Chapter 12
Biocontrol of Pathogens in the Meat Chain

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Introduction

Bacterial foodborne zoonotic diseases are of major concern, impacting public health and causing economic losses for the agricultural-food sector and the wider society. In the United States (US) alone foodborne illness from pathogens is responsible for 76 million cases of illnesses each year (Mead et al., 1999). *Salmonella*, *Campylobacter jejuni* and Enterohaemorraghic *Escherichia coli* (EHEC; predominately serotype O157:H7) and *Listeria monocytogenes* are the most predominant foodborne bacterial pathogens reported in the developed world (United States Department of Agriculture, 2001). The importance of meat and meat products as a vehicle of foodborne zoonotic pathogens cannot be underestimated (Center for Disease Control, 2006; Gillespie, O'Brien, Adak, Cheasty, & Willshaw, 2005; Mazick, Ethelberg, Nielsen, Molbak, & Lisby, 2006; Mead et al., 2006). Pathogen carriage in food animals, such as livestock and poultry can lead to both direct and indirect contamination of raw and processed meats. Hide contamination and fecal pathogen shedding contribute to the contamination of the beef carcass (Elder et al., 2000; Koohmaraie et al., 2005), while skin and feathers contaminated with feces serve as major sources of poultry contamination (Doyle & Erickson, 2006). Processing of meat can further spread microbial contamination, while inadequate temperature control can allow pathogens to increase in numbers. Eradication of pathogens from farm livestock and the environment is not yet an achievable goal. However, risk reduction measures can be implemented on the farm to minimize the risk of infection. During meat slaughter and processing, methods to ensure food safety and preservation may include a range of chemical preservative agents and/or physical processing intervention strategies. However, increased consumer demand for healthier and minimally processed food with lower amounts of additives as well as concerns regarding antibiotic resistance in foodborne bacteria has led to a greater interest and demand for natural, biological methods of food preservation and safety.

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Extensive research on alternative biological methods (biocontrol) for biopreservation and reduction of foodborne pathogens is an active area of research. Numerous methods have been reported for the reduction of food pathogens at the pre-harvest stage (in the animal) and post-harvest and processing stages of the meat chain. These approaches include the use of organic compounds, vaccines and bacteriophages as well as the use of antagonistic bacteria (Callaway et al., 2004; Joerger, 2003; Koohmaraie et al., 2005; LeJeune & Wetzel, 2007). Antagonism refers to the inhibition of other (e.g., undesired or pathogenic) microorganisms through either competitive exclusion or by the production of one or more antimicrobial active metabolites such as organic (lactic and acetic) acids, hydrogen peroxide, or bacteriocins (Holzapfel, Geisen, & Schillinger, 1995; Kostrzynska & Bachand, 2006). The criteria for biocontrol agents is that they must be (1) efficacious, (2) practical, and (3) safe and not interfere with animal growth or development (Doyle & Erickson, 2006). This chapter reviews various biocontrol agents and their applications to reduce the carriage of key food pathogens *Salmonella*, EHEC, *Listeria* and *Campylobacter* in animals or levels of these pathogens on carcasses or processed meats.

Organic Compounds

Organic Acids

Organic acid solutions are the most frequently used decontaminants in meat processing. Organic acids including acetic and lactic acid are widely used in the USA to decontaminate carcasses. The use of these organic acid solutions at concentrations of up to 2.5% has been approved by the US Department of Agriculture's Food Safety and Inspection service (USDA-FSIS) (1996). Such solutions are usually applied to a carcass following hide removal when the carcass is still warm. Numerous studies have reported the effects of various organic acid solutions on general microflora and pathogenic organisms on meat products (Table 12.1). The effectiveness of organic acid solutions in inactivating or removing bacterial pathogens on a carcass and meat product varies considerably (Table 12.1) (Dorsa, 1997; Huffman, 2002; Smulders & Greer, 1998). Hardin et al. (1995) reported that a beef carcass wash followed by a 2% acid spray was more effective than either trimming or washing with water alone in the reduction of *E. coli* O157:H7 and *S*. Typhimurium. In many studies, however, sanitizing rinses were either ineffective in reducing the level of *E. coli* O157:H7 on beef tissues or only reduced the bacterial counts by 1 to 2 log CFU cm^{-2} (Brackett, Hao, & Doyle, 1994; Fratamico, Schultz, Benedict, & Buchanan, 1996).

Recently, concerns have been expressed about whether acidic decontamination may induce acid resistance in pathogens (Samelis, Sofos, Kendall, & Smith, 2002). The efficiency of such compounds can also be dependent on a number of environmental factors which can influence application in various food products. For example, the antimicrobial effect of organic acids can be influenced by pH, tissue type and

Table 12.1 Examples of organic acids used in the decontamination of meat products

Organic acid	Concentration tested (%)	Meat type	Target pathogen(s)	Reduction (log_{10} CFU)	Reference
Acetic acid	2	Chicken breast	*L. monocytogenes*, *S. enteritidis* *E. coli* O157:H7	1.8–2.0 0.9–1.7 1.8–2.6	Anang, Rusul, Bakar, & Ling (2007)
Acetic acid, lactic acid, citric acid	1.5	Lean beef	*E. coli* O157:H7	0.3–0.5	Brackett et al. (1994)
Acetic acid	2	Beef tenderloin and adipose tissue	*E. coli* O157:H7 and *E. coli* K12	No effect compared to control	Fratamico et al. (1996)
Acetic or lactic acid	3	Lean beef	*L. monocytogenes* *S.* Typhimurium *E. coli* *C. jejuni*	~1.0–1.2 ~0.5–1.0 ~0.5 ~1.0–1.2	Greer and Dilts (1992)
Lactic acid	4	Chilled beef carcasses	Natual microflora	3.0–3.3 on aerobic plate counts.	Castillo, Lucia, Mercado, and Acuff (2001)
			Total coliforms	Undetectable levels	Castillo, Lucia, Mercado, and Acuff (2001)
Lactic acid	2.5–5	Lean beef	*E. coli* O157:H7	0.9–1.1	Heller et al. (2007)
Fumaric acid	1	Lean beef	*L. monocytogenes*	0.1–0.9	Podolak, Zayas, and Kastner (1996)
Lactic acid	1			0.1–0.5	
Acetic acid	1			0.1–0.5	
Fumaric acid	1		*E. coli* O157:H7	0.4–1.3	
Lactic acid	1			0.1–0.8	
Acetic acid	1			0.1–0.8	

bacterial microorganisms (Smulders & Greer, 1998). Sensory effects such as color, flavor or odor can also be a major concern. Dilute organic acid solutions of up to 3%, generally, have no effect on the desirable sensory properties of meat when used as a carcass decontaminant. However, some treatment conditions using lactic and acetic acid can produce adverse sensory and appearance changes when applied directly to meat cuts or products (Smulders & Greer, 1998). Further evaluation of organic acids and their effects in meat processing and their wider impact on pathogens continues to be researched and improved.

Essential Oils

Essential oils (EOs) are aromatic oily liquids obtained from plant materials. The greatest use of EOs is as flavorings in food; however, their antimicrobial potential has attracted increased attention and research. EOs can comprise more than 60 individual components, where the major components make up 85% of the EO and other components are present as trace (Burt, 2004). The mode of action of EOs is relatively unknown (Burt, 2004; Lambert, Skandamis, Coote, & Nychas, 2001). However, the extensive range of EOs and components suggest that there is most likely to be many different modes of action (Burt, 2004). The phenolic components of EOs are chiefly responsible for the antibacterial properties of the EOs and usually EOs containing a high percentage of phenolic compounds have the strongest activity (Lambert et al., 2001). Many studies have reported the antimicrobial effect of EOs or their components against foodborne pathogens (Burt, 2004; Burt & Reinders, 2003; Hammer, Carson, & Riley, 1999; Lambert et al., 2001; Si et al., 2006). EOs have a broad spectrum of activity, but are slightly more active against Gram-positive than Gram-negative bacteria (Lambert et al., 2001). Application of EOs and their components have been reported in many foods, including meat products (Table 12.2). The physical structure and composition of the food matrix as well as the environmental conditions of the food (e.g. temperature, vacuum/gas/air packaging) can affect the efficiency of the EO against pathogens in foods (Tassou, Drosinos, & Nychas, 1995; Tsigarida, Skandamis, & Nychas, 2000). For example, high levels of fat and/or protein in foods such as meat products can protect the bacteria from the antimicrobial activity of the EO, whereby the EO can dissolve in the lipid phase of the food and will therefore be less available against bacteria (Gill, Delaquis, Russo, & Holley, 2002; Tassou et al., 1995). Many studies have attempted to improve microbial quality of meats by combining the use of EOs with other preservation techniques, such as different packaging environments, radiosensitization, bacteriocins, and incorporation of EOs into packaging films (Brashears, Reilly, & Gilliland, 1998; Chiasson, Borsa, Ouattara, & Lacroix, 2004; Ghalfi, Benkerroum, Doguiet, Bensaid, & Thonart, 2007; Gill et al., 2002; Tsigarida et al., 2000). An important aspect to consider in the application of EOs to foods is the effect on the organo-leptic properties of the food product. Low concentrations of EOs in meat products have been reported to be acceptable after storage and

Table 12.2 Examples of essential oils used to control foodborne pathogens in meat products

Essential oil or component	Meat type	Concentration tested	Target pathogen(s)	Storage conditions	Reduction (log_{10}CFU g^{-1})	Reference
Carvacrol	Ground beef	1.0%	*E. coli* *S*. Typhimurium	Treated with radiosensitization for 24 h at 4°C	Relative sensitivity increased by 2.2 times.	Chiasson et al. (2004)
Oregano oil Savory oil	Pork meat	50 ul /100 g^{-1}	*L. monocytogenes*	42 d, 4°C	0.12–4.8 0.2–2.7	Ghalfi et al. (2007)
Cilantro oil	Ham	0.1–0.6%	*L. monocytogenes*	Vacuum-packed. Oil incorporated into gelatin film 28 d, 10°C.	1.3 in first week but no effect after this	Gill et al. (2002)
Eugenol	Cooked chicken breast	0.1 ml spread onto 25 g chicken pieces	*L. monocytogenes*	14 d, 5°C 15°C	0.79–1.73 0.7–2.2	Hao, Brackett, and Doyle (1998a)
	Cooked beef	0.1 ml spread onto 25 g beef pieces	*L. monocytogenes*	14 d, 5°C 15°C	0.61–0.96 0.60–1.77	Hao, Brackett, and Doyle (1998a)
Mustard oil	Cooked acidified chicken meat	0.10%	*E. coli*	14 d, 22°C	~ 2.0	Lemay et al. (2002)
Oregano oil	Beef fillets	0.80%	*S*. Typhimurium	Aerobic, vacuum package or MAP, 20–25 d, 5°C.	1–2 all conditions.	Skandamis, Tsigarida, and Nychas (2002)
		0.80%	*L. monocytogenes*	Aerobic, vacuum package or MAP, 12–15d, 5°C.	2–3 all conditions.	Tsigarida et al. (2000)
Clove oil	Minced mutton	0.5–1.0%	*L. monocytogenes*	15 d, 7°C 30°C	0.2–2.5 0.5–2.7	Vrinda Menon and Garg (2001)

cooking (Tsigarida et al., 2000). Oregano oil (1% v/w) was also found to improve the flavor and quality of minced meat following storage in modified atmospheres (Skandamis & Nychas, 2001). Further studies with respect to the interactions of EOs and their components with food constituents is required to improve the efficacy of EOs against food spoilage and pathogenic organisms whilst minimizing impact on the organo-leptic properties of the product.

Antagonistic Bacteria

Bacterial Metabolites

The production of one or more antimicrobial active metabolites is part of the complex mechanisms by which a culture becomes established in the presence of other competing organisms (Holzapfel et al., 1995). Along with bacteriocins, bacteria can produce many types of substances or metabolites that are inhibitory to other bacteria. These can include clinical or therapeutic low-molecular weight antibiotics, lytic agents, toxins, bacteriolytic enzymes, bacteriophages and other metabolic products such as hydrogen peroxide and diacetyl (Holzapfel et al., 1995; Kostrzynska & Bachand, 2006). These substances can act as bio-preservatives by inhibiting spoilage or pathogenic microorganisms (Deegan, Cotter, Hill, & Ross, 2006). The main mechanism by which lactic acid bacteria (LAB) inhibit microorganisms is through the production of organic acids such as lactic acid and acetic acid. Organic acids produced by LAB, including lactic, acetic and propionic acid, exert antimicrobial effects due to their action on the bacterial cytoplasmic membrane, which interferes with the maintenance of membrane potential and inhibits active transport (Kostrzynska & Bachand, 2006). The use of LAB is common in sausage fermentations, in which accumulating lactic acid levels inhibit meat-borne pathogenic bacteria (Lucke, 2000). In addition, the inhibitory property of hydrogen peroxide has also been reported. *Lactobacillus lactis* can produce hydrogen peroxide which in effect can reduce the numbers of *E. coli* O157 (0.37–1.09 $\log_{10}$ CFU ml^{-1} lower counts compared to controls) on refrigerated raw chicken (Brashears et al., 1998). Senne & Gilliland (2003) reported that the application of *Lb. delbrueckii* subsp. *lactis* (also found to produce hydrogen peroxide) could reduce the numbers of *E. coli* O157:H7 (0.8–1.3 $\log_{10}$ CFU cm^{-2}) and *S*. Typhimurium (0.8–1.5 $\log_{10}$ CFU cm^{-2}) on pork and beef carcasses kept in refrigerated storage. Reuterin, a broad-spectrum low-molecular weight antimicrobial substance produced by *Lb. reuteri* during glycerol conversion has also been reported as a potential biopreservative for food. One study found that *Lb. reuteri* in the presence of glycerol was highly effective against inocula of *E. coli* O157:H7 of 3 $\log_{10}$ CFU g^{-1} and 6 $\log_{10}$ CFU g^{-1} levels in ground beef during refrigerated storage in modified atmosphere packages (Muthukumarasamy, Han, & Holley, 2003). Reuterin was also reported to inhibit **the** growth of *L. monocytogenes* but not *Salmonella* spp. on the surface of sausages (Kuleasan & Cakmakci, 2002).

