INHIBITION OF *ESCHERICHIA COLI* O157:H7 BY *LACTOBACILLUS ACIDOPHILUS* ISOLATED FROM CALVES

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Story in Brief

Of six strains of *Lactobacillus acidophilus* tested in preliminary experiments only four produced significant inhibition of two strains of *Escherichia coli* O157:H7 and *Staphlococcus aureus*. Further tests of these four strains revealed significant inhibitory effects on three different strains of *E. coli* O157:H7. All strains of *L. acidophilus* tested were originally isolated from intestinal contents of calves. The presence of *E. coli* O157:H7 in meat and dairy products has unfortunately become a great threat to the producer and the consumer. A possible site of prevention of this deadly pathogen from getting into the food supply lies at the very beginning of the food production process. Since *E. coli* O157:H7 has been associated with the digestive system of beef and dairy cattle, supplementation of cattle feed with a selected strain of *Lactobacillus acidophilus* has the potential of inhibiting growth of *E. coli* O157:H7 in the intestine of cattle and thus minimizing its presence at slaughter.

(Key Words: *Lactobacillus acidophilus, E. coli* O157:H7.)

Introduction

In the past decade, *Escherichia coli* O157:H7 has become a serious foodborne pathogen capable of producing severe illness and even death. The majority of notable outbreaks in the United States have been related to consumption of contaminated bovine products such as milk and beef. Rarely has the actual reservoir of *E. coli* O157:H7 been investigated. A few investigations have indicated that the primary sources of the *E. coli* O157:H7 are beef and dairy cattle. Specifically, O157:H7 was found to inhabit the bovine rumen and can be easily shed in the feces (Rasmussen et al., 1993). Therefore, the prevention of subsequent outbreaks of this pathogen should begin at the farm/feedlot. In addition to proper cattle management systems, the supplementation of cattle feed with probiotics may influence the microflora in the intestine. *L. acidophilus* has been indicated as a possible antagonist to several pathogens occurring in the intestines.

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Due to host specificity and strain to strain variation, it is of primary importance to select a host specific strain of lactobacilli that will produce maximum inhibition of *E. coli* O157:H7 if it is to aid in control of this pathogen. The objectives of this study were to compare the antagonistic activity of several strains of *L. acidophilus* isolated from calves toward different strains of *E. coli* O157:H7 and *S. aureus* (another pathogen of concern).

Materials and Methods

Initial experiments involved two strains of *E. coli* O157:H7 (933 and 43895), one strain of *S. aureus* (305) and six strains of *Lactobacillus acidophilus* (38-IL-28, C-28, FR-3, R-2, 30-S-C and 53545). The cultures were maintained in MRS broth and subcultured (1% inocula and 18 hr incubation at 37 ^{o}C) as needed.

MRS broth (100 ml) was inoculated with 1 ml of a 1:100 dilution of a freshly prepared broth culture of *E. coli* 933, 43895 or *S. aureus* 305. After thorough mixing, the inoculated broth media were aseptically dispensed into sterile tubes (10 ml/tube) followed by additional inoculation with 0.1 ml of a 1:100 dilution of the cultures of *L. acidophilus* (one tube per culture for each pathogen). A control tube for each pathogen (i.e., without lactobacilli) was also prepared. After six hours of incubation at 37^{o}C the tubes of associative cultures and control cultures were immediately placed in an ice water bath to stop any further growth. Numbers of *E. coli* were determined by plating the samples on Violet Red Bile Agar (VRBA) followed by incubation at 37^{o}C for 24 hr. The numbers of *S. aureus* were enumerated by plating the samples on Mannitol Salt Agar (MSA) followed by incubation for 24 hr at 37^{o}C. Percentages of inhibition caused by each culture of lactobacilli were calculated by the following formula:

$$\%\text{Inhibition} = 100\left[\frac{(\#\text{pathogens in control}) - (\#\text{pathogens in presence of lactobacilli})}{(\#\text{pathogens in control})}\right]$$

The most inhibitory strains of *L. acidophilus* were selected for further testing. The procedure was the same as described above except that *S. aureus* was not included and three different strains of *E. coli* O157:H7 were used in place of the two listed above. The three strains tested were *E. coli* O157:H7 strains 35150, 43890, and 43894 all obtained from the American Type Culture Collection (ATCC). All three had been involved in causing hemorrhage colitis in humans.

