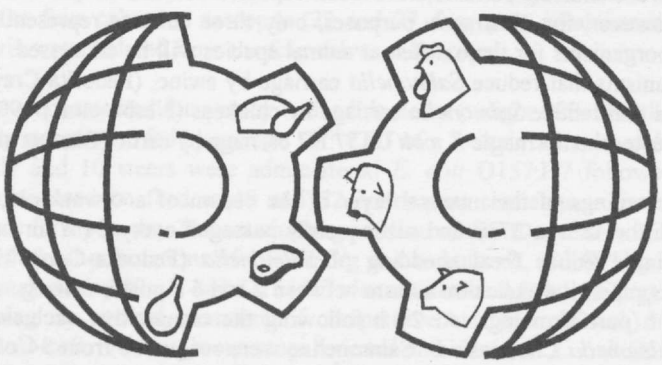
Session 6.I

Meat safety

46th ICoMST



Argentina, 27 August - 1 September, 2000

Use of Microorganisms in Non-Traditional Methods to Increase Meat Safety

Michael P. Doyle

Center for Food Safety and Quality Enhancement
University of Georgia
Griffin, GA 30223-1797
USA

The world of microorganisms is in a constant state of tension with those microbes developing superior mechanisms of survival out competing others vying for the same niche. Public health microbiologists have the challenging role of controlling the proliferation of microorganisms that have evolved virulence factors detrimental to human hosts. Many of these pathogens which are harmful to humans, including enterohemorrhagic *Escherichia coli* O157:H7, *Salmonella*, and *Campylobacter jejuni*, are carried in the intestinal tract of animals as harmless commensals. However, not all animals within a herd or flock of pathogen carriers are infected or colonized by the human pathogen. There are many protective mechanisms that can affect intestinal carriage of microorganisms, ranging from immunity to availability of colonization sites. However, one very important protective mechanism that is receiving increased attention is the involvement of beneficial microorganisms that are harmless to their hosts but can eliminate or reduce populations of undesirable microorganisms in the intestinal tract.

Probiotic microorganisms include those microbes that suppress the carriage of harmful microorganisms. Many descriptions of probiotic microorganisms have been offered; however, they are often very limited in their scope. For purposes of this discussion, probiotic microorganisms will be defined as live microbial food or feed ingredients that benefit the health of humans or animals.

Probiotic microorganisms appear to be effective in benefitting the health of humans or animals by a variety of different mechanisms. Examples include: (1) assimilating, degrading or binding undesirable toxicants or anti-nutritional compounds that negatively affect human or animal health, (2) enhancing immune responses by an adjuvant or antigenic effect, (3) producing growth-promoting metabolites, (4) preventing undesirable microorganisms from colonizing animals or humans by binding at or covering receptor sites required for colonization by undesirable microbes, and (5) excluding or eliminating undesirable microorganisms from animals or humans by localizing in common sites and producing antimicrobials active against undesirable microbes.

Among probiotic microorganisms, competitive exclusion bacteria are showing great promise as treatments to reduce intestinal carriage and fecal shedding of foodborne bacterial pathogens by animal hosts. Competitive exclusion bacteria can be grouped in two general categories, i.e. undefined cultures and defined cultures, based on strategies used to obtain and characterize the microbes which constitute the culture. Undefined microbial cultures are those in which many different microorganisms are present but they have not been fully identified and may vary from batch to batch. In contrast, defined microbial cultures are comprised of microorganisms that previously have been isolated, identified and characterized for various attributes which influence their usefulness. Both undefined and defined competitive exclusion bacteria are usually obtained from the intestinal tract of pathogen-free animals of the same species as their intended use.

Several different competitive exclusion cultures have been reported for use in meat animal production to reduce fecal shedding of human pathogens; however, for illustrative purposes, only three cultures representing three different approaches to obtain competitive exclusion microorganisms for three different animal species will be addressed. These include: (1) an undefined culture of mucosal cecal microorganisms that reduce *Salmonella* carriage by swine, (Fedorka-Cray, 1999), (2) a defined culture of 29 identified cecal bacterial isolates that reduce *Salmonella* carriage by chickens (Nesbit et al., 1996), and (3) a defined culture of 3 *E. coli* colonic isolates that reduce enterohemorrhagic *E. coli* O157:H7 carriage by cattle (Zhao et al., 1998).