Limitations of Using Metabolites

Although some metabolites of LAB have been shown to inhibit Gram-negative bacteria their use in maintaining the safety and stability of meat products is not ideal as some metabolites can interfere with the sensory properties (e.g. hydrogen peroxide) of the food or may not be produced in sufficient amounts (e.g. reuterin). Research may provide methods to engineer metabolic pathways to give better control of the rate and extent of formation of lactic and acetic acids, and to eliminate unwanted properties such as formation of biogenic amines. Depending on the product and processing situation, one or more of these metabolites may constitute a basis for the selection of a protective culture (Holzapfel et al., (1995). It must be emphasized that LAB used to reduce pathogens in foods should not affect the sensory characteristic of the foods, ensuring that foods are palatable to consumers (Kostrzynska & Bachand, 2006).

Competitive Exclusion Technology

Competitive exclusion (CE) as a technology, involves the addition of a non-pathogenic bacterial culture to the intestinal tract of food animals in order to reduce colonization or decrease populations of pathogenic bacteria in the gastrointestinal tract (Callaway et al., 2004). A CE culture may be composed of one or many strains or species of bacteria, but should ideally be composed of species normally resident in the animal intestinal microflora. The use of CE is similar to using probiotics which are defined as 'a preparation of or a product containing viable, defined microorganisms in sufficient numbers which alter the microflora of the host and exert beneficial health effects in this host' (de Vrese, & Schrezenmeir, 2002). In contrast to CE, probiotic preparations generally consist of individual species or mixtures of LAB or yeasts that are not necessarily of animal origin (Callaway et al., 2004). The use of probiotics in food animals have been extensively reviewed (Callaway et al., 2004; Nava, Bielke, Callaway, & Castaneda, 2005; Wagner, 2006). This section will only focus on the use of CE cultures as a food safety strategy in food animals.

The precise mechanism by which CE microorganisms reduce pathogens in the animal intestine is unclear, however, the main role of CE cultures is to attach to the surface of the intestinal epithelium and establish itself within the gut. This direct binding of the CE culture to the intestinal wall prevents potentially pathogenic strains from attaching to it. Some of the bacteria may produce antimicrobial compounds such as acids or bacteriocins to eliminate species competing within the same niche (Callaway et al., 2004). Some of these antimicrobial compounds have been specifically investigated for use as biopreservatives and for food safety applications in meat and their products and will be discussed later in this chapter. CE cultures may be composed of **defined** microbial strains (known and characterized) or undefined (incompletely characterized or unknown) microbial strains. The use of CE cultures has been used extensively in poultry to reduce *Salmonella* and *Campylobacter* carriage (Chen & Stern, 2001; La Ragione & Woodward, 2003;

Wagner, 2006; Zhang, Ma, & Doyle, 2007a, 2007b). The use of CE cultures, including commercial products in poultry has been reported to reduce the colonization of poultry with *Salmonella* spp. by up to 70% or by 7–9 $\log_{10}$ cycles (Davies & Breslin, 2003; Hoszowski & Truszczynski, 1997; Schneitz & Hakkinen, 1998). Reductions of between 3–100% **of** *Campylobacter* spp. colonization on poultry has also been reported (Schoeni & Wong, 1994). The use of CE cultures in cattle and pigs to eliminate *E. coli* O157:H7 and/or *Salmonella* from rumen and gastrointestinal tract have also shown potential for further commercial development (Brashears, Jaroni, & Trimble, 2003; Genovese et al., 2003; Zhao et al., 2003).

Limitations of Using CE Cultures

The most important property of a CE culture is the establishment of a complex intestinal microbiota that resists colonization by human and animal pathogens. While CE has been shown to work in several animal species, the benefits have not been consistent. The differing results between studies involving CE in animals may be due to the difference between host animals, cultures, or experimental designs. Although the benefit of using CE to reduce human pathogen carriage in animals has enormous potential, many of the effective and commercially available CE products have their disadvantages. Firstly, the use of CE culture in which the microbial isolates are unknown requires regulation as an animal drug due to the cultures' effects on animal health and the risk of transfer of undesirable bacteria to humans. There is also the potential of these component bacteria of CE to transfer virulence genes onto the animal or human microbiota resulting in enhanced antimicrobial resistance (Wagner, 2006). Ongoing research in this area aims to characterize the bacteria present in CE products which will lead to safe assurance of use of the product and will allow optimization of their efficacy.

Bacteriocin-Producing Bacteria and Bacteriocins

Bacteriocins are ribosomally synthesized antimicrobial peptides or proteins produced by bacteria that kill or inhibit the growth of other bacteria, either in the same species (narrow spectrum), or across genera (broad spectrum) (Cotter, Hill, & Ross, 2005). The use of bacteriocins in biopreservation is a growing area of research. Bacteriocins are a heterogeneous group, characteristically selected for evaluation and used as specific antagonists against problematic bacteria. However, their effectiveness in foods can become limited for various reasons, and cost remains an issue impeding the broader use of bacteriocins as food additives. Evaluation of methods to improve the use of bacteriocins in various food products is continually being investigated and new developments are frequently reported. Although, Gram-negative and Gram-positive bacteria can produce bacteriocins, LAB continues to be the preferred source of food-use bacteriocins as they are generally regarded as safe (GRAS) bacteria (Chen & Hoover, 2003). The structure, biosynthesis, and application of LAB bacteriocins in many different food products have been reviewed

by others (Cleveland, Montville, Nes, & Chikindas, 2001; Cotter et al., 2005; Deegan et al., 2006; Galvez, Abriouel, Lopez, & Omar, 2007; Kostrzynska & Bachand, 2006; O'Sullivan, Ross, & Hill, 2002). The use of bacteriocin-producing bacteria (BPB) or the derived bacteriocins in food safety strategies in meat applications will be further discussed below.

Bacteriocins generally act by creating pores in the membrane of their target cells and may be cytotoxic to the target cell as a result of disturbance of the bacterial inner or outer membranes. Alternatively, bacteriocins may pass through the membrane to reach a target inside the cell causing major disruptions in cell functions. Different mechanisms of action by bacteriocins have been described but interaction with the bacterial membrane is an important requirement for most, if not all, bacteriocins (Cleveland et al., 2001).

Application of BPB and Bacteriocins in Animals

Rather than applying the bacteriocin directly as a biocontrol, the bacteriocin-producing bacteria (BPB) may be applied. BPB have been isolated from rumen environments and some have been applied to manipulate the rumen environment in poultry and cattle (Cole, Farnell, Donoghue, Stern, & Evetoch, 2006; Diez-Gonzalez, 2007; Etcheverria, Arroyo, Perdigon, & Parma, 2005; Nava et al., 2005; Russell & Mantovan, 2002; Svetoch et al., 2005; Zhao et al., 1998). BPB can be administered to animals by mixing dried or wet cultures with feed or drinking water, and depending on the ability of the bacterial strain to colonize the gastrointestinal tract may be fed sporadically or continuously. The feeding of BPB can have a direct effect on reducing the existing populations of foodborne pathogens such as *Salmonella* and *E. coli* O157:H7 and long-term colonization with BPB would prevent further re-introduction of the pathogenic bacteria. Few studies have addressed the fate of bacteriocins in the intestinal tract, but some data suggests that some of the low molecular weight bacteriocins can survive at least some of the intestinal environments and possibly could be administered through feed (Ganzle, Hertel, van der Vossen, & Hammes, 1999).

The use of colicins (bacteriocin produced by *E. coli*), as a pre-harvest control, has been actively evaluated to reduce pathogenic *E. coli* in cattle populations (Diez-Gonzalez, 2007; Schamberger, Phillips, Jacobs, Diez-Gonzalez, 2004). Calves fed with a colicin-producing *E. coli* yielded an overall reduction of 1.1 $\log_{10}$ $CFUg^{-1}$ of *E. coli* O157:H7 and a maximum decrease of 1.8 $\log_{10}$ $CFUg^{-1}$ of *E. coli* O157:H7 over 24 days (Schamberger et al., 2004). Colicins specific for *E. coli* are particularly advantageous in the rumen intestine in that it will only inhibit one type of bacterial strain or species while not disrupting the other microbial populations in the intestine (Diez-Gonzalez, 2007). Studies continue to investigate whether expression of colicins could be incorporated into bacteria which are normally present in the animal rumen, which can decrease the chances of transfer of colicin genes to other potentially pathogenic *E. coli* strains, such as *E. coli* O157:H7 (McCormick, Klaenhammer, & Stiles, 1999). In poultry, a few studies have investigated the use

of BPB to control foodborne pathogens. Bacteriocin-like compounds were shown to have direct antimicrobial activity, in vitro against *Campylobacter* (Morency, Mota-Meira, LaPointe, Lacroix, & Lavoie, 2001; Schoenis & Doyle, 1992; Svetoch et al., 2005). A purified bacteriocin produced by *Paenibacillus polymyxa* microencapsulated and administered via feed was reported to reduce cecal *Campylobacter* colonization in young broiler chickens and turkeys (Cole et al., 2006; Stern et al., 2005). Treatment with bacteriocin eliminated detectable *Campylobacter* concentrations in turkey cecal contents (detection limit, 1×10^2 CFU g^{-1}) compared to controls (1.0×10^6 CFU g^{-1} of cacal contents) (Cole et al., 2006). In chicken cacel samples, use **of bacteriocin** resulted in significant reductions in colonization by *C. jejuni* for *C. jejuni*. These studies reported significant reductions of both intestinal levels and frequency of chicken and turkey colonization by *C. jejuni*, suggesting that this on-farm application would be an alternative to chemical disinfection of contaminated carcasses (Stern et al., 2005). In order for BPB and their bacteriocins to be used successfully in animal production, more research is required to improve their efficacy in different animal systems.

Application of Bacteriocin-Producing Bacteria and Bacteriocins in Meat Products

Bacteriocins are potentially valuable biological tools to improve food preservation and food safety by reducing the prevalence of undesirable spoilage microorganisms or foodborne pathogens in a food product (Deegan et al., 2006). Although the use of LAB is common in many food processes, the use of these microorganisms in different meat products and storage conditions continues to be evaluated (Kostrzynska & Bachand, 2006). The application of bacteriocins is not recommended as a primary processing step or barrier to prevent the growth or survival of pathogens, but should form part of a system with multiple hurdles. Bacteriocins can be incorporated into food as an ingredient in the form of a purified/semi-purified bacteriocin preparation (Table 12.3). Alternatively, a BPB can be introduced as a 'protective culture' in the form of a live culture which produces the bacteriocins *in situ* in the food (Table 12.4). In this case, the BPB is either substituted for all or part of the starter or is subsequently applied to the food to improve the safety of the culture (O'Sullivan et al., 2002). A major criterion for a protective culture in meat products is to inhibit pathogens and/or prolong shelf life, while not changing the sensory properties of the product (Lucke, 2000). The use of purified bacteriocins is not always attractive as some can be inactivated in meats and may also required regulatory approval or be labeled as an additive on the meat product (Aasen et al., 2003). The use of BPB as a protective culture is a more practical approach as it does not require regulatory approval or preservative label declarations and can be substituted into the product as a starter culture (Deegan et al., 2006; Jacobsen, Budde, & Koch, 2003; Katla et al., 2002).

