International Journal of Food Microbiology 280 (2018) 46–56

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Phenotypic and genotypic diversity of *Lactobacillus buchneri* strains isolated from spoiled, fermented cucumber☆

Katheryne V. Daughtry[a,b], Suzanne D. Johanningsmeier[b,*], Rosemary Sanozky-Dawes[a], Todd R. Klaenhammer[a], Rodolphe Barrangou[a]

[a] Department of Food, Bioprocessing and Nutrition Sciences, North Carolina State University, 400 Dan Allen Drive, Raleigh, NC 27695-7642, USA
[b] USDA-ARS, SEA Food Science Research Unit, 322 Schaub Hall, Box 7624, North Carolina State University, Raleigh, NC 27695-7624, USA

ARTICLE INFO

Chemical compounds studied in this article:
Lactic Acid (PubChem CID: 612)
Fucose (PubChem CID: 17106)
Trehalose (PubChem CID: 7427)
Gentiobiose (PubChem CID: 441422)
Cellobiose (PubChem CID: 10712)
Inulin (PubChem CID: 18772499)
Arabinose (PubChem CID: 66308)
Xylose (PubChem CID: 644160)
Ribose (PubChem CID: 10975657)
Maltose (PubChem CID: 6255)

Keywords:
Lactic acid bacteria
Pickled vegetables
Spoilage organisms
Lactobacillus buchneri
Strain diversity

ABSTRACT

Lactobacillus buchneri is a Gram-positive, obligate heterofermentative, facultative anaerobe commonly affiliated with spoilage of food products. Notably, *L. buchneri* is able to metabolize lactic acid into acetic acid and 1,2-propanediol. Although beneficial to the silage industry, this metabolic capability is detrimental to preservation of cucumbers by fermentation. The objective of this study was to characterize isolates of *L. buchneri* purified from both industrial and experimental fermented cucumber after the onset of secondary fermentation. Genotypic and phenotypic characterization included 16S rRNA sequencing, DiversiLab® rep-PCR, colony morphology, API 50 CH carbohydrate analysis, and ability to degrade lactic acid in modified MRS and fermented cucumber media. Distinct groups of isolates were identified with differing colony morphologies that varied in color (translucent white to opaque yellow), diameter (1 mm–11 mm), and shape (umbonate, flat, circular or irregular). Growth rates in MRS revealed strain differences, and a wide spectrum of carbon source utilization was observed. Some strains were able to ferment as many as 21 of 49 tested carbon sources, including inulin, fucose, gentiobiose, lactose, mannitol, potassium ketogluconate, saccharose, raffinose, galactose, and xylose, while others metabolized as few as eight carbohydrates as the sole source of carbon. All isolates degraded lactic acid in both fermented cucumber medium and modified MRS, but exhibited differences in the rate and extent of lactate degradation. Isolates clustered into eight distinct groups based on rep-PCR fingerprints with 20 of 36 of the isolates exhibiting > 97% similarity. Although isolated from similar environmental niches, significant phenotypic and genotypic diversity was found among the *L. buchneri* cultures. A collection of unique *L. buchneri* strains was identified and characterized, providing the basis for further analysis of metabolic and genomic capabilities of this species to enable control of lactic acid degradation in fermented plant materials.

1. Introduction

Lactobacillus buchneri is a Gram-positive, facultative anaerobe that has been isolated from a wide range of food, feed, and bioprocessing environments due to its broad range of metabolic capabilities. Notably, *L. buchneri*'s ability to anaerobically metabolize lactic acid into acetic acid and 1,2-propanediol (Oude Elferink et al., 2001) makes this organism both detrimental to the cucumber pickling industry and suitable as an adjunct starter culture for the silage industry. In addition to fermented cucumber (Franco et al., 2012; Johanningsmeier et al., 2012) and silage (Cooke, 1995; Muck, 1996), *L. buchneri* has also been isolated from human intestines (Zeng et al., 2010), fermented sorghum product (Yousif et al., 2010), wine (de Orduña et al., 2001), beer wort (Sakamoto and Konings, 2003), tomato pulp (Hammes and Hertel, 2015) and spoiled Swiss cheese (Sumner et al., 1985). Considering the various ecological niches that *L. buchneri* strains occupy, diversity within the species is likely.

Pickling cucumbers are fermented in a sodium chloride (NaCl) solution in open-top, 10–40 kL vats where naturally occurring lactic acid bacteria, principally homolactic *Lactobacillus* species, convert sugars to lactic acid to preserve the fruits (Franco et al., 2016). Prior to processing, it is common for fermented cucumbers to be held in bulk storage for up to 1 year. During bulk storage, secondary fermentation may ensue, particularly when the salt concentration is low (Fleming et al., 1989; Johanningsmeier et al., 2012; Kim and Breidt, 2007). Spoilage-associated secondary cucumber fermentation occurs during bulk

☆ Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture or North Carolina Agricultural Research Service, nor does it imply approval to the exclusion of other products that may be suitable. USDA is an equal opportunity provider and employer.

* Corresponding author.
E-mail address: Suzanne.Johanningsmeier@ars.usda.gov (S.D. Johanningsmeier).

https://doi.org/10.1016/j.ijfoodmicro.2018.04.044
Received 30 August 2017; Received in revised form 6 March 2018; Accepted 28 April 2018
Available online 09 May 2018
0168-1605/

storage after residual carbohydrates have been depleted (Fleming et al., 1989; Franco et al., 2012, 2016). This particular spoilage is characterized by an increase in pH and decrease in lactic acid with a concomitant increase in acetic and propionic acids (Fleming et al., 1989; Franco et al., 2012; Johanningsmeier et al., 2012). In numerous studies, *L. buchneri* has been shown to initiate spoilage in fermented cucumber by degrading lactate to acetate and 1,2-propanediol under aerobic or anaerobic conditions, causing a rise in pH (Johanningsmeier et al., 2012; Johanningsmeier and McFeeters, 2013). As lactate is depleted and pH rises, spoilage occurs through a succession of microbial metabolic activity of less acid-tolerant species. *Propionibacterium* species, *Pectinatus sotaceto*, *Enterobacter cloacae*, *Clostridium tertium*, and *Clostridium bifermentans* produce additional propionic acid and butyric acid, creating undesirable off-odors and compromising food safety (Breidt et al., 2013; Caldwell et al., 2013; Fleming et al., 1989; Franco et al., 2012; Franco and Pérez-Díaz, 2012). Ultimately, this renders the final product unfit for consumption and leads to significant economic loss (Franco et al., 2012).

The unique metabolic activities of *L. buchneri* that make it a robust spoilage organism in foods also hold potential for industrial applications in food and feed technology. Strains of *L. buchneri* sourced from a multitude of environments have been proposed for a variety of industrial applications. One particular strain isolated from pickle juice was shown to have potential probiotic effects, such as the reduction of serum cholesterol levels (Zeng et al., 2010). This strain of *L. buchneri* produces a bile salt hydrolase, which catalyzes the hydrolysis of conjugated bile salts into amino acid residues and bile acids. A strain of *L. buchneri* isolated from sauerkraut produced bacteriocins targeting several Gram-positive bacteria, including species from *Listeria*, *Bacillus*, *Micrococcus*, *Enterococcus*, *Leuconostoc*, *Lactobacillus*, *Streptococcus*, and *Pediococcus* (Yildirim and Yildirim, 2001; Yildirim et al., 2002). *L. buchneri* strain NRRL B-30929, originally isolated as a contaminant in a commercial ethanol facility, has since been exploited for bioethanol production and was the first of this species to have its genome fully sequenced and annotated (Liu et al., 2011). Anaerobic lactic acid utilization by *L. buchneri* in silage results in the production of acetic and propionic acids, which act as natural antimicrobials for the inhibition of aerobic spoilage yeasts and fungi that are often responsible for heating and degradation of dry matter in silage upon exposure to air. This has been demonstrated in various silage fodder, including grass (Driehuis et al., 2001), corn (Kleinschmit et al., 2005; Muck, 1996; Ranjit and Kung, 2000), barley (Kung and Ranjit, 2001; Taylor et al., 2002) and alfalfa (Kung et al., 2003). Relying on this same metabolic pathway, *L. buchneri* was used in concert with *Lactobacillus diolivorans* to naturally produce propionate in sourdough bread, extending product shelf life without added preservatives (Zhang et al., 2010). Despite common features observed in several cases, it is not yet clear whether the ability to metabolize lactic acid is strain-dependent.

To date, diversity within the *L. buchneri* species has not been fully explored. Understanding the genotypic and phenotypic differences among *L. buchneri* strains will facilitate the development of strategies to eliminate the onset of secondary cucumber fermentation by this species. Furthermore, this knowledge may also be exploited to develop improved starter culture adjuncts for the silage industry. The objective of this study was to characterize *L. buchneri* cultures isolated from spoiled, fermented cucumber to determine the phenotypic and genotypic diversity of this species from a single environmental niche.

2. Materials and methods

2.1. Bacterial strain cultivation

L. buchneri cultures isolated from spoiled, fermented cucumber (Franco and Pérez-Díaz, 2012; Johanningsmeier et al., 2012) and the type strain for *L. buchneri* (ATCC® 4005™, American Type Culture Collection, Manassas, VA, USA) were obtained from the USDA-ARS Food Science Research Unit Culture Collection (Raleigh, NC, USA). The sources of the isolates are described in Table 1. Species determination was confirmed using 16S rRNA sequencing. Frozen stocks were prepared in de Man-Rogosa-Sharpe (MRS) broth and 30% vol/vol glycerol, and stored at −80 °C. Prior to experiments, isolates were streaked onto MRS agar and incubated at 30 °C under anoxic conditions for 4–5 days followed by anaerobic propagation in MRS broth for 2–3 days at 30 °C.

