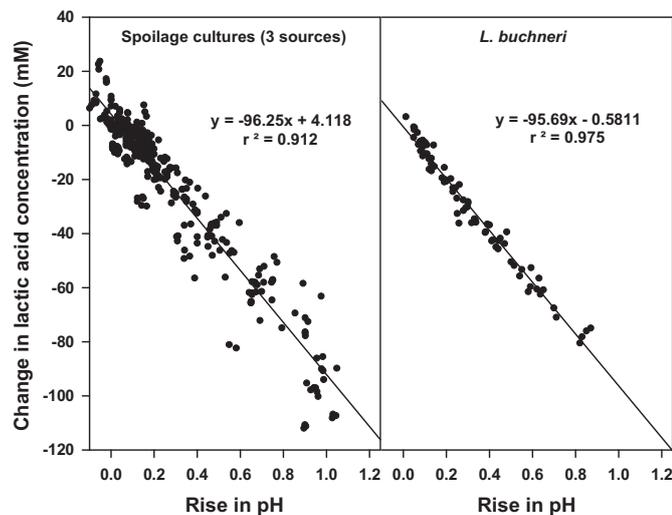
Table 2
Chemical changes in fermented cucumber medium (6% NaCl, pH 3.8) after 77 days incubation with spoilage cultures.

Environment	Treatment	D/L-lactic acid (mM)	Acetic acid (mM)	Propionic acid (mM)	Glucose (mM)	1,2-Propanediol (mM)
Aerobic	Control	115.5 ± 2.0	7.8 ± 0.2	ND	3.1 ± 0.2	ND
	L. buchneri	72.9 ± 3.7	42.8 ± 3.8	ND	ND	14.8 ± 1.6
	Commercial spoilage	48.9 ± 1.0	14.2 ± 12.3	12.2 ± 8.1	ND	ND
	Reduced NaCl spoilage	51.4 ± 4.0	68.9 ± 3.0	16.7 ± 1.1	ND	ND
Anaerobic	Control	117.8 ± 1.2	7.9 ± 0.2	ND	3.3 ± 0.1	ND
	L. buchneri	70.2 ± 8.0	38.0 ± 3.5	ND	ND	20.3 ± 3.9
	Commercial spoilage	67.0 ± 3.4	40.6 ± 0.7	16.1 ± 0.3	ND	ND
	Reduced NaCl spoilage	74.3 ± 6.4	37.9 ± 3.4	17.1 ± 2.8	ND	ND

available online in Supplemental Fig. 1), indicating that oxygen is not required, nor does it influence the lactate degrading ability of *L. buchneri*. Therefore, both air purged and anaerobic cucumber fermentations would be equally susceptible to spoilage by *L. buchneri*. Studies of lactate utilization by *L. plantarum* have shown differences in the rate of lactate utilization (Bobillo and Marshall, 1991) and end products formed (Lindgren et al., 1990) in the presence of oxygen. Oude Elferink et al. (2001) proposed that the anaerobic conversion of lactic acid to acetic acid and 1,2-propanediol did not require external electron acceptors. We have found that there is also no enhancement of this reaction when oxygen is present, and that 1,2-propanediol is still formed (Table 2).
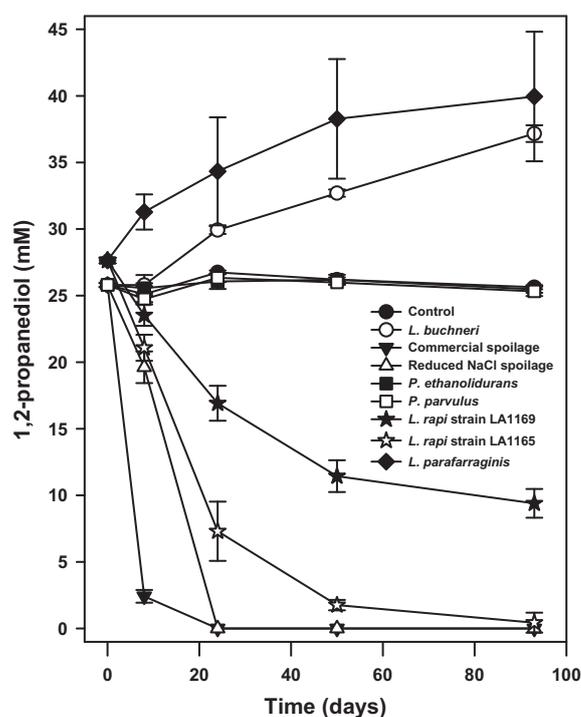
Under anaerobic conditions, the rates of lactic acid degradation by *L. buchneri* and spoilage cultures from reduced NaCl and commercial sources were similar, and both D- and L- forms of lactic acid were metabolized (Data not shown. Information available online in Supplemental Fig. 2). D- and L-lactic acid that were produced during normal cucumber fermentation (55 mM and 45 mM, respectively) were simultaneously metabolized by this *L. buchneri* spoilage isolate, indicating that the isomeric form is not a limiting factor for lactic acid utilization in fermented cucumbers. Furthermore, the rise in pH that was observed during anaerobic lactic acid utilization in fermented cucumber media was strongly correlated with the decrease in lactic acid concentration for mixed spoilage cultures ($R^2 = 0.912$) and pure cultures of *L. buchneri* ($R^2 = 0.975$) across multiple independent experiments with varying NaCl concentrations and initial pH (Fig. 3). The pathway proposed for lactic acid degradation by *L. buchneri* includes decarboxylation of pyruvate and formation of acetyl-CoA by a pyruvate dehydrogenase complex (Oude Elferink et al., 2001). The reaction mechanism for this

