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Investigation of the Lactic Acid Bacteria in Sauerkraut Fermentations by DNA fingerprinting

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ABSTRACT

Previous studies using traditional biochemical identification methods to study the ecology of commercial sauerkraut fermentations revealed that four lactic acid bacteria species, *Leuconostoc mesenteroides*, *Lactobacillus plantarum*, *Pediococcus pentosaceus*, and *Lactobacillus brevis* were the primary microorganisms in the fermentation. In this study 686 isolates were collected for analysis by DNA fingerprinting, from four commercial fermentations. The results indicate that the species of lactic acid bacteria present in sauerkraut fermentations are more diverse than previously reported, including *Leuconostoc citreum*, *Leuconostoc argentinum*, *Lactobacillus paraplantarum*, *Lactobacillus coryniformis*, and *Weissella* sp. The newly identified species, *Leuconostoc fallax* was also found. Unexpectedly, only 2 isolates of *Pediococcus pentosaceus*, and 15 isolates of *Lactobacillus brevis* were recovered during this study. A better understanding of the microbiota may aid the development of low salt fermentations, which may have altered microflora and altered sensory characteristics.

Keywords: sauerkraut, fermentation, lactic acid bacteria, PCR, microbial diversity

INTRODUCTION

Sauerkraut fermentation involves many physical, chemical and microbiological changes that influence quality and safety of the product. The fermentation can be broadly categorized as having successive stages, including an initial heterofermentative stage, followed by a homofermentative stage (11, 23). Historically, four species of lactic acid bacteria have been identified as present in sauerkraut fermentations: *Leuconostoc mesenteroides, Lactobacillus brevis, Pediococcus pentosaceus, Lactobacullus plantarum.* The identification of these microorganisms has been based on morphological and biochemical criteria (22). Several species of lactic acid bacteria (LAB), other than the four species mentioned above, have been found in cabbage fermentations, including *Lactobacillus curvatus*, *Lactobacillus sake*, *Lactococcus lactis* subsp. *lactis*, and *Leuconostoc fallax* (3, 19, 25). Recently, six *L. fallax* strains have been isolated from brine samples obtained from sauerkraut fermentations (2). The taxonomic characterization of LAB has been modified, and new species have been described using molecular techniques (1, 10, 20, 24) . Improvements in molecular identification techniques for the study of microbial ecology have created new opportunities for the analysis of food fermentations.

This study was carried out because of the need to reduce sodium chloride (salt) waste from commercial vegetable fermentations. It is well documented that the concentration of salt has a controlling influence on the microbial succession in a typical sauerkraut fermentation (11, 12, 22) . It may be possible to reduce salt waste by fermenting cabbage with 1% instead of the typical 2% salt concentration. The introduction of a *L. mesenteroides* starter culture to the fermentation could help ensure that the initial stage of the fermentation produces the desirable flavor compounds (11). A method has been developed (23) to determine the ability of an unmarked starter culture predominate over the indigenous microbiota in sauerkraut

fermentations. In that study (23) however, the effect of the starter culture on the indigenous microbiota was not determined.

During the sauerkraut fermentation there is a rapid turnover of LAB species. The dominant species present in the fermentation shifts within two to three days from less acid-tolerant heterolactic LAB species to more acid-tolerant homolactic fermenting LAB species, with sequential populations each reaching 10^8 to 10^9 CFU/g *(11)*. Under normal conditions, the fermentation is essentially complete within two weeks, with the most acid-tolerant species *L. plantarum* predominating. Our objective was to characterize the dominant LAB species in the successive stages of fermentation. There is strong evidence that the genetic diversity of many ecosystems, as assessed by molecular techniques, exceeds the microbial diversity determined by traditional culture based identification methods (26). However, due to the rapid succession of LAB in sauerkraut, and the potential impact of large numbers of dead cells on culture-free nucleic acid-based methods, we carried out this study using bacterial isolates. In this report we characterized isolates from the microbiota of commercial sauerkraut fermentations using a rDNA intergenic spacer PCR method (ITS-PCR) with a database of known ITS-PCR patterns for LAB (4, and this report), supplemented with 16S rDNA sequence analysis. Several species of LAB not previously found in sauerkraut were observed. Notably, *Weissella* and *Leuconostoc citrium* species were found in the heterolactic phase of the fermentations, and two (*P. pentosaceus* and *L. brevis*) of the four LAB species expected to be present were apparently minor constituents of the microbiota. A better understanding of the microbial ecology of sauerkraut fermentations may aid in the development of low salt fermentation technology, which may alter normal microflora and sauerkraut flavor (Breidt and coworkers, unpublished).