Numerous studies have attempted to isolate BPB, particularly LAB from meat products (Albano et al., 2007; Arlindo et al., 2006; Budde, Hornbaek, Jacobsen,

Table 12.3 Examples of bacteriocins used to control pathogens in meat products

Bacteriocin	Producer strain	Meat product	Target pathogen(s)	Reduction $\log_{10}$ CFU g^{-1})	Reference
Sakacin P	*Lb. sakei*	Chicken cuts, fillet	*L. monocytogenes*	3.0–5.1 (from 35 µg g^{-1})	Aasen et al. (2003)
Enterocin AS-48	*Enterococcus faecalis*	Pork sausage mixture	*S. aureus*	5.31 (from 40 µg g^{-1})	Ananou, Maqueda, Martinez-Bueno, Galvez, and Valdivia (2005)
Piscicocin CS526	*Carnobacterium piscicola* CS526	Ground meat (beef and pork mixture)	*L. monocytogenes*	to $<3.0\times10^3$ at 4 and 12°C.	Azuma, Bagenda, Yamamoto, Kawai, and Yamazaki (2006)
Lactocin 705 and Lactocin AL705	*Lb. curvatus* CRL705	Beef meat	*L. innocua* *B. thermosphata*	no reduction over 36 d (2°C). 2.8	Castellano and Vignolo (2006)
Plantaricin UG1	*Lb. plantarum* UG1	Cooked and uncooked chicken, turkey and beef and dry mortadella.	*L. monocytogenes* *C. perfringens*	<1(3 d, 15°C). zero within 7–14 d.	Enan (2006a, 2006b)
Sakacin K	*Lb. sakei* CTC494	Raw minced pork	*L. innocua*	from 50 MPN g^{-1} to <3MPN g^{-1} (8 d, 7C).	Hugas, Pages, Garriga, and Monfort (1998)
Sakacin P	*Lb. sakei*	Chicken cold cuts	*L. monocytogenes*	Inhibition of growth (28 d, 10°C).	Katla, Moretro, Sreen, Aasen, Axelsson, Rolvik and Waterstad (2002)
Pediocin AcH	*Lb. plantarum*	Sliced cooked sausage	*L. monocytogenes*	~0.7(6 d, 6°C).	Mattila, Saris, and Tyopponen (2003)
Bacteriocin not identified	*Pediococcus acidilactici*	Raw pork meat	*L. monocytogenes* *C. perfringens*	3 (over 72 h, 15°C). 0.5	Nieto-Lozano, Reguera-Useros, Pelaez-Martinez, and Hardisson de la Torre (2006)
Lactocin 705, Enterocin CRL35 and Nisin.	*Lb. casei* CRL35, *Enterococcus faecium* CRL35	Mince meat	*L. monocytogenes* *L. innocua*	~4.8–5.2 (from individual bacteriocins) -~7.0–9.9 (from bacteriocin combinations)	Vignolo et al. (2000)

Table 12.4 Examples of use of bacteriocin-producing bacteria as protective cultures to control pathogens in meat products

Culture	Meat product	Target pathogen(s)	Reduction (Log_{10}CFU g^{-1})	Reference
Lb. sakei CECT 4808 and/or *Lb. curvatus* CECT904	Pork sausage mixture	*L. monocytogenes*	to $<1\times10^2$ (over 9 d, 22°C).	Ananou et al. (2005)
Enterococcus faecalis A-48-32	Dry-fermented sausage	*L. monocytogenes*	to <1(over 15 d ~15°C).	Benkerroum et al. (2005)
Lactococcus lactis subsp. *lactis* LMG21206 and *Lb. curvatus* LBPE	Raw sausage (mergeuz)	*L. monocytogenes*	2.4 during fermentation	Benkerroum et al. (2003)
Five uncharacterised Lactic acid bacteria	Ham and servelat sausage	*L. monocytogenes*	Inhibited growth (at 8 and 4°C over 28 d).	Bredholt, Nesbakken, and Holck (2001)
Leuconostoc carnosum 4010	Pork meat sausage slices	*L. monocytogenes*	to <10 CFU (21 d, 5°C).	Budde et al. (2003)
Lb. sakei TH1	Beef meat	*L. innocua*	No reduction (36 d, 2°C).	Castellano and Vignolo (2006)
		B. thermosphata	2.8 (14 d).	
Lb. casei CRL705	Beef meat slurry	*L. innocua*	No reduction (21 d, 4°C)	Castellano, Holzapfel, and Vignolo (2004)
		Lb sakei		
Lb. curvatus CRL705	Pork adipose tissue	*B. thermosphacta*	~2–3 less than controls (7 or 13 d, 4°C)	Greer and Dilts (2006)
		L. monocytogenes	~2–3 less than controls (7 or 13 d, 4°C)	
Lb. sakei CTC494	Poultry breast		0.8 (7 d, 7°C)	
Leuconostoc carnosum 4010	Sliced pork saveloys	*L. monocytogenes*	No reduction spray (28 d, 10°C).	Jacobsen et al. (2003)
	Cooked pork		No reduction.	

Barkholt, & Koch, 2003; Noonpakdee, Santivarangkna, Jumriangrit, Sonomoto, & Panyim, 2003; Prema, Bharathy, Palavesam, Sivasubramanian, & Immanuel, 2006; Schneider et al., 2006; Yin, Wu, & Jiang, 2003), with the aim of identifying a potential protective culture that can not only be used as a starter culture but also possess bacteriocin activity to eliminate or reduce foodborne pathogens (Benkerroum et al., 2005; Benkerroum, Daoudi, & Kamal, 2003; Leroy & De Vuyst, 2005). Many LABs are used to combat other spoilage organisms in aerobic or vacuum packed meat products (Barakat, Griffiths, & Harris, 2000; Lucke, 2000). With respect to meat safety, majority of **the** studies investigating the use of bacteriocins in meat products have targeted the pathogen *L. monocytogenes* due to its ability to grow at refrigeration temperature and survive in fermented foods (Tables 12.3 and 12.4). The disadvantage of many LAB bacteriocins is that although they are active against Gram-positive organisms they are not as effective against Gram-negative foodborne pathogens such as *E. coli* (particularly *E. coli* O157:H7) or *Salmonella* spp. which are also a concern in meat products (Lucke, 2000). This is due to the fact that Gram-negative bacteria are protected by their outer membrane, which prevents bacteriocins from reaching the cytoplasmic membrane (Abee, Krockel, & Hill, 1995). Studies have therefore attempted to find alternative bacteriocins with broader spectrums or have used other combinations of strategies to improve bacteriocin activity.

The most commonly used bacteriocin in foods, including meat products is Nisin (produced by *Lactococcus lactis* subsp. *lactis*). Nisin is one of the commercially available bacteriocins with US Food and Drug Administration (FDA) approval. It inhibits the growth of a wide range of Gram-positive bacteria including *L. monocytogenes*. Nisin has been shown to be effective in a number of food systems but is predominantly used in canned foods and dairy products, particularly in cheese production, where it protects against heat-resistant, spore forming organisms such as *Bacillus* and *Clostridium* spp (Cotter et al., 2005; Deegan et al., 2006). Nisin has been used as a food safety agent in beef (Ariyapitipun, Mustapha, & Clarke, 2000; Barboza De Martinez, Ferrer, & Marquez Salas, 2002; Zhang & Mustapha, 1999), sausages (Patel, Sanglay, Sharma, & Solomon, 2007), ground mince (Castillo, Meszaros, & Kiss, 2004) and poultry (Yuste, Pla, Capellas and Mor-Mur, 2002; Zuckerman & Abraham, 2002), but is not as effective in the preservation of meat as it is in dairy products. The inhibitory activity of Nisin is reduced by interference from meat components such as phospholipids, especially where there may be a high fat content (Leroy & De Vuyst, 2005). In addition, Nisin has a low solubility at normal meat pH and its interaction with phospholipids results in the uneven distribution of the bacteriocin in the meat (De Martinis, Publio, Santarosa, & Freitas, 2001; Stergiou, Thomas, & Adams, 2006).

Substantial research has been done to evaluate and improve the effectiveness of Nisin and other bacteriocins activity against Gram-negative pathogens and its applicability in different food products. Several bacteriocins show additive or synergistic effects when used in combination with other antimicrobial agents including, organic acids, other antimicrobials or sublethal treatments such as mild heat or high pressure (Ananou, Galvez, Martinez-Bueno, Maqueda, & Valdivia, 2005;

Ariyapitipun et al., 2000; Arques et al., 2004; Ganzle, Weber, & Hammes, 1999; Rodriguez, Nunez, Gaya, & Medina, 2005).

An alternative use of bacteriocins is as the agent incorporated into packaging materials (Cooksey, 2005; Quintavalla & Vicini, 2002). Combining the bacteriocin directly into a plastic material provides a number of advantages for delivery of the bacteriocin to the food product. Firstly, only the necessary amount of bacteriocin would be required. Secondly, the agent would not be a direct additive to the food product and would therefore avoid labeling and regulatory approval. Thirdly, if the plastic material were made from an edible and/or biodegradable plastic benefits for the environment would be apparent (Siragusa, Cutter, & Willett, 1999). Bacteriocins such as Nisin and Pediocin among others have been incorporated into different films and have been successful in inhibiting spoilage microorganisms, such as LAB and *Brochothrix thermosphacta* on beef carcass tissue, as well as *Listeria* spp. on meat and poultry samples (Marcos, Aymerich, Monfort, & Garriga, 2007; Mauriello, Ercolini, La Storia, Casaburi, & Villani, 2004; Ming, Weber, Ayres, & Sandine, 1997; Scannell et al., 2000; Siragusa et al., 1999). Further development of packaging technologies may prove to be an effective way of delivering bacteriocins to the surface of food to improve food safety and preservation.

Limitations of Using Bacteriocins and Future Prospects

The extensive use of bacteriocins in foods is hindered by a number of factors. One of the main limitations is the narrow spectrum of activity of most bacteriocins, though the specificity of a bacteriocin can be advantageous for applications in which a single bacterial strain of species is targeted without disrupting other microbial populations. Research continues to identify alternative bacteriocins which may extend **the** bacteriocin applications either through their use alone or in combination with other antimicrobials or hurdle technologies. Considerable studies are also required to investigate the factors influencing the applicability of certain bacteriocins in various food systems. Bacteriocin activity is difficult to maintain in a range of foods particularly in meat products. Another potential problem associated with using bacteriocins in foods is the development of resistant populations of problematic bacteria. Resistance can occur naturally and it has been reported especially with regard to Class IIa bacteriocins (Naghmouchi, Kheadr, Lacroix, & Fliss, 2007). Consequently, studies have examined the possibility of generating multiple bacteriocin producers to limit the potential of bacteriocin-resistant populations (O'Sullivan, Ryan, Ross, & Hill, 2003). Some BPB strains may also spontaneously loss their ability to produce bacteriocin(s) due to genetic instability (Holzapfel et al., 1995; Riley & Wertz, 2002) and can also become ineffective if the cell membrane of a target organism changes in response to a particular environmental condition. Although there are some limitations for the use of bacteriocins in meat applications, the ongoing study of existing bacteriocins as well as the identification of new bacteriocins and improvements of application will only optimize the potential of these agents in many different

food applications which will lead to further improvements of food safety and quality of meat products.

Antimicrobial Peptides

Antimicrobial peptides (AMPs) are generally short cationic peptides produced by both animals and plants that have potent killing activity against a range of bacteria, fungi, viruses, and protozoa (Higgs et al., 2007). They are ubiquitous in nature and they play an important role in host defence and microbial control. Their exact mode of action is not completely understood, but it is thought that cationic AMPs are attracted to negatively charged phospholipids in the cell membrane and interact with the membrane, displacing lipids and altering the membrane structure. A number of hypotheses have been put forward as to how AMPs kill microbes and these are discussed elsewhere (Zasloff, 2002). Over 850 AMPs have been identified from a host of species and a catalogue of these can be found online (http://www.bbcm.univ.trieste.it/~tossi/amsdb.html). AMPs can also be designed and chemically synthesized and have been shown to possess antimicrobial activity (Anzai et al., 1991; Appendini & Hotchkiss, 1999; Haynie, Crum, & Doele, 1995).

Many studies have demonstrated the activity of AMPs and derivatives from a variety of species, including host species, against pathogens including *Salmonella, E. coli* O157, and *Listeria*. A selection of these studies is shown in Table 12.5. AMPs are now emerging as a solution to the development of antibiotic resistance by bacterial pathogens, and therefore, the focus at present is on understanding their mode of action and potential application from a clinical point of view (Hancock & Sahl, 2006). Investigations of AMPs in food systems to date are limited. Some studies have been performed in food products or suggest the potential of the AMP in a food product (Table 12.5). Yaron, Rydlo, Shachar, and Mor (2003) studied the antimicrobial activity of dermaseptin S4 and its derivative K_4-S4 which come from tree frogs. It was found that K_4-S4 reduced the population of *E. coli* O157:H7 in apple juice by more than 7 log units in less than 2 hours, indicating its potential usefulness as a biocontrol agent. The authors put forward potential advantages for the use of animal derived AMPs in food safety and preservation measures, such as their activity over a wide range of conditions. Another study in apple juice showed that a synthetic AMP could reduce the *E. coli* O157:H7 numbers by 3.5 log units in 8 hours. However, the same peptide had no effect on *E. coli* O157:H7 when grown in skim milk indicating that components from the milk could be interacting with the peptide causing it to lose its antibacterial activity (Appendini & Hotchkiss, 1999). A different study showed that peptides produced by *Lb. acidophilus* fermentation of sodium caseinate had bacteriocidal activity against *Enterobacter sakazakii, L. innocua* and *E. coli*, and it was suggested that such peptides could be used as a protection mechanism against *E. sakazakii* in infant formula by producing a casein-cased milk ingredient by fermentation (Hayes, Ross, Fitzgerald, Hill, & Stanton, 2006).