2.2. 16S rRNA sequencing

All isolates were subjected to 16S rRNA sequencing for species verification prior to characterization studies. Genomic DNA was isolated from broth cultures of each of the 36 isolates using a DNA purification kit (Ultra-Clean Microbial DNA Isolation Kit, MoBio laboratories, Carlsbad, CA, USA). Polymerase Chain Reaction (PCR) was conducted to amplify a 500 bp hypervariable region with 16S rRNA primers. The primers used in this study were plb16 5′ AGAGTTTGAT CCTGGCTCAG 3′ and mlb16 5′ GGCTGCTGGCACGTAGTTAG 3′ (Kullen et al., 2000). DNA amplification was performed in a BioRad MyCycler Thermocycler programmed for 5 min at 94 °C (initial denaturation), and 30 cycles of 30 s at 94 °C (denaturation), 30 s at 55 °C (annealing), 40 s at 72 °C (extension) and 7 min 72°C (final extension). Amplicons were visualized with ethidium bromide in a 1% agarose gel and sequenced using Eton Bioscience Inc. (Durham, NC, USA). The NCBI Basic Local Alignment Search Tool was used to identify the bacterial cultures. All isolates were confirmed to be *L. buchneri* based on alignment matches with ≥99.7% identity.

2.3. Colony and cellular morphology

Each of the isolates was streaked onto MRS agar, and incubated anaerobically for 4 days at 30 °C. Colony morphology was evaluated based on diameter, pigmentation, form, elevation, margin, texture and opacity of each colony for all isolates of *L. buchneri* using the method found in the Manual of Microbiological Methods (Pelczar Jr., 1957). Isolates were further propagated anaerobically in MRS broth for 36 h at 30 °C prior to cellular morphology observation. Cellular morphology was visualized using a Nikon Eclipse E600 phase contrast microscope with a Q-Imaging Micropublisher Camera attachment (Nikon, Japan). Isolates that represented a range of distinct colony morphologies and differed in DiversiLab® genotype fingerprints were selected for detailed photographs (Fig. 1).

2.4. Growth curves in De Man Rogosa and Sharpe (MRS) broth

Growth curves were generated by measuring optical density (OD_{600nm}) of cultures grown in standard MRS medium for 64 h using a microtiter plate reader (Spiral Biotech QCount; Advanced Instruments, Inc.; MA, USA). A 5 µL aliquot of a 10^8 CFU/mL culture was inoculated into a single well of 245 µL of sterile MRS broth for each of the 36 isolates and incubated at 30 °C under modified anaerobic conditions using a mineral oil overlay. Readings were taken every 30 min after a 5-s vibrational shake of the microtiter plate. This experiment was independently conducted in duplicate. Lag times, growth rates, and max OD were calculated using a published algorithm (Breidt et al., 1994). Isolates that varied in DiversiLab® genotype and colony morphology were selected for presentation.

2.5. Carbohydrate fermentation profiles

The ability to ferment a variety of carbon sources was determined by the API 50 CHL method per the manufacturer's guidelines (bioMerieux, Marcy l'Etoile, France). Each of the 36 cultures of *L. buchneri* was cultivated in standard MRS broth under anaerobic conditions at 30 °C for 4 days prior to inoculation.

Table 1
Lactobacillus buchneri isolates characterized in this study.

Isolate ID[a]	Isolation source[b]	Salt content	Original source[c]	GenBank accession #	CRISPR genotype[d]	% ID[e]
ATCC 4005	American Type Culture Collection *Lactobacillus buchneri* type strain	N/A	Tomato Pulp	KF624610	I	99.9
LA1181	Spoiled, fermented cucumber brine	2% NaCl	Reduced salt cucumber fermentation	JQ249043	A	100
LA1187B	Spoiled, fermented cucumber brine	2% NaCl	Reduced salt cucumber fermentation	N/A	A	100
LA1173	Spoiled, fermented cucumber brine	2% NaCl	Reduced salt cucumber fermentation	JQ249034	B	99.8
LA1175A	Spoiled, fermented cucumber brine	2% NaCl	Reduced salt cucumber fermentation	JQ249037	B	100
LA1175D	Spoiled, fermented cucumber brine	2% NaCl	Reduced salt cucumber fermentation	JQ249037	B	100
LA1178	Spoiled, fermented cucumber brine	2% NaCl	Reduced salt cucumber fermentation	JQ249040	C	99.9
LA1185A	Spoiled, fermented cucumber brine	2% NaCl	Reduced salt cucumber fermentation	N/A	E	99.7
LA1185B	Spoiled, fermented cucumber brine	2% NaCl	Reduced salt cucumber fermentation	N/A	E	99.9
LA1188	Spoiled, fermented cucumber brine	2% NaCl	Reduced salt cucumber fermentation	N/A	E	100
LA1147	Spoiled, fermented cucumber brine	2% NaCl	Reduced salt cucumber fermentation	JQ249035	G	100
LA1184	Spoiled, fermented cucumber brine	2% NaCl	Reduced salt cucumber fermentation	JQ24904	J	99.8
LA1160A	Anaerobic reproduction of spoilage	None	Reduced salt cucumber fermentation	JQ249058	F	100
LA1160B	Anaerobic reproduction of spoilage	None	Reduced salt cucumber fermentation	JQ249058	F	100
LA1157A	Anaerobic reproduction of spoilage	2% NaCl	Reduced salt cucumber fermentation	JQ249055	F	100
LA1157B	Anaerobic reproduction of spoilage	2% NaCl	Reduced salt cucumber fermentation	JQ249055	F	99.9
LA1155A	Anaerobic reproduction of spoilage	2% NaCl	Reduced salt cucumber fermentation	JQ249053	G	100
LA1155B	Anaerobic reproduction of spoilage	2% NaCl	Reduced salt cucumber fermentation	JQ249053	G	99.9
LA1155C	Anaerobic reproduction of spoilage	2% NaCl	Reduced salt cucumber fermentation	JQ249053	G	100
LA1156A	Anaerobic reproduction of spoilage	2% NaCl	Reduced salt cucumber fermentation	JQ249054	H	100
LA1156B	Anaerobic reproduction of spoilage	2% NaCl	Reduced salt cucumber fermentation	JQ249054	H	100
LA1154	Anaerobic reproduction of spoilage	2% NaCl	Reduced salt cucumber fermentation	JQ249052	J	99.8
LA1151	Anaerobic reproduction of spoilage	4% NaCl	Reduced salt cucumber fermentation	JQ249047	A	99.9
LA1152	Anaerobic reproduction of spoilage	4% NaCl	Reduced salt cucumber fermentation	JQ249048	A	100
LA1158A	Anaerobic reproduction of spoilage	4% NaCl	Reduced salt cucumber fermentation	JQ249056	F	99.8
LA1158B	Anaerobic reproduction of spoilage	4% NaCl	Reduced salt cucumber fermentation	JQ249056	F	100
LA1138	Anaerobic reproduction of spoilage	6% NaCl	Reduced salt cucumber fermentation	FJ867641	–	100
LA1159A	Anaerobic reproduction of spoilage	6% NaCl	Reduced salt cucumber fermentation	JQ249057	G	100
LA1167	Anaerobic reproduction of spoilage	2% NaCl	Commercial cucumber fermentation	JQ249065	D	99.8
LA1161A	Anaerobic reproduction of spoilage	4% NaCl	Commercial cucumber fermentation	JQ249060	D	100
LA1161B	Anaerobic reproduction of spoilage	4% NaCl	Commercial cucumber fermentation	JQ249060	D	99.9
LA1161C	Anaerobic reproduction of spoilage	4% NaCl	Commercial cucumber fermentation	JQ249060	D	100
LA1163	Anaerobic reproduction of spoilage	4% NaCl	Commercial cucumber fermentation	JQ249062	G	100
LA1164	Anaerobic reproduction of spoilage	4% NaCl	Commercial cucumber fermentation	JQ249063	G	100
LA1166	Anaerobic reproduction of spoilage	4% NaCl	Commercial cucumber fermentation	JQ249064	G	100
LA1149[f]	Spoiled, fermented cucumber brine	1% $CaCl_2$	Commercial cucumber fermentation in calcium chloride brine	JQ086334	G	100

[a] Isolates described by Johanningsmeier et al. (2012) (unless otherwise specified).
[b] Isolation Source: Isolates were obtained directly from spoiled, fermented cucumber brines or after controlled reproduction of spoilage under various conditions (NaCl, calcium chloride).
[c] The original source for the spoilage isolates was the brine from 1) a reduced salt fermentation conducted in the laboratory with cucumbers sourced from a commercial pickling facility; 2) a commercial cucumber fermentation in the Southeast USA that had begun to spoil; or 3) an experimental cucumber fermentation in calcium chloride brine conducted at a commercial pickling facility in the Northeast USA.
[d] CRISPR (Clustered Regularly Interspaced Short Palindromic Repeat) genotype determined by Briner and Barrangou (2014).
[e] % ID: Isolate identity to the reference *L. buchneri* 16S sequence, over a sequence that spanned between 569 bp and 754 bp.
[f] LA1149 was isolated from a commercial, low salt cucumber fermentation that spoiled (Franco et al., 2012).

2.6. Screening for lactate utilization in fermented cucumber media

Pickling cucumbers (size 2B) were fermented at 25 °C in brines containing $CaCl_2$ (100 mM equilibrated). Fermentation was conducted in a 5 gal (~18.9 L) sealed container, with a pack-out ratio (weight cucumbers: volume of cover brine solution) of 60:40. A mixed starter culture of *Lactobacillus plantarum* strains (Culture Collection ID LA0445, LA0085 and LA0219, USDA-ARS Food Science Research Unit, Raleigh NC, USA) was inoculated at 10^6 CFU/mL in the fermentation container and allowed to ferment at ambient temperature until a brine pH of 3.4 had been reached. Fermented cucumbers and brine were blended into slurry and stored at −4 °C until subsequent use. Frozen fermented cucumber slurry was thawed and pressed through multi-layered-cheesecloth to remove large particulate matter and then centrifuged for 15 min at 17,568 × *g* at 4 °C (Sorvall; RC-5B centrifuge, DuPont Instruments, Wilmington, DE, USA). The supernatant was filter-sterilized using a 500 mL bottle-top Nalgene apparatus (Nalgene FAST PES, 0.2 um pore size, Daigger, Vernon Hills, IL, USA) to prepare the fermented cucumber slurry media (FCS). FCS was aseptically aliquoted into sterile conical tubes then transferred to a Coy anaerobic chamber (Coy Laboratory Products, Inc., Grass Lakes, MI, USA) to allow for removal of oxygen in the media for 5 days prior to inoculation with *Lactobacillus buchneri* isolates (~10^6 CFU/mL). Inoculated medium was incubated anaerobically at 25 °C. Measurements of pH were recorded as an indication of lactate utilization. This experiment was conducted in duplicate. An Accumet AR25 pH meter equipped with a gel-filled pH electrode (Fisher Scientific, Pittsburgh, PA, USA) was used for pH measurements. The pH electrode was calibrated prior to each use using certified standards of pH 2.0, 4.0, and 7.0 (Fisher Scientific).