MATERIALS AND METHODS

Bacterial strains: Bacterial strains were obtained from the Food Fermentation Culture Collection, US Department of Agriculture, Agriculture Research Service, Raleigh, NC (Suppl. Table 1), or isolated as described below. LAB were maintained at –80°C in MRS broth (Difco Laboratories) supplemented with 16% glycerol, and grown on MRS broth or agar plates (MRS broth supplemented with 1.5% agar, Difco). To inhibit aerobic yeast or mold growth on MRS agar plate with sauerkraut isolates, MRS agar was supplemented with 0.2% sodium azide (Sigma Chemical Co., St. Louis, MO), or plates were incubated in an anaerobic hood (Coy Laboratories, Detroit, MI).

Commercial sauerkraut fermentation and sample collection: Sauerkraut fermentations at a commercial processing plant in Wisconsin consisting of approximately 100 megagrams of cabbage were sampled for this study, including one tank in the first year of the study (Y1), and three different tanks in the second year (Y2). Only one sauerkraut production facility in Wisconsin was used in this study, because there are currently very few commercial production facilities in the US. The fermentation tanks were of cement construction, with the approximate dimensions: 5 m length by 5 m width by 4 m deep. Each fermentation was carried out with approximately 2.3 % NaCl (final equilibrated concentration), which was added by a dry salting process using shredded cabbage from mixed cultivars. The cabbage was manually spread in the tanks, covered with plastic sheeting, and initially weighted down with water on top of the plastic sheeting. The fermentation temperature was not controlled, but the temperature in these commercial fermentations typically averaged 18˚C. For analysis of cabbage prior to ferementation, shredded cabbage samples (approximately 500 g) were collected in sterile plastic bags prior to salting. Brine samples (100 ml each) from the fermentations were obtained for

111 microbial and biochemical analysis with a one-cm-diameter, perforated stainless steel tube that
112 was sealed at the bottom, from a depth of approximately 60 cm from the tops of the fermentation
113 tanks, and about 60 cm from the sides. The sampling apparatus was sanitized with dilute (10%)
114 Clorox solution and rinsed with sterile water prior to use. Brine and cabbage samples were
115 obtained on days 1, 3, 7, and 14 after the start of fermentation and were placed in 2 x 50 ml
116 sterile plastic tubes (#430829 Corning Inc., Corning, NY). The cabbage and brine samples were
117 transported to the laboratory by overnight mail in insulated boxes containing wet-ice packs to
118 maintain a temperature between 0 and 5°C. Samples were processed immediately upon arrival.
119 **Chemical and microbial analysis of cabbage samples.** For chemical and
120 microbiological analysis, 100 g of the shredded cabbage was blended in sterile glass blender jars
121 with 200 g of water for 3 min in a Waring blender (Waring Products, Torrington, CT) and then
122 homogenized in a stomacher (Stomacher 400, Tekmar, Cincinnati, OH) for 3 min at the
123 maximum speed using bags containing an internal filter. Filtrate from the extract, approximately
124 30 mL, was transferred to a 50-mL sterile plastic tube and frozen at -20˚C for subsequent
125 chemical analysis. Prior to freezing, one mL of the cabbage extract was removed for
126 microbiological analysis (see below). Chemical analysis was carried out using high-performance
127 liquid chromatography. Organic acids and ethanol were analyzed using an anion-exchange
128 column (Aminex HPX-87H, Bio-Rad Laboratories) with a 0.8 mL/min flow rate of 0.03 N
129 H_2SO_4 at 75˚C. A UV detector (UV-6000, Thermo Separation Products Inc., San Jose, CA) and a
130 differential refractometer (Waters 410, Waters, Milford, MA) were connected in series for
131 detection of organic acids (at 210 nm) and ethanol. Sugars and mannitol were separated by a
132 Carbopac PA1 column (Dionex Corp., Sunnyvale, CA) with a 0.8 mL/min flow rate of 0.12 N
133 NaOH at room temperature, and detected by a pulsed amperometric detector (model PAD-2;

Dionex). The salt (NaCl) content in brine was determined by titration with $AgNO_3$ solution using 4'5'-dichlorofluorescencein as the indicator (13).