Studies with swine using scrapings of the mucosal layer of the cecum of a 6-week-old, *Salmonella*-free pig cultured in modified brain heart infusion broth for 48 h at 37°C and subsequently passaged every 24 h for up to 7 days revealed the cultures when fed to pigs could substantially reduce fecal shedding of *Salmonella* (Fedorka-Cray, 1999). Sucking pigs were each administered perorally 5 ml of the competitive exclusion culture between 2 and 6 h postfarrowing. An additional 5 ml of culture was administered to each piglet at 24 h postfarrowing. At 24 h following the competitive exclusion culture dose, each piglet was administered intranasally $5x10^3$ *Salmonella* Cholerasuis. Salmonellae were recovered from 34 of 110 (31%) and 20 of 80 (25%) intestinal tissues and contents of piglets treated with the competitive exclusion bacteria, for Trials 1 and 2, respectively. In contrast, salmonellae were recovered from 24 of 30 (80%) and 35 of 45 (78%) intestinal tissues and contents of control piglets that did not receive competitive exclusion bacteria, for Trials 1 and 2, respectively. Analysis of rectal swabs and tissues of tonsil, bronchiole lymph node, lung, liver, spleen, colon, ileocolic junction, cecum, cecal contents, and stomach wall revealed salmonellae was present in 20% and 41% of samples from piglets administered the competitive exclusion culture compared with 63% and 63% of control piglets for Trials 1 and 2, respectively. Although *S.* Cholerasuis was not eliminated from all piglets by the competitive exclusion cultures, these bacteria substantially decreased the occurrence of salmonellae tissues and intestinal contents of a large portion of piglets.

A defined culture of competitive exclusion bacteria (PREEMPT) to reduce cecal colonization of chickens by *Salmonella* was developed by Nisbet et al. (1996). The competitive exclusion culture was obtained by homogenizing in an anaerobic chamber the intact ceca from several adult broilers and culturing these in VL broth at 39°C anaerobically until the pH was 5.5. This batch culture was then used as the inoculum for a continuous flow culture. Continuous flow cultures were grown in VL broth in a 2-L chemostat vessel flushed with a stream of carbon dioxide at 39°C with agitation (200 rpm) for 365 days. The pH of the culture was maintained at 5.9. A total of 29 different bacterial isolates were obtained from and identified from the continuous flow culture. They included one or more strains of the following genera: *Enterococcus, Lactobacillus, Escherichia, Enterobacter, Serratia, Citrobacter, Eubacterium, Pseudomonas, Bifidobacterium, Propionibacterium, Fuscobacterium,* and *Veillonella*. Chicks were treated with the competitive exclusion bacteria obtained from the continuous flow culture at 5, 30, 100, or 365 days. Thereafter, chicks were each administered perorally 10^4 *Salmonella* Typhimurium. The cecal contents of a substantially lower percentage of chicks (5 to 35%) treated with the defined competitive exclusion bacteria were positive for *Salmonella* compared to 90 to 100% of *Salmonella*-positive control chicks. Furthermore, those chicks treated with the competitive exclusion bacteria that were *Salmonella*-positive had considerably less *Salmonella* in cecal contents (average 0.08 to 0.61 $\log_{10}$ *Salmonella*/g) compared to *Salmonella*-positive control chicks (average 4.11 to 6.35 $\log_{10}$ *Salmonella*/g).

Zhao et al. (1998) developed a systematic approach for isolating highly effective, defined competitive exclusion bacteria for reducing carriage and fecal shedding of *E. coli* O157:H7 by cattle. The approach involved: (1) identifying the sites of localization of *E. coli* O157:H7 in cattle, (2) isolating bacteria from principal site (colonic tissue) of *E. coli* O157:H7 localization from cattle that do not shed *E. coli* O157:H7 in feces, (3) screen bacterial isolates from their ability to secrete extracellular metabolites that inhibit or kill *E. coli* O157:H7, (4) identify the sites of localization and persistence of potential competitive exclusion bacteria in sites in cattle where *E. coli* O157:H7 persist, and (5) conduct challenge studies with combinations of potential competitive exclusion bacteria and isolate the dominant competitive exclusion strains from primary sites of localization in cattle from which *E. coli* O157:H7 was eliminated.

Using this approach, we initially determined the primary sties of *E. coli* O157:H7 in weaned 8- to 12-week-old calves were the forestomachs (rumen, reticulum, omasum, and colon) (Brown et al. 1997). Subsequently, Zhao et al. (1998) obtained 1,200 bacterial isolates from feces and colonic tissue and contents from cattle that did not fecally shed *E. coli* O157:H7. From these 1,200 isolates, 18 potential probiotic bacteria were obtained that secreted extracellular metabolites to *E. coli* O157:H7. Seventeen were *E. coli* and pulsed-field gel electrophoresis DNA subtyping revealed there were 13 different DNA profiles among the isolates suggesting there were only 13 distinct isolates among the 18.

All 18 potential probiotic strains at 10^{10} CFU/calf were fed weaned, cannulated calves followed 2 days later with a peroral dose of 10^{10} CFU of a 5-strain mixture of *E. coli* O157:H7 per calf. Within 3 weeks, *E. coli* O157:H7 was eliminated from the rumen and no longer shed in feces of 5 of 6 calves whereas 9 of 9 control calves continued to harbor *E. coli* O157:H7 in the colon and shed in feces up to the time of necropsy (up to 1 month post administration of *E. coli* O157:H7). Four probiotic *E. coli* were the dominant strains isolated from probiotic-treated calves at necropsy.

These four dominant probiotic *E. coli* were used to treat four weaned, cannulated calves 1 or 3 days after peroral administration of 10^{10} CFU of a 5-strain mixture of *E. coli* O157:H7. Results revealed that 11 of the 12 control calves perorally administered only *E. coli* O157:H7 shed the pathogen continuously in feces throughout the study (up to 28 days), with *E. coli* O157:H7 isolated from the rumen of 9 of 12 calves and from the colon of 10 of 12 calves at necropsy. *E. coli* O157:H7 was detected in calves receiving probiotic bacteria for up to 9 days in the rumen and for up to 15 days in feces. *E. coli* O517:H7 was not detected in the rumen or colon of any of the four calves at necropsy (22 and 27 days post administration of *E. coli* O157:H7). Three dominant strains of probiotic bacteria were isolated from the four calves at necropsy.

These three probiotic strains were selected for a study with adult steers (weight 980-1160 lbs) fed a grain diet containing 30 grams of monensin per ton. Twenty steers were fed by gavage 10^{10} CFU of a 5-strain mixture of *E. coli* O157:H7. Ten steers were administered *E. coli* O157:H7 only and 10 steers were administered *E. coli* O157:H7 followed by a 3-strain mixture of 10^{10} competitive exclusion *E. coli* administered on feed at 48 and 72 hours post challenge. *E. coli* O157:H7 fecal shedding was monitored periodically in all animals for up to day 33 post challenge. Nine of the 10 steers fed *E. coli* O157:H7 only shed *E. coli* O157:H7 in feces up to day 33, with fecal populations of 10^5 CFU/g in two animals, 10^4 CFU/g in one animal, 10^2 CFU/g in two animals, and 10^1 CFU/g in four animals at day 30. *E. coli* O157:H7 was not detected (<1.1 CFU/g) at 12 days post challenge with *E. coli* O157:H7 (9 days after receiving the second treatment of competitive exclusion *E. coli*) in the feces of any of the steers administered competitive exclusion *E. coli*. Although one steer reverted and shed 10^2 *E. coli* O157:H7/g of feces at day 21, none had detectable *E. coli* O157:H7 at day 30 or 33. One steer had 10^2 *E. coli* O157:H7/g in its rumen at necropsy (day 33), but in no other location of the gastrointestinal tract. However, none of the other steers had detectable *E. coli* O157:H7 at any gastrointestinal site, including the rumen, at necropsy (day 33). Two important findings were obtained from this study. First, the competitive exclusion *E. coli* could reduce or eliminate fecal shedding of *E. coli* O157:H7 to undetectable levels in most adult grain-fed cattle within 9 days after receiving the beneficial bacteria. Secondly, 7 or 10 steers administered only *E. coli* O157:H7 harbored 10^3-10^5 *E. coli* O157:H7/g in their gastrointestinal tract at necropsy (day 33). This is an unusually large percentage of animals having an unusually large population of *E. coli* O157:H7 in their gastrointestinal tract at 33 days post challenge.

In conclusion, selected probiotic cultures can effectively reduce carriage and fecal shedding of foodborne pathogens in

animal reservoirs. Undefined bacterial cultures from mucosal surfaces of ceca from "pathogen-free" animals have been proven to be effective. However, limitations of undefined probiotic cultures include: (1) some bacteria may carry transferrable antibiotic resistance genes and (2) undetected pathogens may be present. Defined bacterial cultures that localize in the same gastrointestinal sites as pathogens and produce antimicrobials against pathogens also have been proven to be effective. However, depending on the culture many strains (e.g., more than 25) of defined bacteria may be required to be an effective probiotic culture. It can be difficult and impractical to commercially produce defined cultures with many combinations of microorganisms. An ideal probiotic culture would contain only one to three strains that are easy to cultivate, have good survival characteristics, under the conditions used for commercial sale, and are highly effective in eliminating the pathogens targeted from animal carriers. Studies to date indicate that probiotic microorganisms have great potential in serving as critical control points on the farm during animal production. Such treatments could greatly increase fresh meat safety by reducing the likelihood of pathogen contamination of hides prior to slaughter and meat during slaughter.

References

Brown, C.A., B.G. Harmon, T. Zhao, and M.P. Doyle. 1997. Experimental *Escherichia coli* O157:H7 carriage in calves. Appl. Environ. Microbiol. 63:27-32.

Fedorka-Cray, P.J., J.S. Bailey, N.J. Stern, N.A. Cox, S.R. Ladely, and M. Musgrove. 1999. Mucosal competitive exclusion to reduce *Salmonella* in swine. J. Food Protect. 62:1376-1380.

Nisbet, D.J., D.E. Corrier, S.C. Ricke, M.E. Hume, J.A. Byrd II, and J.R. DeLoach. 1996. Maintenance of the biological efficacy in chicks of a cecal competitive-exclusion culture against *Salmonella* by continuous flow fermentation. J. Food Protect. 59:1279-1283.

Zhao, T., M.P. Doyle, B.G. Harmon, C.A. Brown, P.O.E. Mueller, and A.H. Parks. 1998. Reduction of carriage of enterohemorrhagic *Escherichia coli* O157:H7 in cattle by inoculation with probiotic bacteria. J. Clin. Microbiol. 36:641-647.