2.7. Lactate utilization in a modified MRS medium (mMRS)

Seven unique spoilage strains and the *L. buchneri* type strain (shown in Fig. 1) were evaluated for the ability to degrade lactic acid over time. A modified MRS (mMRS) medium (without added glucose) was prepared with 50 mM lactic acid, and pH adjusted to 3.8 (Oude Elferink et al., 2001). The mMRS medium (~12 mL) was aliquotted into 15 mL sterile conical tubes and placed in the Coy anaerobic chamber for 5 days

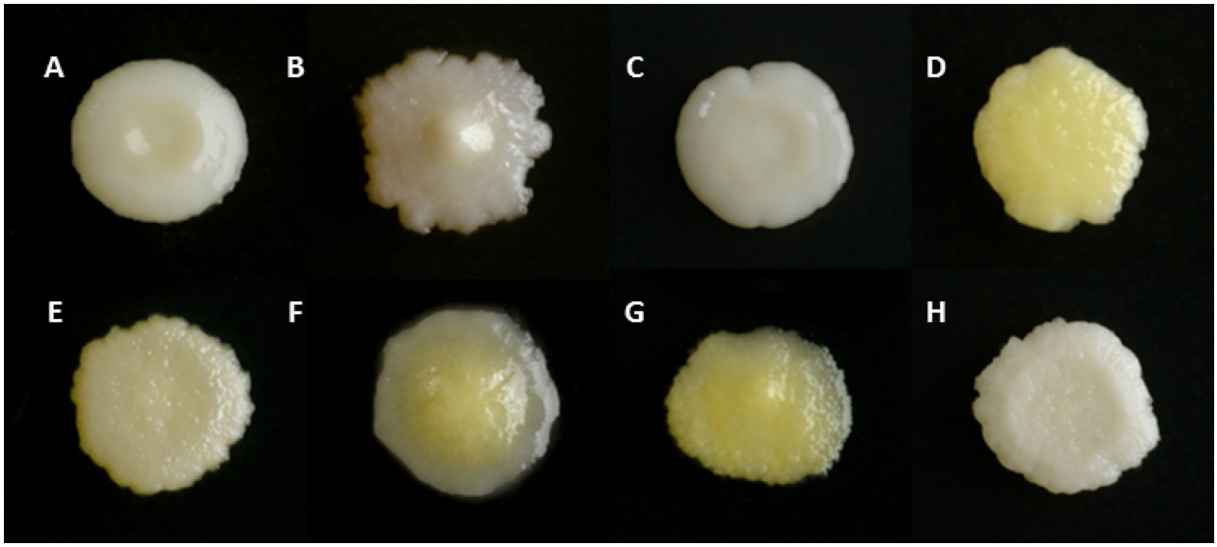


Fig. 1. Morphological diversity of selected isolates of *Lactobacillus buchneri* illustrating distinct phenotypic differences among the strains: (A) ATCC 4005 (LA 0030), (B) LA1147 (C) LA1161B (D) LA1184 (E) LA1167 (F) LA1175D (G) LA1181 (H) LA1161C.

to allow for oxygen removal. Each *L. buchneri* isolate was individually grown in standard MRS broth under anaerobic conditions at 25 °C for 4 days prior to inoculation at 1.5 ± 0.4 × 10^6 CFU/mL into the mMRS medium. Inoculated media was incubated anaerobically at 25 °C; samples were aseptically collected over a time course of 107 days; and media pH was measured for each of the isolates. HPLC analysis of samples was used to quantify lactic acid concentration in modified MRS (mMRS) nutrient media inoculated with eight strains of *L. buchneri*. An Aminex Fast-Acid Analysis column (100 × 7.8 mm, Bio-Rad Laboratories, Hercules, CA, USA) with 0.03 N H_2SO_4 eluent at a flow rate of 0.6 mL/min was used for separation of organic acids, and lactic acid was quantified using a refractive index detector with external standard calibration. This experiment was conducted in duplicate with independently grown cultures of each *L. buchneri* strain.

2.8. DiversiLab® strain typing

The DiversiLab® System (bioMerieux, Marcy I'Etoile, France) was used for rep-PCR-based strain typing. Genomic DNA from each of the isolates was extracted using an Ultraclean Microbial DNA Isolation Kit (MO BIO Laboratories, Inc., CA, USA) and used with the DiversiLab® *Lactobacillus* Primer Kit for DNA fingerprinting according to the manufacturer's instructions (bioMerieux). Outward-facing, genus-specific primers amplify the sequence between DNA fragments, as opposed to inward-facing primers that amplify the repetitive element itself, generating amplicons of varying sizes to create a virtual gel image to visualize similarities and differences between each isolate. (Shutt et al., 2005). Data was analyzed with automated DiversiLab® web-based statistical software (version 3.4) using the Pearson Correlation Coefficient to generate a proximity matrix and calculate pair-wise similarities between the isolates (Shutt et al., 2005; Tenover et al., 2009).

3. Results

3.1. Colony morphology

Images of selected *L. buchneri* isolates with eight distinct morphotypes, including variances in shape, pigmentation, size, and texture are shown in Fig. 1. While sizes ranged from 1 to 11 mm (Table 2), the colonies pictured ranged in size from 2.5–6 mm (Fig. 1). The neotype strain, ATCC 4005 (LA0030), was originally isolated many decades ago from tomato pulp (Hammes and Hertel, 2015) and contrasts entirely with the other seven strains. ATCC 4005 colonies have a slight depression in the center of each colony. This was also the only strain displaying a complete circular form and convex margin. Pigmentation also varied among the isolates, ranging from white and opaque to yellow and translucent. One strain, LA1147, arguably has the most distinct morphology, with sharp, irregular edges and a small elevation directly in the center of the colony. Two of the strains, ATCC 4005 (LA0030) and LA1161B had a mucoid appearance and viscous texture, suggesting production of exopolysaccharides. Alternatively, isolates LA1181 and LA1167 had a dry, powdery texture, while LA1147, LA1161C, LA1175D, and LA1184 presented a butyrous-like texture.

3.2. Cellular morphology

Cellular morphology visualized after 36 h of anaerobic incubation revealed two distinct morphotypes illustrated by LA1167 and LA1184, which displayed an aggregative phenotype, compared to the non-aggregating strains ATCC 4005 (LA0030) and LA1181 (Fig. 2). Aggregative phenotypes may be influenced by proteinaceous components on the cell surface (Kos et al., 2003). Several surface layer (S-layer) proteins have been putatively identified in the genome of *L. buchneri* strain CD034 (Heinl et al., 2012), and differences in S-layer and associated proteins may contribute to differences in cell morphologies between strains (Hynönen and Palva, 2013). Indeed, variation in the aggregative phenotype was attributed to differences in specific S-layer associated proteins in *Lactobacillus acidophilus* (Johnson et al., 2017), suggesting that there may be other functional differences among these strains of *L. buchneri*.

3.3. Growth curves in De Man, Rogosa and Sharpe (MRS) medium

L. buchneri isolates displayed differences in their growth rates in MRS broth (Table 3). Eight of the isolates are displayed in Fig. 3, divided into two general growth patterns. Fig. 3A displays the slower growing strains, with a longer exponential phase. Comparably, strains shown in Fig. 3B entered into stationary phase just after 24 h of growth, while the three strains LA1167, LA1161B, and LA1175D entered stationary phase after 36 h.

3.4. Carbohydrate utilization

Each of the *L. buchneri* isolates was screened using the API 50 CHL

Table 2
Colony morphologies of 7 *Lactobacillus buchneri* strains isolated from spoiled, fermented cucumber and the ATCC type strain.

	ATCC 4005	LA1147	LA1161B	LA1161C	LA1167	LA1175D	LA1181	LA1184
Size (mm)	3.5	3	4.5	5	3	2.5	6	2.5
Color	White	White	White	White	White	Yellow	Yellow	Yellow
Form	Circular	Irregular	Circular	Irregular	Irregular	Irregular	Irregular	Irregular
Elevation	Convex	Umbonate	Raised	Umbonate	Umbonate	Umbonate	Flat	Flat
Margin	Entire	Undulate	Entire	Undulate	Undulate	Entire	Lobate	Undulate
Surface	Mucoid	Bumpy	Dull	Granular	Granular	Smooth	Matte	Matte
Texture	Butyrous	Butyrous	Powdery	Powdery	Powdery	Butyrous	Powdery	Butyrous
Opacity	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Translucent	Opaque

fermentation kit. Substrates including carbohydrates, polyalcohols, uronic acids, and heterosides were divided into cupules on separate strips and elicited a color-change when partially or fully fermented by the inoculated organism. This method has been exploited for carbohydrate profiling of various species of lactobacilli to elucidate physiological capabilities. Fig. 4 displays two-way hierarchical clustering of the API 50 CHL results for 36 *L. buchneri* cultures and 26 of the 49 carbon sources. A total of 26 carbon sources were fermented (partial or complete reaction) over 3 days of incubation, while the remaining 23 carbohydrates were not metabolized by any of the isolates in this timeframe. Fully fermented carbohydrates are shown in dark green; white is displayed in the absence of a reaction; and partial metabolism of a carbon source is indicated in light green. The obligate heterofermentative nature of *L. buchneri* allowed for 100% of the isolates to ferment glucose and maltose, while 72% of the isolates were also able to metabolize arabinose, ribose, xylose and fructose (Fig. 4). Approximately 85% and 64% of *L. buchneri* strains were previously reported to ferment potassium gluconate and potassium-5-ketogluconate, respectively. Similarly, after 3 days of anaerobic incubation at 30 °C, 81% of the *L. buchneri* strains in this study partially fermented potassium gluconate. However, complete fermentation of this substrate was observed only in strain LA1161B, and substantial fermentation of potassium-5-ketogluconate was only observed in two isolates in the present study, LA1161B and LA1161C.