For microbial analysis, samples were diluted in sterile saline (0.85% NaCl) and plated on: plate count agar (PCA, Difco Laboratories, Detroit, MI), modified violet red bile agar VRBG (VRB agar, Difco, supplemented with 1% glucose), modified De Man, Rogosa and Sharpe (MMRS) agar (MRS, Difco, supplemented with 0.2% sodium azide), and yeast extract and malt agar (YM, Difco), containing chlortetracycline and chloramphenicol at 250 mg/L each, (Sigma Aldrich, St. Louis, MO), to enumerate total aerobic microbiota, *Enterobacteriaceae*, LAB, yeasts and molds, respectively. In addition, each brine sample was plated on unmodified MRS agar (without sodium azide) for collection of LAB isolates. Plating and plate counting were performed using a spiral plater (Model 4000, Spiral Biotech, Norwood, MA) with an automated colony counter (Protos Plus, Microbiology International, Frederick, MD). From each of the five sampling times (days 1, 3, 5, 7, and 14 after the start of fermentation) in Y1, 96 isolated colonies from MRS agar were randomly selected and isolated on MRS agar, then cells were frozen in MRS broth containing 16% glycerol at –80°C. For the four sampling times in Y2 (days 1, 3, 7, and 14), 20 isolates were similarly obtained from each of three fermentation tanks, resulting in a combined total for both years of the study of 720 possible isolates, although only 686 isolates were recovered. The isolates were screened for gas production using Durham tubes (6 x 50 mm, Kimble) inverted in 5 ml MRS broth. In addition, cells were cultured on homolactic-heterolactic differentiation (HHD) medium (17).

DNA extraction and PCR amplification: MRS broth cultures from each fermentation isolate were incubated at 30°C 12-16 h and then subjected to DNA extraction. Genomic DNA was isolated using a Wizard™ Genomic DNA Purification kit (Promega Corporation, Madison,

WI) in accordance with the manufacturer's instructions, with minor modification. Twenty microliters of mutanolysin (2.4 mg/ml, Sigma-Aldrich) was substituted for lysostaphin. The method of Breidt and Fleming (4) was used to amplify the intergenic transcribed spacer (ITS) region between 16S and 23S rRNA genes. Each 100 µl of reaction mixture consisted of 10 µl of 10X PCR buffer (500mM KCl and 100mM Tris-Cl, pH 8.0), 10 µl of 25 mM $MgCl_2$, 1 µl of dNTP mixture (25mM each dNTP, Stratagene), 4 µl of DNA preparation as described above, 70 µl of water, 1 µl of *Taq* DNA polymerase (5 U/µl), and 2 µl of each primer. The primers used for PCR amplification (4) were GAAGTCGTAACAAGG and GGGTTTCCCCATTCGGA. All primers in this report were obtained from Sigma-Genosys (Sigma-Aldrich, St. Louis, MO). PCR reactions were carried out using a model GTC-2 Genetic Thermal Cycler with a model LTM-2 refrigeration unit (Precision Scientific Inc., Chicago, IL). The temperature program consisted of an initial heat denaturation step at 94°C for 5 min, 25 cycles of 1 min at 94°C, 5 min at 55°C, and 2 min at 72°C followed by a final 5 min interval at 72°C. DNA products from the PCR reaction were treated (without purification) with 1 µl of *Rsa* I enzyme solution (16 U/µl. No. 500890, Stratagene, La Jolla, CA) for 1 h at 37°C. The restriction digest samples were analyzed by electrophoresis in 5% nondenaturing polyacrylamide gels using a vertical gel electrophoresis apparatus (BRL Model V16, Invitrogen, Carlsbad, CA). The DNA-banding profiles were identified by ethidium bromide staining and were subsequently analyzed using the GelCompar II software (Applied Maths, Inc., Austin, TX). For sequencing the 16S rDNA variable regions V1 and V2 (21), the primers (2) used for amplification of the 5' end (approximately 300 bases) of the 16S gene were 5'-AGAGTTTGATCCTGGCTCAG-3'and 5'-GTCTCAGTCCCAATGTGGCC-3'. The cycle times and temperatures were: 10 min at 94˚C; 25 cycles of 1 min at 94˚C, 2 min at 61˚C, and 2 min at 72˚C; and 5 min at 72˚C. Alternatively,