enzyme includes the uptake of one hydrogen ion per molecule of pyruvate that is decarboxylated (Combs, 1998), which could contribute substantially to the observed deacidification. This reaction and the production of less acidic end products explains the rise in pH that was correlated with lactic acid utilization. Furthermore, this rise in pH in a commercial fermentation environment would allow undesirable metabolic activity by a host of other organisms that are usually controlled by low pH.

The major difference in lactic acid degradation between *L. buchneri* and mixed culture spoilages was the formation of 1,2-propanediol by *L. buchneri* and the production of propionic acid by the latter (Table 2). This difference in end products indicates that *L. buchneri* cannot be solely responsible for the fermented cucumber spoilage that has been observed. Krooneman et al. (2002) showed that *L. dioliverans* isolated from silage prepared with *L. buchneri* as a fermentation adjunct was able to metabolize 1,2-propanediol to propionic acid and propanol. Therefore, FC (6% NaCl, pH 3.8) supplemented with 1,2-propanediol was used to test individual LAB isolates and spoilage cultures from two different sources for the ability to metabolize 1,2-propanediol to propionic acid and propanol. *L. buchneri* exhibited the same behavior in the presence of additional 1,2-propanediol as in FC media, resulting in a decrease in lactic acid and increases in acetic acid and 1,2-

Fig. 3. Rise in pH correlated with anaerobic D/L-lactic acid utilization in fermented cucumber media (initial pH 3.1–3.8) regardless of NaCl concentration (0–6%) by spoilage cultures from three sources ($n = 366$) and *L. buchneri* ($n = 77$).

Fig. 4. Utilization of 1,2-propanediol in fermented cucumber medium (6% NaCl, pH 3.8) by spoilage cultures and *Lactobacillus rapi*. Control samples were not inoculated.

Table 3
Chemical changes in 1,2-propanediol supplemented fermented cucumber medium (6% NaCl, pH 3.8) after 93 days anaerobic incubation with spoilage cultures and selected lactic acid bacteria.

Treatment	pH	1,2-Propanediol (mM)	D/L-lactic acid (mM)	Acetic acid (mM)	Propionic acid (mM)	n-Propanol (mM)
Control	3.77 ± 0.01	25.6 ± 0.2	113.2 ± 1.2	70.4 ± 0.4	ND	ND
Commercial spoilage	3.77 ± 0.01	ND	111.0 ± 0.4	71.1 ± 0.1	16.9 ± 1.0	12.9 ± 0.6
Reduced NaCl spoilage	3.89 ± 0.01	ND	99.7 ± 0.8	78.5 ± 1.2	15.6 ± 0.5	17.9 ± 0.4
L. buchneri strain LA1147	4.04 ± 0.01	37.2 ± 0.6	80.7 ± 3.7	87.3 ± 1.5	ND	ND
P. ethanolidurans strain LA1139	3.75 ± 0.01	25.5 ± 0.3	112.2 ± 0.7	69.3 ± 0.3	ND	ND
P. parvulus strain LA1140	3.76 ± 0.01	25.3 ± 0.4	112.7 ± 0.8	69.4 ± 0.6	ND	ND
L. rapi strain LA1169	3.84 ± 0.01	9.4 ± 1.1	108.5 ± 1.5	64.7 ± 0.9	ND	2.3 ± 0.3
L. rapi strain LA1165	3.83 ± 0.01	0.4 ± 0.8	109.9 ± 1.2	66.0 ± 0.6	9.7 ± 0.7	6.2 ± 0.6
L. parafarraginis strain LA1153	4.15 ± 0.09	40.0 ± 4.9	73.0 ± 9.3	84.7 ± 4.2	ND	ND

