Lactic Acid Bacteria–Friend or Foe? Lactic Acid Bacteria in the Production of Polysaccharides and Fuel Ethanol

Presented in the 3rd International Conference for Value Added Agricultural Products (3rd FerVAAP Conference)

Joseph O. Rich[1], Kenneth M. Bischoff[1], Timothy D. Leathers[1], Gregory L. Côté[1] and Siqing Liu[1]*

Abstract

Lactic acid bacteria (LAB) are generally considered to be beneficial microorganisms, as they have been widely used in the production of fermented foods and as probiotics. In this article we offer examples of research from our own laboratory that contrast the roles of LAB as either useful or undesirable agents. Specifically, we discuss the production of a valuable polysaccharide by *Leuconostoc mesenteroides* and the role of *Lactobacillus* sp. as contaminants of fuel ethanol production. Alternan is a glucan with a distinctive backbone structure of alternating α-(1,6) and α-(1,3) linkages produced by the LAB *L. mesenteroides.* In recent years, we have developed improved strains for production of alternan and novel derivatives, including a lower molecular weight form that more closely mimics gum arabic. Alternan oligosaccharides were produced using enzyme-catalyzed acceptor reactions and have potential as second generation prebiotics. This alternan technology led to the development of sucromalt, a low-glycemic index sweetener produced by Cargill that appears in the Glucerna line of diabetic foods. More recently, LAB have been investigated as microbial catalysts for production of fuels and chemicals from lignocellulosic biomass. LAB are well suited for ethanol production due to their ability to utilize a range of mixed substrates and their high tolerance to environmental ethanol concentrations. Nevertheless, these same characteristics also make LAB prolific contaminants of fuel ethanol fermentations, creating a costly problem for the ethanol industry. We have isolated bacterial contaminants from a variety of US fuel ethanol production facilities and determined their antibiotic susceptibility. These strains were used to develop a reliable "stuck" fermentation model that will be useful in developing effective intervention strategies to control bacterial contamination in commercial fermentation cultures.

Keywords: *biofuels*, Leuconostoc, *polysaccharides*, Lactobacillus

[1] *US Department of Agriculture, Agricultural Research Service, National Center for Agricultural Utilization Research, Bioproducts and Biocatalysis Research, 1815 N. University St, Peoria, IL 61604 USA*
**Corresponding author, e-mail: joseph.rich@ars.usda.gov*

Introduction

Lactic acid bacteria are Gram-positive bacteria that have found widespread use in food fermentation and preservation (Scott and Sullivan, 2008), as well as probiotics that promote human health (Soomro et al., 2002). These bacteria produce lactic acid as the primary fermentation end product, as well as other carboxylic acids, ethanol, bacteriocins, and carbohydrate-based biopolymers (Kandler, 1983; Kumar et al., 2007; Pfeiler and Klaenhammer, 2007; Makarova et al., 2006). Foods from many cultures, including Thailand, are produced using these microorganisms, including various dairy products (cheeses, yogurt, and kefir), sourdough, sauerkraut and kimchi, sausages, vinegar, pickles (*phak-sian-dong*), and fish sauce (*nam-pla*).

Microbial polysaccharides are well known and the subject of many reviews (Kumar et al., 2007; Behare et al., 2009). There have been many successful commercial applications of these polysaccharides, including food (xanthan, pullulan), industrial products (xanthan), cosmetics (hyaluronic acid), and pharmaceutical (dextran, heparin). Dextran (Leathers, 2002) and alternan (Cote, 2002) are related polysaccharides that are produced by selected LAB strains. Dextrans are α-D-glucans produced by LAB, particularly *Streptococcus* sp. and *L. mesenteroides.* They feature a substantial number of consecutive α-(1➔6) linkages (typically more than 50%) and contain sidechains that branch from α-(1➔2), α-(1➔3), or α-(1➔4) linkages. Alternan, however, is produced from sucrose by alternansucrase from *L. mesenteroides* and contains D-glucopyranosyl units linked by alternating α-(1➔6) and α-(1➔3) linkages (Figure 1).