primers 5'-AGTTTGATCMTGGCTCAG-3' (M=A or C), and 5'-AGGAGGTGATCCARCCGCA-3' (R=A or G) were used to amplify the entire 16S rDNA gene (7) substituting an annealing temperature of 55°C. PCR products were purified using a QIAquick® PCR purification kit (Qiagen Inc., Valencia, CA.) and the DNA fragments were sequenced commercially (Davis Sequencing, Davis, CA). Sequences were analyzed by BLAST (Basic Local Alignment Search Tool) search of the NCBI (National Center for Biotechnology Information) non-redundant DNA sequence database (http://www.ncbi.nlm.nih.gov/).

RESULTS

Fermentation microbiology and chemistry: The shredded cabbage used for all fermentations in this study contained between 4 x 10^6 and 6 x 10^6 cfu/g total aerobes, 2 to 3 x 10^6 cfu/g total *Enterobacteriaceae*, and less than10^2 cfu/g yeast and mold. However, the initial LAB populations from the shredded cabbage varied from 10^4 cfu/g in the Y1 cabbage to 10^6 cfu/g from two of the Y2 cabbage samples. It is possible some growth of LAB occurred during transport, prior to analysis. As previously reported (11, 22) , during the first week of each fermentation a rapid increase in total and lactic acid bacteria occurred, as well as a rapid decrease in *Enterobacteriaceae* (data not shown).

Glucose and fructose were the primary fermentable sugars in the cabbage (between 1.5% and 2.2%, respectively). Sucrose made up only a small amount of the fermentable sugars, less than 0.2% of the cabbage by weight, and was not detectable in Y2 samples. Overall, the cabbage used in Y1 contained more sugar than the Y2 samples. However, sugar utilization, acid production, and pH profiles were similar in the commercial tanks from Y1 and Y2. The results from chemical analysis indicated that the fermentation in the four commercial sauerkraut

fermentation tanks from Y1 (Fig. 1) and Y2 (not shown) were normal and consistent with those described by Fleming et. al. (14) and Peterson and Albury (22). During the first week of fermentation lactic acid, acetic acid and mannitol were produced. The pH on day 14 for all fermentations was in the range of 3.4 to 3.7 (data not shown).

ITS-PCR database and 16S rDNA sequence analysis: A database of ITS-PCR gel banding patterns was generated using 64 LAB strains from 42 different species (Suppl. Table 1 and Suppl. Fig. 1), using both the *Rsa*I digested and undigested PCR products. The 686 fermentation isolates obtained from brine during the two year study were grouped by ITS-PCR banding patterns using the GenCompar II software, for both the *Rsa*I digested and undigested PCR products. Banding patterns for the isolates were grouped by unique ITS-PCR banding patterns, and 652 isolates tentatively identified using the ITS-PCR database. One or more representative cultures from each group with unique banding patterns were then further characterized by sequencing the 5' end of the 16S rDNA. The sequence accession numbers, along with the corresponding bacterial strain identification by BLAST analysis, are shown in Suppl. Data Table 2. By this method *L. mesenteroides* was identified as the predominant species (179 of 686 isolates) in the early heterofermentative stage of fermentation and *L. plantarum* was the predominant species involved in the late homofermentative stage (280 of 686 isolates). Together, these two species made up 2/3 of all isolates (67%). *Weissella* sp. and *L. curvatus* were the next most common species among the isolates, with 57 (8.3%) and 40 (5.8%) total isolates from the two year study. The majority of LAB isolates (90% to 100%) taken from days 1 and 3 after the start of fermentation for the Y1 and Y2 samples were heterofermentative species. Homofermentative LAB made up a similar majority of the isolates from day 14 (Figure 2A and 2B).