Three replicate trials were conducted for each experiment. Tests for variations in percentages of inhibition of each pathogen among strains of *L. acidophilus* were done by ANOVA using SAS General Linear Models procedure. Means were separated by least significant difference analysis.

Results and Discussion

Data obtained from the initial experiments revealed significant variation in the inhibitory action (% inhibition) among strains of calf *L. acidophilus* towards *E. coli* O157:H7 and *S. aureus* cultures. *L. acidophilus* 381-IL-28, C-28, and FR-3 produced a significantly greater ($P<.05$) inhibitory effect toward all three pathogens than did 30-S-C and 53545 (Table 1). However, *L. acidophilus* R-2 was significantly less inhibitory to *S. aureus* than were strains 381-IL-28, C-28, and FR-3. The lower levels of inhibition caused by *L. acidophilus* R-2, 30-SC and 53545 was probably, in part, related somewhat to their slower growth. These results clearly show strain to strain variation and the importance of carefully selecting a strain with the greatest potential toward the inhibition of the pathogens growth.

Based on the data in Table 1, four strains, *L. acidophilus* 381-IL-28, C-28, FR-3, and R-2 were selected for further testing against three additional strains of *E. coli* O157:H7 obtained from ATCC. All four selected strains of the lactobacilli statistically inhibited all three strains of *E. coli* O157:H7 although no difference ($P>.05$) occured among strains (Table 2). Inhibition of *Escherichia coli* O157:H7 ranged from 93.6 to 96.0% indicating the potential benefit of using these four strains of *L. acidophilus* as dietary feed supplements for controlling this pathogen in cattle.

Feeding trials in which cattle, being fed a selected strain of *L. acidophilus*, are challenged with *E. coli* O157:H7 will be necessary to confirm the potential role of the lactobacilli in controlling the pathogen in vivo. Plans are underway for such studies using one of the selected strains of *L. acidophilus*.

An important note regarding the strain of *L. acidophilus* FR-3: This strain, after the completion of these experiments, did not remain active and was lost from our collection. This too illustrates the importance of strain selection in that the culture must survive under routine laboratory and production procedures.

Literature Cited

Rasmussen, M. A. et al. 1993. FEMS Microbiology-Letters. 114:1, p79.
SAS. 1985. SAS Inst. Inc., Cary NC.

Table 1. Antagonistic action of *Lactobacillus acidophilus* toward *Escherichia coli* O157:H7 and *Staphylococcus aureus* 305 in associative cultures.[1]

	% Inhibition[2]		
	E. coli O157:H7		
L. acidophilus	strain 43895	strain 933	*S. aureus* 305
381-IL-28	91.8[a]	92.7[a]	82.6[a]
C-28	89.7[a]	93.5[a]	80.6[a]
FR-3	88.7[a]	91.0[a]	84.8[a]
R-2	64.9[a]	89.4[a]	23.3[b]
30-SC	-4.1[b]	28.1[b]	-15.6[c]
53545	16.8[b]	-3.4[c]	-22.1[c]
SEM	14.8	9.9	10.5

[a,b,c] Means within the same column with different superscripts differ ($P<.05$).

[1] During six hours of growth at 37°C.

[2] Each value represents the mean from 3 trials.

Table 2. Antagonistic action of selected strains of *Lactobacillus acidophilus* toward three strains of *Escherichia coli* O157:H7 in associative cultures.[1]

	% Inhibition of *E. coli* O157:H7[2]		
L. acidophilus	35150	43890	43894
381-IL-28	96.0[a]	95.0[a]	95.5[a]
C-28	95.3[a]	95.0[a]	94.7[a]
FR-3	95.4[a]	93.6[a]	95.6[a]
R-2	95.2[a]	94.9[a]	94.7[a]
SEM	.45	.54	.61

[a] Means within the same column with different superscripts differ (P<.05).

[1] During six hours of growth at 37°C.

[2] Each value represents the mean from 3 trials.