Evaluation of Lactic Acid Bacteria for a Direct-Fed-Microbial Against Shigatoxigenic *Escherichia coli* in Food Animals

Kaylee Rumbaugh, Jordan Drake, and Divya Jaroni*

Food & Agricultural Products Center, and the Dept. of Animal and Food Sciences, Oklahoma State University

INTRODUCTION

Shigatoxigenic *Escherichia coli* (STEC) is an important foodborne pathogen that lives commensally in the rumen of cattle and other food animals such as sheep and goat [1,3]. Direct or indirect contact with animals or manure of animals carrying STEC could mediate its transfer to water and food products, which could result into human infections upon consumption. In the US, it causes an estimated 63,000 foodborne illnesses, 2,100 hospitalizations and 20 deaths, imposing an economic burden of $271 million [2, 4]. Some high-risk food commodities associated with these illnesses include beef and meat products; fresh produce; unpasteurized apple-juice; and dairy products [4]. Reduction of this pathogen at the pre-harvest level could play a significant role in preventing the introduction of this pathogen into the food chain. Direct-fed-microbials (DFM) consist of live microbial cultures that exhibit antagonistic effects against specific groups of organisms, resulting in a decrease in their numbers in the intestinal tract. Several lactic acid bacteria (LAB), most commonly strains from the genera, *Lactobacillus, Enterococcus*, and *Streptococcus*, have been tested as probiotic agents for livestock. Additionally, LAB are fed to cattle to improve animal performance, hence a DFM could be easily integrated into current management strategies.. *Lactobacillus acidophilus* is one common lactic acid bacteria that may be utilized effectively [5]. However, in order to produce the desired effects, strains of this group must be carefully selected and screened to maximize their inhibitory activity.

OBJECTIVE

To evaluate lactic acid bacteria strains for direct-fed-microbials against STEC in food animals.

MATERIALS & METHODS

Viability of LAB Cultures

- Several strains (n=205) of lactic acid bacteria were tested for their viability (growth in de Man, Rogosa, Sharpe (MRS) broth at 37°C for 24-48 h).
- Revived cultures were initially identified using the Gram-stain technique.
- Frozen concentrated cultures (FCC) of the revived cultures were prepared for future use.

Inhibition of STEC

- Selected LAB strains, that showed good growth at 24 h during revival, were further tested for inhibition capability against STEC serotypes (O157:H7, O111, O26, O103, O145, O45, O121).
- Overnight cultures of LAB isolates (MRS broth at 37°C; 18-20 h) were spot-inoculated onto MRS agar plates and incubated anaerobically at 37°C for 24 h.
- Overnight cultures of STEC isolates were prepared in Tryptic Soy Broth (TSB) and incubated at 37°C for 16 h.
- Cocktails (1:1:1:1) of STEC O157:H7 and nonO157 at ~5x10^5 CFU/mL) were inoculated into 9 ml of soft TSA and poured onto the LAB-agar spots, and plates incubated for 24 h at 37°C.
- Inhibition zones (mm) around LAB colonies were measured the next day using Vernier Calipers.

Acid and Bile Tolerance

- Selected (n=16) strains of LAB, that showed excellent (>15 mm) or very-good (>10 mm) inhibition against STEC, were tested for acid and bile tolerance.
- Growth of each isolate (1x10^8 CFU/ml) was determined in MRS broth adjusted to pH (2, 4, 5) and bile (0.1, 0.3, 0.5%), by incubating at 37°C for 0, 1, 3, and 6h. Growth was measured by measuring absorbance at 560 nm for acid and bile tolerance.

Statistical Analysis

- A 2-way ANOVA was performed using treatment (pH or bile concentrations) x time (0, 1, 3, 6 h) factorial at P<0.05.

RESULTS & DISCUSSION

Of the 205 strains revived, 41 (20%) showed excellent growth and 160 (78%) showed very-good growth after 24 h. Fifty of those were further tested for inhibition against STEC (Table 1 and Fig. 1). Among these, 15% showed excellent (>15 mm), 32% very-good (>10 mm), and 29% good (>5 mm) inhibition against STEC. Of these, 16 were further screened for acid and bile tolerance.

Acid Tolerance (Fig. 2a-f): All strains showed increased growth in control experiments. Compared to 0h (A_{560}=0.213-0.597), all isolates showed stable growth up to 6h (A_{560}=0.263-0.991) at all pH values. While the rest of the strains showed good tolerance, *L. acidophilus* C-2 showed excellent tolerance to acidic conditions. Among the non *L. acidophilus* strains, *L. plantarum* E-16 and *L. reuteri* X-18 grew significantly at pH 2 and pH 5, respectively over 6h. This could mean that *L. plantarum* E-16 performs well in more acidic conditions while L. reuteri X-18 performs better at slightly higher pH. The *L. acidophilus* strains: C28, GP2A, 381-IL27 and 6-L4 showed decreased growth at 3 h but increased growth at 6 h, indicating that they were able to recover from the acid shock, and probably developed acid tolerance.

