Bacteriocins Reduce *Campylobacter* Colonization and Alter Gut Morphology in Turkey Poults1,2


*Department of Poultry Science, University of Arkansas, Fayetteville 72701; †Poultry Production and Product Safety Research Unit, USDA-ARS, Fayetteville, AR 72701; ‡State Research Center for Applied Microbiology, Obolensk, Russian Federation; and §Poultry Microbiological Safety Research Unit, Russell Research Center, USDA-ARS, Athens, GA 30604

**ABSTRACT** *Campylobacter* is a leading cause of foodborne illness in the United States. Recent evidence has demonstrated that bacteriocins produced by *Bacillus circulans* and *Paenibacillus polymyxa* reduce cecal *Campylobacter* colonization in broiler chickens infected with *Campylobacter jejuni*. As *Campylobacter coli* is the most prevalent *Campylobacter* isolate recovered in turkeys, the objectives of the present study were to evaluate the efficacy of these bacteriocins against *C. coli* colonization and their influence on the gastrointestinal architecture of young turkeys. In 3 separate trials, a total of 135 day-of-hatch poults (n = 45/trial) were orally challenged on d 3 with approximately 10^6 cfu of a mixture of 3 *C. coli* isolates. Immediately before bacteriocin treatment (d 10), cecal *Campylobacter* concentrations averaged 1.1 × 10^7 cfu/g of cecal contents (n = 15/trial). On d 10 to 12 posthatch, 2 bacteriocin treatment groups were given free access to feed supplemented with purified, microencapsulated bacteriocins, whereas the positive control treatment group had access to untreated feed (n = 10/treatment group per trial). At the end of the 3-d dosing period, ceca and duodenal loops were collected for analysis. In each of the 3 separate trials, treatment with bacteriocin eliminated detectable ceca *Campylobacter* concentrations (detection limit, 1 × 10^2 cfu/g of cecal contents) vs. controls (1.0 × 10^6 cfu of *Campylobacter* /g of cecal contents). Duodenum crypt depth and goblet cell numbers were also reduced in turkeys treated with either bacteriocin vs. controls (P < 0.05). The dynamic reduction in crypt depth and goblet cell density in turkeys dosed with bacteriocin may provide clues to how bacteriocins inhibit enteric *Campylobacter*.

**Key words:** *Campylobacter*, ceca, bacteriocin, turkey, gastrointestinal tract

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**INTRODUCTION**

*Campylobacter* is one of the leading bacterial causes of human foodborne illness in the United States (Centers for Disease Control and Prevention, 2005). A substantial number of poultry and retail poultry products are contaminated with *Campylobacter*, with isolation rates approaching 100% (Stern et al., 2001; Zhao et al., 2001; Newell and Fearnley, 2003). Epidemiological evidence has emphasized the importance of poultry products as a significant source of human *Campylobacter* infection (Jacobs-Reitsma, 2000; Corry and Attabay, 2001). Therefore, the reduction or elimination of this organism in commercial poultry flocks should greatly reduce the incidence of human *Campylobacter* infection (Jacobs-Reitsma, 1997; Sahin et al., 2002).

One approach to reduce *Campylobacter* colonization in preharvest poultry is the use of competitive exclusion (CE) cultures (Stern et al., 2001). Competitive exclusion is the administration of nonpathogenic enteric microflora that may compete with and reduce enteric pathogens. Competitive exclusion, first described by Nurmi and Ran tala (1973) has been used to successfully control *Salmonella* contamination in poultry (Corrier et al., 1995; Bielke et al., 2003). Unfortunately, the use of CE cultures has not consistently reduced *Campylobacter* colonization (Stern et
al., 2001; Mead, 2002). Through efforts to improve the effectiveness of CE cultures against Campylobacter, researchers have observed that certain bacteria produce metabolites that are inhibitory to Campylobacter growth in vitro (Schoeni and Doyle, 1992; Newell and Wagenaar, 2000; Svetoch et al., 2005). These metabolites, identified as bacteriocins, are proteins naturally produced by bacteria that kill or inhibit the growth of other bacteria (Cleveland et al., 2001). Unlike antibiotics, bacteriocins have no known toxic effects and have a narrow killing spectrum (Riley and Wertz, 2002). The use of bacteriocins as antimicrobials has already been applied in food preservation; as the bacteriocin nisin is considered a generally recognized as safe compound and is approved for use in foods (Joeger, 2003).

Recently, Svetoch et al. (2005) found that bacteriocins produced by certain strains of Bacillus circulans and Paenibacillus polymyxa were inhibitory to Campylobacter growth in vitro. In a follow-up study, these purified bacteriocins, microencapsulated and administered via feed, reduced cecal Campylobacter colonization in young broiler chickens experimentally infected with Campylobacter jejuni (Stern et al., 2005). However, the efficacy of these bacteriocins in turkeys has not been determined. Furthermore, the influence of bacteriocins on enteric histology has not been evaluated. Therefore, the objectives in the present study were to evaluate the efficacy of these bacteriocins against Campylobacter coli colonization and to evaluate the influence of bacteriocins on the gastrointestinal morphology in turkeys.

**MATERIALS AND METHODS**

**Bacteriocins**

Bacteria producing the bacteriocins were recovered from the intestinal tracts of broiler chickens. Associated bacteriocin purification and microencapsulation procedures have been previously described in detail (Stern et al., 2005; Svetoch et al., 2005). Briefly, bacteriocin B602 was secreted by the isolate P. polymyxza (NRRL B-30509), whereas bacteriocin OR7 was secreted by the isolate Lactobacillus salivarius (NRRL B-35014; N. J. Stern, E. A. Svetoch, B. V. Eruslanov, V. V. Perelygin, E. V. Mitsevich, I. P. Mitsevich, V. D. Pokhilenko, V. P. Levchuk, and O. E. Svetoch, unpublished data). Each bacteriocin was precipitated with saturated ammonium sulfate, dissolved, dialyzed, and purified by Superose 12HR 16/50 column chromatography (Pharmacia, Uppsala, Sweden), followed by passing the protein over a 300-mL SP Sepharose Fast Flow column (GE Healthcare Bio-Sciences Corp., Piscataway, NJ). The purified bacteriocins were then mixed with polyvinylpyrrolidone powder to produce microencapsulated bacteriocins, which were used to produce a medicated feed. The final concentration of each bacteriocin was 250 mg/kg of feed.

**Campylobacter Isolates and Growth Conditions**

Poults used in this study were challenged with a solution containing an equal combination of 3 C. coli isolates, 2 wild-type turkey isolates, and an American Type Culture Collection isolate 43481. A frozen culture of each isolate was inoculated into 9.0 mL of Campylobacter enrichment broth and grown individually for 24 h at 42°C in a microaerobic environment (5% O₂, 10% CO₂, 85% N₂), as previously described (Cole et al., 2004). After 24 h, 10 μL of each culture was passed into another 9.0 mL of Campylobacter enrichment broth and grown for 24 h in a microaerophilic environment. After 24 h, each culture was combined in a 50-mL conical tube and used for poult inoculation (see below).

**Experimental Design**

A total of 135 poults were used in this study. In each of 3 separate trials, 45 day-of-hatch poults were obtained from a local commercial hatchery and randomly allocated to 1 of 3 treatment groups: positive control, bacteriocin B602, or bacteriocin OR7 (n = 15/pen). Each treatment group was housed in an individual floor pen on fresh pine litter and provided water and feed ad libitum. Three days posthatch, all poults in each treatment group were inoculated, via oral gavage, with 0.25 mL of a solution containing a mixture of 3 C. coli isolates (approximately 10⁶ cfu/mL), as described previously (Farnell et al., 2005). Immediately before bacteriocin treatment (d 10), 5 of the 15 birds from each of the 3 treatment pens (n = 15/trial) in each trial were euthanized and ceca was collected for Campylobacter enumeration. On d 10 to 12 posthatch, the 2 bacteriocin treatment groups were given free access to feed supplemented with purified, microencapsulated bacteriocins, whereas the positive controls had access to untreated feed. At the end of the 3-d dosing period, ceca were collected from all remaining turkeys (n = 10/pen; 30/trial) for Campylobacter enumeration, and their duodenal loops were collected for morphometric analysis.

