ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

International Journal of Poultry Science 10 (12): 940-945, 2011
ISSN 1682-8356

In vitro Selection of Enteric Microflora for Potential Use as a Competitive Exclusion Culture Against *Campylobacter* in Poultry†

H.P. Bhaskaran[1], A.M. Donoghue[2], K. Arsi[1], A. Wooming[1], I. Reyes-Herrera[1], L.R. Bielke[1], G. Tellez[1], J.A. Byrd[3], P.J. Blore[1], B.M. Hargis[1] and D.J. Donoghue[1]
[1]Department of Poultry Science, University of Arkansas, Fayetteville, AR 72701, USA
[2]Poultry Production and Product Safety Research Unit, ARS, USDA, Fayetteville, AR, 72701, USA
[3]Food and Feed Safety Unit, ARS, USDA, College Station, TX, 77845, USA

Abstract: The administration of nonpathogenic microflora in neonatal poultry has been employed to reduce or eliminate the colonization of enteric pathogens. This concept, also called Competitive Exclusion (CE), although effective against *Salmonella*, has not consistently worked against *Campylobacter*. Most CE cultures are developed by randomly collecting enteric bacteria without any preselection criteria for bacteria. It may be possible to enhance the efficacy of a CE against *Campylobacter* by preselecting enteric microflora with the ability to inhibit *Campylobacter*, *in vitro*. With this goal, an assay was developed to test individual isolates with the ability to reduce or eliminate *Campylobacter* growth, *in vitro*. Individual isolates (n = 137) were collected from ceca of both juvenile and adult poultry and tested for efficacy against *Campylobacter*. Isolates were serially diluted (10^4 or 10^5 CFU/well) and added to 96 well polystyrene plates containing 1 x 10^4 CFU *C. jejuni or C. coli*/well. Plates were incubated at 42°C in a microaerophilic environment for 24-48 h. Following incubation, a 1 µl loop from each well was streaked onto Campy-Cefex agar plate and incubated at 42°C in a microaerophilic environment for 24-48 h. Twenty-three isolates were identified with the ability to inhibit *C. jejuni* or *C. coli* growth *in vitro*. This research demonstrates that it is possible to pre-screen enteric isolates for *Campylobacter* inhibition for use as competitive exclusion cultures.

Key words: *Campylobacter*, enteric microflora, competitive exclusion, foodborne pathogens

INTRODUCTION

Campylobacter and *Salmonella* are the most commonly reported pathogens causing food borne infections in the United States (CDC, 2011). An estimated 2.1 to 2.4 million cases are reported annually (Altekruse *et al*., 1999; CDC, 2010). High proportions of retail chicken and turkey product are contaminated with *Campylobacter* and *Salmonella* (Norkrans and Svedhem, 1982; Genigeorgis *et al*., 1986, Stern and Line, 1992; Zhao *et al*., 2001; Fratamico, 2003) and epidemiological evidence has implicated raw poultry products as a significant source of human infection (Blaser, 1997; Friedman *et al*., 2004).

One approach to reducing pathogen contamination in poultry is to administer Competitive Exclusion (CE) cultures as a preharvest preventative measure. Competitive exclusion can broadly be defined as the oral administration of non-pathogenic intestinal bacteria which are able to establish and eventually colonize the digestive tract and maintain or increase the natural flora to prevent or reduce colonization of pathogenic organisms (Fuller, 1989; Vanbelle *et al*., 1990; Griggs and Jacob, 2005). First described by Nurmi and Rantala (1973), CE has been an effective method of control for salmonellosis in commercial poultry flocks. Since this initial report, numerous researchers have described the beneficial effects of CE against *Salmonella* (Snoeyenbos *et al*., 1978; Rigby and Pettit, 1980; Impey *et al*., 1982, 1984; Stavric *et al*., 1987; Schneitz *et al*., 1990; Cox *et al*., 1991; Schneitz and Nuotio, 1992; Bailey, 1993; Bielke *et al*., 2003; Nisbet *et al*., 1993, 1994, 1996; Corrier *et al*., 1994, 1995a,b; Hume *et al*., 1996a,b; Methner *et al*., 1997; Mead, 2000; Stern *et al*., 2001; Schneitz, 2005; Wolfenden *et al*., 2007; Zhang *et al*., 2007a; Mountzouris *et al*., 2009; Higgins *et al*., 2010).