The API 50 CHL data clustered into 11 main groupings of isolates. The top cluster, which included ATCC 4005 (LA0030), LA1158A, and LA1158B, metabolized the fewest of the carbohydrates as compared to the rest of the 36 cultures. In particular, LA1158A only metabolized eight of the 49 carbohydrates tested, and only fully metabolized six of these. Conversely, isolate LA1161B found within the 10th and largest cluster encompassing 12 isolates, fully fermented a total of 16 carbon sources, the largest number of any isolate screened. Also in the 10th cluster was isolate LA1167, which was the only isolate able to metabolize cellobiose during the 3-day incubation period. LA1159A and LA1156B were in separate clusters, but were the only isolates able to metabolize trehalose. One of two isolates in the 11th cluster, LA1185B, demonstrated the most unique carbon source utilization profile. LA1185B fully metabolized 15 substrates including the rarely

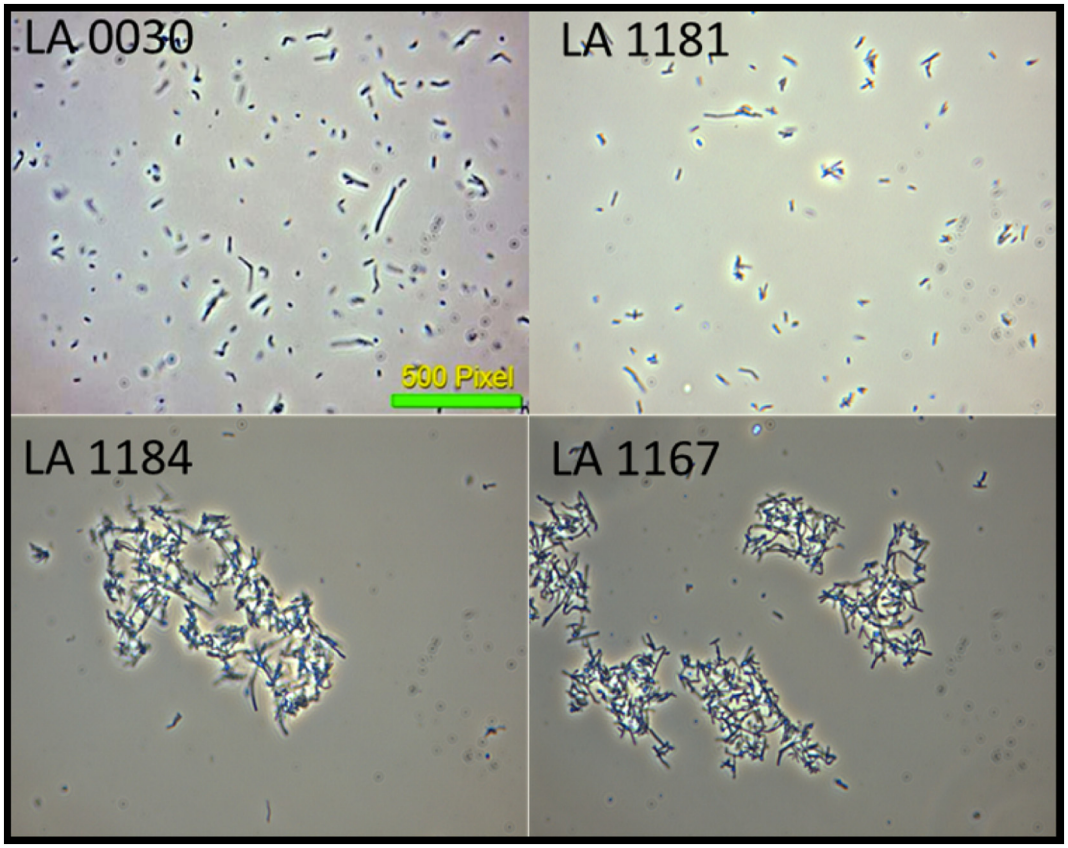


Fig. 2. Cellular morphology of selected *Lactobacillus buchneri* isolates. Presence of aggregative phenotype in strains LA1184 and LA1167 after 36 h of growth in MRS media was observed using phase contrast microscopy. Aggregative phenotype was not observed in strains ATCC 4005 (LA0030) and LA1181.

Table 3
Growth characteristics of eight strains of *Lactobacillus buchneri*.

Strain	Growth rate (μ/h)[1]	Doubling time[2] (h)	Max OD_{600nm}	Lag time (h)
ATCC 4005	0.13 ± 0.02 a b	5.4 ± 0.7 a b	1.67 ± 0.05 a	2.9 ± 0.4 a
LA1147	0.11 ± 0.01 a b	6.2 ± 0.7 a b	1.79 ± 0.18 a	2.3 ± 0.6 a
LA1161B	0.03 ± 0.04 c	10.5 ± 2.2 a b	1.67 ± 0.00 a	3.9 ± 0.4 a
LA1161C	0.15 ± 0.01 a	4.7 ± 0.2 a	1.63 ± 0.05 a	4.6 ± 0.1 a
LA1167	0.11 ± 0.01 a b c	6.7 ± 0.4 a b	1.67 ± 0.15 a	5.5 ± 3.3 a
LA1175D	0.07 ± 0.02 b c	9.8 ± 2.7 a b	1.68 ± 0.07 a	3.0 ± 0.9 a
LA1181	0.12 ± N/A a b	5.7 ± N/A a b	1.61 ± N/A a	1.9 ± N/A a
LA1184	0.11 ± 0.00 a b	6.4 ± 0.1 a b	1.91 ± 0.01 a	3.3 ± 0.8 a

1,2 Data shown as means ± standard deviation of two independent growth experiments. Statistically significant differences in means by Tukey HSD are indicated by different lowercase letters within each column ($P < 0.05$).

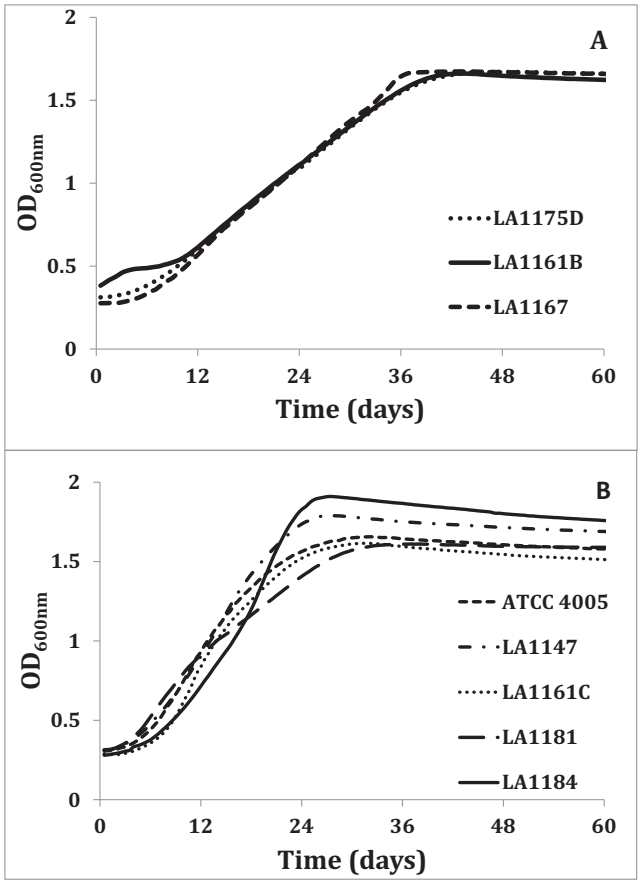


Fig. 3. Growth curves in MRS broth for 8 distinct strains of *Lactobacillus buchneri*. Panel A shows growth curves for 3 slower growing strains, and panel B shows growth curves for the other 5 strains, which had a higher growth rate but were more variable in overall growth patterns.