Table 12.5 Examples of antimicrobial peptides (AMPs) which have antimicrobial activity against pathogens

AMP	Source	Antimicrobial activity	Reference
Synthetic cathelicidins	ovine cathelicidins	*E. coli* O157:H7	Anderson (2005)
Synthetic PR-26	porcine neutrophils AMP	*E. coli* O157:H7 and *L. monocytogenes*	Annamalai, Venkitanarayanan, Hoagland, and Khan (2001)
6K8L	Synthetic	*B. subtilis, E. coli* O157:H7, *L. monocytogenes, Pseudomonas fluorescens, S.* Typhimurium, *Serratia liquefasciens, Staphylococcus aureus, Kluyveromyces marxianus*	Appendini & Hotchkiss (1999)
L. acidophilus fermentation of sodium caseinate	Bovine α_{s1}-casein	*Enterobacter sakazakaii, L. innocua, E. coli*	Hayes et al. (2006)
Synthetic modified AvBD8	chicken avian β-defensin-8	*E. coli, S.* Typhimurium and *L. monocytogenes*	Higgs et al. (2007)
Pepsin digest of casein	Bovine kappa-casein	*E. coli, L. innocua*	Lopez-Exposito, Minervini, Amigo, and Recio (2006)
Recombinant gallinacins	Chicken	*S.* Typhimurium, *S. enteriditis*	Milona, Townes, Bevan, and Hall (2007)
Purified aurelin	*Aurelia aurita* jellyfish	*E. coli, L. monocytogenes*	(2006)
Synthetic cathelicidin Bac7 and derivatives	Granulocytes of mammalian species	*S.* Typhimurium, *E. coli*	Podda et al. (2006)
Synthetic cathelicidin K9CATH	Canine	*L. monocytogenes, S. aureus, E. coli, Klebsiella pneumoniae, S.* Typhimurium, *Ps. aeroginosa, Proteus mirabilis, S. enteriditis*, *Neisseria gonorrhoeae*	Sang et al. (2007)
Lactoferrin, pepsin hydrolysed lactoferrin, Lactoferricin®	Bovine	*E. coli* O157:H7	Shin et al. (1998)
Synthetic dermaseptin S4 and derivative K_4-S4	Tree frogs of the *Phyllomedusa* species	*E. coli, E. coli* O157:H7, *S.* Typhimurium, *S.* Stanley, *L. monocytogenes, L. innocua*	Yaron et al. (2003)

Very little research has focused on the use of AMPs in the meat industry. One study looked at the effect of a synthetic peptide on the microflora in meat exudates, but aerobic and anaerobic counts were reduced by less than one log indicating a small degree of inhibition (Appendini & Hotchkiss, 1999). Although studies in this area are limited at the moment, the potential advantages provided by AMPs, such as their wide spectrum of activity and lack of bacterial resistance to AMPs, suggests that **the** interest in their use as biocontrol agents in foods can only grow in the years to come.

Bacteriophage

Bacteriophages are viruses that infect and kill bacterial cells by reproducing within **the** bacteria and disrupting the host metabolic pathways, causing the bacterium to lyse. Bacteriophage are ubiquitous in the environment; they specifically target bacterial cells and do not infect mammalian cells, hence the reason why they are proposed as biocontrol agents in human, animal, clinical and industrial applications.

Classification and Mode of Action

Most bacteriophages range in size from 24–200 nm in length and are classified into 13 families. The bacteriophage structure consists of a head or capsid; composed of protein that acts as a protective barrier in which either DNA or RNA is stored. Some bacteriophages possess a tail through which the nucleic acid is delivered to the bacterial cell during infection.

Bacteriophages are classified as either lytic or lysogenic. Lytic bacteriophages bring about rapid lysis and death of the bacterial cell, whereas a lysogenic bacteriophage does not result in immediate lysis, but instead enters a quiescent state and is known as a prophage during this period (Hanlon, 2007). A lysogenic bacteriophage integrates into the genome of the host bacterial cell, it undergoes replication with the host chromosome and the viral DNA is passed onto the daughter cells. Lysogenic bacteriophages have the ability to transfer genes for toxin production or pathogenicity factors between bacterial communities (Wagner & Waldor, 2002). Therefore, lytic bacteriophages are preferred for the purpose of bacteriophage therapy.

Bacteriophage first come in contact with bacterial host cells during Brownian motion. Infection of a bacterial cell by a bacteriophage involves attachment of the bacteriophage to the bacterial membrane. This is accomplished by the tail fibers or an equivalent structure, attaching to specific receptors on the surface of the bacterial cell. These receptors may be protein, peptidoglycan, teichoic acid, lipopolysaccharide and oligosaccharide (Lenski, 1988).

Application of Bacteriophage

There has been a vast amount of research into the application of bacteriophage to control foodborne pathogens such as *Campylobacter*, *E. coli* O157:H7, *Listeria* and *Salmonella* in animal food products, and food processing environments. Bacteriophage has also been applied to fruit and vegetables to control against bacterial pathogens pre-harvest, (Balogh et al., 2003; Pao, Randolph, Westbrook, & Shen, 2004). Another aspect of bacteriophage biocontrol is the use of bacteriophages as indicators for detection of pathogens in foods and fecal contamination of animal feeds (Goodridge, Chen, & Griffiths, 1999; Hsu, Shieh, & Sobsey, 2002; Maciorowski, Pillai, & Ricke, 2001). Detection of bacterial contamination can be achieved quickly by using bacteriophage rather than using bacteria (Hsu et al., 2002). An area of interest is the use of bacteriophage enzymes in foods and food grade bacteria (LAB). Endolysins are bacteriophage enzymes that are synthesized late during virus replication. They target bacterial peptidoglycan which results in the release of progeny virions (Gaeng, Scherer, Neve, & Loessner, 2000; Loessner, 2005). Gaeng et al. (2000) applied endolysin encoding genes from *Listeria* bacteriophage to lactococcal starter organisms to obtain organisms with biopreservation properties against *L. monocytogenes*.

Application of Bacteriophage in Animals: Preharvest Control

Wagenaar, Van Bergen, Mueller, Wassenaar, and Carlton (2005) applied a bacteriophage (bacteriophage strain 71 and 69) with a wide host range against *C. jejuni* strains to control *C. jejuni* colonization in broiler chickens. They observed a 3 $\log_{10}$ $CFUg^{-1}$ reduction in *C. jejuni* counts initially in the therapeutic group; however, counts stabilized after 5 days to 1 $\log_{10}$ $CFUg^{-1}$ lower than the control group. They concluded that this bacteriophage treatment could be an alternative method for reducing *C. jejuni* colonisation in broiler chickens. Loc Carrillo et al. (2005) reported a reduction in *C. jejuni* counts of between 0.5 and 5 $\log_{10}$ $CFUg^{-1}$ when broiler chickens were orally administered bacteriophage CP8 and CP34 in an antacid suspension to reduce *C. jejuni* colonization in broiler chickens.

The literature, till date, suggests that application of a cocktail of bacteriophage yields more successful results in biocontrol of *E. coli* than bacteriophage administered singly (Bach, McAllister, Viera, Gannon, & Holley, 2002). Waddell et al. (2000) administered a cocktail of bacteriophage to control shedding of *E. coli* O157:H7 in calves and showed that shedding of the pathogen was observed for 6 to 8 days in bacteriophage treated calves, compared to 6 to 14 days in the control calves. Kudva, Jelacic, Tarr, Youderian, and Hovde (1999) isolated three coliphages (KH1, KH4, and KH5) and applied them to a number of O157 and non-O157 strains to determine their ability to lyse laboratory cultures. The three coliphages were capable of lysing the O157 serotype, and did not have any effect on the non-O157 strains. A high multiplicity of infection (MOI) of 10^3 plaque forming units (PFU) and aeration

were required for successful control of *E. coli* O157:H7. Multiplicity of infection is the ratio of infectious agents to infection targets. A difference between the three coliphages in their ability to kill host cells was observed. KH1 was the most effective coliphage in reducing host cell numbers; however, the three coliphages were unable to eliminate *E. coli* O157:H7.

Sheng, Knecht, Kudva, & Hovde (2006) applied bacteriophage to determine their ability to control intestinal *E. coli* 0157:H7 in ruminants. Bacteriophage KH1 and SH1 were applied orally and rectally to sheep and cattle respectively. No reduction in intestinal carriage of *E. coli* O157:H7 in sheep was observed when KH1 was administered orally. An equal mixture of KH1 and SH1 were administered rectally to cattle. Combination of the two bacteriophages reduced the numbers of *E. coli* O157:H7; however, they were unable to clear the *E. coli* O157:H7 infection from the cattle.

Raya et al. (2006) demonstrated that a single oral dose of *E. coli* O157:H7 specific bacteriophage (CEV1) applied to sheep reduced shedding of the pathogen by 2 $\log_{10}$ $CFUg^{-1}$. Recently, the US Department of Agriculture approved a bacteriophage for hide washing. The product produced by OmniLytics is administered on the hides of live animals prior to slaughter to minimize contamination of *E. coli* O157:H7 onto beef carcasses.

Application of Bacteriophage in Meat and Meat Products

The application of bacteriophage has been reported for a range of pathogens on poultry and meat. Atterbury, Connerton, Dodd, Rees, and Connerton (2003) showed that when bacteriophage Φ 02 was applied to chicken skin inoculated with *C. jejuni* and stored at 4 and −20°C a reduction of approximately 1 $\log_{10}$ CFU ml^{-1} was obtained. They also observed a reduction in Campylobacteraceae on the frozen samples by 2 $\log_{10}$ CFU ml^{-1} after day 1, and remained at similar levels thereafter. Goode, Allen, and Barrow (2003), also showed that three bacteriophages, specific for *C. jejuni* and *Salmonella* gave a 1 $\log_{10}$ CFU ml^{-1} reduction of both pathogens, at a MOI of 1. When other bacteriophages were applied at a MOI of 100 to 1000, a reduction of 2 $\log_{10}$ CFU ml^{-1} in *S*. Enteritidis was observed over 48 hours.

The effectiveness of a three bacteriophage cocktail in reducing *E. coli* O157:H7 on inoculated meat samples has been demonstrated by O'Flynn et al. (2004). The three bacteriophage**s** (e11/2, pp01 e41c) reduced *E. coli* O157:H7 from initial numbers of approximately 3 $\log_{10}$ CFU ml^{-1} to undetectable levels during a 2 hour enrichment **process**. The effectiveness may be due to lysis from outside the cell as the MOI used was 10^6 – fold, the bacterial cells may have been overwhelmed by the number of bacteriophage attaching to the cell surface causing the bacterial cell to lyse.

Dykes and Moorhead (2002), applied listeriophage LH7 to beef inoculated with *L. monocytogenes*, which was vacuum packed and stored at 4°C. **T**hey also applied LH7 to mixed population of *L. monocytogenes* stored in PBS. A combination of listeriophage and nisin had no effect when applied to vacuum packed beef. Listeriophage

alone had no effect when applied to broth, however, when nisin was combined with LH7 and applied to broth a decrease in *L. monocytogenes* counts was observed. In 2006, the US Food and Drug Administration approved a bacteriophage preparation to be applied on Ready-To-Eat (RTE) meat and poultry products as an antimicrobial agent against *L. monocytogenes* (Federal Register, 2006). The bacteriophage preparation consists of six *Listeria* specific bacteriophages, which were combined to reduce the possibility of *L. monocytogenes* developing resistance to the agent. Once *L. monocytogenes* is no longer present in the product the bacteriophage remains dormant. The regulation specifies that the cocktail of bacteriophage must be negative for *L. monocytogenes* and listeriolysin O, a toxin produced by *L. monocytogenes*. Another commercially available product is Listex ^{TM}P100, produced by EBI Food Safety. It is a bacteriophage **used** for **the** control of *L*. monocytogenes in meat and cheese products. It is recognized as GRAS by the FDA and has a wide host range against *Listeria* strains (Carlton, Noordman, Biswas, de Meester, & Loessner, 2005).

Whichard, Sriranganathan, & Pierson (2003) investigated the ability of bacteriophage Felix 01 (wild type) and a variant of Felix 01, to control *Salmonella* growth on chicken frankfurters. A 1.8 and 1.2 $\log_{10}$ CFU ml^{-1} reduction of *S*. Typhimurium in artificially contaminated chicken frankfurters was reported for the wild type Felix 01 and the variant respectively. Another study artificially inoculated broiler carcasses with *S*. Enteritidis and stored them at 4°C for 2 hours. Carcasses were then sprayed with 5.5 ml of saline containing bacteriophage PHL 4 at various concentrations. This resulted in a 93% reduction in recovery of *S*. Enteritidis when inoculated with 10^8 or 10^{10} PFU. They also applied PHL 4 to carcass rinse water at concentrations of 10^6 and 10^{10} PFU. When bacteriophage was applied at 10^{10} PFU recovery of *S*. Enteritidis was reduced to between 50 and 100% (Higgins et al., 2005).