Although selection of bacteria for CE cultures against *Salmonella* are effective in poultry, efforts to develop consistent CE cultures against *Campylobacter*, have produced limited or inconsistent success (Shanker *et al*., 1990; Stern *et al*., 2001; Mead, 2002). In an effort to overcome these problems, pre-screening enteric bacteria for the ability to inhibit *Campylobacter*, may enhance the efficacy of competitive exclusion products. Therefore, the purpose of this study was to develop an *in vitro* screening method capable of identifying bacterial candidates against *Campylobacter*.

MATERIALS AND METHODS

Collection of cecal isolates: Cecal samples from 6 to 74 wk of age chickens (n = 46) were collected aseptically

Corresponding Author: D.J. Donoghue, Department of Poultry Science, University of Arkansas, Fayetteville, AR 72701, USA

at the University of Arkansas poultry farm. The cecal contents were squeezed into sterile tubes and diluted with Butterfield's Phosphate Diluent (BPD - Difco, Becton Dickison, MD) in three ten-fold dilutions. One hundred microliters of each solution was spread on Blood Agar Plates (BAP - Difco, Becton Dickinson, MD) and the plates were aerobically incubated for 24 h at 37°C. Isolated colonies were picked and streaked onto Tryptic Soy Agar (TSA; EMD, NJ) to assure purity and incubated aerobically at 37°C for 24 h. Single, isolated colonies were individually grown in 5 mL of Tryptic Soy Broth (TSB - Difco, Becton Dickinson, MD) for 8 h or until turbid. Stocks of those bacteria were prepared by centrifuging TSB at 3000 rpm for 10 min, pouring off supernatant and resuspending in 2 mL TSB with 20% filter-sterilized glycerol, the suspensions were dispensed into 1 mL aliquots and stored at -80°C.

Screening for *in vitro* efficacy against Campylobacter: American Type Culture Collection (ATCC) 33291 *Campylobacter jejuni* and a wild type poultry *Campylobacter coli* strain were used in separate experiments. An aliquot of frozen *Campylobacter* culture was taken up by sterile loop and inoculated into 5 ml *Campylobacter* Enrichment Medium (CEM) without antibiotics and incubated for 24-48 h at 42°C in a micro-aerophilic environment (5% O_2, 10% CO_2 and 85% N) and pipetted into 96 well plates. For the bacterial challenge, a total of 137 cecal isolates were tested for their ability to inhibit *Campylobacter*, *in vitro*. A frozen culture of each isolate was thawed, picked up with a sterile plastic loop (10 µL Bac-Loops®) and inoculated into 10 mL CEM without antibiotics and allowed to grow for 12 h at 37°C. Isolates exhibiting turbidity were diluted with CEM and diluted to a final concentration of 10^5 or 10^6 CFU/mL and dispensed in 96 well NUNC™ brand flat bottomed polystyrene plates.

The treatments included: 1) *Campylobacter* alone (positive control), 2) cecal bacterial isolates alone (negative control) or 3) co-incubation with *Campylobacter* and individual cecal isolates. The final concentration in each well was 10^5 CFU/mL *Campylobacter* and either 10^5 or 10^6 CFU/mL of each enteric isolate. The 96 well plates were incubated in micro-aerophilic condition at 42°C for 24-48 h.

Following incubation, the contents of the individual challenge or control were streaked onto Campy-Cefex plates with 1 µl Bac-Loops® for comparison. The plates were incubated in a micro-aerophilic environment (5% O_2, 10% CO_2 and 85% N) at 42°C and were read after 24-48 h. This allowed a subjective assessment of *Campylobacter* survival following co-incubation with the cecal bacteria. The *Campylobacter* positive control plates were defined as 0% inhibition. *Campylobacter* inhibition following cecal bacterial challenges were placed into 5 categories. Those being, 0%, 10-24%, 25-49%, 50-74% or 75-100% inhibition of *Campylobacter* growth.