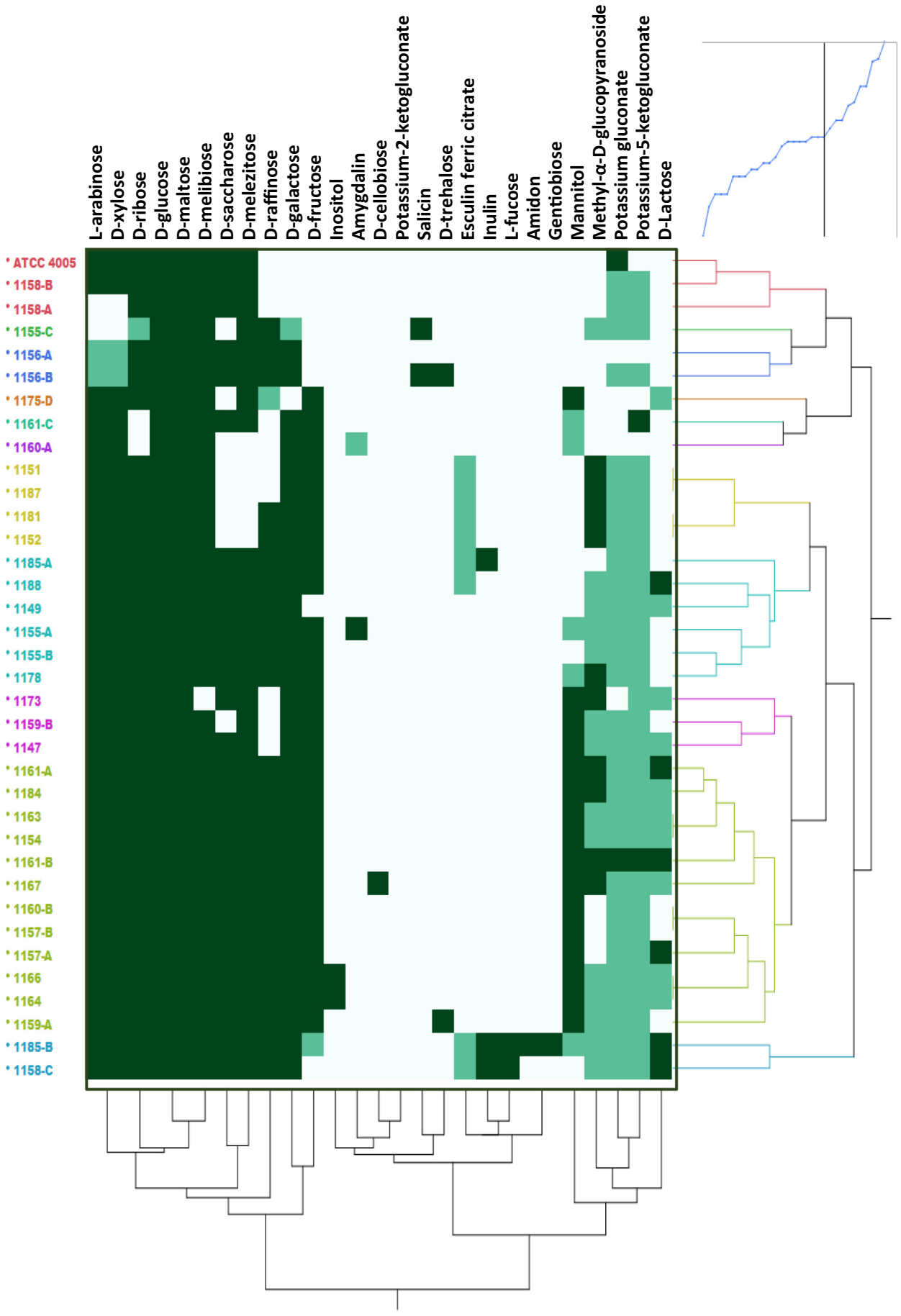
metabolized fucose, partially metabolized an additional six substrates, and was the only isolate to ferment gentiobiose and amidon. It was also one of only three isolates that fermented the prebiotic fiber, inulin. Cellobiose and trehalose were previously shown to be depleted by *L. buchneri* strain LA1147 in fermented cucumber prior to the onset of lactic acid degradation (Johanningsmeier and McFeeters, 2015). However, only one isolate, LA1167, metabolized enough cellobiose during the short term incubation to acidify the medium and produce a color change. A recent study by Sanders et al. (2015) showed that *L. buchneri* metabolized various carbon sources at different rates. When grown in media containing L-arabinose, potassium-gluconate, D-melezitose, or D-melibiose, *L. buchneri* strains reached an OD_{600nm} of 0.7 within 2.4 days. Other carbohydrates such as D-lactose, methyl-D-glucopyranoside, methyl-D-mannopyranoside, raffinose, D-sorbitol, D-turanose, and D-xylose were metabolized more slowly, requiring 7 days for an increase in OD_{600nm} to 0.7, or as long as 12 days in the case of D-trehalose (Sanders et al., 2015). The API 50 CHL panel clearly delineated strains with varying abilities to metabolize carbon sources. These data indicate strains that may be technologically useful but may not accurately reflect the ability of these strains to degrade carbohydrates during long-term bulk storage of commodities or during shelf storage of finished products.

3.5. Lactic acid utilization

In this study, we investigated the variation in lactic acid utilization among each of the *L. buchneri* isolates. Conversion of lactic acid to acetic acid and 1,2-propanediol by *L. buchneri* strains was indicated by a rise in medium pH (Johanningsmeier and McFeeters, 2013) for two different types of media; a modified MRS (mMRS) containing 50 mM lactic acid and FCS. Each medium was incubated under anaerobic conditions at ambient temperature (25 °C). Within 3 days post-inoculation, all strains had initiated lactate metabolism in the mMRS medium (data not shown). However, lactate metabolism in the FCS media did not begin until > 21 days after inoculation (Table 4). This delay in lactic acid degradation was congruent with similar studies conducted in fermented cucumber media (Franco and Pérez-Díaz, 2012; Johanningsmeier et al., 2012; Johanningsmeier and McFeeters, 2013, 2015). Fig. 5 displays lactate degradation in mMRS over a 107-day time course for eight distinct strains of *L. buchneri*. Among these strains, there were significant differences in lactic acid utilization ($P < 0.0001$). The three strains displayed in Fig. 5A, ATCC 4005, LA1147, and LA1184, each had a slower rate of lactate degradation as compared to the five strains shown in Fig. 5B. Between 3 and 42 days of incubation, ATCC 4005, LA1147, and LA1184 utilized approximately 0.5–0.6 mM lactic acid per day compared to ~0.9–1.0 mM per day for strains LA1161B, LA1161C, LA1167, LA1175D, and LA1181. The latter strains also degraded over 8 mM more lactic acid over the course of 107 days (Fig. 5B).

3.6. DiversiLab® strain typing

DiversiLab® is a commercially available repetitive-PCR-based method to differentiate bacterial isolates at the subspecies and strain level through non-coding, recurring elements interspersed throughout the genome (Healy et al., 2005). DiversiLab® results showed clustering of the isolates into eight distinct groups based on their respective rep-PCR fingerprints. The first cluster consisted of twenty isolates with > 97% similarity (Fig. 6A). Isolates within this cluster also had similar colony morphology, with a flat surface, irregular edges, powdery texture and yellow opacity (Fig. 1G). The remaining 17 isolates were separated into seven clusters with > 80% similarity (Fig. 6B). The isolates within each of these clusters were more diverse in carbohydrate metabolism and colony morphologies. Particularly, isolates LA1161A and LA1161C were the most distinct (Fig. 6B), with only 80% similarity to



(caption on next page)

Fig. 4. Two-way hierarchical cluster analysis of carbohydrate utilization by *Lactobacillus buchneri* cultures isolated from spoiled, fermented cucumber. Dark green indicates carbon sources with a positive reaction in the API50 panel, white indicates a negative reaction, and light green indicates an intermediate reaction. Isolates were clustered by similarity in pattern of carbon source utilization and color coded by cluster. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the other isolate groups. ATCC 4005, LA1167, and LA1161B, which had very distinct colony morphologies (Fig. 1) and varied in lactate degradation (Fig. 5), were also distinct in DiversiLab® typing. However, isolates within a genotypic cluster (≥97% similarity) were not always phenotypically identical.

4. Discussion

Characterization of secondary cucumber fermentation has revealed a diverse population of microorganisms associated with different stages throughout the spoilage process (Breidt et al., 2013). While it is difficult to study the progression of industrial secondary fermentation because of its irregular manifestation, reproduction of anaerobic spoilage under controlled laboratory conditions has been successful in isolating some of the responsible organisms. In previous studies, 26 cultures of *L. buchneri* were isolated from spoiled, fermented cucumber and laboratory-scale reproductions of fermented cucumber spoilage (Franco et al., 2012; Johanningsmeier et al., 2012), and *L. buchneri* was shown to initiate spoilage under various conditions present in fermented cucumber (Johanningsmeier and McFeeters, 2013). Factors such as sodium chloride (NaCl) concentration and pH influenced lactic acid metabolism by *L. buchneri* with the most substantial lactic acid degradation observed in reduced salt fermented cucumbers (Franco et al., 2012; Johanningsmeier et al., 2012; Johanningsmeier and McFeeters, 2013). Efforts by the pickle industry to reduce the amount of NaCl in wastewater streams have led to the development of fermentation processes in reduced or no NaCl brines (Fleming et al., 1995, 2002; Guillou et al., 1992; Guillou and Floros, 1993) as well as substitution with calcium chloride ($CaCl_2$) alone (McFeeters and Pérez-Díaz, 2010; Pérez-Díaz et al., 2015). However, these fermented cucumbers have an increased risk of spoilage by *L. buchneri* during bulk storage, limiting the commercial implementation of these more environmentally-friendly processes. The present study evaluated 35 *L. buchneri* spoilage cultures to determine the diversity in phenotypic and genotypic characteristics of the species from this industrially relevant environmental niche.

Lactate utilization varied among the *L. buchneri* spoilage isolates, indicating that some strains may be better suited for this metabolic activity. The conversion of lactic acid to 1,2-propanediol and acetic acid under anaerobic conditions was first documented by Oude Elferink et al. (2001), and the genes for this novel pathway of lactate assimilation by *L. buchneri* were later verified in the genome of strain CD034 (Heinl et al., 2012). *L. buchneri* appears to use this pathway for both D-

Table 4
Rise in pH associated with lactate degradation in fermented cucumber media (100 mM calcium chloride, 28 days) by *Lactobacillus buchneri* spoilage isolates.