**Enumeration of Campylobacter in Cecal Contents**

The cecal contents of each poult were serially diluted 1:9 in buffered phosphate diluent, and 100 μL of each dilution was plated onto Campylobacter Line agar plates (Line, 2001). The plates were incubated for 48 h at 42°C in a microaerobic environment. After incubation, characteristic colonies were confirmed as Campylobacter using a commercial latex agglutination test kit (Panbio Inc., Columbia, MD). The direct counts were converted to log₁₀ colony-forming units per gram of cecal contents. The detection limit for Campylobacter was 1 × 10² cfu/g of cecal contents.

**Morphometric Analysis of the Gut**

The gastrointestinal morphometric variables evaluated were villus height, villus surface area, lamina propria...
In the present study, oral administration of the purified microencapsulated bacteriocins eliminated detectable cecal Campylobacter colonization in young turkeys in 3 separate trials. These findings are consistent with previous studies in which treatment with these same bacteriocins

One of the possible mechanisms by which bacteriocins reduce *Campylobacter* colonization in poultry is by direct bactericidal or bacteriostatic activity. Bacteriocins have been demonstrated to inhibit or kill other foodborne pathogens, such as *Listeria*, *Clostridium*, and *Salmonella*, and are used in food processing and preservation (Daly et al., 1970; Tagg et al., 1976; Natrajan and Sheldon, 2000). Bacteriocin-like compounds have also been shown to have direct antimicrobial activity, in vitro, against *Campylobacter* (Schoeni and Doyle, 1992; Morency et al., 2001; Chaveerach et al., 2004), including the bacteriocins used in this study (Svetoch et al., 2005; N. J. Stern, E. A. Svetoch, B. V. Eruslanov, V. V. Perelygin, E. V. Mitsevich, I. P. Mitsevich, V. D. Pokhilenko, V. P. Levchuk, and O. E. Svetoch, unpublished data).

Another possible mechanism of action of the bacteriocins is physical or functional alteration of *Campylobacter* colonization sites. Use of either bacteriocin in this study reduced both duodenal crypt depth and goblet cell numbers. To our knowledge, this is the first study demonstrating that altering the gastrointestinal tract eliminated detectable *Campylobacter* colonization. Previous research has demonstrated that the mucus layer of intestinal crypts is an important niche for *Campylobacter* colonization in poultry (Beery et al., 1988; Meinersmann et al., 1991). The ability of *Campylobacter* to sequester itself within these crypts may be an important strategy to avoid intervention efforts, such as the use of antibiotics or CE cultures (Mead, 2002; Zhang et al., 2003; Bywater, 2004; Mead, 2004; Farrell et al., 2005). The reduction in crypt depth may have multiple effects on *Campylobacter* colonization. For example, it is possible the smaller crypt size, and subsequent greater exposure to the lumen, may change the nutrient or chemical environment (e.g., increased oxygen tension), limiting *Campylobacter* growth and colonization. It is also possible that different microflora will colonize these smaller crypts, with the ability to outcompete *Campylobacter* (CE).

Another potentially important affect on *Campylobacter* colonization is the reduction in goblet cell numbers following bacteriocin treatment. Mucin glycoproteins are synthesized and secreted from goblet cells, which arise from stem cells at the base of the crypts and migrate toward the villus tip, in which they enter into the lumen.