propanediol. Two pediococci that have been observed in fermented cucumber spoilage, but do not use lactic acid in FC (Franco et al., 2012; Franco and Pérez-Díaz, 2012b; Johanningsmeier et al., 2012) were evaluated for their ability to be syntrophic with *L. buchneri* in the production of the components found with mixed culture spoilage. Neither *P. ethanolidurans* nor *P. parvulus* used 1,2-propanediol that was added to FC (Fig. 4) or that which was naturally produced in co-culture with *L. buchneri* (data not shown). However, both reduced NaCl and commercial spoilage cultures contained organisms that were able to utilize 1,2-propanediol in FC with 6% NaCl at pH 3.8 under anaerobic conditions (Fig. 4). Decreases in 1,2-propanediol were observed as early as 8 days after inoculation, and 1,2-propanediol was no longer detected after 24 days. Utilization of the added 1,2-propanediol by these unidentified spoilage microorganisms preceded lactic acid utilization, which may explain why this intermediate has not been detected in the normal course of fermented cucumber spoilage. Either 1,2-propanediol is a preferred substrate or the organism(s) that use(s) 1,2-propanediol cannot metabolize lactic acid. Two strains of *L. rapi*, newly isolated LAB from fermented cucumber spoilage (Johanningsmeier et al., 2012), were able to degrade 1,2-propanediol in FC when inoculated in pure culture (Fig. 4). Consistent with the mixed spoilage cultures, decreases in 1,2-propanediol accompanied increases in propionic acid and propanol for *L. rapi* strain LA1165 (Table 3). *L. rapi* strain LA1169 utilized 1,2-propanediol more slowly and did not produce propionic acid, indicating that strain differences influence this metabolic activity. Another recently isolated spoilage LAB, *L. parafarraginis* strain LA1153 (Johanningsmeier et al., 2012) behaved similarly to *L. buchneri* in FC supplemented with 1,2-propanediol. Within 24 days of anaerobic incubation, a significant decrease in lactic acid with concurrent increases in acetic acid and 1,2-propanediol and rise in pH were observed (Table 3). More research is needed to determine the prevalence of this lactic acid degrading bacterium in fermented cucumbers. Based on these collective results, we hypothesize that *L. buchneri* or genetically related LAB such as *L. parafarraginis* initiate lactic acid utilization in fermented cucumbers, producing acetic acid and 1,2-propanediol. The 1,2-propanediol is used by one or more syntrophic organisms, including *L. rapi*, at a rate that does not allow accumulation of 1,2-propanediol, resulting in acetic acid, propionic acid and propanol as the spoilage metabolites detected in fermented cucumber brine. Furthermore, the utilization of lactic acid by *L. buchneri* and *L. parafarraginis* results in a rise in pH that may allow other propionic and butyric acid producing organisms to contribute to spoilage of fermented cucumbers.

4. Conclusions

Lactic acid utilization by *L. buchneri* occurred under a variety of environmental conditions that may be encountered in commercial cucumber fermentations. At an initial pH of 3.8, *L. buchneri* was able to degrade lactic acid in FC under both aerobic and anaerobic atmospheres and in the presence of 0–6% NaCl. The enhanced degradation of lactic acid observed under reduced salt conditions indicates that this organism could be of significant concern in efforts by the pickling industry to reduce the NaCl used for fermentation and storage of cucumbers. Regardless of environmental conditions, utilization of lactic acid by *L. buchneri* was related to increases in acetic acid and 1,2-propanediol and a significant rise in pH that could compromise the quality and safety of the product. *L. rapi* and unidentified spoilage organisms were able to convert 1,2-propanediol to propionic acid and propanol, suggesting that one or more organisms work in concert with *L. buchneri* to produce the spoilage metabolites that have been observed in fermented cucumber brines.

Appendix A. Supplementary data

Supplementary data related to this article can be found at doi:10.1016/j.fm.2013.03.004.

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