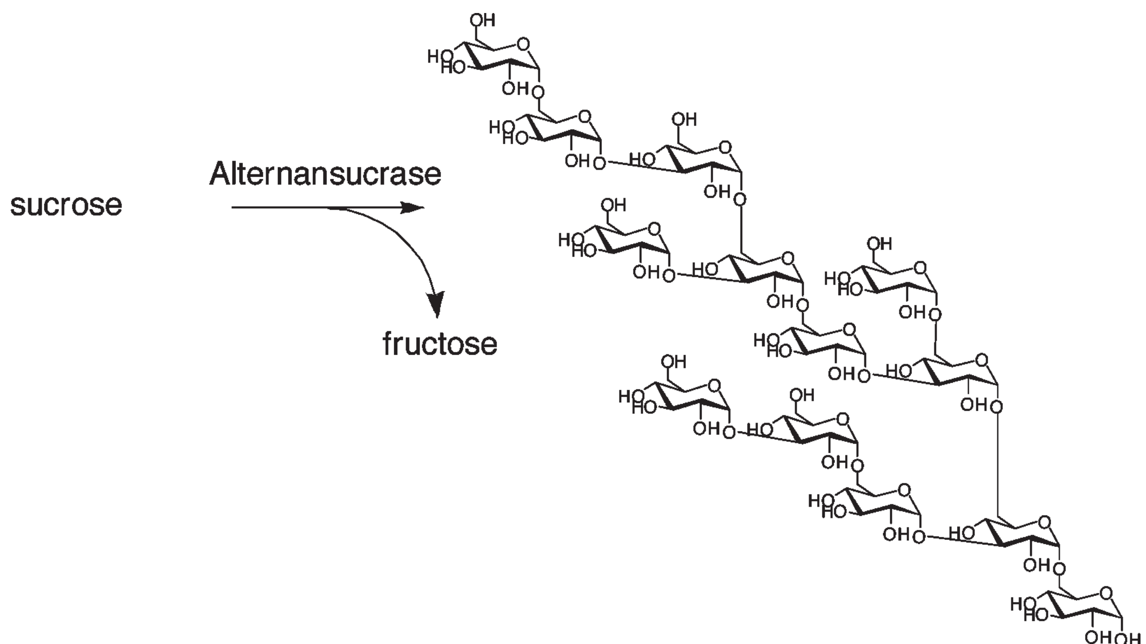

Figure 1. Production of Alternan from sucrose by *Leuconostoc mesenteroides*

While there are clearly many positive uses for LAB, these organisms have also been found to be problematic in the ethanol fermentation industry. LAB have been long linked to incomplete alcoholic fermentations more commonly known as "stuck" fermentations in the winemaking industry (Bisson, 1999). Recently, LAB have also been shown to significantly contaminate fuel ethanol fermentations (Skinner and Leathers, 2004). The US fuel ethanol industry uses antibiotics, including virginiamycin and penicillin, to minimize the impact of the contamination on productivity (Bischoff et al., 2007). These antibiotics result in increased resistance to treatment (Bischoff et al., 2007) and may be present in the primary by-product, dried distillers grains, that are used as animal feed (Basaraba et al., 1999, Benjamin, 2008, McChesney, 2007).

In this work we illustrate the dual nature of LAB as either beneficial or troublesome organisms. Two specific examples from our own research are offered. These are the role of the LAB *Leuconostoc mesenteroides* in the production of the valuable polysaccharide, alternan, and the role of *Lactobacillus* sp. as contaminants of fuel ethanol production.

Materials and Methods

1. Alternan Modification

Polysaccharides and enzymes

Native alternan was produced as previously described from *Leuconostoc mesenteroides* strain NRRL B-21297 (Leathers et al., 1995, Leathers et al., 1997a, Leathers et al., 1997b). Bioconversion-modified alternan was produced using novel strains of *Penicillium* sp. as previously described (Leathers et al., 2002a, Leathers et al., 2002b). Specifically, a basal liquid medium ("WW") of Koenig and Day (1989) containing 1% (w/v) native alternan was inoculated to 10^5 spores/ml and incubated at 28°C and 200 rpm for 7 days. Commercial dextranases were from *Penicillium* sp. (Sigma) or from *Chaetomium erraticum* (Bio-Cat Inc., Troy, VA).

Analytical methods

The absorbance of appropriate dilutions of alternan solutions was monitored at 225 nm as a measure of alternan modification (Leathers et al., 2002b). Polysaccharide molecular weight distributions were analyzed by size exclusion chromatography using a Shodex SB-806 M HQ column (Showa Denko, Tokyo, Japan) eluted with 0.05 M sodium nitrate at room temperature at 0.5 ml/min. Separations were monitored using a Shodex OR-1 optical rotation detector (Showa Denko K.K.). Dextranase activity was measured using a reducing sugar assay with 1 U representing the release of 1 μmol isomaltose (measured as maltose) per min. Solution viscosities were measured as previously described (Cote, 1992), using a Brookfield LVTDV-1 digital viscometer. Methylation analysis was carried out using the method of Ciucanu and Kerek (1984) in which hydrolysis was accomplished using 2 M trifluoroacetic acid. The permethylated derivatives were analyzed by capillary GC-MS as the peracetylated aldononitrile (PAAN) derivatives (Seymour et al., 1975).