RESULTS

Development of *in vitro* screening procedure for cecal microflora efficacy against *Campylobacter*: One hundred and thirty-seven isolates were collected and tested for their ability to inhibit the *in vitro* growth of *Campylobacter*. Of the 137 isolates, 14 were highly efficacious in reducing growth of both *C. jejuni* or *C. coli* at concentrations of 10^5 and 10^6 CFU/mL per bacterial isolate *in vitro* (Table 1). Furthermore, for most of these isolates, efficacy was improved at the 10^6 CFU/mL versus 10^5 CFU/mL concentration. An additional nine isolates demonstrated a consistent inhibition in *Campylobacter* growth (both *C. jejuni and C. coli*) at the 10^6 CFU/mL per bacterial isolate treatment. For these nine isolates, reduced efficacy was observed, defined as a reduction in 25-50% *Campylobacter* growth, at the 10^5 CFU/mL concentration (Table 1). The remaining 114 isolates had either inconsistent or no inhibition of *Campylobacter* growth rates when tested in our *in vitro* system.

Table 1: Percent inhibition of *Campylobacter jejuni* or *Campylobacter coli* when co-incubated with individual poultry enteric isolates, *in vitro**

Number of bacterial isolates	Enteric isolate (10^5 CFU/mL)		Enteric isolate (10^6 CFU/mL)	
	C. jejuni	*C. coli*	*C. jejuni*	*C. coli*
3	75-100%	75-100%	75-100%	75-100%
5	50-74%	75-100%	75-100%	75-100%
2	75-100%	50-74%	75-100%	75-100%
4	50-74%	50-74%	75-100%	75-100%
3	---------- Less than 25-49% ----------		50-74%	75-100%
2	---------- Less than 25-49% ----------		75-100%	50-74%
4	---------- Less than 25-49% ----------		50-74%	50-74%
114	-------- No or inconsistent efficacy ---------		---------- No or inconsistent efficacy ----------	

*Percent *Campylobacter* inhibition was determined following co-incubation with cecal bacteria *in vitro* for 24-48 h at 42°C. *Campylobacter* inhibition (%) was estimated by comparing the growth of *Campylobacter* co-incubated with individual isolates versus *Campylobacter* alone (0% inhibition). *Campylobacter* inhibition was placed into 5 categories: 0%, 10-24%, 25-49%, 50-74% or 75-100%

DISCUSSION

Previous efforts to develop Competitive Exclusion (CE) cultures against *Campylobacter* colonization in poultry have had limited success (Mead, 2002). These poor results may be due to the lack of pre-selection criteria used for collection of bacteria for development of CE cultures. Most CE cultures are obtained by randomly collecting cecal micro flora from healthy poultry (Nurmi and Rantala, 1973; Stavric *et al*., 1987; Nisbet *et al*., 1993; Corrier *et al*., 1995a,b). This strategy has successfully been used for developing effective CE cultures against *Salmonella* (Bailey *et al*., 1988; Aho *et al*., 1989; Qin *et al*., 1995; Stern *et al*., 2001; Heres *et al*., 2003), but not against *Campylobacter* (Soerjadi-Liem *et al*., 1984; Stern *et al*., 2001; Mead, 2002; Patterson and Burkholder, 2003).

Using a novel approach, we have successfully developed a technique to pre-select for cecal microbes with the potential to reduce *Campylobacter* growth *in vitro*. This technique is selective, with varying degrees of efficacy against *Campylobacter*. Of the 137 isolates tested, 23 bacterial isolates demonstrated the ability to consistently reduce *Campylobacter* growth and 14 of these isolates were highly efficacious in our system. Several investigators have attempted to improve CE cultures by mimicking properties of efficacious bacteria, defining cultures or quantitating the effect within the gastrointestinal tract. Schoeni and Doyle (1992) isolated cecum-colonizing bacteria that produced anti-*Campylobacter* metabolites from *C. jejuni*-free hens and demonstrated that these isolates could provide some protection against *C. jejuni* in chicks. These investigators focused on mechanisms of *Campylobacter* colonization in birds, specifically the chemoattraction of *Campylobacter* to mucin and its ability to use mucin as a substrate. They isolated potential CE bacteria that could grow on mucin as a sole substrate and that occupied the same niche in the cecum as *Campylobacter*. Zhang and co-workers (2007b) have also developed a unique method for collecting isolates with *in vitro* efficacy against *Campylobacter*. This group identified chickens that did not become colonized with *Campylobacter* and isolated bacteria from the enteric tracts of these birds. Using this strategy they have also demonstrated *in vivo* efficacy of defined bacterial isolates against *Salmonella* (Zhang *et al*., 2007a).