Isolate ID	pH
Un-inoculated control	3.74 ± 0.01
ATCC 4005	4.29 ± 0.40
1138	4.04 ± 0.14
1147	4.48 ± 0.11
1149	3.96 ± 0.02
1151	4.21 ± 0.05
1152	4.38 ± 0.04
1154	3.76 ± 0.01
1155A	4.01 ± 0.16
1155B	3.97 ± 0.06
1155C	4.06 ± 0.07
1156A	3.91 ± 0.02
1156B	3.97 ± 0.05
1157A	4.01 ± 0.00
1157B	4.02 ± 0.00
1158A	3.93 ± 0.01
1158B	3.93 ± 0.11
1159A	3.95 ± 0.09
1160A	3.94 ± 0.00
1160B	3.97 ± 0.01
1161A	4.01 ± 0.01
1161B	3.91 ± 0.01
1161C	3.96 ± 0.02
1163	4.10 ± 0.16
1164	4.63 ± 0.17
1166	4.70 ± 0.00
1167	3.90 ± 0.00
1173	4.32 ± 0.22
1175A	3.95 ± 0.09
1175D	4.07 ± 0.06
1178	3.91 ± 0.07
1181	4.15 ± 0.05
1184	3.94 ± 0.00
1185A	3.96 ± 0.02
1185B	4.06 ± 0.01
1187	4.30 ± 0.04
1188	3.91 ± 0.02

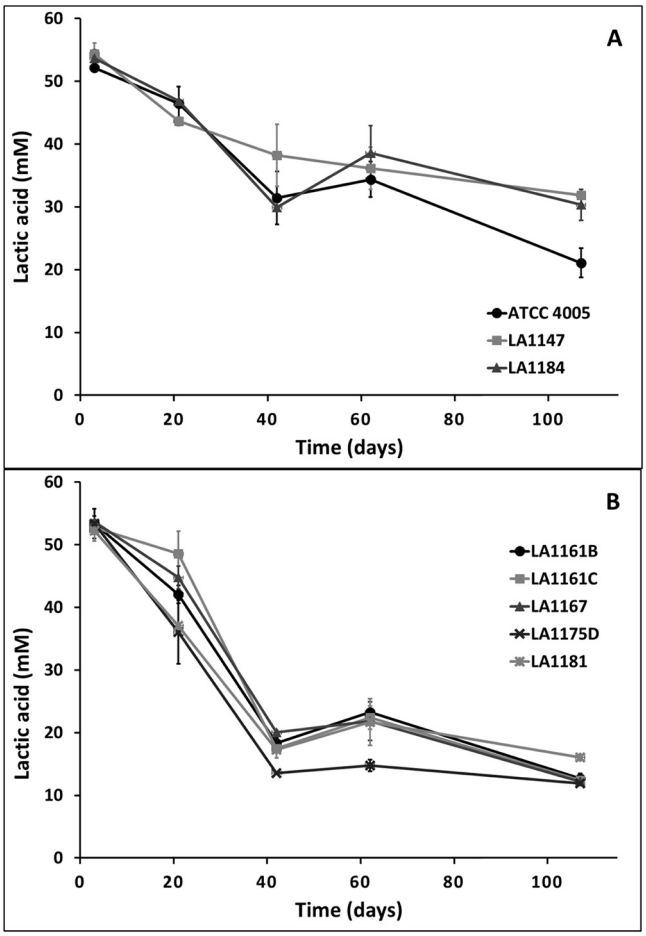


Fig. 5. Lactic acid degradation by eight strains of *Lactobacillus buchneri* in modified MRS media supplemented with 50 mM lactic acid. Panel A shows three strains that slowly and partially metabolized lactic acid, while panel B depicts the more rapid and almost complete metabolism of lactic acid by 5 spoilage strains.

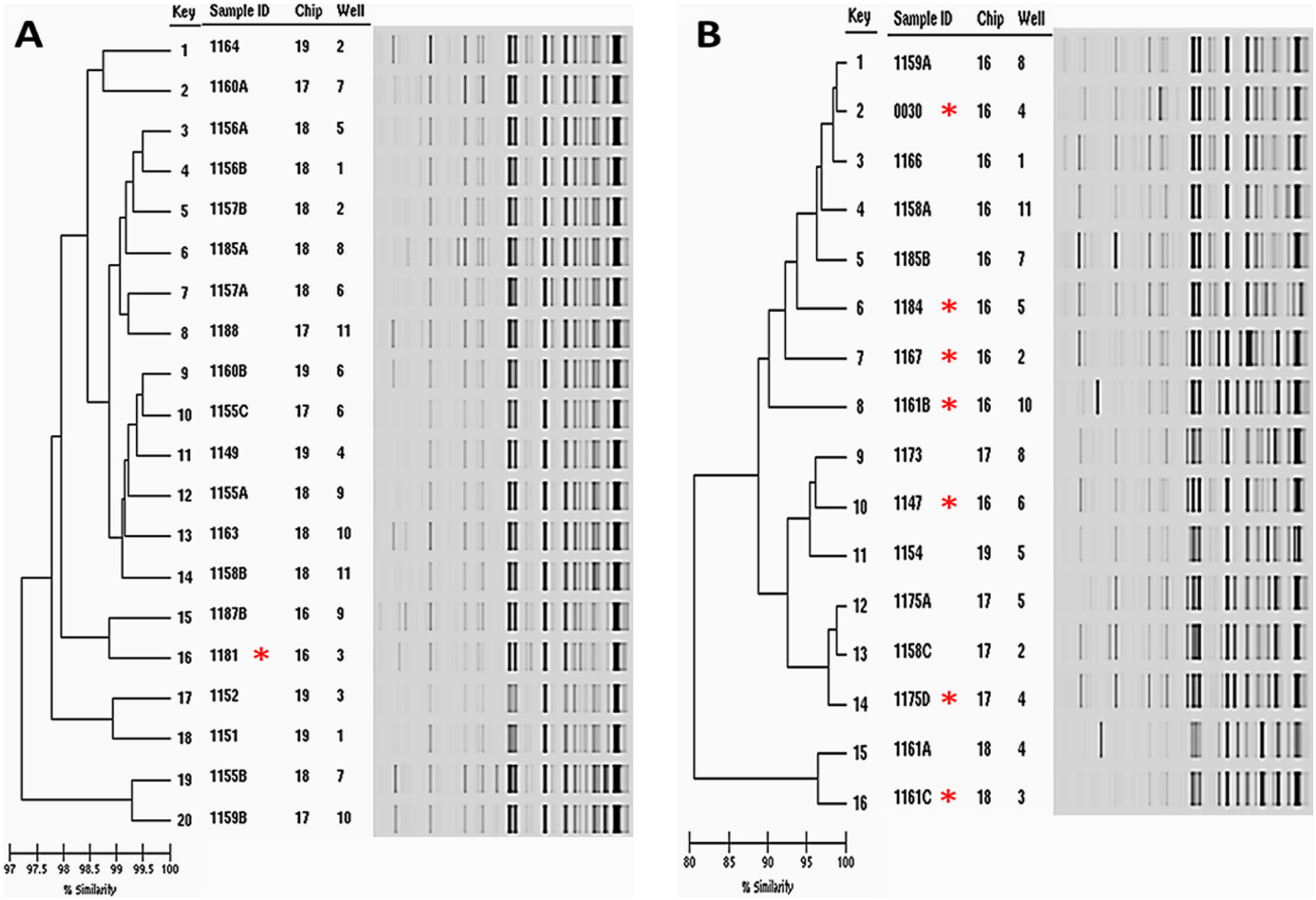


Fig. 6. DiversiLab® genotyping of 36 *Lactobacillus buchneri* isolates. Dendrogram showing isolates clustered with > 97% similarity (Panel A) and > 80% similarity (Panel B). Asterisks denote unique strains selected for further characterization.

and L-lactate degradation in fermented cucumber in the absence of fermentable sugars (Johanningsmeier and McFeeters, 2013), but the regulatory mechanism for this metabolism in fermented plant materials is not yet fully understood. No consistent relationships were observed between growth rate and ability to metabolize lactate among the eight unique strains identified in this study, which is in accordance with the idea that lactic acid degradation under acidic conditions is an acid resistance mechanism for *L. buchneri* (Oude Elferink et al., 2001). Lactic acid conversion to 1,2-propanediol and acetic acid only occurred at acidic pH's (3.2–4.3), and no increase in cell counts were observed during lactate utilization experiments in fermented cucumber media or in nutrient growth media supplemented with lactic acid (Johanningsmeier et al., 2012; Johanningsmeier and McFeeters, 2013; Oude Elferink et al., 2001). Furthermore, lactate utilization is not initiated until residual glucose has been depleted from the environment (Oude Elferink et al., 2001), and onset of lactate utilization typically occurs ≥21 days after the end of a primary cucumber fermentation (Johanningsmeier and McFeeters, 2013). Specifically, depletion of cellobiose, trehalose, and citrulline may signify imminent lactate degradation by *L. buchneri* strains at the initiation of fermented cucumber spoilage (Johanningsmeier and McFeeters, 2015). However, LA1167 was the only isolate to metabolize cellobiose as a sole carbon source in carbohydrate profiling assays. In a previous metabolomics study, *L. buchneri* LA1147 degraded cellobiose prior to the onset of secondary fermentation (Johanningsmeier and McFeeters, 2015), but this metabolic activity was not observed with the standard API 50 assay. Overall, we found that lactate metabolism is strain-dependent, and not specifically associated with the organism's ability to grow in complex media or rapidly ferment specific carbon sources.

Although the strains were isolated from a similar environmental niche (Table 1), significant phenotypic differences existed with regard to colony type, cellular morphology and ability to ferment various carbon sources. CD034, a strain of *L. buchneri* isolated from silage fermentation most closely resembles the colony phenotype of the type strain of the organism, ATCC 4005 (unpublished observation). However, these are both morphologically different than all *L. buchneri* isolated from spoiled, fermented cucumber. Aggregation of selected strains of *L. buchneri* was visualized under a microscope. Three of eight of the unique strains displayed an aggregative phenotype, suggesting differences in surface proteins between strains.

L. buchneri is classified under the Group III obligately-heterofermentative lactobacilli, capable of growth by metabolizing hexoses and pentoses to lactic acid, acetic acid and traces of ethanol and carbon dioxide. In this study, the API 50 CHL method was utilized to examine specific carbohydrate profiles for each of the 36 isolates. Results of the assay were compared to the documented API sugar profiles for *L. buchneri*. D-glucose and D-maltose were the only substrates that were universally metabolized by the *L. buchneri* strains in this study. The type strain is documented with the ability to fully utilize melibiose, maltose, arabinose, gluconate, melezitose, and ribose as sole carbon sources (Pot et al., 2014). Most of the *L. buchneri* isolates from spoiled, fermented cucumber followed this pattern with only a few unable to grow rapidly on some of the aforementioned substrates. The exception was gluconate, which was rapidly metabolized by the type strain (ATCC 4005) and LA 1161B, but only partially fermented by the majority of the spoilage isolates. Although reported as variable for the type strain (Pot

Table 5
Phenotypic and genotypic characteristics of 8 unique *Lactobacillus buchneri* strains.