Limitations of Bacteriophage Use

Adsorption of a bacteriophage to receptors on a bacterial cell occurs during Brownian motion. This process may be obstructed by the existence of considerable numbers of non host bacterial cells. This initial interaction may also be hindered by a viscous environment, for example, the rumen environment (Joerger, 2003). This type of environment may protect the bacterial cell from bacteriophage infection. Host bacterial cells may also be protected by biofilms present in a food environment as bacteriophage would be incapable of penetrating and accessing the bacterial cell within the biofilm. Many meat products are distributed and stored at refrigeration temperatures, conditions under which many pathogens may not grow. This poses a problem with bacteriophage use as replication can not occur under conditions where the host is not dividing. In addition, other food conditions can also affect bacteriophage use, these include visible and UV light, osmotic shock and pressure and thermotolerence. Bacteriophages may be destroyed in food processing environments as they are effectively cleaned and sanitized. **There are r**eports of dairy bacteriophage**s** be**ing** destroyed by sodium hypochlorite (100 ppm free chlorine) and peracetic acid (Quiberoni, Suarez, & Reinheimer, 1999). Some pathogens can survive **the** low pH

environments while bacteriophages would be instable at such low acidity. Smith, Huggins, and Shaw (1987) reported the stability of bacteriophage over a pH range of 3.5 to 6.8, however a decrease in bacteriophage titer was observed at pH 3, and a large decrease was observed below pH 3.

The need for high bacterial populations for phage replication to occur and lyse the bacterial cells has been reported in the literature. Berchieri, Lovell, and Barrow (1991), stated that approximately 10^4 CFU ml^{-1} of the host cell was required for the bacteriophage to have an effect on the host cell.

Bacteriophages also have the capability to transfer unfavorable genes from one bacterium to another; these could be virulence genes or antibiotic resistance genes (Alisky, Iczkowski, Rapoport, & Troitsky, 1998; Figueroa-Bossi & Bossi, 1999; Mirold, Rabsch, Tschape, & Hardt, 2001; Schmieger & Schicklmaier, 1999). This is a major concern and much effort must be applied to characterize and determine the potential of a bacteriophage to transfer virulence factors and antibiotic resistance genes to commensal or pathogenic bacteria before it is commercialised.

The emergence of host resistance to bacteriophages due to DNA mutations has been reported in pathogens in pre-harvest (Sklar & Joerger, 2001) and post**-** harvest environments (Greer & Dilts, 2002). A cocktail of bacteriophage may settle the issues of host resistance (Barrow & Soothill, 1997; Leverentz et al., 2003; Tanji et al., 2004). Desirably, bacteriophages with a broad host spectrum are favourable for biocontrol as there are many subtypes in each pathogen species that may possess different cell surface receptors. Finally, if bacteriophages are to be used in food produc**ts**, consumer acceptance will be a major factor. The marketing strategy involved **for** bacteriophage products will be a critical area to ensure consumer acceptance.

Vaccines

An option which has been investigated as a potential biocontrol agent at the pre**-**harvest stage is vaccination of food animals. In this way the animal's own immune system is used to reduce pathogen loads by producing antigens against particular pathogens. A successful vaccine would prevent colonization of the host by the pathogen; i.e. when the animal ingests the pathogen it can not colonize and multiply and therefore less of the pathogen would be present at slaughter or in the feces thus reducing the likelihood of the pathogen entering the food chain. There are obstacles to be overcome; however, a major challenge being the ability to prime the mucosal immune response of animals to mount a protective response against an otherwise commensal organism (LeJeune & Wetzel, 2007). Vaccinations have been developed with varying degrees of success for a number of zoonotic pathogens. The following is by no means an exhaustive list but provides an overview of the different types of vaccine, currently either in development or in use.

Intimin from *E. coli* O157:H7 has been identified as a potential vaccine candidate. Intimin is an outer membrane protein encoded by the *eae* gene that is required for intestinal colonization and attaching and effacing activity of *E. coli* O157:H7 in

piglets and calves (Dean-Nystrom, Bosworth, Moon, & O'Brien, 1998). A vaccine containing intimin$_{O157}$ was tested using neonatal piglets as a challenge model and it was demonstrated that piglets that ingested maternal antibodies against intimin$_{O157}$ were protected from colonization with an intimin producing *E. coli* O157:H7 strain (Dean-Nystrom, Gansheroff, Mills, Moon, & O'Brien, 2002). This provides evidence that this may be a viable candidate for an anti-*E. coli* O157:H7 vaccine in cattle, the main reservoir of enterohaemorrhagic *E. coli.* Another study looked at the use of the cell-binding domain of intimin or a truncated EHEC factor for adherence (Efa-1) as potential vaccines. Both were found to induce humoral immunity in calves but did not protect against intestinal colonization by *E. coli* O157:H7 and O26:H- upon subsequent challenge (van Diemen et al., 2007). Similarly, the same study showed that an inactivated vaccine comprising of formalin-killed *E. coli* O157:H7 was ineffective, despite IgG responses. Nonetheless, the authors concluded that it may be possible to use these antigens, provided appropriate exposure to the intestinal immune system can be achieved.

Potter et al. (2004) looked at the use of proteins involved in intestinal colonization as possible vaccine targets. In this case, the authors used the proteins Tir, EspA and EspB, which are part of a type III secretion system involved in bovine intestinal colonization. Cattle were immunised with supernatant proteins containing Esps and Tir and subsequently challenged with *E. coli* O157:H7 and **the** fecal shedding was monitored. It was found that fecal shedding of *E. coli* O157:H7 was significantly reduced following vaccination, and the number of animals in the group shedding the pathogen and the duration of shedding were also reduced. It was also suggested that this vaccine could be used as a vaccine for non-O157 serotypes as the type III secreted antigens are relatively conserved among non-O157 serotypes. However, a subsequent field trial of the vaccine in nine feedlots showed no significant association between vaccination and pen prevalence of fecal *E. coli* O157:H7 (Van Donkersgoed, Hancock, Rogan, & Potter, 2005). A number of possible reasons were suggested for this, including different preparation of the vaccine, different vaccination strategies, different pen sizes and different timeframes. Another study also investigated the use of highly purified recombinant EspA as a vaccine for calves, and while this induced antigen specific IgG and salivary IgA responses, these responses did not protect against intestinal colonization upon subsequent challenge with *E. coli* O157:H7 (Dziva et al., 2007).

Salmonella is another zoonotic pathogen where substantial efforts have been invested to develop an effective vaccine. The success of this is dependent on understanding how *Salmonella* infect their hosts and the host response. However, a major obstacle is that *Salmonella* pathogenicity is both serotype-dependent and host-dependent and the factors influencing serotype-host specificity are not well known (Barrow, 2007).

Salenvac® is a commercially available, killed iron-restricted *S. enteriditis* PT4 vaccine which has been used as part of control programmes to reduce the burden of *S. enteriditis* infection of poultry flocks. A laboratory trial showed that the vaccine was successful in decreasing egg contamination (5.4–7.4% had culture positive shells in comparison to 16.7% for unvaccinated birds) and tissue colonization

subsequent to intravenous *S. enteriditis* challenge (Woodward, Gettinby, Breslin, Corkish, & Houghton, 2002). However, it has been suggested that oral or respiratory challenge would have been more relevant (Barrow, 2007). Another study evaluated the efficacy of Salenvac® T which is made up of inactivated *S*. Typhimurium and *S. enteriditis* which have been grown under iron restriction. In this case the chickens were orally challenged with *S*. Typhimurium. Vaccination resulted in a significant reduction in the shedding of *S*. Typhimurium (Clifton-Hadley et al., 2002). Liu, Yang, Chung, & Kwang (2001) used formalin-inactivated *S. enteriditis* encapsulated in biodegradable microspheres as a vaccine and dosed chickens either orally or via an intramuscular route and also challenged them by these routes. It was found that shedding and colonization by *S. enteriditis* was significantly decreased in the vaccinated birds in comparison to the control birds. When challenged intramuscularly vaccinated birds were 27.9% feces positive and 18.7% organ positive for *S. enteriditis* in comparison to 59.3% feces positive and 44% organ positive for nonvaccinated chickens.

A number of live *Salmonella* vaccines have also been developed, some of which are commercially available. TAD Salmonella vac® E and TAD Salmonella vac® T are metabolic drift mutants of *S. enteriditis* and *S*. Typhimurium respectively, which were produced by chemical mutagenesis (Linde, Beer, & Bondarenko, 1990). It was shown in laboratory studies that these vaccines used either singly or in combination reduced organ and reproductive tract colonization and internal egg contamination, in comparison to control birds. This indicates cross protection provided by the TAD Salmonella vac® T vaccine (Gantois et al., 2006). Another example of a live *Salmonella* vaccine for use in chickens was described by Cerquetti and Gherardi (2000). Trials showed that in the case of *S. enteriditis* and *S*. Gallinarum there was a significant decrease in colonization of the cecum and also reduced colonization by *S*. Typhimurium but not to a significant degree. A recent study has looked at using strains harbouring mutants in the *Salmonella* pathogenicity islands 1 and 2 (either *hilA, sipA,* or *ssrA*) as protective vaccines against *S. enteriditis* challenge in newly hatched chicks. While the *sipA* and *ssrA* mutants were found to protect against challenge strain colonization of the cecum and internal organs they were not deemed to be useful due to the vaccine strains' persistent colonization throughout the study. However, the *hlyA* mutant strain was found to confer protection against colonization by the challenge strain and the vaccine strain could not be detected in the cecum four weeks post inoculation. The authors proposed that the longer the vaccine strain colonized the intestine, the longer the protection against virulent *Salmonella.* Therefore, the challenge is to provide a balance between colonization of the vaccine and clearance of the vaccine before slaughter to exploit colonization inhibition as a protection mechanism (Bohez et al., 2007).

The investigation of *Salmonella* vaccines has not just been limited to poultry with numerous studies being carried out in other animal models. One such example is the use of an attenuated *S*. Typhimurium strain which has a mutation in DNA adenine methylase in calves. It was found that it provided protection against subsequent virulent *S*. Typhimurium challenge via adaptive immunity and competitive exclusion (Dueger, House, Heithoff, & Mahan, 2003a). This strain was also found

to provide protection in avian models (Dueger, House, Heithoff, & Mahan, 2001, 2003b). Pigs usually do not develop clinical salmonellosis, but as they can be carriers and shedders they can be a reservoir for the disease in humans. A metabolic drift mutant of *S*. Typhimurium was used as an oral vaccine for piglets that were subsequently challenged with a highly virulent *S*. Typhimurium strain. It was found that vaccinated animals shed substantially smaller amounts of the challenge strain and for a shorter period of time (Roesler et al., 2004). A similar trend was seen when the same strain was tested in poultry (Linde, Hahn, & Vielitz, 1996).

Subunit vaccines, i.e., vaccines which only contain individual proteins which will act as antigens, have received much less attention as a control agent for *Salmonella* and appear to have focused on using outer membrane proteins. Two studies of such vaccines were shown to either reduce colonization of *S. enteriditis* to the chicken intestinal mucosa following challenge (Khan, Fadl, & Venkitanarayanan, 2003) or had significantly reduced shedding of the challenge organism (Meenakshi et al., 1999).

There are presently no commercially available vaccines against *Campylobacter* in poultry. The development of such vaccines is hampered by a number of factors including the antigenic variety of strains and the lack of knowledge of antigens which induce a protective immune response. Also, there is a need to provide protection in the very early days of life as *Campylobacter* infection can occur at a very early stage (Wagenaar, Mevius, & Havelaar, 2006). However, a number of strategies have been or are being investigated and are reviewed by de Zoete, van Putten, & Wagenaar (2007). Killed whole cell vaccines have been examined and have shown mixed results. In one study, formalin-inactivated *C. jejuni* was administered with and without *E. coli* heat-labile toxin and after challenge by the homologous strain it was found that lower numbers of *C. jejuni* were isolated from the cecum (Rice, Rollins, Mallinson, Carr, & Joseph, 1997). However, another study which also used formalin-inactivated *C. jejuni* did not result in reduced cecal colonization upon subsequent homologous strain challenge (Cawthraw, Ayling, Nuijten, Wassenaar, & Newell, 1998). Subunit vaccines have also been looked at, and again have had variable levels of success. A number of studies have looked at using flagellin, which is involved in colonization of *Campylobacter* in the chicken gut. One such study by Widders et al. (1998) found that birds immunized twice intraperitoneally with killed *C. jejuni* and purified flagellin showed a significant reduction in cecal colonization, whereas, birds that received a second immunization orally, or birds immunized twice with flagellin alone did not show significant reductions in cecal colonization of *C. jejuni*. Another study created a fusion protein where flagellin was fused to the B-subunit of the labile toxin of *E. coli* and was administered as a vaccine to chickens that were subsequently challenged with *C. jejuni.* It was found that there was significantly less colonization in comparison to the control birds (Khoury & Meinersmann, 1995). A cocktail of attenuated live *C. jejuni* strains has also been used, but subsequent challenge with the parent strain did not result in reduced colonization compared to control birds (Ziprin, Hume, Young, & Harvey, 2002). These studies clearly indicate that there is a long way to go in designing an efficient *Campylobacter* vaccine.