The mechanisms by which the enteric isolates from this study inhibited *Campylobacter* in our system are probably due to either out-competing *Campylobacter* for nutrients and/or producing products (i.e. bacteriocins) that inhibit the pathogen's ability to grow. Although competition for nutrients has been proposed for microorganisms (Grover, 2009; Grover *et al*., 2011; Saito and Miki, 2010), to our knowledge, this type of interaction has not been demonstrated in the poultry gut. For bacteriocins, however, previous research has shown efficacy against *Campylobacter* in poultry. Bacteriocins are defined as biologically active proteinaceous compounds produced by certain strains of bacteria which are bactericidal to other closely related bacteria (Tagg *et al*., 1976). It has been demonstrated that bacteriocins can significantly reduce *Campylobacter* colonization in broiler chickens and turkeys (Stern *et al*., 2005, 2006; Cole *et al*., 2006). It has been suggested that bacteriocin treatment may be an effective and feasible strategy to reduce *Campylobacter* in poultry (Lin, 2009; Svetoch and Stern, 2010). In conclusion, we have developed an *in vitro* method capable of identifying bacterial CE isolates with the potential to reduce *Campylobacter* in poultry.

REFERENCES

Aho, M., M. Kurki, H. Rautelin and T.U. Kosunen, 1989. Waterborne outbreak of *Campylobacter* enteritis after an outdoors infantry drill in Utti, Finland. Epidemiol. Infect., 103: 133-141.

Altekruse, S.F., N.J. Stern, P.I. Fields and D.L. Swerdlow, 1999. *Campylobacter jejuni*-an emerging foodborne pathogen. Emerg. Infect. Dis., 5: 28-35.

Bailey, J.S., 1993. Control of *Salmonella* and *Campylobacter* in poultry production. A summary of work at Russell Research Center. Poult. Sci., 72: 1169-1173.

Bailey, J.S., L.C. Blankenship, N.J. Stern, N.A. Cox and F. McHan, 1988. Effect of anticoccidial and antimicrobial feed additives on prevention of *Salmonella* colonization of chicks treated with anaerobic cultures of chicken feces. Avian Dis., 32: 324-329.

Bielke, L.R., A.L. Elwood, D.J. Donoghue, A.M. Donoghue, L.A. Newberry, N.K. Neighbor and B.M. Hargis, 2003. Approach for selection of individual enteric bacteria for competitive exclusion in turkey poults. Poult. Sci., 82: 1378-1382.

Blaser, M.J., 1997. Epidemiologic and clinical features of *Campylobacter jejuni* infections. J. Infect. Dis., 176(Suppl. 2): S103-S105.

CDC, 2010. Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases, http://www.cdc.gov/nczved/divisions/dfbmd/diseases/campylobacter/ (Accessed 10 November 2011).

CDC, 2011. Centers for Disease Control and Prevention, CDC Estimates of Foodborne Illness in the United States. http://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html (Accessed 10 November 2011).

Cole, K., M.B. Farnell, A.M. Donoghue, N.J. Stern, E.A. Svetoch, B.N. Eruslanov, L.I. Volodina, Y.N. Kovalev, V.V. Perelygin, E.V. Mitsevich, I.P. Mitsevich, V.P. Levchuk, V.D. Pokhilenko, V.N. Borzenkov, O.E. Svetoch, T.Y. Kudryavtseva, I. Reyes-Herrera, P.J. Blore, F. Solis de los Santos and D.J. Donoghue, 2006. Bacteriocins reduce *Campylobacter* colonization and alter gut morphology in turkey poults. Poult. Sci., 85: 1570-1575.