Microbial diversity in commercial sauerkraut fermentations: Three different bacterial species were found in the Y1, day one samples: *L. mesenteroides, Weissella* sp., and *L. citreum* (Table 2, Figure 3). As expected, *L. mesenteroides* was the predominant organism associated with the fermentation, comprising 88% (84 out of 95) of the isolates from the first day. *L. mesenteroides* was the most frequently isolated species in the brine samples from day 3, but it contributed only 41% (38 out of 93) of total isolates. Other species found on day 3 included: *L. curvatus* (15%), *Weissella* sp. (14%), *L. fallax* (9%) and others (Table 2). Homofermentative *L. plantarum* strains were observed in the fermentation samples from day 3, but only accounted for 9.7% of the isolates. We observed variation in the pattern of hetero- and homofermentative LAB between the Y1 and three Y2 fermentations. Samples from Tank 3 of Y2 were unique when compared to the samples from other tanks, because both heterofermentative and homofermentative LAB were present in all samples.

As expected, bacterial isolates from Y1 days 7, 9 and 14 contained no heterofermentative *L. mesenteroides* or *Weissella* sp. isolates. Overall, the diversity of species was greatest from the day 9 samples from Y1, when 9 different species were isolated, out of a total of 90 isolates. The majority of isolates from the brine samples taken during days 7, 9 and 14, were identified as *L. plantarum* (68.8%, 56.7%, and 78.1%, respectively). *L. argentinum*, which has not previously been found in sauerkraut fermentations, accounted for 10% of total isolates recovered from Y1 day 9. Interestingly, only one isolate of *P. pentosaceus* and 14 isolates of *L. brevis* were found among the 467 total Y1 isolates. Of the 279 Y1 isolates from days 7, 9 and 14 a total of 28 were not identified by ITS-PCR pattern or 16S rDNA sequencing.

Isolates from the three Y2 fermentation tanks had bacterial species profiles as described in Table 3. In tank one, three heterofermentative LAB species were recovered from day one, *L.*

249 *mesenteroides, Weissella* sp., and *L. citreum.* However *Weissella* sp. represented the majority of
250 isolates recovered (12 of 20 in day 1, and 7 of 19 in day 3). *L. curvatus*, *L. fallax*, and *L.*
251 *plantarum* were also recovered from day 3 brine samples. *L. plantarum* was the major LAB
252 species isolated from the Y2 tank one sample from days 7 and 14, representing over 46% and
253 94% of isolates, respectively. The bacterial species from tank two profiles were similar to the
254 tank one fermentation (Table 3). At day 14, *L. plantarum* was the only LAB isolated from the
255 brine sample, accounted for 100% (18 of 18) of the isolates. In tank three, however, 8 different
256 species were identified from 17 isolates from day one. Only 1 *P. pentosaceus* and 1 *L. brevis*
257 isolate was recovered from the 219 total Y2 isolates.
258
259 **DISCUSSION**
260 We used the ITS-PCR database to identify major bacterial species present in commercial
261 sauerkraut fermentations. It is possible that different bacterial species could be present that have
262 very similar or indistinguishable ITS patterns, or that some bacterial species did not grow on
263 MRS medium. Because we only sequenced 16S DNA from isolates with representative ITS-PCR
264 patterns, we may have failed to identify some species that were present among the isolates. The
265 data presented, therefore, may underreport the microbial diversity in the sauerkraut
266 fermentations. Analysis of ITS-PCR patterns and 16S rDNA sequences from the isolates,
267 however, agreed with the biochemical analysis indicating that heterofermentative LAB species
268 dominated the first week of the commercial sauerkraut fermentations, and homofermentative
269 species were predominate in the second week. *Weissella* sp. were recovered together with *L.*
270 *mesenteroides* both in day one and three. We could not identify species of any *Weissella* sp.
271 isolates by 16S rDNA sequence comparisons with the GenBank database because of the

sequence similarity of *Weissella confusa, Weissella cibraria,* and *Weissella kimchii*. *Weissella* species have previously been recovered from plant products (5, 8, 18), and have very similar 16S rDNA sequences (3, 5). Complete sequencing of the 16S genes from selected *Weissella* isolates did not allow conclusive identification with known species (data not shown). *Weissella* sp. were previously characterized as members of the *Leuconostoc* genus (6). There was variation between the three Y2 fermentations in microbiota from the heterolactic (days one and three) stage of fermentation, which is believed to be vital to the quality of sauerkraut. This variation may be due in part to the small sampling size (20 isolates from each sampling time, and from each fermentation in Y2) but could represent differences in microbiota. These data indicate that the use of *L. mesenteroides* starter cultures may help improve consistency in flavor development in commercial sauerkraut.