Strain ID	Colony morphology color (type)[a]	Aggregative property	Growth profile in MRS	Lactate degradation in mMRS	API 50 cluster	Sole carbon sources metabolized (#)[b]	DiversiLab® cluster
ATCC 4005	White (A)	No	Fast	Low	1	9	2
LA1161C	White (H)	No	Fast	High	5	11	8
LA1161B	White (C)	No	Slow	High	10	16	5
LA1167	White (E)	Yes	Slow	High	10	17	4
LA1147	White (B)	Yes	Fast	Low	9	15	6
LA1175D	Yellow (F)	No	Slow	High	4	11	7
LA1181	Yellow (G)	No	Fast	High	7	13	1
LA1184	Yellow (D)	Yes	Fast	Low	10	16	3

[a] Letter code for type corresponds to panel in Fig. 1.
[b] Total number of carbon sources partially or fully fermented in 3 day API 50 screening.

et al., 2014), D-xylose was fully metabolized by the type strain and 31 of 35 spoilage isolates in this study. This is consistent with broth culture studies by Liu et al. for the type strain and *L. buchneri* strains NRRL B-30929 and DSM 5987 (Liu et al., 2008; Liu et al., 2009), suggesting that the ability to metabolize D-xylose into lactic and acetic acids is a common feature among strains isolated from plant materials. Along with the more readily metabolized carbohydrates (glucose, maltose, arabinose, ribose, xylose, and melibiose), several *L. buchneri* spoilage isolates were able to metabolize other carbon sources, such as saccharose, raffinose, galactose, fructose, mannitol, methyl-D-glucopyranoside, potassium-5-ketogluconate, and lactose. According to previous studies, over 90% of *L. buchneri* strains should readily metabolize arabinose, while there is more diversity in strains (11–89%) that will ferment galactose, ribose, and sucrose (Hammes and Vogel, 1995). Furthermore, particular strains isolated from spoiled, fermented cucumber were unique in their ability to rapidly metabolize a wider range of substrates, including inositol, amygdalin, D-cellobiose, salicin, D-trehalose, esculin ferric citrate, inulin, L-fucose, amidon, and gentiobiose. Aside from residual glucose and fructose, fermented cucumber contains xylose, lyxose and disaccharides, trehalose, cellobiose, and gentiobiose, (Johanningsmeier and McFeeters, 2015), which may give certain strains of *L. buchneri* an advantage for growth and survival in this environment.

L. buchneri strain LA1175D is arguably one of the more distinctive strains characterized in this study. Isolated from spoiled, reduced salt fermented cucumber, LA1175D was one of four of the 36 isolates examined that did not catabolize galactose, one of eight unable to degrade saccharose, and was the sole isolate in cluster 4 (Fig. 4). Furthermore, LA1175D degraded lactate more rapidly between days 21 and 42 compared to six of the other strains examined (Fig. 5). In appearance, this isolate has slightly translucent edges, with a darker yellow, opaque center and displayed a slower growth rate, similar to LA1167 and LA1161B.

Interestingly, phenotypic differences among the isolates only loosely correspond with the genotyping tools employed in this study. In a previous study, cultures of *L. buchneri* isolated from spoiled, fermented cucumber along with two annotated genomes of *L. buchneri* (strains CD034 and NRRL B-2099) were investigated for the occurrence of CRISPR-Cas systems (Briner and Barrangou, 2014). CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is a form of adaptive bacteriophage immunity that is present in most archaea and in many species of bacteria, which could be used as a genotyping method for tracking food-associated bacteria (Barrangou and Dudley, 2016). Comparative genomic analysis of the strains revealed the universal occurrence of a type II-A CRISPR-Cas system in each of the *L. buchneri* genomes and fermented cucumber spoilage isolates. The type II-A locus was defined by a highly conserved 36-nt repeat sequence, along with the *cas1* and *cas2* genes and the type II signature nuclease *cas9* (Briner and Barrangou, 2014). Comparative genomic analysis of CRISPR loci from each of the 26 isolates revealed 10 unique CRISPR genotypes containing 9–29 spacers. (Briner and Barrangou, 2014). In this study, it was noted that three strains with the same CRISPR genotype (LA1167, LA1161B, and LA1161C) exhibited various colony morphotypes. However, these strains had differing DiversiLab® rep-PCR fingerprints consistent with the observed differences in colony morphology. A possible explanation for this dichotomy is that DiversiLab® sequencing uses genus-specific primers to amplify regions throughout the genome while CRISPR sequencing is localized to a specific area of the genome. Although both genotyping techniques provided useful information for grouping the *L. buchneri* isolates, neither fully discriminated among isolates with observed phenotypic differences. A detailed examination of the complete genomes of these isolates may be necessary to determine the genetic basis for the plethora of phenotypic differences observed in our study. This may involve searching for the acquisition of mobile genetic elements to possibly explain diverse carbohydrate metabolism profiles and production of EPS, which confers variations in colony morphology and increased acid resistance (Heinl and Grabherr, 2017).

In this study, 35 cultures of *L. buchneri* originally isolated from spoiled, fermented cucumber were characterized using phenotypic and genotypic analyses to explore the diversity within the species from a single environmental niche in comparison to the ATCC type strain. These data present new information for characterization of strain diversity in *L. buchneri*. Variations in colony morphology revealed several characteristic morphotypes that varied in color, size, and shape among the strains. Aggregation phenotypes, growth rates and carbohydrate metabolism also varied significantly among the strains. The unique properties of these strains may be relevant for various applications, such as silage, alternative fuel production, and the biotechnology industry. Specifically, a difference in lactate degradation was noted among the strains, suggesting that some strains may be more robust spoilage organisms in fermented cucumber or perform better as starter culture adjuncts in silage fermentations. Furthermore, this level of intra-species diversity has not been previously explored in *L. buchneri*. Eight strains that represented the phenotypic and genotypic diversity of *L. buchneri* (Table 5) were identified as candidates for whole genome sequencing to further understand the metabolic potential of this organism and identify the genetic basis for the strain differences observed throughout our analyses. Additional insights at both the phenotypic and genomic level are necessary to fully utilize the capacity of *L. buchneri* for industrial application and for development of techniques to prevent spoilage in fermented cucumbers and other foods.

Acknowledgements

We thank Janet Hayes of the USDA-ARS Food Science Research Unit and Brant Johnson and Kurt Selle of the Klaenhammer Lab for excellent technical assistance. We also thank Dr. Fred Breidt for deriving the data from growth curves and Mrs. Sandra Parker for administrative assistance, both of the USDA-ARS Food Science Research Unit.

This study was funded by the United States Department of Agriculture, Agriculture Research Service, Food Science Research Unit (Raleigh, NC) with partial support from the North Carolina Agricultural Foundation (Raleigh, NC).

References

Barrangou, R., Dudley, E.G., 2016. CRISPR-based typing and next-generation tracking technologies. Annu. Rev. Food Sci. Technol. 7, 395–411.

Breidt, F., Romick, T.L., Fleming, H.P., 1994. A rapid method for the determination of bacterial growth kinetics. J. Rapid Methods Autom. Microbiol. 3 (1), 59–68.

Breidt, F., Medina-Pradas, E., Wafa, D., Pérez-Díaz, I.M., Franco, W., Huang, H., Johanningsmeier, S.D., Kim, J., 2013. Characterization of cucumber fermentation spoilage bacteria by enrichment culture and 16S rDNA cloning. J. Food Sci. 78 (3), M470–M476.

Briner, A.E., Barrangou, R., 2014. *Lactobacillus buchneri* genotyping on the basis of clustered regularly interspaced short palindromic repeat (CRISPR) locus diversity. Appl. Environ. Microbiol. 80 (3), 994–1001.

Caldwell, J.M., Juvonen, R., Brown, J., Breidt, F., 2013. *Pectinatus sottacetonis* sp. nov., isolated from commercial pickle spoilage tank. Int. J. Syst. Evol. Microbiol. 63 (10), 3609–3616.

Cooke, L., 1995. New strain slows silage spoilage. Agric. Res. 43 (6), 17.

de Orduña, R.M., Patchett, M.L., Liu, S.-Q., Pilone, G.J., 2001. Growth and arginine metabolism of the wine lactic acid bacteria *Lactobacillus buchneri* and *Oenococcus oeni* at different pH values and arginine concentrations. Appl. Environ. Microbiol. 67 (4), 1657–1662.

Driehuis, F., Oude Elferink, S., Van Wikselaar, P., 2001. Fermentation characteristics and aerobic stability of grass silage inoculated with *Lactobacillus buchneri*, with or without homofermentative lactic acid bacteria. Grass Forage Sci. 56 (4), 330–343.

Fleming, H., Daeschel, M., McFeeters, R., Pierson, M., 1989. Butyric acid spoilage of fermented cucumbers. J. Food Sci. 54 (3), 636–639.

Fleming, H.P., McDonald, L.C., McFeeters, R.F., Thompson, R.L., Humphries, E.G., 1995. Fermentation of cucumbers without sodium chloride. J. Food Sci. 60 (2), 312–315, 319.

Fleming, H.P., Humphries, E.G., Fasina, O.O., McFeeters, R.F., Thompson, R.L., Breidt Jr., F., 2002. BAG-IN-BOX TECHNOLOGY: pilot system for process-ready, fermented cucumbers. Pickle Pak Sci VIII (1), 1–8.

Franco, W., Pérez-Díaz, I., 2012. Role of selected oxidative yeasts and bacteria in cucumber secondary fermentation associated with spoilage of the fermented fruit. Food Microbiol. 32, 338–344.

Franco, W., Pérez-Díaz, I.M., Johanningsmeier, S.D., McFeeters, R.F., 2012. Characteristics of spoilage-associated secondary cucumber fermentation. Appl. Environ. Microbiol. 78 (4), 1273–1284.

Franco, W., Johanningsmeier, S.D., Lu, J., Demo, J., Wilson, E., Moeller, L., 2016. Cucumber fermentation, chapter 7. In: Paramithiotis, S. (Ed.), Lactic Acid Fermentation of Fruits and Vegetables. CRC Press, Boca Raton, FL, pp. 107–155.