Corrier, D.E., D.J. Nisbet, C.M. Scanlan, G. Tellez, B.M. Hargis and J.R. Deloach, 1994. Inhibition of *Salmonella enteritidis* cecal and organ colonization in leghorn chicks by a defined culture of cecal bacteria and dietary lactose. J. Food. Prot., 56: 377-381.

Corrier, D.E., D.J. Nisbet, C.M. Scanlan, A.G. Hollister and J.R. Deloach, 1995a. Control of *Salmonella typhimurium* colonization in broiler chicks with a continuous-flow characterized mixed culture of cecal bacteria. Poult. Sci., 74: 916-924.

Corrier, D.E., D.J. Nisbet, C.M. Scanlan, A.G. Hollister, D.J. Caldwell, L.A. Thomas, B.M. Hargis, T. Tomkins and J.R. Deloach, 1995b. Treatment of commercial broiler chickens with a characterized culture of cecal bacteria to reduce *Salmonellae* colonization. Poult. Sci., 74: 1093-1101.

Cox, N.A., J.S. Bailey and L.C. Blankenship, 1991. Alternative administration of competitive exclusion treatment. In: Blankenship, L.C. (Ed.), Colonization control of human bacterial enteropathogens in poultry. Academic Press, San Diego, CA pp: 105-118.

Fratamico, P.M., 2003. Comparison of culture, polymerase chain reaction (PCR), TaqMan *Salmonella* and Transia Card *Salmonella* assays for the detection of *Salmonella* spp. In naturally-contaminated ground chicken, ground turkey and ground beef. Mol. Cell Probes, 17: 215-221.

Friedman, C.R., R.M. Hoekstra, M. Samuel, R. Marcus, J. Bender, B. Shiferaw, S. Reddy, S.D. Ahuja, D.L. Helfrick, F. Hardnett, M. Carter, B. Anderson and R.V. Tauxe, 2004. Risk factors for sporadic *Campylobacter* infection in the United States: A case-control study in FoodNet sites. Clin. Infect. Dis., 38: S285-296.

Fuller, R., 1989. Probiotics in man and animals. J. Appl. Bacteriol., 66: 365-378.

Genigeorgis, C., M. Hassuney and P. Collins, 1986. *Campylobacter jejuni* infection on poultry farms and its effect on poultry meat contamination during slaughter. J. Food Protect., 49: 895-903.

Griggs, J.P. and J.P. Jacob, 2005. Alternatives to antibiotics for organic poultry production. J. Appl. Poult. Res., 14: 750-756.

Grover, J.P., 2009. Is storage an adaptation to spatial variation in resource availability? Am. Nat., 173: E44-E61.

Grover, J.P., S.B. Hsu and F.B. Wang, 2011. Competition between microorganisms for a single limiting resource with cell quota structure and spatial variation. J. Math. Biol., (Epub ahead of print).

Heres, L., B. Engel, F. Van Knapen, M.C. de Jong, J.A. Wagenaar and H.A. Urlings, 2003. Fermented liquid feed reduces susceptibility of broilers for *Salmonella enteritidis*. Poult. Sci., 82: 603-611.

Higgins, J.P., S.E. Higgins, A.D. Wolfenden, S.N. Henderson, A. Torres-Rodriguez, J.L. Vicente, B.M. Hargis and G. Tellez, 2010. Effect of lactic acid bacteria probiotic culture treatment timing on *Salmonella* Enteritidis in neonatal broilers. Poult. Sci., 89: 243-247.

Hume, M.E., A.G. Hollister, D.J. Nisbet, D.E. Corrier and J.R. DeLoach, 1996a. Effect of a characterized continuous-flow culture of cecal bacteria on *Salmonella typhimurium* crop colonization in broiler chicks. Avian Dis., 40: 391-397.

Hume, M.E., D.E. Corrier, D.J. Nisbet and J.R. Deloach, 1996b. Reduction of *Salmonella* crop and cecal colonization by a characterized competitive exclusion culture in broilers during grow-out. J. Food. Prot., 59: 688-693.

Impey, C.S., G.C. Mead and S.M. George, 1982. Competitive exclusion of *Salmonellas* from the chick caecum using a defined mixture of bacterial isolates from the caecal microflora of an adult bird. J. Hyg., 89: 479-490.