### Table 2. Effects of bacteriocin treatment on duodenal morphology of turkey poults after oral *Campylobacter* challenge

<table>
<thead>
<tr>
<th>Trial</th>
<th>Villus height (μm)</th>
<th>Villus surface area (μm²)</th>
<th>Lamina propria thickness (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Positive control</td>
<td>1,298.6 ± 72.8a</td>
<td>0.16 ± 0.01a</td>
</tr>
<tr>
<td></td>
<td>B602</td>
<td>1,247.5 ± 55.2a</td>
<td>0.15 ± 0.01ab</td>
</tr>
<tr>
<td></td>
<td>OR7</td>
<td>1,193.1 ± 74.3a</td>
<td>0.12 ± 0.01b</td>
</tr>
<tr>
<td>2</td>
<td>Positive control</td>
<td>1,215.1 ± 66.1a</td>
<td>0.11 ± 0.01a</td>
</tr>
<tr>
<td></td>
<td>B602</td>
<td>1,174.4 ± 64.8a</td>
<td>0.10 ± 0.01a</td>
</tr>
<tr>
<td></td>
<td>OR7</td>
<td>1,042.8 ± 48.7a</td>
<td>0.08 ± 0.01b</td>
</tr>
<tr>
<td>3</td>
<td>Positive control</td>
<td>1,288.2 ± 38.7a</td>
<td>0.11 ± 0.004a</td>
</tr>
<tr>
<td></td>
<td>B602</td>
<td>1,204.3 ± 26.6c</td>
<td>0.10 ± 0.003a</td>
</tr>
<tr>
<td></td>
<td>OR7</td>
<td>1,144.8 ± 43.3c</td>
<td>0.10 ± 0.005a</td>
</tr>
</tbody>
</table>

a,bMeans within columns and trials with no common superscript differ significantly (*P* < 0.05).

1Mean ± SEM representing 10 birds per treatment group from 3 separate trials (*n* = 10 poults/treatment per trial; total 30 poults/trial). Ten separate measurements were made for each parameter per poult. In each trial, poults were orally challenged 3 d posthatch with approximately 10⁶ cfu of a mixture of 3 *Campylobacter coli* isolates. On d 10 to 12 posthatch, the 2 treatment groups were fed a diet containing bacteriocins, and the positive control group was fed a commercial diet without bacteriocins. After 72 h of treatment with bacteriocins, turkeys were euthanized, and duodenal loops were collected for morphometric analysis.
(Cheng and Leblond, 1974; Geyra et al., 2001). Previous research has demonstrated that Campylobacter can use mucin as a nutrient source for growth (Hugdahl et al., 1988; Schoeni and Doyle, 1992). This capability may provide a competitive advantage over other microflora. The ability of bacteriocins to reduce goblet cell number and subsequent mucin production may limit Campylobacter colonization. This idea is supported by previous research, reporting that colonization of C. jejuni in chicks can be influenced by diets that alter mucin production and viscosity (Fernandez et al., 2000).

Although bacteriocin treatment eliminated detectable Campylobacter colonization in this study (detection limit, 10^2 cfu/g of cecal contents), it is possible that undetectable numbers of Campylobacter may still persist in these birds. Previous research from our laboratory has demonstrated that even if Campylobacter is eliminated from most, but not all, enteric locations, the remaining enteric Campylobacter can recolonize the gut within a few days (Farnell et al., 2005). If, however, bacteriocins are dosed just before marketing, the ability of any possible Campylobacter to recolonize the tract would be reduced or prevented. Furthermore, even if bacteriocin treatment did not totally eliminate Campylobacter, the approximately 4-log reduction in Campylobacter concentrations obtained in the current study would provide a significant benefit to human food safety. Research by Rosenquist et al. (2003) reported that even a 2-log reduction in carcass contamination would reduce the human incidence of Campylobacteriosis in human by 30-fold.

In the present study, the administration of bacteriocins isolated from B. circulans and P. polymyxa was effective in eliminating detectable Campylobacter colonization in young commercial turkeys. The mechanism of bacteriocin action on Campylobacter colonization may be related to the ability of these compounds to reduce crypt depth and goblet cell density in young turkeys. The use of bacteriocins may be an important strategy to reduce Campylobacter colonization in poultry.

REFERENCES