A major advantage of vaccines as a biocontrol agent is that they can be used as a pre-harvest intervention. Their use has the potential to reduce pathogen carriage, thereby lessening the likelihood of contamination and occurrence of horizontal transfer which allows the pathogen into the food chain. However, the overall results from vaccine trials to reduce the pathogen load in food animals have been mixed and results appear to be very specific to the host used. Arguments also exist about what type of vaccine is best to use-live, killed or subunit vaccines. In the case of *Salmonella*, live vaccines are seen to be more efficacious but concerns such as public acceptability and safety do exist. Killed vaccines are considered safer but appear be less efficient at prompting a protective immune response. Subunit vaccines require an indepth understanding of the pathogen's interaction with its host, in order to choose an effective antigen. Overall, the development of effective vaccines against foodborne pathogens for animals still faces many hurdles.

Concluding Remarks

The key role which meat and meat products play as a vehicle of foodborne zoonotic pathogens is in no doubt. Microbial contamination can occur at all areas of the farm to fork chain. Numerous chemical methods can be employed to remove microbial contamination but more and more there is an increased consumer demand for foods with less additives and an interest in the use of alternative biological agents. It has been shown in this review that numerous potential options are available which can be applied at various points in the food chain and there is substantial research being invested in the area of biocontrol agents. Many of these are in the early stages of development, but some have been employed in food products or in animal trials, with varying degrees of success. What is very clear is that the success of a particular biocontrol agent depends very much on the food matrix, the target pathogen and the conditions used, and a biocontrol which works successfully in one food or animal environment cannot necessarily be extrapolated to another food product type. From a meat perspective, the potential for the use of biocontrol agents is huge as has been exemplified in this review. However, much work remains to be done to tailor these agents for particular products and pathogens and will no doubt be the focus of much research in the years to come.

References

Aasen, I. M., Markussen, S., Moretro, T., Katla, T., Axelsson, L., & Naterstad, K. (2003). Interactions of the bacteriocins sakacin P and nisin with food constituents. *International Journal of Food Microbiology, 87*, 35–43.

Abee, T., Krockel, L., & Hill, C. (1995). Bacteriocins: Modes of action and potentials in food preservation and control of food poisoning. *International Journal of Food Microbiology, 28*, 169–185.

Albano, H., Todorov, S. D., van Reenen, C. A., Hogg, T., Dicks, T. M. T., & Teixeira, P. (2007). Characterization of two bacteriocins produced by *Pediococcus acidilactici* isolated

from “Alheira”, a fermented sausage traditionally produced in Portugal. *International Journal of Food Microbiology, 116*, 239–247.

Alisky, J., Iczkowski, K., Rapoport, A., & Troitsky, N. (1998). Bacteriophages show promise as antimicrobial agents. *The Journal of Infection, 36*, 5–15.

Alves, V. F., Martinez, R. C. R., Lavrador, M. A. S., & De Martinis, E. C. P. (2006). Antilisterial activity of lactic acid bacteria inoculated on cooked ham. *Meat Science, 74*, 623–627.

Anang, D. M., Rusul, G., Bakar, J., & Ling, F. H. (2007). Effects of lactic acid and lauricidin on the survival of *Listeria monocytogenes, Salmonella enteritidis* and *Escherichia coli* O157:H7 in chicken breast stored at 4C. *Food Control, 18*, 961–969.

Ananou, S., Galvez, A., Martinez-Bueno, M., Maqueda, M., & Valdivia, E. (2005). Synergistic effect of enterocin AS-48 in combination with outer membrane permeabilizing against *Escherichia coli* O157:H7. *Journal Applied Microbiology, 99*, 1364–1372.

Ananou, S., Garriga, M., Hugas, M., Maqueda, M., Martinez-Bueno, M., Galvez, A., et al. (2005). Control of *Listeria monocytogenes* in model sausages by enterocin AS-48. *International Journal of Food Microbiology, 103*, 179–190.

Ananou, S., Maqueda, M., Martinez-Bueno, M., Galvez, A., & Valdivia, E. (2005). Control of *Staphylococcus aureus* in sausages by enterocin AS-48. *Meat Science, 71*, 549–556.

Anderson, R. C., & Yu, P.-L. (2005). Factors affecting the antimicrobial activity of ovine-derived cathelicidins against *E. coli* 0157:H7. *International Journal of Antimicrobial Agents, 25*, 205–210.

Annamalai, T., Venkitanarayanan, K. S., Hoagland, T. A., & Khan, M. I. (2001). Inactivation of *Escherichia coli* O157: H7 and *Listeria monocytogenes* by PR-26, a synthetic antibacterial peptide. *Journal of Food Protection, 64*, 1929–1934.

Anzai, K., Hamasuna, M., Kadono, H., Lee, S., Aoyagi, H., & Kirino, Y. (1991). Formation of ion channels in planar lipid bilayer membranes by synthetic basic peptides. *Biochimica et Biophysica Acta, 1064*, 256–266.

Appendini, P., & Hotchkiss, J. H. (1999). Antimicrobial activity of a 14-residue peptide against *Escherichia coli* O157:H7. *Journal of Applied Microbiology, 87*, 750–756.

Ariyapitipun, T., Mustapha, A., & Clarke, A. D. (2000). Survival of *Listeria monocytogenes* Scott A on vacuum-packaged raw beef treated with polylactic acid, lactic acid and nisin. *Journal of Food Protection, 63*, 131–136.

Arlindo, S., Calo, P., Franco, C., Prado, M., Cepeda, A., & Barros-Velazquez, J. (2006). Single nucleotide polymorphism analysis of the enterocin P structural gene of *Enterococcus faecium* strains isolated from nonfermented animal foods. *Molecular Nutrition and Food Research, 50*, 1229–1238.

Arques, J. L., Fernandez, J., Gaya, P., Nunez, M., Rodrigues, E., & Medina, M. (2004). Antimicrobial activity of reuterin in combination with nisin against food-borne pathogens. *International Journal of Food Microbiology, 95*, 225–229.

Atterbury, R. J., Connerton, P. L., Dodd, C. E., Rees, C. E., & Connerton, I. F. (2003). Application of host-specific bacteriophages to the surface of chicken skin leads to a reduction in recovery of *Campylobacter jejuni*. *Applied and Environmental Microbiology, 69*, 6302–6306.

Azuma, T., Bagenda, D. K., Yamamoto, T., Kawai, Y., & Yamazaki, K. (2006). Inhibition of *Listeria monocytogenes* by freeze-dried Piscicocin CS526 fermentate in food. *Letters in Applied Microbiology, 44*, 138–144.

Bach, S. J., McAllister, T. A., Viera, D. M., Gannon, V. P. J., & Holley, R. A. (2002). Transmission and control of *Escherichia coli* O157:H7. A review. *Canadian Journal of Animal Science, 82*, 475–490.

Balogh, B., Jones, J. B., Momol, M. T., Olson, S. M., Obradovic, A., King, P., et al. (2003). Improved efficacy of newly formulated bacteriophages for management of bacterial spot on tomato. *Plant Disease, 87*, 949–954.

Barakat, R. K., Griffiths, M. W., & Harris, L. J. (2000). Isolation and characterization of *Carnobacterium, Lactococcus* and *Enterococcus* spp. from cooked, modified atmosphere packaged, refrigerated, poultry meat. *International Journal of Food Microbiology, 62*, 83–94.

Barboza De Martinez, Y., Ferrer, K., & Marquez Salas, E. (2002). Combined effects of lactic acid and nisin solution in reducing levels of microbiological contamination in red meat carcasses. *Journal of Food Protection, 65*, 1780–1783.

Barrow, P. A. (2007). *Salmonella* infections: Immune and non-immune protection with vaccines. *Avian Pathology, 36*, 1–13.

Barrow, P. A., & Soothill, J. S. (1997). Bacteriophage therapy and prophylaxis: Rediscovery and renewed assessment of potential. *Trends in Microbiology, 5*, 268–271.

Benkerroum, N., Daoudi, A., & Kamal, M. (2003). Behaviour of *Listeria monocytogenes* in raw sausages (merguez) in presence of a bacteriocin-producing lactococcal strain as a protective culture. *Meat Science, 63*, 479–484.

Benkerroum, N., Daoudi, A., Thamraoui, T., Ghalfi, H., Thiry, G., Duroy, M., et al. (2005). Lyophilized preparation of bacteriocinogenic *Lactobacillus curvatus* and *Lactococcus lactis* subsp. *lactis* as potential protective adjuncts to control *Listeria monocytogenes* in dry-fermented sausages. *Journal of Applied Microbiology, 98*, 56–63.

Berchieri, A., Jr., Lovell, M. A., & Barrow, P. A. (1991). The activity in the chicken alimentary tract of bacteriophages lytic for *Salmonella typhimurium*. *Research in Microbiology, 142*, 541–549.

Bohez, L., Ducatelle, R., Pasmans, F., Haesebrouck, F., & Van Immerseel, F. (2007). Long-term colonisation-inhibition studies to protect broilers against colonisation with *Salmonella* Enteritidis, using *Salmonella* Pathogenicity Island 1 and 2 mutants. *Vaccine, 25*, 4235–4243.

Brackett, R. E., Hao, Y. Y., & Doyle, M. P. (1994). Ineffectiveness of hot acid sprays to decontaminate *Escherichia coli* O157:H7 on beef. *Journal of Food Protection, 57*, 198–203.

Brashears, M. M., Jaroni, D., & Trimble, J. (2003). Isolation, selection, and characterization of Lactic acid bacteria for a competitive exclusion product to reduce shedding of *Escherichia coli* O157:H7 in cattle. *Journal of Food Protection, 66*, 355–363.

Brashears, M. M., Reilly, S. S., & Gilliland, S. E. (1998). Antagonistic action of cells of *Lactobacillus lactis* toward *Escherchia coli* O157:H7 on refrigerated raw chicken meat. *Journal of Food Protection, 61*, 166–170.

Bredholt, S., Nesbakken, T., & Holck, A. (1999). Protective cultures inhibit growth of *Listeria monocytogenes* and *Escherichia coli* O157:H7 in cooked, sliced, vacuum- and gas- packaged meat. *International Journal of Food Microbiology, 53*, 43–52.

Bredholt, S., Nesbakken, T., & Holck, A. (2001). Industrial application of an antilisterial strain of *Lactobacillus sakei* as a protective culture and its effect on the sensory acceptibilty of cooked, sliced, vacuum-packaged meats. *International Journal of Food Microbiology, 66*, 191–196.

Budde, B. B., Hornbaek, T., Jacobsen, T., Barkholt, V., & Koch, A. G. (2003). *Leuconostoc carnosum* 4010 has the potential for use as a protective culture for vacuum-packed meats: Culture isolation, bacteriocin identification and meat application experiments. *International Journal of Food Microbiology, 83*, 171–184.

Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in foods – A review. *International Journal of Food Microbiology, 94*, 223–253.

Burt, S. A., & Reinders, R. D. (2003). Antibacterial activity of selected plant essential oils against *Escherichia coli* O157:H7. *Letters in Applied Microbiology, 36*, 162–167.

Callaway, T. R., Anderson, R. C., Edrington, T. S., Genovese, K. J., Harvey, R. B., & Poole, T. L., et al. (2004). Recent pre-harvest supplementation strategies to reduce carriage and shedding of zoonotic enteric bacterial pathogens in food animals. *Animal Health Research Reviews, 5*, 35–47.

Carlton, R. M., Noordman, W. H., Biswas, B., de Meester, E. D., & Loessner, M. J. (2005). Bacteriophage P100 for control of *Listeria monocytogenes* in foods: Genome sequence, bioinformatic analyses, oral toxicity study, and application. *Regulatory Toxicology and Pharmacology, 43*, 301–312.

Castellano, P. H., Holzapfel, W. H., & Vignolo, G. (2004). The control of *Listeria innocua* and *Lactobacillus sakei* in broth and meat slurry with the bacteriocinogenic strain *Lactobacillus casei* CRL705. *Food Microbiology, 21*, 291–298.

Castellano, P., & Vignolo, G. (2006). Inhibition of *Listeria innocua* and *Brochothrix thermosphacta* in vacuum-pakced meat by addition of bacteriocinogenic *Lactobacillus curvatus* CRL705 and its bacteriocins. *Letters in Applied Microbiology, 43*, 194–199.

Castillo, A., Lucia, L. M., Mercado, I., & Acuff, G. R. (2001). In-plant evaluation of a lactic acid treatment for reduction of bacteria on chilled beef carcasses. *Journal of Food Protection, 64*, 738–740.