Impey, C.S., G.C. Mead and S.M. George, 1984. Evaluation of treatment with defined and undefined mixtures of gut microorganisms for preventing *Salmonella* colonization in chicks and turkey poults. Food Microbiol., 1: 143-147.

Lin, J., 2009. Novel approaches for *Campylobacter* control in poultry. Foodborne Pathog. Dis., 6: 755-765.

Mead, G.C., 2000. Prospects for 'competitive exclusion' treatment to control *Salmonellas* and other foodborne pathogens in poultry. Vet. J., 159: 111-123.

Mead, G.C., 2002. Factors affecting intestinal colonization of poultry by *Campylobacter* and role of microflora in control. World's Poult. Sci., 58: 169-178.

Methner, U., P.A. Barrow, G. Martin and H. Meyer, 1997. Comparative study of the protective effect against *Salmonella* colonization in newly hatched SPF chickens using live, attenuated *Salmonella* vaccine strains, wild-type *Salmonella* strains or a competitive exclusion product. Int. J. Food Microbiol., 35: 223-230.

Mountzouris, K.C., C. Balaskas, I. Xanthakos, A. Tzivinikou and K. Fegeros, 2009. Effects of a multi-species probiotic on biomarkers of competitive exclusion efficacy in broilers challenged with *Salmonella* Enteritidis. Br. Poult. Sci., 50: 467-478.

Nisbet, D.J., D.E. Corrier, C.M. Scanlan, A.G. Hollister, R.C. Beier and J.R. Deloach, 1993. Effect of a defined continuous-flow derived bacterial culture and dietary lactose on *Salmonella typhimurium* colonization in broiler chickens. Avian Dis., 37: 1017-1025.

Nisbet, D.J., S.C. Ricke, C.M. Scanlan, D.E. Corrier, A.G. Hollister and J.R. Deloach, 1994. Inoculation of broiler chicks with a continuous-flow derived bacterial culture facilitates early cecal bacterial colonization and increases resistance to *Salmonella typhimurium*. J. Food. Prot., 57: 12-15.

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. Prot., 59: 1279-1283.

Norkrans, G. and A. Svedhem, 1982. Epidemiologic aspects of *Campylobacter jejuni* enteritis. J. Hygiene., 89: 163-70.

Nurmi, E. and M. Rantala, 1973. New aspects of *Salmonella* infection in broiler production. Nature, 241: 210-211.

Patterson, J.A. and K.M. Burkholder, 2003. Application of prebiotics and probiotics in poultry production. Poult. Sci., 82: 627-631.

Qin, Z.R., T. Fukata, E. Baba and A. Arakawa, 1995. Effect of lactose and *Lactobacillus acidophilus* on the colonization of *Salmonella enteritidis* in chicks concurrently infected with *Eimeria tenella*. Avian Dis., 39: 548-553.

Rigby, C.E. and J.R. Pettit, 1980. Observations on competitive exclusion for preventing *Salmonella typhimurium* infection of broiler chickens. Avian Dis., 24: 604-615.

Saito, Y. and T. Miki, 2010. Species coexistence under resource competition with intraspecific and interspecific direct competition in a chemostat. Theor. Popul. Biol., 78: 173-182.

Schneitz, C., M. Hakkinen, L. Nuotio, E. Nurmi and G. Mead, 1990. Droplet application for protecting chicks against *Salmonella* colonization by competitive exclusion. Vet. Rec., 126: 510.

Schneitz, C. and L. Nuotio, 1992. Efficacy of different microbial preparations for controlling *Salmonella* colonization in chicks and turkey poults by competitive exclusion. Br. Poult. Sci., 33: 207-211.

Schneitz, C., 2005. Competitive exclusion in poultry: 30 years of research. Food Control, 16: 657-667.

Schoeni, J.L. and M.P. Doyle, 1992. Reduction of *Campylobacter jejuni* colonization of chicks by cecum-colonizing bacteria producing anti-*C. jejuni* metabolites. Appl. Environ. Microbiol., 58: 664-670.