2. Bacterial Contamination of Fuel Ethanol Fermentation

Strains and Media

For the growth of *S. cerevisiae*, YP/glucose media contained the following per liter: 10 g yeast extract, 20 g peptone, and 170 g glucose. Difco Lactobacilli MRS Broth base was manufactured by Becton, Dickinson, and Co. (Sparks, MD). *S. cerevisiae* strain NRRL Y-2034 was obtained from the ARS Culture Collection maintained at the USDA-ARS National Center for Agricultural Utilization

Research (Peoria, IL) (McGhee et al., 1982). *Lactobacillus delbrueckii* ATCC 4797 was obtained from the American Type Culture Collection (Manassas, VA). It is not an ethanol plant isolate, but was used to compare the response of the ethanol plant strains to a non-adapted strain. *L. fermentum* strains 0315-1, 0315-8, 0315-11, and 0315-25, *L. brevis* strain 84, *L. amylovorus* 0315-7B, and *Weisella confusa* 0216-1 were isolated from fermentors at commercial dry-grind ethanol facilities located within the Midwestern United States. The isolation procedure for planktonic and biofilm isolates was performed as described previously (Skinner and Leathers, 2004; Skinner-Nemec et al., 2007). The strains were identified by sequencing of 16s rRNA genes. Amplicons were generated by polymerase chain reaction using the following primer set: U1 (CCAGCAGCCGCGGTAATACG) and U2 (ATCGGYTACCTTGTTACGACTTC) (Lu et al., 2000). The resulting product (about 1,000 bp) was purified using a Qiagen (Valencia, CA) PCR purification kit and one strand was sequenced by standard methods with the U1 primer. The sequences obtained were compared with those in the GenBank database by using the BLASTN program (Altschul et al., 1997) available at the National Center for Biotechnology Information (available at http://www.ncbi.nlm.nih.gov). More than 98% identity to a known species was considered a positive match.

Shake-Flask Fermentation

Stock cultures of *S. cerevisiae* were grown in YP media supplemented with 5% (w/v) glucose at 32°C and 200 rpm. Stock cultures of lactic acid bacteria were grown in static cultures of MRS media at 37°C. Cells were harvested by centrifugation, and inocula prepared by resuspending cells in sterile saline solution to a density of OD_{600} equivalent to 80 and OD_{600} equivalent to 8.0 for *S. cerevisiae* and bacteria, respectively. One OD_{600} of *S. cerevisiae* corresponds to ca. $6x10^7$ CFU/mL, and one OD_{600} of *Lactobacillus* sp. is equivalent to ca. $1x10^8$ CFU/mL.

Corn mash (ca. 33% solids) was obtained from a commercial dry-grind ethanol facility and stored at -20°C. The facility uses ca. 50% backset in the mash, and the pH was adjusted within the range of 5.0-5.2 with sulfuric acid. The mash was collected after the cooking process with a hydro heater temperature of ca. 107°C. The liquefaction dextrose equivalency range was 8-12, and the initial concentrations of glucose, acetic acid, and lactic acid were 7.1, 0.36, and 0.46 g/L, respectively. Direct plating of mash samples on MRS agar did not detect transient bacteria in the mash ($<10^2$ CFU/mL).

Mash (40 μL) was dispensed in a 50 mL Erlenmeyer flask, and supplemented with ammonium sulfate (0.12%, w/v) and glucoamylase (20 μL of Optidex L-400; Genencor International Inc., Rochester, NY). The culture was inoculated with 0.5 mL of *S. cerevisiae* inoculum, and when indicated, challenged with 0.5 mL of bacterial inoculum. Enzyme, yeast, and when indicated, bacteria and virginiamycin were added sequentially at time 0. Flasks were capped with a rubber stopper and a 20-gauge needle inserted between the stopper and flask to allow for venting of CO_2. Cultures were incubated at 32°C with gentle shaking (100 rpm) for 72 h.

Analytical Methods

Bacterial density was enumerated by colony counting on MRS agar media containing cycloheximide (10 μg/mL). Concentrations of ethanol, glucose, lactic acid, and acetic acid were determined by high-performance liquid chromatography using a 300 mm Aminex HPX 87H column (Bio-Rad Laboratories, Inc., Hercules, CA) on a HP 1100 Series HPLC system equipped with a refractive index detector (Agilent Technologies, Santa Clara, CA).

Samples (10 μL) were injected onto a heated column (65°C) and eluted at 0.6 mL/min using 5 mM H_2SO_4 as mobile phase. Concentrations are reported as mean values (±standard deviation) of at least triplicate cultures. Statistical comparisons of challenged and control cultures were performed using Student's t-test (P<0.05).

Results and Discussion

1. Alternan Modification

While the biosynthesis and characterization of alternan-producing strains of *L. mesenteroides* has been described (Cote, 2002), further modification of the polysaccharide could expand the potential uses of this biopolymer (Leathers et al., 2009). We have previously demonstrated that strains of *Penicillium* sp. that modify alternan produce no alternanase activity (Leathers et al., 2002b). Interestingly, culture supernatants of *Penicillium* sp. strain NRRL 21969 that were germinated on alternan did contain dextranase (Table 1). In fact, dextranase was produced more quickly and in higher yields on alternan than in cultures grown on dextran, despite the fact that growth on alternan was limited (Table 1).