Bile Tolerance (Fig. 3a-f): 80% isolates at 0.1%, 40% at 0.3% and 30% at 0.5% showed increased growth over 6h, indicating that they were better able to tolerate 0.1% compared to the 0.3 and 0.5% bile concentrations. *L. acidophilus* strains, C-28, 22, C-2 and CL3, and *L. cellobiosus* L-4 showed significantly increased growth at 0.5% at 6h, exhibiting better tolerance. Compared to all the strains, *L. acidophilus* C28 exhibited the best growth in all bile concentrations.

RESULTS

ACID TOLERANCE [pH 2, 4, 5, 7 (Control)]

Fig 2 (a-d): *L. acidophilus* Strains

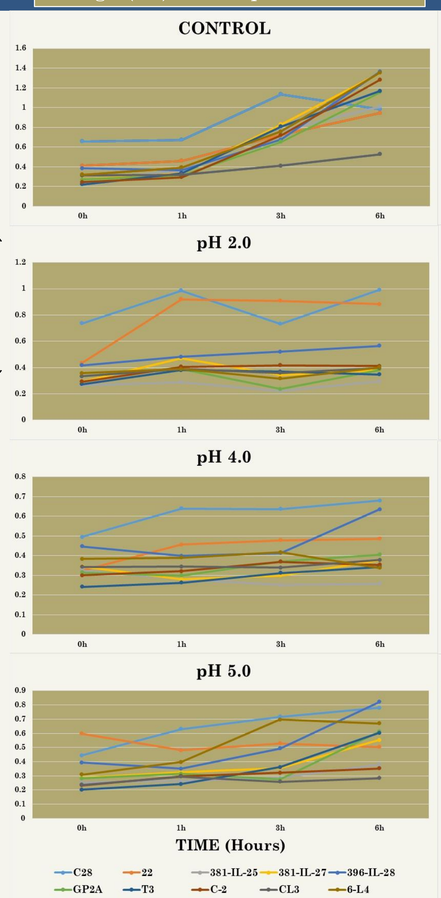
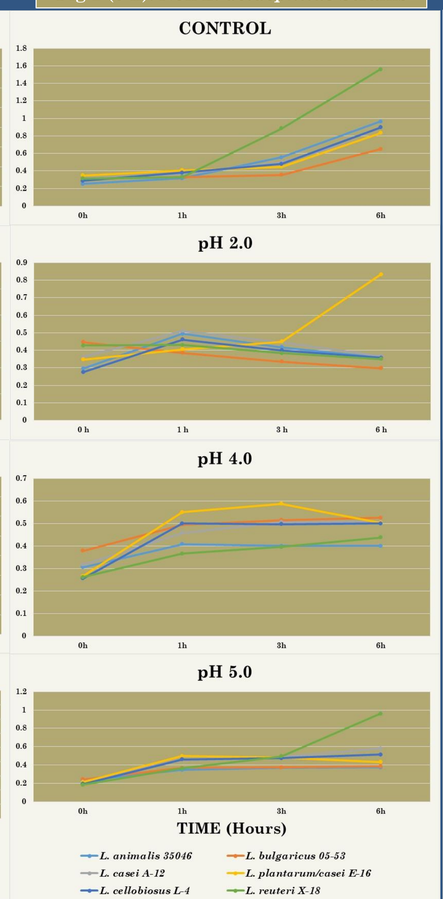


Fig 2 (e-h): Non *L. acidophilus* Strains



BILE TOLERANCE [0, 0.1, 0.3, 0.5%]