During the transition period between the heterofermentation and homofermentation phases of fermentation (days three to nine), a variety of LAB species including *L. curvatus* and *Leuconostoc argentinum* were isolated. *L. argentinum* was originally isolated from raw milk in Argentina (9). *L. plantarum* became the dominant microorganism after seven days into the fermentation when the pH decreased to at least 3.9 or lower in all 4 fermentations. However, we recovered relatively few *L. brevis* (15 total isolates of 686) and only twoisolates of *P. pentosaceus.* This was surprising because the previous research has shown that both were considered to be major bacterial species involved in sauerkraut fermentation (22).

This study and a related study of the bacteriophage ecology of commercial sauerkraut fermentations (15), has significantly revised the classical understanding (11, 22) of the microbiota present in sauerkraut fermentations. Several LABs isolated from the two-year study had never been previously recovered from sauerkraut fermentation including *Weissella* sp., *L.*

295 *argentinum, Lactobacillus coryniformis, Leuconostoc citreum, Lactobacillus paraplantaum,* and
296 *Lactobacillus paracasei.* This study, and a recent study of the comparative genomics of lactic
297 acid bacteria, including *L. mesenteroides* (16), may also aid in the development of starter
298 cultures, and increase our understanding of cabbage fermentation biochemistry and ecology.
299
300

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FIGURE LEGENDS

Figure 1. Biochemistry of the Y1 fermentation. Changes in the concentrations of acids and sugars during the first 14 days after the start of the Y1 fermentation are shown: lactic acid (circles); acetic acid (diamonds); mannitol (triangle up); glucose (squares); fructose (triangle down).

Figure 2. Changes in gas production microrganisms during commercial sauerkraut fermenations. The percent of total isolates that were heterofermentative (A) or homofermentative (B) at each day (1, 3, 7, and 14) for each of the four commercial fermentations sampled (represented by the shaded bars) are shown.

Figure 3. The microbial diversity of the Y1 fermentation as determined by ITS-PCR and 16S rDNA sequencing. For the total number of isolates on days 1, 3, 7, and 14, (95, 93, 93, and 90, respectively), the percent represented by each species is indicated by the bars.

Figure 4. The microbial diversity of the Y2 fermentation as determined by ITS-PCR and 16S rDNA sequencing. For the total number of isolates on days 1, 3, 7, and 14, (95, 93, 93, and 90, respectively), the percent represented by each species is indicated by the bars. Results from tank 1 (A), tank two (B), and tank three (C) are shown.

Table 1. Biochemistry of cabbage used in sauerkraut fermentations

	Glucose		Fructose		Sucrose		Malic acid	
Year/fermentation	mM	%	mM	%	mM	%	mM	%
1/1	119.2	2.15	90.8	1.64	4.8	0.17	5.6	0.08
2/1	92.4	1.66	81.7	1.47	ND[b]	ND	3.9	0.05
2/2	90.8	1.63	83.5	1.50	ND	ND	3.5	0.05
2/3	81.7	1.47	73.8	1.33	ND	ND	4.7	0.06

[a]mM = millimolar, percentage by weight.
[b]ND = not detected.

427

428

Table 2: Identification of Y1 Isolates

Species	Day 1	Day 3	Day 7	Day 9	Day 14
L. mesenteroides	84	38			
Weissella sp.	7	20			
L. citrium	4	1			
L. curvatus		14	14	3	
L. fallax		8	1	1	
L. plantarum		9	64	51	75
L. brevis		3	3	3	5
L. coryniformis			5	1	
L. argentinum			1	11	
P. pentosaceus			1		
L. paraplantarum			3	6	3
NID*			1	14	13
Samples analyzed**	95	93	93	90	96

* NID = Not identified

**Total number of samples analyzed

429

430

431

Table 3. Identification of Y2 isolates

Tank 1

Species	Day 1	Day 3	Day 7	Day 14
L. mesenteroides	6	2	2	
Weissella sp.	12	7		
L. citrium	2			
L. curvatus		5	3	
L. fallax		1	1	
L. plantarum		3	7	16
P. pentosaceus		1		
L. paraplantarum			1	1
ND*			1	
Samples analyzed**	20	19	15	17