Table 1. Growth and dextranase production by *Penicillium* sp. strain NRRL 21969[a]

	Dextranase (IU/ml)		Dry weight (mg/ml)
Carbon source	**4d culture**	**7d culture**	**7d culture**
None	<0.1	<0.1	<0.1
Glucose	<0.1	<0.1	8.8 ± 1.9
Dextran	<0.1	0.6 ± 0.1	3.5 ± 0.2
Alternan	0.7 ± 0.1	1.5 ± 0.2	1.5 ± 0.1

[a] Data are from Leathers et al., 2009

To determine whether dextranase is directly related to the modification of alternan, we treated alternan with commercial dextranase from *Penicillium* sp. under conditions identical to those of the bioconversion process. The extent of alternan modification is conveniently followed over time by monitoring the A_{225} of solutions (Leathers et al., 2002a; Leathers et al., 2003). As shown in Figure 2, dextranase added at 5 U/ml or greater brought about a rapid reduction in A_{225} to approximately 1. This modification by dextranase is equivalent to the maximal amount of modification observed after 17 days in the bioconversion process (Leathers et al., 2002b). No further modification was observed with longer digestion times or higher amounts of enzyme, suggesting that this reaction is complete.

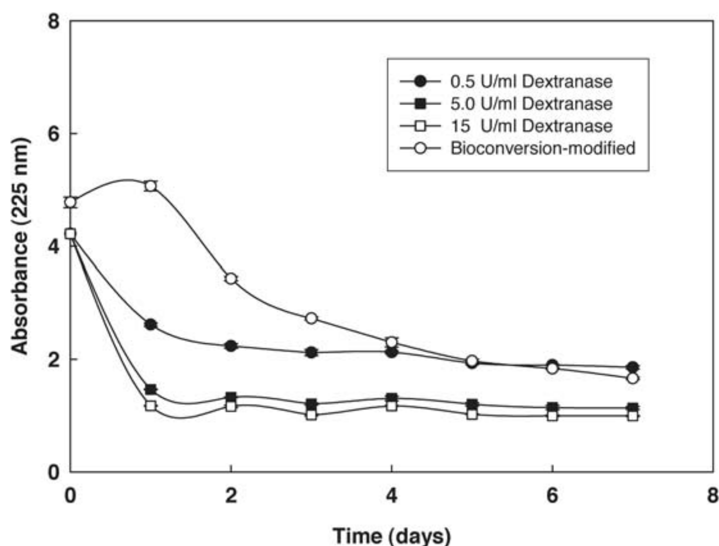

Figure 2. Time course of dextranase-catalyzed modification of alternan. Data are from Leathers et al.(2009)

The molecular weight distribution of native and modified alternan was analyzed by high pressure liquid size exclusion chromatography (HPLC SEC). As previously described, high molecular weight native alternan of approximately 10^6-10^7 Da is modified by bioconversion to a characteristic, lower molecular weight, heterodisperse form with peaks at approximately 5-10 x 10^5 Da and 1-5 x 10^4 Da (Leathers et al., 2002b). In vitro treatment with dextranase results in a nearly identical product (Figure 3). Dextranase-modified alternan also includes a minor low molecular weight peak eluting at about 20 min that consists of free glucose and maltose or isomaltose. Presumably, these sugars are consumed in the bioconversion process in support of the limited spore germination.

Dextranase from *C. erraticum* was equivalent to commercial enzyme from *Penicillium* sp. for modification of alternan (Leathers et al., 2009). Since this enzyme is considered generally regarded as safe (GRAS), it may be useful in the modification of alternan for potential food uses.