Guillou, A.A., Floros, J.D., 1993. Multiresponse optimization minimizes salt in natural cucumber fermentation and storage. J. Food Sci. 58 (6), 1381–1389.

Guillou, A.A., Floros, J.D., Cousin, M.A., 1992. Calcium chloride and potassium sorbate reduce sodium chloride used during natural cucumber fermentation and storage. J. Food Sci. 57 (6), 1364–1368.

Hammes, W.P., Hertel, C., 2015. Lactobacillus. Bergey's Manual of Systematics of Archaea and Bacteria. Published by John Wiley & Sons, Inc., Somerset, NJ in association with Bergey's Manual Trust, pp. 1–76.

Hammes, W.P., Vogel, R.F., 1995. The genus *Lactobacillus*. In: Wood, B.J.B., Holzapfel, W.H. (Eds.), The Genera of Lactic Acid Bacteria. Vol. 2. Published by Springer US, pp. 19–54.

Healy, M., Huong, J., Bittner, T., Lising, M., Frye, S., Raza, S., Schrock, R., Manry, J., Renwick, A., Nieto, R., 2005. Microbial DNA typing by automated repetitive-sequence-based PCR. J. Clin. Microbiol. 43 (1), 199–207.

Heinl, S., Grabherr, R., 2017. Systems biology of robustness and flexibility: *Lactobacillus buchneri*—a show case. J. Biotechnol. 257, 61–69.

Heinl, S., Wibberg, D., Eikmeyer, F., Szczepanowski, R., Blom, J., Linke, B., Goesmann, A., Grabherr, R., Schwab, H., Pühler, A., 2012. Insights into the completely annotated genome of *Lactobacillus buchneri* CD034, a strain isolated from stable grass silage. J. Biotechnol. 161 (2), 153–166.

Hynönen, U., Palva, A., 2013. *Lactobacillus* surface layer proteins: structure, function and applications. Appl. Microbiol. Biotechnol. 97 (12), 5225–5243.

Johanningsmeier, S.D., McFeeters, R.F., 2013. Metabolism of lactic acid in fermented cucumbers by *Lactobacillus buchneri* and related species, potential spoilage organisms in reduced salt fermentations. Food Microbiol. 35 (2), 129–135.

Johanningsmeier, S.D., McFeeters, R.F., 2015. Metabolic footprinting of *Lactobacillus buchneri* strain LA1147 during anaerobic spoilage of fermented cucumbers. Int. J. Food Microbiol. 215, 40–48.

Johanningsmeier, S.D., Franco, W., Perez-Diaz, I., McFeeters, R.F., 2012. Influence of sodium chloride, pH, and lactic acid bacteria on anaerobic lactic acid utilization during fermented cucumber spoilage. J. Food Sci. 77 (7), M397–M404.

Johnson, B.R., O'Flaherty, S., Goh, Y.J., Carroll, I., Barrangou, R., Klaenhammer, T.R., 2017. The S-layer associated serine protease homolog PrtX impacts cell surface-mediated microbe-host interactions of *Lactobacillus acidophilus* NCFM. Front. Microbiol. 8, 1185. http://dx.doi.org/10.3389/fmicb.2017.01185.

Kim, J., Breidt, F., 2007. Development of preservation prediction chart for long term storage of fermented cucumber. Korean J. Life Sci. 17 (12), 1616–1621.

Kleinschmit, D., Schmidt, R., Kung, L., 2005. The effects of various antifungal additives on the fermentation and aerobic stability of corn silage. J. Dairy Sci. 88 (6), 2130–2139.

Kos, B., Susković, J., Vuković, S., Simpraga, M., Frece, J., Matosić, S., 2003. Adhesion and aggregation ability of probiotic strain *Lactobacillus acidophilus* M92. J. Appl. Microbiol. 94 (6), 981–987.

Kullen, M., Sanozky-Dawes, R., Crowell, D., Klaenhammer, T., 2000. Use of the DNA sequence of variable regions of the 16S rRNA gene for rapid and accurate identification of bacteria in the *Lactobacillus acidophilus* complex. J. Appl. Microbiol. 89 (3), 511–516.

Kung, L., Ranjit, N., 2001. The effect of *Lactobacillus buchneri* and other additives on the fermentation and aerobic stability of barley silage. J. Dairy Sci. 84 (5), 1149–1155.

Kung, L., Taylor, C., Lynch, M., Neylon, J., 2003. The effect of treating alfalfa with *Lactobacillus buchneri* 40788 on silage fermentation, aerobic stability, and nutritive value for lactating dairy cows. J. Dairy Sci. 86 (1), 336–343.

Liu, S., Skinner-Nemec, K.A., Leathers, T.D., 2008. *Lactobacillus buchneri* strain NRRL B-30929 converts a concentrated mixture of xylose and glucose into ethanol and other products. J. Ind. Microbiol. Biotechnol. 35 (2), 75–81.

Liu, S., Bischoff, K., Hughes, S., Leathers, T., Price, N., Qureshi, N., Rich, J., 2009. Conversion of biomass hydrolysates and other substrates to ethanol and other chemicals by *Lactobacillus buchneri*. Lett. Appl. Microbiol. 48 (3), 337–342.

Liu, S., Leathers, T.D., Copeland, A., Chertkov, O., Goodwin, L., Mills, D.A., 2011. Complete genome sequence of *Lactobacillus buchneri* NRRL B-30929, a novel strain from a commercial ethanol plant. J. Bacteriol. 193 (15), 4019–4020.

McFeeters, R.F., Pérez-Díaz, I.M., 2010. Fermentation of cucumbers brined with calcium chloride instead of sodium chloride. J. Food Sci. 75 (3), C291–C296.

Muck, R., 1996. A Lactic Acid Bacteria Strain to Improve Aerobic Stability of Silages. US Dairy Forage Res. Center, pp. 42–43.

Oude Elferink, S.J.O., Krooneman, J., Gottschal, J.C., Spoelstra, S.F., Faber, F., Driehuis, F., 2001. Anaerobic conversion of lactic acid to acetic acid and 1, 2-propanediol by *Lactobacillus buchneri*. Appl. Environ. Microbiol. 67 (1), 125–132.

Pelczar Jr., M.J., 1957. Manual of Microbiological Methods. McGraw-Hill Book Co., New York, NY.

Pérez-Díaz, I.M., McFeeters, R.F., Moeller, L., Johanningsmeier, S.D., Hayes, J.S., Fornea, D., Gilbert, C., Custis, N., Beene, K., Bass, D., 2015. Commercial scale cucumber fermentations brined with calcium chloride instead of sodium chloride. J. Food Sci. 80 (12), M2827–M2836. http://dx.doi.org/10.1111/1750-3841.13107.

Pot, B., Felis, G. E., De Bruyne, K., Tsakalidou, E., Papadimitriou, K., Leisner, J., Vandamme, P., 2014. The genus *Lactobacillus*. Chapter 19 in Lactic Acid Bacteria: Biodiveristy and Taxonomy, First Edition, Wilhelm H. Holzapfel and Brian J. B. Wood, John Wiley & Sons, Ltd, Hoboken, NJ.

Ranjit, N.K., Kung, L., 2000. The effect of *Lactobacillus buchneri*, *Lactobacillus plantarum*, or a chemical preservative on the fermentation and aerobic stability of corn silage. J. Dairy Sci. 83 (3), 526–535.

Sakamoto, K., Konings, W.N., 2003. Beer spoilage bacteria and hop resistance. Int. J. Food Microbiol. 89 (2), 105–124.

Sanders, J.W., Oomes, S.J.C.M., Membre, J.M., Wegkamp, A., Wels, M., 2015. Biodiversity of spoilage lactobacilli: phenotypic characterisation. Food Microbiol. 45, 34–44.

Shutt, C.K., Pounder, J.I., Page, S.R., Schaecher, B.J., Woods, G.L., 2005. Clinical evaluation of the DiversiLab microbial typing system using repetitive-sequence-based PCR for characterization of *Staphylococcus aureus* strains. J. Clin. Microbiol. 43 (3), 1187–1192.

Sumner, S., Speckhard, M., Somers, E., Taylor, S., 1985. Isolation of histamine-producing *Lactobacillus buchneri* from Swiss cheese implicated in a food poisoning outbreak. Appl. Environ. Microbiol. 50 (4), 1094–1096.

Taylor, C., Ranjit, N., Mills, J., Neylon, J., Kung, L., 2002. The effect of treating whole-plant barley with *Lactobacillus buchneri* 40788 on silage fermentation, aerobic stability, and nutritive value for dairy cows. J. Dairy Sci. 85 (7), 1793–1800.

Tenover, F.C., Gay, E.A., Frye, S., Eells, S.J., Healy, M., McGowan, J.E., 2009. Comparison of typing results obtained for methicillin-resistant *Staphylococcus aureus* isolates with the DiversiLab system and pulsed-field gel electrophoresis. J. Clin. Microbiol. 47 (8), 2452–2457.

Yildirim, Z., Yildirim, M., 2001. Characterization of buchnericin LB produced by *Lactobacillus buchneri* LB. Turk. J. Biol. 25, 73–82.

Yildirim, Z., Avs, Y.K., Yıldırım, M., 2002. Factors affecting the adsorption of buchnericin LB, a bacteriocin produced by *Lactocobacillus buchneri*. Microbiol. Res. 157 (2), 103–107.

Yousif, N.M.K., Huch, M., Schuster, T., Cho, G.-S., Dirar, H.A., Holzapfel, W.H., Franz, C.M.A.P., 2010. Diversity of lactic acid bacteria from Hussuwa, a traditional African fermented sorghum food. Food Microbiol. (6), 757–768.

Zeng, X., Pan, D., Guo, Y., 2010. The probiotic properties of *Lactobacillus buchneri* P2. J. Appl. Microbiol. 108 (6), 2059–2066.

Zhang, C., Brandt, M.J., Schwab, C., Gänzle, M.G., 2010. Propionic acid production by cofermentation of *Lactobacillus buchneri* and *Lactobacillus diolivorans* in sourdough. Food Microbiol. 27 (3), 390–395.