Tank 2

Species	Day 1	Day 3	Day 7	Day 14
L. mesenteroides	12	16	7	
Weissella sp.	2			
L. citrium			1	
L. fallax		2	1	
L. plantarum	1		9	18
L. lactis		1		
L. argentinum	4			
L. mali			1	
Samples analyzed	19	19	19	18

Tank 3

Species	Day 1	Day 3	Day 7	Day 14
L. mesenteroides	1	9	2	
Weissella sp.	1	7	1	
L. citrium	4	1		
L. curvatus			1	
L. fallax	2	2	4	
L. plantarum	1		10	16
L. brevis				1
L. argentinum	1			
L. paracasei	4			
NID	3	1		1
Samples analyzed	17	20	18	18

* NID = Not identified
** Total number of samples analyzed

432
433

Figures for Eddie paper 1

Figure 1

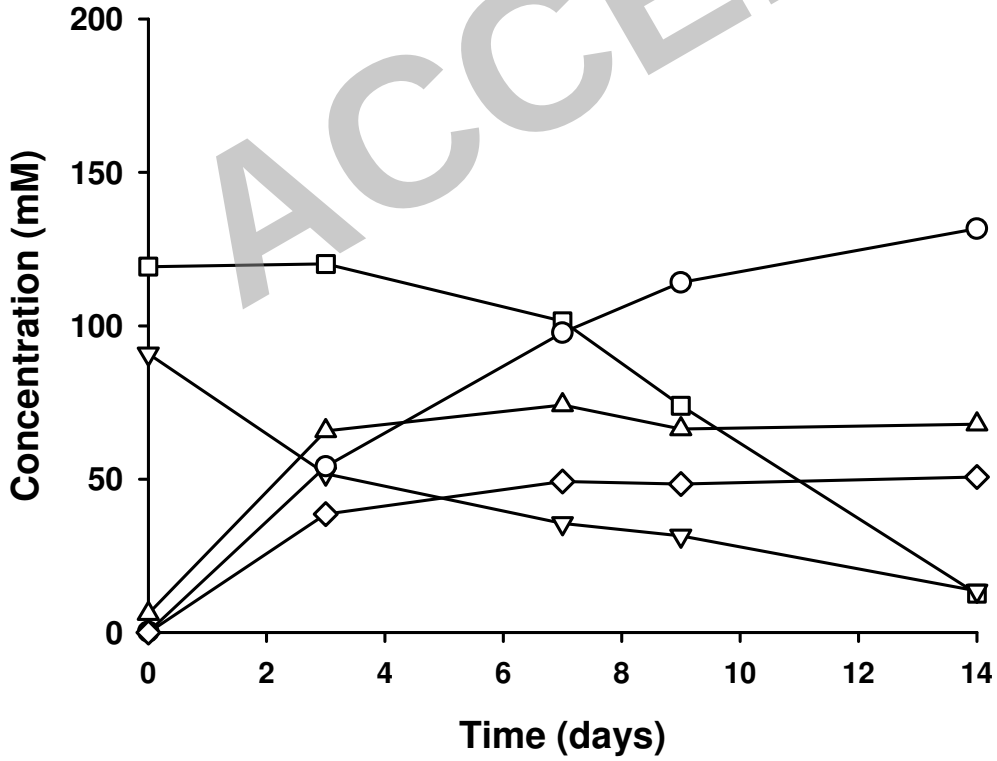

Figure 2

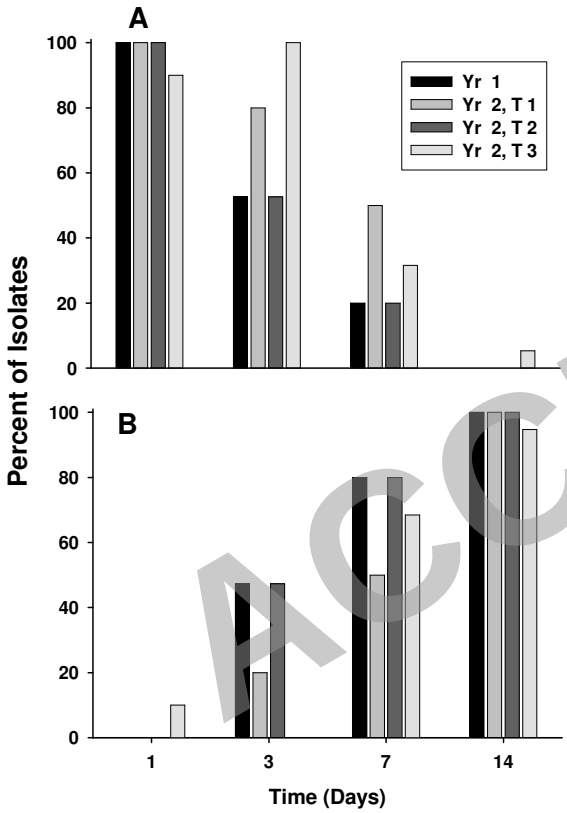

Figure 3

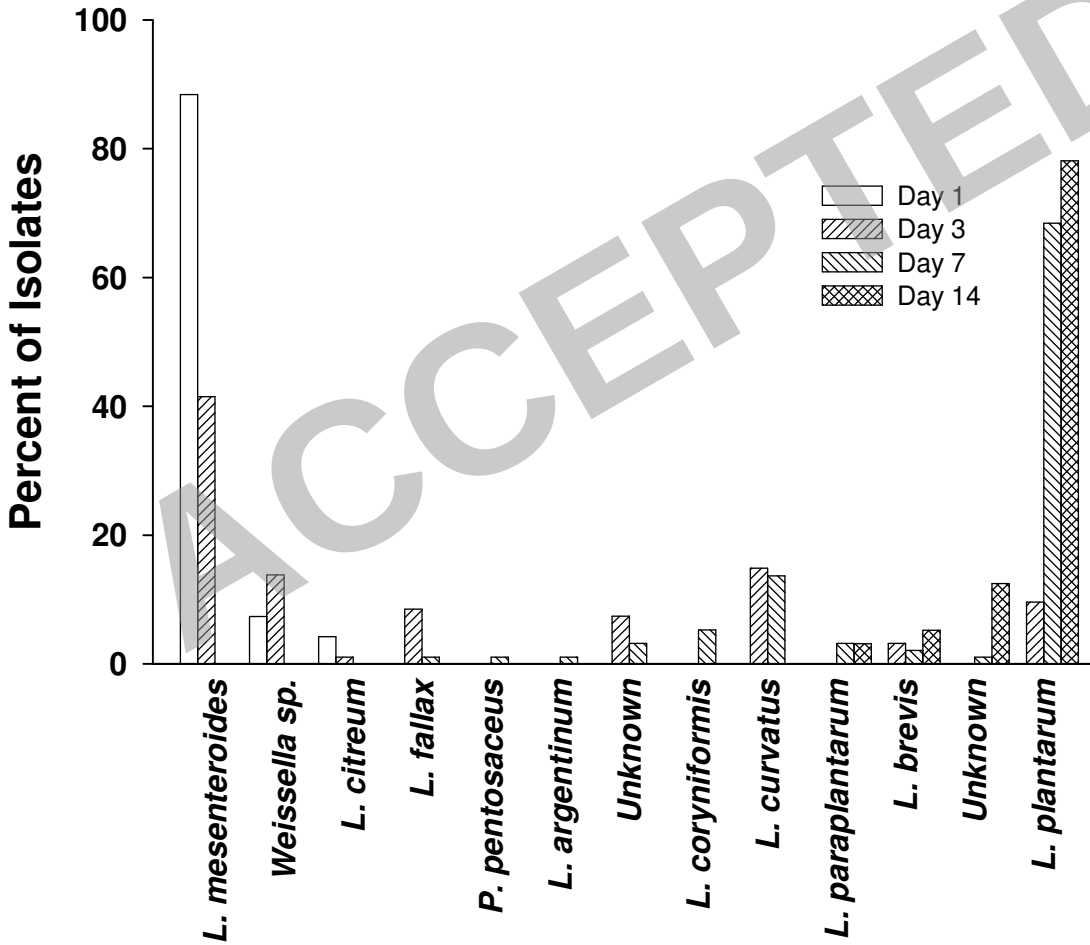

Figure 4