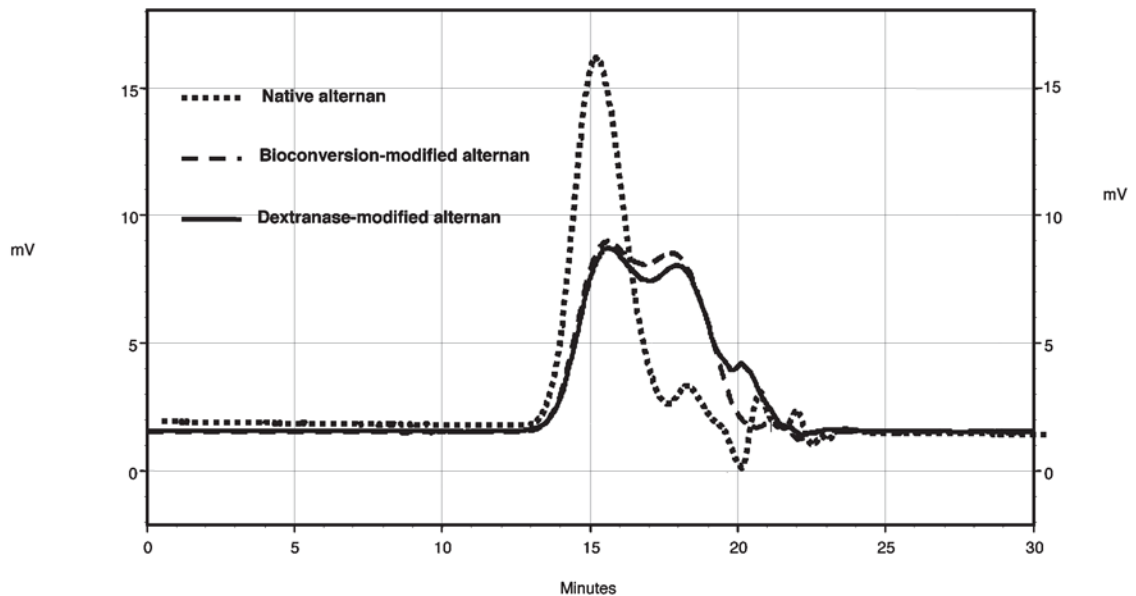

Figure 3. Molecular weight distributions of native alternan, bioconversion-modified alternan, and dextranase-modified alternan, determined by HPLC size exclusion chromatography (HPSEC). Data are from Leathers et al.(2009)

The action of endodextranase on alternan may be analogous to the limited susceptibility of pullulan to amylase. Pullulan is generally resistant to α-amylase because of its regular structure of α-(1➔6) linked maltotriose subunits, as maltotriose is not a substrate for this enzyme. However, pullulan contains a minor percentage of maltotetraose subunits that are substrates for α-amylase (Catley, 1970; Catley and Whelan, 1971). The action of amylases reduces the molecular weight of pullulan in late cultures. In nature, such limited hydrolysis of extracellular polysaccharides may serve to reduce viscosity in response to environmental conditions, or perhaps to facilitate the detachment of microcolonies during the propagation of biofilms. The use of LAB to produce polysaccharides is well known and they have expressed enzymes that have been useful in making commercial food ingredients. While LAB and their associated products have found widespread use in many food applications, these robust bacteria are problematic for the fermentative production of fuel ethanol.

2. Bacterial Contamination of Fuel Ethanol Fermentation

Bacterial contamination is a chronic problem in commercial fermentation cultures, particularly in fuel ethanol fermentations, which are not performed under sterile, pure-culture conditions (Connolly, 1997). Bacterial infections pose a constant drain on the sugar available for conversion to ethanol, and the bacteria scavenge essential micronutrients required for optimal yeast growth and ethanol production. Acute infections occur unpredictably, and bacterial byproducts such as acetic and lactic acids inhibit yeast growth and may result in “stuck” fermentations

that require costly shut-downs of facilities for cleaning (Makanjuola et al., 1992; Narendranath et al., 1997). Despite efforts to prevent contamination with extensive cleaning and disinfecting procedures, saccharification tanks, continuous yeast propagation systems, and notoriously drug-resistant biofilms can act as reservoirs of bacteria that continually reintroduce contaminants (Skinner and Leathers, 2004; Skinner-Nemec et al., 2007).

Insoluble matter and protein in corn mash increases its buffering capacity. Using exogenously added acids, Abbott and Ingledew (2004) concluded that the buffering capacity of whole corn mash mitigates the inhibitory effects of lactic and acetic acids on *S. cerevisiae* (Abbott and Ingledew, 2004). In our model, however, the deleterious effect caused by experimentally infected contaminants was much greater with saccharified corn mash as feedstock than with glucose. Ethanol yields were reduced by 27% and residual glucose increased from 13.6 g/l to 61.7 g/l at 48 h in control and challenged cultures, respectively (Table 2). Lactic and acetic acid concentrations in challenged cultures increased to 6.9 g/l and 3.3 g/l, respectively, and the final pH dropped from 4.5 in control cultures to 4.0 in experimentally infected ones. This suggests that with the strains used here, the mash's buffering capacity was not sufficient to encumber the effects of the acids. A synergistic inhibitory effect on yeast-catalyzed fermentations has previously been observed when both lactic and acetic acids are present (Narendranath et al., 2001), which may explain the response difference observed in the present study between the glucose and corn mash feedstocks. It is also possible that lactic acid bacteria outcompete yeast by effectively scavenging the limited micronutrients available in corn mash. It should be noted that final bacterial load in the corn mash fermentations were 10-fold higher than in the glucose fermentations ($3.8 \pm 1.5 \times 10^9$ CFU/ml versus $1.5 \pm 0.5 \times 10^8$ CFU/ml). Thus, the corn mash provided a more hospitable environment for the lactic acid bacteria relative to *S. cerevisiae*, allowing the contaminants to effectively compete with the yeast.