Castillo, L. A., Meszaros, L., & Kiss, I. F. (2004). Effect of high hydrostatic pressure and nisin on microorganisms in minced meats. *Acta Alimentaria, 33*, 183–190.

Cawthraw, S., Ayling, R., Nuijten, P., Wassenaar, T., & Newell, D. G. (1998). Prior infection, but not a killed vaccine, reduces colonization of chickens by *Campylobacter jejuni.* In A. J. Lastovica, D. G. Newell, and E. E. Lastovica (Eds.), *Campylobacter, Helicobacter and related organisms* (pp. 364–372). Cape Town: Institute of Child Health, University of Cape Town.

Center for Disease Control and Prevention (2006). Multistate outbreak of *Salmonella typhimurium* infections associated with eating ground beef–United States, 2004. *MMWR. Morbidity and Mortality Weekly Report, 55*, 180–182.

Cerquetti, M. C., & Gherardi, M. M. (2000). Orally administered attenuated *Salmonella* enteritidis reduces chicken cecal carriage of virulent *Salmonella* challenge organisms. *Veterinary Microbiology, 76*, 185–192.

Chen, H.-C., & Stern, N. J. (2001). Competitive exclusion of heterologous *Campylobacter* spp. in chicks. *Applied And Environmental Microbiology, 67*, 848–851.

Chen, H., & Hoover, D. G. (2003). Bacteriocins and their food applications. *Comprehensive Reviews in Food Science and Food Safety, 2*, 82–100.

Chiasson, F., Borsa, J., Ouattara, B., & Lacroix, M. (2004). Radiosensitization of *Escherichia coli* and *Salmonella* Typhi in ground beef. *Journal of Food Protection, 67*, 1157–1162.

Cleveland, J., Montville, T. J., Nes, I. F., & Chikindas, M. L. (2001). Bacteriocins: Safe, natural antimicrobials for food preservation. *International Journal of Food Microbiology, 71*, 1–20.

Clifton-Hadley, F. A., Breslin, M., Venables, L. M., Sprigings, K. A., Cooles, S. W., Houghton, S., et al. (2002). A laboratory study of an inactivated bivalent iron restricted *Salmonella enterica* serovars Enteritidis and Typhimurium dual vaccine against Typhimurium challenge in chickens. *Veterinary Microbiology, 89*, 167–179.

Cole, K., Farnell, M. B., Donoghue, A. M., Stern, N. J., Svetoch, E. A., Eruslanov, B. N., Volodina, L. I., Kovalev, N., Perelygin V. V., Mitsevich, E. V., Mitserich, P., Levchuk, V. P., Pokhilenkov, D., Borzenkov, V. N., Svetoch, O. E., Kudrgavtseva, T. Y., Reyes-Herreia, I., Blore, P. J., Solis De Los Santos, F., & Donoghue, D. J. (2006). Bacteriocins reduce *Campylobacter* colonization and alter gut morphology in turkey poults. *Poultry Science, 85*, 1570–1575.

Cooksey, K. (2005). Effectiveness of antimicrobial food packaging materials. *Food Additives and Contaminants, 22*, 980–987.

Cotter, P. D., Hill, C., & Ross, R. P. (2005). Bacteriocins: Developing innate immunity for food. *Nature Reviews. Microbiology, 3*, 777–788.

Davies, R. H., & Breslin, M. F. (2003). Observations on the distribution and persistence of *Salmonella enterica* serovar Enteritidis phage type 29 on a cage layer farm before and after the use of competitive exclusion treatment. *British Poultry Science, 44*, 551–557.

De Martinis, E. C. P., Publio, M. R. P., Santarosa, P. R., & Freitas, F. Z. (2001). Antilisterial activity of lactic acid bacteria isolated from vacuum-packaged Brazilian meat and meat products. *Brazilian Journal of Microbiology, 32*, 32–37.

de Vrese, M., & Schrezenmeir, J. (2002). Probiotics and non-intestinal infectious conditions. *The British Journal of Nutrition, 88*, S59–S66.

de Zoete, M. R., van Putten, J. P. M., & Wagenaar, J. A. (2007). Vaccination of chickens against *Campylobacter*. *Vaccine, 25*, 5548–5557.

Dean-Nystrom, E. A., Bosworth, B. T., Moon, H. W., & O'Brien, A. D. (1998). *Escherichia coli* O157:H7 requires intimin for enteropathogenicity in calves. *Infection and Immunity, 66*, 4560–4563.

Dean-Nystrom, E. A., Gansheroff, L. J., Mills, M., Moon, H. W., & O'Brien, A. D. (2002). Vaccination of pregnant dams with intimin (O157) protects suckling piglets from *Escherichia coli* O157:H7 infection. *Infection and Immunity, 70*, 2414–2418.

Deegan, L. H., Cotter, P. D., Hill, C., & Ross, R. P. (2006). Bacteriocins: Biological tools for bio-preservation and shelf-life extension. *International Dairy Journal, 16*, 1058–1071.

Diez-Gonzalez, F. (2007). Applications of bacteriocins in livestock. *Current Issues in Intestinal Microbiology, 8*, 15–24.

Dorsa, W. J. (1997). New and established carcass decontamination procedures commonly used in the beef-processing industry. *Journal of Food Protection, 60*, 1146–1151.

Doyle, M. P., & Erickson, M. C. (2006). Reducing the carriage of foodborne pathogens in livestock and poultry. *Poultry Science, 85*, 960–973.

Dueger, E. L., House, J. K., Heithoff, D. M., & Mahan, M. J. (2001). *Salmonella* DNA adenine methylase mutants elicit protective immune responses to homologous and heterologous serovars in chickens. *Infection and Immunity, 69*, 7950–7954.

Dueger, E. L., House, J. K., Heithoff, D. M., & Mahan, M. J. (2003a). *Salmonella* DNA adenine methylase mutants elicit early and late onset protective immune responses in calves. *Vaccine, 21*, 3249–3258.

Dueger, E. L., House, J. K., Heithoff, D. M., & Mahan, M. J. (2003b). *Salmonella* DNA adenine methylase mutants prevent colonization of newly hatched chickens by homologous and heterologous serovars. *International Journal of Food Microbiology, 80*, 153–159.

Dykes, G. A., & Moorhead, S. M. (2002). Combined antimicrobial effect of nisin and a listeriophage against *Listeria monocytogenes* in broth but not in buffer or on raw beef. *International Journal of Food Microbiology, 73*, 71–81.

Dziva, F., Vlisidou, I., Crepin, V. F., Wallis, T. S., Frankel, G., & Stevens, M. P. (2007). Vaccination of calves with EspA, a key colonisation factor of *Escherichia coli* O157:H7, induces antigen-specific humoral responses but does not confer protection against intestinal colonisation. *Veterinary Microbiology, 123*, 254–261.

Elder, R. O., Keen, J. E., Siragusa, G. R., Brarkocy-Gallagher, G. A., Koohmaraie, M., & Lagreid, W. W. (2000). Correlation of enterohemorrhagic *Escherichia coli* O157 prevalance in feces, hides and carcasses of beef cattle during processing. *Proceedings of the National Academy of Sciences of the United States of America, 97*, 2999–3003.

Enan, G. (2006a). Inhibition of *Clostridium perfringens* LMG 11264 in meat samples of chicken, turkey and beef by the bacteriocin Plantarcin UG1. *International Journal OF Poultry Science, 5*, 195–200.

Enan, G. (2006b). Behaviour of *Listeria monocytogenes* LMG 10470 in poultry meat and its control by the bacteriocin Plantaricin UG 1. *International Journal of Poultry Science,5*, 355–359.

Etcheverria, A. I., Arroyo, G., Perdigon, G., & Parma, A. E. (2005). *Escherichia coli* with anti-O157:H7 activity isolated from bovine colon. *Journal of Applied Microbiology, 100*, 384–389.

Federal Register (2006). Food additives Permitted for Direct Addition to Food for Human Consumption; Bacteriophage Preparation. *Federal Register, 71*, 47729–47732.

Figueroa-Bossi, N., & Bossi, L. (1999). Inducible prophages contribute to *Salmonella* virulence in mice. *Molecular Microbiology, 33*, 167–176.

Fratamico, P. M., Schultz, F. J., Benedict, R. C., & Buchanan, R. L. (1996). Factors influencing attachment of *Escherichia coli* O157:H7 to beef tissues and removal using selected sanitizing rinses. *Journal of Food Protection, 59*, 453–459.

Gaeng, S., Scherer, S., Neve, H., & Loessner, M. J. (2000). Gene cloning and expression and secretion of *Listeria monocytogenes* bacteriophage-lytic enzymes in *Lactococcus lactis*. *Applied Environmental Microbiology, 66*, 2951–2958.

Galvez, A., Abriouel, H., Lopez, R. L., & Omar, N. B. (2007). Bacteriocin-based strategies for food biopreservation. *International Journal of Food Microbiology,* In press.

Gantois, I., Ducatelle, R., Timbermont, L., Boyen, F., Bohez, L., Haesebrouck, F., et al. (2006). Oral immunisation of laying hens with the live vaccine strains of TAD Salmonella vac E and

TAD Salmonella vac T reduces internal egg contamination with *Salmonella* Enteritidis. *Vaccine, 24*, 6250–6255.

Ganzle, M. G., Hertel, C., van der Vossen, J. M. B. M., & Hammes, W. P. (1999). Effect of bacteriocin-producing lactobacilli on the survival of *Escherichia coli* and *Listeria* in a dynamic model of the stomach and the small intestine. . *International Journal of Food Microbiology, 48*, 21–35.

Ganzle, M. G., Weber, S., & Hammes, W. P. (1999). Effect of ecological factors on the inhibtory spectrum and activity of bacteriocins. *International Journal of Food Microbiology, 46*, 207–217.

Genovese, K. J., Anderson, R. C., Harvey, R. B., Callaway, T. R., Poole, T. L., Edrington, T. S., et al. (2003). Competitive exclusion of *Salmonella* from the cut of neonatal and weaned pigs. *Journal of Food Protection, 66*, 1353–1359.

Ghalfi, H., Benkerroum, N., Doguiet, D. D. K., Bensaid, M., & Thonart, P. (2007). Effectiveness of cell-adsorbed bacteriocin produced by *Lactobacillus curvatus* CWBI-B28 and selected essential oils to control *Listeria monocytogenes* in pork meat during cold storage. *Letters in Applied Microbiology, 44*, 268–273.

Gill, A. O., Delaquis, P. J., Russo, P., & Holley, R. A. (2002). Evaluation of antilisterial action of cilantro oil on vacuum packed ham. *International Journal of Food Microbiology, 73*, 83–92.

Gillespie, I. A., O'Brien, S. J., Adak, G. K., Cheasty, T., & Willshaw, G. (2005). Foodborne general outbreaks of Shiga toxin-producing *Escherichia coli* O157 in England and Wales 1992–2002: where are the risks? *Epidemiology and Infection, 133*, 803–808.

Goode, D., Allen, V. M., & Barrow, P. A. (2003). Reduction of experimental *Salmonella* and *Campylobacter* contamination of chicken skin by application of lytic bacteriophages. *Applied Environmental Microbiology, 69*, 5032–5036.

Goodridge, L., Chen, J., & Griffiths, M. (1999). The use of a fluorescent bacteriophage assay for detection of *Escherichia coli* O157:H7 in inoculated ground beef and raw milk. *International Journal of Food Microbiology, 47*, 43–50.

Greer, G. G., & Dilts, B. D. (1992). Factors affecting the susceptibilitiy of meatborne pathogens and spoilage bacteria to organic acids. *Food Research International, 25*, 355–364.

Greer, G. G., & Dilts, B. D. (2002). Control of *Brochothrix thermosphacta* spoilage of pork adipose tissue using bacteriophages. *Journal of Food Protection, 65*, 861–863.

Greer, G. G., & Dilts, B. D. (2006). Control of meatborne *Listeria monocytogenes* and *Brochothrix thermosphacta* by a bacteriocinogenic *Brochothrix campestris* ATCC 43754. *Food Microbiol, 23*, 785–790.

Hammer, K. A., Carson, C. F., & Riley, T. V. (1999). Antimicrobial activity of essential oils and other plant extracts. *Journal of Applied Microbiology, 86*, 985–990.

Hancock, R. E., & Sahl, H. G. (2006). Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. *Nature Biotechnology, 24*, 1551–1557.

Hanlon, G. W. (2007). Bacteriophages: An appraisal of their role in the treatment of bacterial infections. *International Journal of Antimicrobial Agents, 30*, 118–128.

Hao, Y. Y., Brackett, R. E., & Doyle, M. P. (1998a). Efficacy of plants extracts in inhibiting *Aeromonas hydrophilia* and *Listeria monocytogenes* in refrigerated cooked poultry. *Food Microbiol, 15*, 367–378.

Hao, Y. Y., Brackett, R. E., & Doyle, M. P. (1998b). Inhibition of *Listeria monocytogenes* and *Aeromonas hydrophila* by plant extracts in refrigerated cooked beef. *Journal of Food Protection, 61*, 307–312.