Fig 3 (a-d): *L. acidophilus* Strains

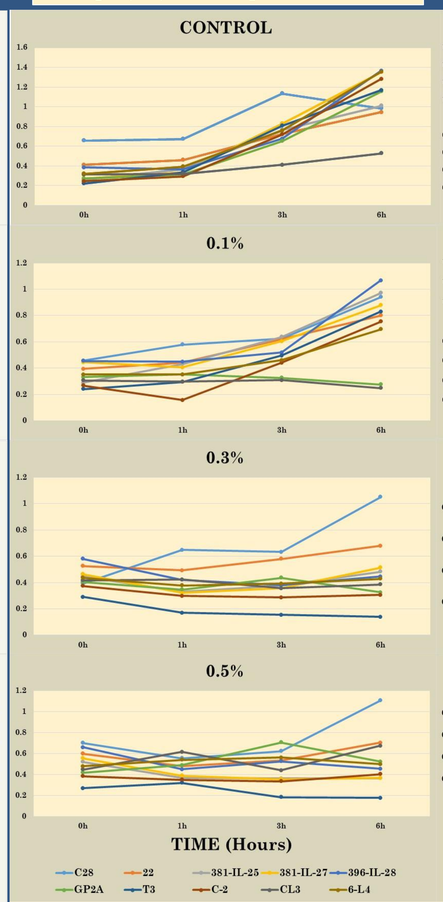


Fig 3 (e-h): Non *L. acidophilus* Strains

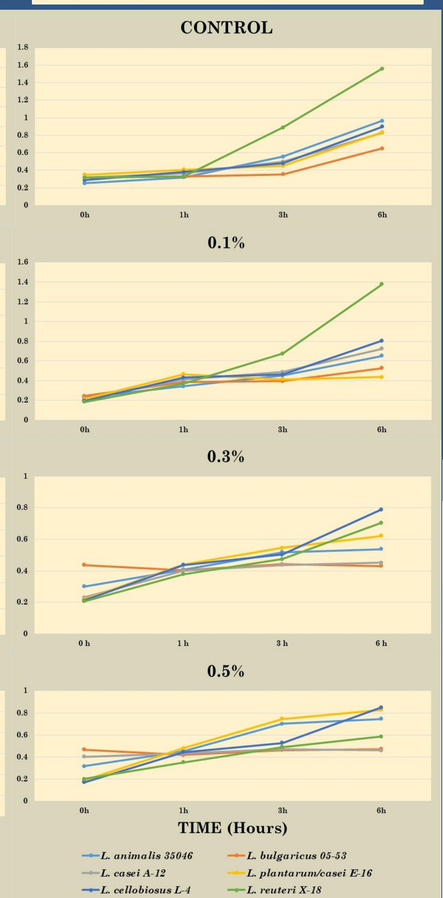


Fig. 1a. No Inhibition Zones

STEC INHIBITION Agar-Spot Test

Fig. 1b. Inhibition Zones

TABLE 1. Growth, Gram Identification, and Inhibition Capability of LAB Strains

LAB	GROWTH (24 h)	GRAM ID	INHIBITON (mm)		TOLERANCE (A_{560})	
			O157	NonO157	Acid	Bile
L. acidophilus C28	Very Good	+ Rods	12.4	15.8	Good	Excellent
L. acidophilus 223	Excellent	+ Rods	13.6	11.2	Good	Very good
L. acidophilus 381-IL25	Very Good	+ Rods	12.2	12.8	Good	Good
L. acidophilus 381-IL27	Very Good	+ Rods	13.4	16.6	Good	Good
L. acidophilus 396-IL28	Very Good	+ Rods	14.8	19.1	Good	Good
L. acidophilus GP2A	Very Good	+ Rods	7.0	-	Good	Excellent
L. acidophilus T-3	Very Good	+ Rods	17.7	12.8	Good	Good
L. acidophilus C-2	Very Good	+ Rods	14.8	12.1	Excellent	Fair
L. acidophilus CL3	Excellent	+ Rods	10.2	13.7	Good	Fair
L. acidophilus 6-L4	Very Good	+ Rods	12.4	11.6	Good	Good
L. animalis 35046	Excellent	+ Rods	15.0	13	Good	Excellent
L. bulgaricus 05-53	Very Good	+ Rods	12.8	8.5	Good	Excellent
L. casei A-12	Excellent	+ Rods	15.5	16.5	Good	Excellent
L. plantarum E-16	Excellent	+ Rods	14.3	15.1	Good	Excellent
L. cellobiosus L-4	Good	+ Rods	13.7	11.6	Good	Excellent
L. reuteri X-18	Very Good	+ Rods	11.5	14.6	Good	Good

CONCLUSIONS

Selected LAB strains indicated survival capabilities for the GI-tract environment by showing good growth at acidic pH and various bile concentrations. Majority of the strains also significantly inhibited STEC. They show promise for use as direct-fed-micobials in cattle or other food animals.

REFERENCES

1. Rasmussen, M. A., W. C. Cray, T. A. Casey, and S. C. Whipp. 1993. Rumen contents as a reservoir of enterohemorrhagic *Escherichia coli*. FEMS Micro. Lett., 114, 79-84.
2. Su, C. and L. J. Brandt. 1995. *Escherichia coli* O157: H7 infection in humans. Ann. Int. Med., 123, 698-707.
3. Grauke, L. J., I. T. Kudva, J. W. Yoon, C. W. Hunt, C. J. Williams, and C. J. Hovde. 2002. Gastrointestinal tract location of *Escherichia coli* O157: H7 in ruminants. App. Environ. Micro. 68, 2269-2277.
4. Centers for Disease Control and Prevention (CDC). 2022. Report of selected *E. coli* outbreak investigations.
Available at *https://www.cdc.gov/ecoli/outbreaks.html*. Accessed March 3, 2022.
5. Younts, S. M., G.D. Osborn, M. Galyean, J. D. Rivera, G. Loneragan, and M. Brashears. 2005. Reduction of *Escherichia coli* O157 in Finishing Beef Cattle by Various Doses of *Lactobacillus acidophilus* in Direct-Fed Microbials. J. Food Prot., 68, 6–10.