Table 2. Challenge of *S. cerevisiae* fermentations with *L. fermentum* [a]

Feedstock	Challenge	Ethanol, g/l	Glucose, g/l	Lactic Acid, g/l	Acetic Acid, g/l
YP/glucose	-	75.7 ± 0.353	0.396 ± 0.324	0.129 ± 0.005	0.459 ± 0.037
	+	74.1 ± 0.477	0.501 ± 0.324	2.79 ± 0.135	1.15 ± 0.032
Corn mash	-	112 ± 2.00	6.2 ± 1.3	1.0 ± 0.1	0.8 ± 0.1
	+	81.8 ± 2.40	45.5 ± 1.18	5.2 ± 0.1	3.1 ± 0.1

[a]Initial density of *S. cerevisiae* was approximately 10^7 CFU/mL. Cultures were challenged with the following *L. fermentum* strains each at a density of 10^7 CFU/ml: 0315-1, 0315-8 and 0315-11. Data are reported for 48 h fermentations (YP/glucose) or 72 h fermentations (corn mash). Data are mean values ± standard deviation from three replicate cultures. Data are from Bischoff et al., (2009).

Based on a previous survey of bacterial contaminants of corn-based fuel ethanol facilities in the United States that found the bacterial load in dry-grind plants could reach 10^8 CFU/mL (Skinner and Leathers, 2004), we initially challenged the shake-flask cultures with 10^8 CFU/mL *L. fermentum.* In yeast-catalyzed fermentations of wheat mash in bioreactors, Narendranath et al. (1997) reported that challenging with up to 10^9 CFU/mL lactobacilli (corresponding to a 1000:1 ratio of lactobacilli to yeast) resulted in as much as a 7.6% decrease in final ethanol concentration. In the present study, we challenged the corn mash fermentations with varying concentrations of *L. fermentum* 0315-1 ranging from 10^4 to 10^8 CFU/ml, which corresponds to lactobacilli:yeast ratios of 1:1000 to 10:1. Final ethanol decreased and residual glucose increased as the inoculation of *L. fermentum* increased (Figure 4). The final ethanol concentration was 17% lower with 10^8 CFU/ml *L. fermentum* inoculum, but even the 10^5 CFU/mL challenge decreased ethanol by 8%. There were no significant differences ($P > 0.05$) in final ethanol and residual glucose between the 10^4 CFU/mL challenge and unchallenged controls. Narendranath and Power (2004) concluded that increasing the yeast inoculation rate reduces the growth of contaminating lactic acid bacteria, with a rate of 3 x 10^7 cells/mL able to outcompete a 10-fold increase in bacterial inoculation. Thus, the effect of lactobacilli contamination observed in the present study is more pronounced than that reported previously (Narendranath et al., 1997; Narendranath and Power, 2004). While previous studies used strains of *L. plantarum* and *L. paracasei* to challenge corn mash fermentations in bioreactors, the isolates used here may be more potent antagonists of yeast-catalyzed fermentation.

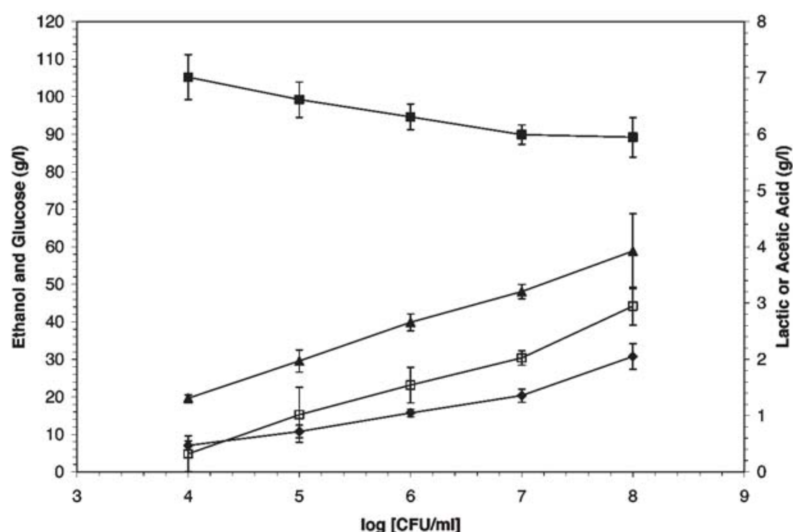

Figure 4. Dose-response on ethanol fermentation by varying inoculum of *L. fermentum* 0315-1. Cultures of *S. cerevisisae* grown on corn mash feedstock were challenged with the indicated inoculum of *L. fermentum* 0315-1. The following products were measured after 72 hr incubation: ethanol (■), residual glucose (□), lactic acid (▲), acetic acid (◆). Data are reported as mean values ± standard deviation of triplicate cultures. Final concentrations for the control culture (no bacterial challenge) were as follows: ethanol = 108 ± 3 g/l, glucose = 4.8 ± 3.6 g/l, lactic acid = 0.63 ± 0.01 g/l, acetic acid = 0.31 ± 0.04 g/L. Data are from Bischoff et al., (2009).

We have demonstrated the ability to produce infections of corn mash in shake-flask cultures using selected strains of lactic acid bacteria. The magnitudes of ethanol loss and residual glucose were dependent on the infecting strain and on the initial bacterial load. The simplicity of the model allows for miniaturization into multi-well plate formats, and given the dynamic range of the residual glucose response and the availability of commercial devices for rapid glucose monitoring, it may be developed for high-throughput screening processes to test new antibacterial agents.

Currently the treatment for contamination of commercial ethanol production is often prophylactic, necessitating the addition of antibiotics to each fermentor. Thus control and treatment of bacterial contamination is a critical control point for decreasing costs and improving efficiency of both starch and cellulosic ethanol production. New antibacterial agents and new drug management methods will need to be developed to effectively control bacterial infections.

Finally, it is clear that LAB are both "friend and foe" depending upon your perspective. They can be successfully used in the synthesis of a variety of healthful foods and useful commercial products, including polysaccharides. We have shown that a novel modified alternan, previously produced by a bioconversion process, was produced *in vitro* using commercial dextranases, including a GRAS enzyme. On the other hand, LAB remain a chronic problem for the fuel ethanol fermentation industry and successful control of their proliferation remains an unresolved challenge. We have also demonstrated a reliable model for bacterial contamination in fuel ethanol fermentation that will help develop a fundamental understanding of acute and chronic bacterial contamination events.

Acknowledgements

We would like to thank Eric Hoecker, Imran Khan, Melinda Nunnally, Suzanne Unser, and Jacqueline Zane for excellent technical assistance. We also acknowledge Drs. Ting Carlson and Anton Woo for work with alternan and Prof. Ron Worthington for his collaboration on bacterial contamination of fuel ethanol fermentations.

References

Abbott, D.A. & Ingledew, W.M. 2004. Buffering capacity of whole corn mash alters concentrations of organic acids required to inhibit growth of Saccharomyces cerevisiae and ethanol production. **Biotechnology Letters** 26: 1313-1316.

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D.J. 1997. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. **Nucleic Acids Research** 25: 3389-3402.

Basaraba, R.J., Oehme, F.W., Vorhies, M.W. & Stokka, G.L.1999. Toxicosis in cattle from concurrent feeding of monensin and dried distiller's grains contaminated with macrolide antibiotics. **Journal of Veterinary Diagnostic Investigation** 11: 79-86.

Behare, P. V., Singh, R., Kumar, M., Prajapati, J. B. & Singh, R. P. 2009. Exopolysaccharides of lactic acid bacteria: A review. **Journal of Food Science and Technology** 46: 1-11.

Benjamin, L. A. 2008. *Overview: biofuel co-products.* Lake Region State College. [Online].[Cite 17 July 2009]Available from: http://www.lrsc.nodak.edu/upload/resources/838/

benjamin-lrst-talk.pdf.

Bischoff, K.M., Liu, S., Leathers, T.D., Worthington, R.E., Rich, J.O.2009. Modeling bacterial contamination of fuel ethanol fermentation. **Biotechnology and Bioengineering** 103: 117-122.

Bischoff, K.M., Skinner-Nemec, K.A. & Leathers, T.D. 2007. Antimicrobial susceptibility of Lactobacillus species isolated from commercial ethanol plants. **Journal of Industrial Microbiology and Biotechnology** 34: 739-744.

Bisson, L.F. 1999. Stuck and sluggish fermentations. American Journal of Enology and Viticulture 50: 107-119.

Catley, B.J. 1970. Pullulan, a relationship between molecular weight and fine structure. **FEBS Letters** 10: 190-193.

Catley, B. J. & Whelan, W. Y. 1971. Observations on the structure of pullulan. **Archives of Biochemistry and Biophysics** 143: 138-142.

Ciucanu, I. & Kerek, F. 1984. A simple and rapid method for the permethylation of carbohydrates. **Carbohydrate Research** 131: 209-217.

Connolly, C. 1997. Bacterial contaminants and their effects on alcohol production. *In:* JACQUES, K.A., LYONS, T.P. & KELSALL, D.R. (eds.) **The Alcohol Textbook.** 3rd. ed. Nottingham, UK: Nottingham University Press.

Cote, G.L. 1992. Low-viscosity a-D-glucan fractions derived from sucrose which are resistant to enzymatic digestion. **Carbohydrate Polymers** 19: 249-252.

Cote, G.L. 2002. Alternan. *In:* VANDAMME, E.J., DEBAETS, S. & STEINBUCHEL, A. (eds.) **Biopolymers, Vol. 5. Polysaccharides I: Polysaccharides from Prokaryotes.** Weinheim: Wiley-VCH.

Kandler, O. 1983. Carbohydrate metabolism in lactic acid bacteria. **Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology** 49: 209-224.

Koenig, D.W. & Day, D.F. 1989. Induction of *Lipomyces starkeyi* dextranase. **Applied and Environmental Microbiology** 55: 2079-2081.

Kumar, A.S., Mody, K. & Jha, B. 2007. Bacterial exopolysaccharides - A perception. **Journal of Basic Microbiology** 47: 103-117.

Leathers, T.D. 2002. Dextran. *In:* VANDAMME, E.J., DEBAETS, S. & STEINBUCHEL, A. (eds.) **Biopolymers, Vol. 5. Polysaccharides I: Polysaccharides from Prokaryotes.** Weinheim: Wiley-VCH.

Leathers, T.D., Ahlgren, J.A. & Cote, G.L. 1997a. Alternansucrase mutants of Leuconostoc mesenteroides strain NRRL B-21138. **Journal of Industrial Microbiology and Biotechnology** 18: 278-283.

Leathers, T.D., Hayman, G.T. & Cote, G.L. 1995. Rapid screening of Leuconostoc mesenteroides mutants for elevated proportions of alternan to dextran. **Current Microbiology** 31: 19-22.

Leathers, T.D., Hayman, G.T. & Cote, G.L. 1997b. Microorganism strains that produce a high proportion of alternan to dextran. US patent application.

Leathers, T.D., Nunnally, M.S., Ahlgren, J.A. & Côté, G.L. 2003. Characterization of a novel modified alternan. **Carbohydrate Polymers** 54: 107-113.

Leathers, T.D., Nunnally, M.S. & Cote, G.L. 2002a. *Penicillium* isolates for modifying alternan. US patent application.

Leathers, T.D., Nunnally, M.S. & Côté,, G.L. 2009. Modification of alternan by dextranase. **Biotechnology Letters** 31: 289-293.

Leathers, T.D., Nunnally, M.S. & Côté, G.L. 2002b. Modification of alternan by novel Penicillium spp. **Journal of Industrial Microbiology and Biotechnology** 29: 177-180.

Lu, J.J., Perng, C.L., Lee, S.Y. & Wan, C.C. 2000. Use of PCR with universal primers and restriction endonuclease digestions for detection and identification of common bacterial pathogens in cerebrospinal fluid. **Journal of Clinical Microbiology** 38: 2076-2080.

Makanjuola, D.B., Tymon, A. & Springham, D.G. 1992. Some effects of lactic acid bacteria on laboratory-scale yeast fermentations. **Enzyme and Microbial Technology** 14: 350-357.

Makarova, K., Slesarev, A., Wolf, Y., Sorokin, A., Mirkin, B., Koonin, E., et al. 2006. Comparative genomics of the lactic acid bacteria. **Proceedings of the National Academy of Sciences of the United States of America** 103: 15611-15616.

Mcchesney, D.G. 2007. **Nationwide Survey of Distillers Grains for Antibiotic Residues**. US Food and Drug Administration. [Online]. [Cite 17 July 2009].Available from: http://www.fda.gov/downloads/AnimalVeterinary/Products/AnimalFoodFeeds/Contaminants/UCM151206.pdf.

McGhee, J.E., Julian ST, G., Detroy, R.W. & Bothast, R.J. 1982. Ethanol production by immobilized *Saccharomyces cerevisiae*, *Saccharomyces uvarum*, and *Zymomonas mobilis*. **Biotechnology and Bioengineering** 24: 1155-1163.

Narendranath, N.V., Hynes, S.H., Thomas, K.C. & Ingledew, W.M. 1997. Effects of lactobacilli on yeast-catalyzed ethanol fermentations. **Applied and Environmental Microbiology** 63: 4158-4163.

Narendranath, N.V. & Power, R. 2004. Effect of yeast inoculation rate on the metabolism of contaminating lactobacilli during fermentation of corn mash. **Journal of Industrial Microbiology and Biotechnology** 31: 581-584.

Narendranath, N.V., Thomas, K.C. & Ingledew, W.M. 2001. Effects of acetic acid and lactic acid on the growth of Saccharomyces cerevisiae in a minimal medium. **Journal of Industrial Microbiology and Biotechnology** 26: 171-177.

Pfeiler, E.A. & Klaenhammer, T.R. 2007. The genomics of lactic acid bacteria. **Trends in Microbiology** 15: 546-553.

Scott, R. & Sullivan, W.C. 2008. Ecology of fermented foods. **Human Ecology Review** 15: 25-31.

Seymour, F.R., Plattner, R.D. & Slodki, M.E. 1975. Gas-liquid chromatograhpy-mass spectrometry of methylated and deuteriomethylated per-O-acetyl-aldononitriles from D-mannose. **Carbohydrate Research** 44.

Skinner-Nemec, K.A., Nichols, N.N. & Leathers, T.D. 2007. Biofilm formation by bacterial contaminants of fuel ethanol production. **Biotechnology Letters** 29: 379-383.

Skinner, K.A. & Leathers, T.D. 2004. Bacterial contaminants of fuel ethanol production. **Journal of Industrial Microbiology and Biotechnology** 31: 401-408.

Soomro, A.H., Masud, T. & Anwaar, K. 2002. Role of lactic acid bacteria (LAB) in food preservation and human health-a review. **Pakistan Journal of Nutrition** 1: 20-24.