Shanker, S., A. Lee and T.C. Sorrell, 1990. Horizontal transmission of *Campylobacter jejuni* amongst broiler chicks: experimental studies. Epidemiol. Infect., 104: 101-110.

Snoeyenbos, G.H., O.M. Weinack and C.F. Smyser, 1978. Protecting chicks and poults from *Salmonellae* by oral administration of normal gut microflora. Avian Dis., 22: 273-287.

Soerjadi-Liem, A.S., G.H. Snoeyenbos and O.M. Weinack, 1984. Comparative studies on competitive exclusion of three isolates of *Campylobacter fetus* subsp. *jejuni* in chickens by native gut microflora. Avian Dis., 28: 139-146.

Stavric, S.T.M.G., B. Blanchfield and H. Pivnick, 1987. Role of adhering microflora in competitive exclusion of *Salmonella* from young chicks. J. Food. Prot., 50: 928-932.

Stern, N.J. and J.E. Line, 1992. Comparison of three methods for recovery of *Campylobacter* from broiler carcasses. J. Food Anim. Protect., 55: 663-666.

Stern, N.J., N.A. Cox, J.S. Bailey, M.E. Berrang and M.T. Musgrove, 2001. Comparison of mucosal competitive exclusion and competitive exclusion treatment to reduce *Salmonella* and *Campylobacter* sp. colonization in broiler chickens. Poult. Sci., 80: 156-160.

Stern, N.J., E.A. Svetoch, B.V. Eruslanov, Y.N. Kovalev, L.I. Volodina, V.V. Perelygin, E.V. Mitsevich, I.P. Mitsevich and V.P. Levchuk, 2005. *Paenibacillus polymyxa* purified bacteriocin to control *Campylobacter jejuni* in chickens. J. Food Prot., 68: 1450-1453.

Stern, N.J., E.A. Svetoch, B.V. Eruslanov, V.V. Perelygin, E.V. Mitsevich, I.P. Mitsevich, V.D. Pokhilenko, V.P. Levchuk, O.E. Svetoch and B.S. Seal, 2006. Isolation of a *Lactobacillus salivarius* strain and purification of its bacteriocin, which is inhibitory to *Campylobacter jejuni* in the chicken gastrointestinal system. Antimicrob. Agents Chemother., 50: 3111-3116.

Svetoch, E.A. and N.J. Stern, 2010. Bacteriocins to control *Campylobacter* sp. in poultry - A review. Poult. Sci., 89: 1763-1768.

Tagg, J.R., A.S. Dajani and L.W. Wannamaker, 1976. Bacteriocins of gram-positive bacteria. Bacteriol. Rev., 40: 722-775.

Vanbelle, M., E. Teller and M. Focant, 1990. Probiotics in animal nutrition: A review. Arch. Tierernahr., 40: 543-567.

Wolfenden, A.D., J.L. Vicente, L.R. Bielke, C.M. Pixley, S.E. Higgins, D.J. Donoghue, A.M. Donoghue, B.M. Hargis and G. Tellez, 2007. Effect of a defined competitive exclusion culture for prophylaxis and reduction of horizontal transmission of *Salmonella Enteritidis* in broiler chickens. Int. J. Poult. Sci., 6: 489-492.

Zhao, C., B. Ge, J. De Villena, R. Sudler, E. Yeh, S. Zhao, D.G. White, D. Wagner and J. Meng, 2001. Prevalence of *Campylobacter* sp., *Escherichia coli* and *Salmonella* serovars in retail chicken, turkey, pork and beef from the greater Washington D.C. area. Appl. Environ. Microbiol., 67: 5431-5436.

Zhang, G., M.A. Li and M.P. Doyle, 2007a. *Salmonellae* reduction in poultry by competitive exclusion bacteria *Lactobacillus salivarius* and *Streptococcus cristus*. J. Food Protect., 70: 874-878.

Zhang, G., M.A. Li and M.P. Doyle, 2007b. Potential competitive exclusion bacteria from poultry inhibitory to *Campylobacter jejuni* and *Salmonella*. J. Food Protect., 70: 867-873.

[†]Mention of trade names or commercial products in this report is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture