Antimicrobial Activity of *Bifidobacterium longum* (NCFB 2259) as Influenced by Spices

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ABSTRACT

Common food spices that have antibacterial properties have potential for controlling *Escherichia coli* O157:H7, one of the leading causes of bacterial food borne diseases in the United States. This potential may be even greater with the addition of bifidobacteria. The objective of this research was to determine the effectiveness of combinations of bifidobacteria and spices on inactivation of *E. coli* O157:H7 (380-94) in ground beef. Lean ground beef was inoculated to the level of 2 log/ml with *E. coli* O157:H7. These samples were then subjected to one of three conditions: spice alone, bifidobacteria alone (*Bifidobacterium longum* (NCFB 2259)), and spice and bifidobacteria combined. Spice treated samples were mixed with one of the following spices: origanox, jalapeno pepper, ginger or garlic at 2% (w/v) levels. Bifidobacteria treated samples received levels of 5.0 log CFU/ml. Samples without any treatments served as controls. Samples were maintained at 37° C for 48 hrs. Changes in the populations of *E. coli* were examined using modified eosin methylene blue (EMB) agar plates. The results showed that origanox had the highest inhibitory effect against *E. coli* O157:H7 ($P < 0.05$), followed by jalapeno pepper and garlic. The synergistic effect of the spices and bifidobacteria on *E. coli* was higher than the effect of any single spice ($p < 0.05$). With origanox or bifidobacteria alone, a gradual decline of *E. coli* O157:H7 counts (2-log reduction) was detected. However, the combination of origanox and bifidobacteria resulted in at least a 5-log reduction. This study suggests that combinations of bifidobacteria and origanox could be used as an effective method to eliminate *E. coli* O157:H7 in meat products, and ultimately improve the biosafety of these foods.

INTRODUCTION

The need for better control of foodborne pathogens has been paramount in recent years. Within the last five years, considerable interest has been developed in the United States with respect to the use of bifidobacteria, as natural bio-preservatives in food. Bifidobacteria have the ability to suppress the growth of pathogenic bacteria by producing organic acids (Ibrahim and Bezkorovainy, 1993) and other antimicrobial compounds such as bacteriocins (Gibson and Wang. 1994, Gomes and Xavier Malcata. 1999; Ibrahim and Salameh, 2001).

Escherichia coli 0157:H7 is one of the leading causes of bacterial food borne disease outbreaks in the United States. According to the USDA reports, an estimated 73,000 cases of infection and 61 deaths occur each year in the USA (Buzby, 1996). Many of which are associated with meat products such as ground beef and ground beef patties. Spices are usually added to meat products to improve the sensory quality. Spices are also well known as antimicrobial agents. Spices are rich in manganese (Mn^{2+}) (Zaika and Kissinger,1984). Kang and Fung (2000) and Zaika and Kissinger (1984) indicated that manganese ions are strong stimulants for starter cultures. To date, little information is available about the indirect influence of manganese ion on the production of antimicrobial agent, bifidogenic compound, by bifidobacteria. Therefore, the stimulatory effect of selected spices on the production of bifidogenic compound by bifidobacteria needs to be understood with greater precision and accuracy. The objectives of this research were to: 1. determine the stimulatory effect of selected spices (garlic, ginger, cloves, jalapeno pepper and origanox) on the production of bifidogenic compound by *B. longum* (NCFB 2259), and 2. determine the effectiveness of combinations of bifidobacteria and origanox on inactivation of *E. coli* O157:H7 in ground beef.

MATERIALS AND METHODS

Bacterial strain and culture conditions:

E. coli O157:H7 (380) and *Bifidobacterium* species (Table 1) were obtained from the food microbiology culture collection at North Carolina A&T State University. *E. coli* O157:H7 was transferred into brain heart infusion (BHI) broth and incubated at 37°C for 24 hrs. The *Bifidobacterium* species were transferred into trypticase peptone-yeast extract (TPY) broth and incubated anaerobically at 37°C for 24 hrs. Overnight cultures were centrifuged and then washed with peptone, before use.

Table 1. Antimicrobial activity of different strains of bifidobacteria

Strain No.	Bacterial Strain	Source/Reference	Antimicrobial Activity
B1	*B. adolescentis*	ATCC 15704	Negative
B2	*B. animalis*	ATCC 27672	Negative
B3	*B. bifidum*	ATCC 15696	Negative
B4	*B. bifidum*	ATCC 29521	Negative
B5	*B. breve*	ATCC 15698	Negative
B6	*B. breve*	ATCC 15701	Negative
B7	*B. infantis*	ATCC 15697	Negative
B8	*B. infantis*	ATCC 15702	Negative
B9	*B. infantis*	ATCC 25962	Negative
B10	*B. infantis*	NCFB 2205	Negative
B11	*B. infantis*	ATCC 15708	Negative
B12	***B. longum***	**NCFB 2259**	**Positive**
B13	*B. Sp.* Strain BF-1	Commercial Source	Negative
B14	*B. Sp.* Strain BF-6	Commercial Source	Negative
B15	*B. Sp.* Strain BF-7	Commercial Source	Negative

Antimicrobial activity:

Antimicrobial activity of *Bifidobacterium* strains (Table 1) and several spices (garlic, onion, cinnamon, pepper, cloves, sage, rosemary, oregano, and origanox) against *E. coli* O157:H7 was tested using agar diffusion assay (Ibrahim and Salameh, 2001).

Meat samples:

Ground beef (93% lean meat) samples were obtained from a local grocery market. Samples were kept in refrigerator until used within 24hr.

Experimental design:

Ground beef was inoculated with *E. coli* O157:H7 to make the initial inoculum level of 2.0 log CFU/ml. Inoculated ground beef samples were then subjected to one of the three conditions: spice alone, bifidobacteria alone (*B. longum* (NCFB 2259)), and spice and bifidobacteria. Spice treated samples were mixed with one of the following spices: origanox, jalapeno pepper, or garlic at 2% (w/v) levels. Bifidobacteria treated samples received levels of 5.0 log CFU/ml. Samples without any treatments served as controls. Beef samples were then held at 37° C for 48 hrs. Changes in the populations of *E. coli* O157: H7 in meat samples were followed on modified eosin methylene blue (EMB) agar plates at 0, 24, and 48 hrs.

Effect of Origanox on the growth of bacteria:

E . coli O157:H7 and *B. longum* (NCFB 2259) was individually inoculated at the level of 2.0 log CFU/ml in BHI broth containing origanox at the following concentrations: 0, 0.1, 0.2, 0.3, 0.4, 0.5, 1.0, 1.5, and 2% w/w. Samples were held at 37° C. The microbial growth of *E . coli* O157:H7 and bifidobacteria was followed on EMB and modified BIM 25 plates respectively.

RESULTS AND DISCUSSION

Table 1 shows the antimicrobial activity of the tested strains of bifidobacteria against *E. coli* O157:H7 using the agar diffusion assay. Strain *B. longum* (NCFB 2259) showed a strong inhibitory effect against *E. coli* O157:H7, as observed by the formation of a large inhibition zone. This strain was used to conduct this research work.

Among the spices tested for their antimicrobial activity against the foodborne pathogen, *E. coli* O157:H7, origanox, a natural, anti-oxidant, extracted from *Origanum,* showed a strong inhibitory activity. This herb along with other spices were selected in this study to determine the synergistic effect of bifidobacteria and spices. Figure 1 shows the antimicrobial effect of spices against *E. coli* O157:H7 in ground beef. Origanox had the highest inhibitory effect against *E. coli* O157:H7 ($p < 0.05$), followed by jalapeno pepper and garlic. Ginger had little effect on the growth of *E. coli* O157:H7 in ground beef. The synergistic effect of spices and

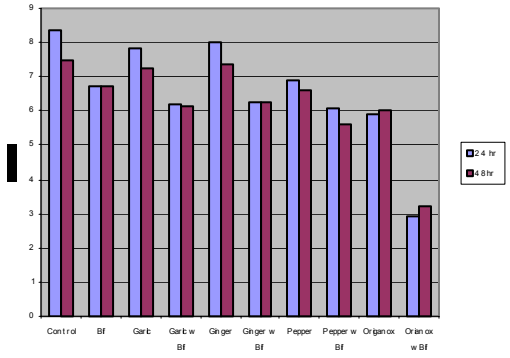
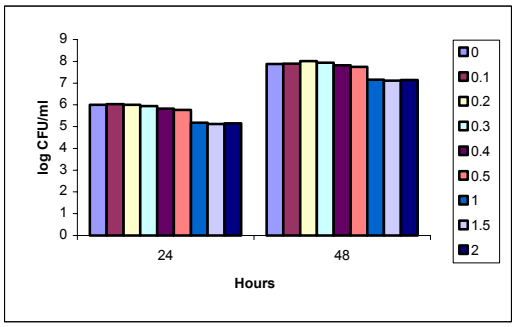


Figure 1. Effect of spices alone or in combination with *B. longum* (NCFB 2259) on the survival and growth of *E. coli* O157: H7

bifidobacteria on *E. coli* O157:H7 was higher than the effect of spices alone (Figure 1). Figure 2 and 3 shows the effect of origanox on the survival and growth of bifidobacteria and *E. coli* O157:H7. Origanox had little effect on the survival and growth of *B. longum* (NCFB 2259) whereas; origanox reduced the growth rate of *E. coli* O157:H7 when used at 0.3% or higher.

CONCLUSIONS

Our results demonstrated that ground beef treated with origanox had the highest inhibitory effect against *E. coli* O157:H7 ($p < 0.05$), followed by jalapeno pepper and garlic. Ginger had little effect on the growth of *E. coli* O157:H7 in ground beef. The synergistic effect of spices and bifidobacteria on *E. coli* O157:H7 was higher than the effect of a spice alone.



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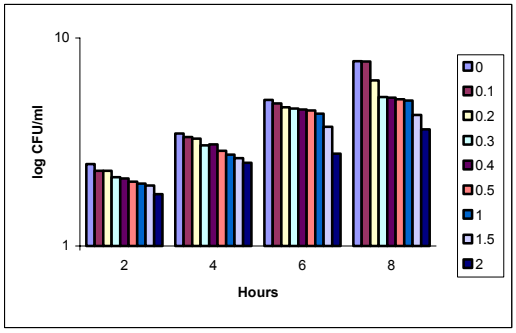

Figure 2. Growth of bifidobacteria (1) and *E. coli* O157 : H7 (2) at 37°C in BHI broth containing different concentrations of Origanox

Results also indicated that origanox has little effect of the survival and growth of bifidobacteria, while it did inhibit the growth of *E. coli* O157:H7. Combination of bifidobacteria and spices could be used to control the growth of *E. coli* O157:H7 in food and to increase the bio-safety of many consumable food products.

Acknowledgements:

This study was supported, in part, by a grant from the USDA CSREES program. Authors would like to acknowledge the dean associate for research, Dr. C.Turner, at North Carolina A&T State University for her continuous support while conducting this work.

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