Hardin, M. D., Acuff, G. R., Lucia, L. M., Oman, J. S., & Savell, J. W. (1995). Comparison of methods for decontamination from beef carcass surfaces. *Journal of Food Protection, 58*, 368–374.

Hayes, M., Ross, R. P., Fitzgerald, G. F., Hill, C., & Stanton, C. (2006). Casein-derived antimicrobial peptides generated by *Lactobacillus acidophilus* DPC6026. *Applied and Environmental Microbiology, 72*, 2260–2264.

Haynie, S. L., Crum, G. A., & Doele, B. A. (1995). Antimicrobial activities of amphiphilic peptides covalently bonded to a water-insoluble resin. *Antimicrobial Agents and Chemotherapy, 39*, 301–307.

Heller, C. E., Scanga, J. A., Sofos, J. N., Belk, K. E., Warren-Serna, W., Bellinger, G. R., et al. (2007). Decontamination of beef subprimal cuts intended for blade tenderization or moisture enhancement. . *Journal of Food Protection, 70*, 1174–1180.

Higgins, J. P., Higgins, S. E., Guenther, K. L., Huff, W., Donoghue, A. M., Donoghue, D. J., et al. (2005). Use of specific bacteriophage treatment to reduce *Salmonella* in poultry products. *Poultry Science, 84*, 1141–1145.

Higgs, R., Lynn, D., Cahalane, S., Alaña, I., Hewage, C., James, T., et al. (2007). Modification of chicken avian β-defensin-8 at positively selected amino acid sites enhances specific antimicrobial activity. *Immunogenetics, 59*, 573–580.

Holzapfel, W. H., Geisen, R., & Schillinger, U. (1995). Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. *International Journal of Food Microbiology, 24*, 343–362.

Hoszowski, A., & Truszczynski, M. (1997). Prevention of *Salmonella typhimurium* caecal colonisation by different preparations for competitive exclusion. *Comparative Immunology, Microbiology and Infectious Diseases, 20*, 111–117.

Hsu, F. C., Shieh, Y. S., & Sobsey, M. D. (2002). Enteric bacteriophages as potential fecal indicators in ground beef and poultry meat. *Journal of Food Protection, 65*, 93–99.

Huffman, R. D. (2002). Current and future technologies for the decontamination of carcasses and fresh meat. *Meat Science, 62*, 285–294.

Hugas, M., Pages, F., Garriga, M., & Monfort, J. M. (1998). Application of the bacteriocinogenic *Lactobacillus sakei* CTC494 to prevent growth of *Listeria* in fresh and cooked meat products packed with different atmospheres. *Food Microbiology, 15*, 639–650.

Jacobsen, T., Budde, B. B., & Koch, A. G. (2003). Application of *Leuconostoc carnosum* for biopreservation of cooked meat products. *Journal of Applied Microbiology, 95*, 242–249.

Joerger, R. D. (2003). Alternatives to antibiotics: Bacteriocins, antimicrobial peptides and bacteriophages. *Poultry Science, 82*, 640–647.

Katla, T., Moretro, T., Sveen, I., Assen, I. M., Axelsson, L., Rorvik, L. M., & Naterstad, K. (2002). Inhibition of *Listeria monocytogenes* by addition of Sakacin P and Sakacin P-producing *Lactobacillus sakei. Journal of Applied Microbiology, 83*, 191–196.

Khan, M. I., Fadl, A. A., & Venkitanarayanan, K. S. (2003). Reducing colonization of *Salmonella* Enteritidis in chicken by targeting outer membrane proteins. *Journal of Applied Microbiology, 95*, 142–145.

Khoury, C. A., & Meinersmann, R. J. (1995). A genetic hybrid of the *Campylobacter jejuni flaA* gene with LT-B of *Escherichia coli* and assessment of the efficacy of the hybrid protein as an oral chicken vaccine. *Avian Diseases, 39*, 812–820.

Koohmaraie, M., Arthur, T. M., Bosilevac, J. M., Guerini, M., Shackelford, S. D., & Wheeler, T. L. (2005). Post-harvest interventins to reduce/eliminate pathogens in beef. *Meat Science, 71*, 79–91.

Kostrzynska, M., & Bachand, A. (2006). Use of microbial antagonism to reduce pathogen levels on produce and meat products: A review. *Canadian Journal of Microbiology, 52*, 1017–1026.

Kudva, I. T., Jelacic, S., Tarr, P. I., Youderian, P., & Hovde, C. J. (1999). Biocontrol of *Escherichia coli* O157 with O157-specific bacteriophages. *Applied Environmental Microbiology, 65*, 3767–3773.

Kuleasan, H., & Cakmakci, M. L. (2002). Effect of reuterin produced by *Lactobacillus reuteri* on the surface of sausages to inhibit the growth of *Listeria monocytogenes* and *Salmonella* spp. *Nahrung/Food, 46*, 408–410.

La Ragione, R. M., & Woodward, M. J. (2003). Competitive exclusion by *Bacillus subtilis* spores of *Salmonella enterica* serotype Enteritidis and *Clostridium perfringens* in young chickens. *Veterinary Microbiology, 94*, 245–256.

Lambert, R. J. W., Skandamis, P., Coote, P. J., & Nychas, G.-J. E. (2001). A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *Journal of Applied Microbiology, 91*, 453–462.

LeJeune, J. T., & Wetzel, A. N. (2007). Preharvest control of *Escherichia coli* O157 in cattle. *Journal of Animal Science, 85*, E73–80.

Lemay, M. J., Choquette, J., Delaquis, P. J., Claude, G., Rodrigue, N., & Saucier, L. (2002). Antimicrobial effect of natural preservatives in a cooked and acidified chicken meat model. *International Journal of Food Microbiology, 78*, 217–226.

Lenski, R. E. (1988). Dynamics of interactions between bacteria and virulent bacteriophage *Advances in Microbial Ecology, 10*, 1–44.

Leroy, F., & De Vuyst, L. (2005). Simulation of the effect of sausage ingredients and technology on the functionality of the bacteriocin-producing *Lactobacillus sakei* CTC 494 strain. *International Journal of Food Microbiology, 100*, 141–152.

Leverentz, B., Conway, W. S., Camp, M. J., Janisiewicz, W. J., Abuladze, T., Yang, M., et al. (2003). Biocontrol of *Listeria monocytogenes* on fresh-cut produce by treatment with lytic bacteriophages and a bacteriocin. *Applied Environmental Microbiolgy, 69*, 4519–4526.

Linde, K., Beer, J., & Bondarenko, V. (1990). Stable *Salmonella* live vaccine strains with two or more attenuating mutations and any desired level of attenuation. *Vaccine, 8*, 278–282.

Linde, K., Hahn, I., & Vielitz, E. (1996). Entwicklung von optimal fur das Huhn attenuierten *Salmonella*-Lebendimpfstoffen. *Tierärztliche Umschau, 51*, 23–31.

Liu, W., Yang, Y., Chung, N., & Kwang, J. (2001). Induction of humoral immune response and protective immunity in chickens against *Salmonella* Enteritidis after a single dose of killed bacterium-loaded microspheres. *Avian Diseases, 45*, 797–806.

Loc Carrillo, C., Atterbury, R. J., El-Shibiny, A., Connerton, P. L., Dillon, E., Scott, A., et al. (2005). Bacteriophage Therapy To Reduce *Campylobacter jejuni* Colonization of Broiler Chickens. *Applied Environmental Microbiology, 66*: 220–225.

Loessner, M. J. (2005). Bacteriophage endolysins – current state of research and applications. *Current Opinion in Microbiology, 8*, 480–487.

Lopez-Exposito, I., Minervini, F., Amigo, L., & Recio, I. (2006). Identification of antibacterial peptides from bovine kappa-casein. *Journal of Food Protection, 69*, 2992–2997.

Lucke, F. K. (2000). Utilization of microbes to process and preserve meat. *Meat Science, 56*, 105–115.

Maciorowski, K. G., Pillai, S. D., & Ricke, S. C. (2001). Presence of bacteriophages in animal feed as indicators of fecal contamination. *Journal of Environmental Science and Health, 36*, 699–708.

Marcos, B., Aymerich, T., Monfort, J. M., & Garriga, M. (2007). Use of antimicrobial biodegradable packaging to control *Listeria monocytogenes* during storage of cooked ham. *International Journal of Food Microbiology,* In press.

Mattila, K., Saris, P., & Tyopponen, S. (2003). Survival of *Listeria monocytogenes* on sliced cooked sausage after treatment with pediocin AcH. *International Journal of Food Microbiology, 89*, 281–286.

Mauriello, G., Ercolini, D., La Storia, A., Casaburi, A., & Villani, F. (2004). Development of polythene films for food packaging activated with an antilisterial bacteriocin from *Lactobacillus curvatus* 32Y. *Journal of Applied Microbiology, 97*, 314–322.

Mazick, A., Ethelberg, S., Nielsen, E. M., Molbak, K., & Lisby, M. (2006). An outbreak of *Campylobacter jejuni* associated with consumption of chicken, Copenhagen, 2005. *Euro Surveillance, 11*, 137–139.

McCormick, J. K., Klaenhammer, T. R., & Stiles, M. E. (1999). Colicin V can be produced by lactic acid bacteria. *Letters in Applied Microbiology, 29*, 37–41.

Mead, P. S., Dunne, E. F., Graves, L., Wiedmann, M., Patrick, M., Hunter, S., et al. (2006). Nationwide outbreak of listeriosis due to contaminated meat. *Epidemiology and Infection,* 134, 744–751.

Mead, P. S., Slutsker, L., Dietz, V., McCaig, L. F., Bresee, J. S., Shapiro, C., et al. (1999). Food-related illness and death in the United States. *Emerging Infectious Diseases, 5*, 607–625.

Meenakshi, M., Bakshi, C. S., Butchaiah, G., Bansal, M. P., Siddiqui, M. Z., & Singh, V. P. (1999). Adjuvanted outer membrane protein vaccine protects poultry against infection with *Salmonella* Enteritidis. *Veterinary Research Communications, 23*, 81–90.

Milona, P., Townes, C. L., Bevan, R. M., & Hall, J. (2007). The chicken host peptides, gallinacins 4, 7, and 9 have antimicrobial activity against *Salmonella* serovars. *Biochemical and Biophysical Research Communications, 356*, 169–174.

Ming, X., Weber, G. H., Ayres, J. W., & Sandine, W. E. (1997). Bacteriocins applied to food packaging material to inhibit *Listeria monocytogenes* on meats. *Journal of Food Science, 62*, 413–415.

Mirold, S., Rabsch, W., Tschape, H., & Hardt, W. D. (2001). Transfer of the *Salmonella* type III effector *sopE* between unrelated phage families. *Journal of Molecular Biology, 312*, 7–16.

Morency, H., Mota-Meira, M., LaPointe, G., Lacroix, C., & Lavoie, M. C. (2001). Comparison of the activity spectra against pathogens of bacterial strains producing a mutacin or a lantibiotic. *Canadian Journal of Microbiology, 47*, 322–331.

Muthukumarasamy, P., Han, J. H., & Holley, R. A. (2003). Bactericidal effects of *Lactobacillus reuteri* and allyl isothiocyanate on *Escherichia coli* O157:H7 in refrigerated ground beef. *Journal of Food Protection, 66*, 2038–2044.

Naghmouchi, K., Kheadr, E., Lacroix, C., & Fliss, I. (2007). Class I/IIa bacteriocin cross-resistance phenomenon in *Listeria monocytogenes. Food Microbiology, 24*, 718–727.

Nava, G. M., Bielke, L. R., Callaway, T. R., & Castaneda, M. P. (2005). Probiotic alternatives to reduce gastrointestinal infections: The poultry experience. *Animal Health Research Reviews, 6*, 105–118.

Nieto-Lozano, J. C., Reguera-Useros, J. I., Pelaez-Martinez, M. C., & Hardisson de la Torre, A. (2006). Effect of a bacteriocin produced by *Pediococcus acidilactici* against *Listeria monocytogenes* and *Clostridium perfringenes* on Spanish raw meat. *Meat Science, 72*, 57–61.

Noonpakdee, W., Santivarangkna, C., Jumriangrit, P., Sonomoto, K., & Panyim, S. (2003). Isolation of nisin-producing *Lactococcus lactis* WNC20 strain from *nham*, a traditional Thai fermented sausage. *International Journal of Food Microbiology, 81*, 137–145.

O'Flynn, G., R. P.Ross, Fitzgerald, G. F., & Coffey, A. (2004). Evaluation of a Cocktail of Three Bacteriophages for Biocontrol of *Escherichia coli* O157:H7. *Applied Environmental Microbiology, 70*, 3417–3424.

O'Sullivan, L., Ross, R. P., & Hill, C. (2002). Potential of bacteriocin-producing lactic acid bacteria form improvements in food safety and quality. *Biochimie, 84*, 593–604.

O'Sullivan, L., Ryan, M. P., Ross, R. P., & Hill, C. (2003). Generation of food-grade Lactococcal starters which produce the Lantibiotics Lacticin 3147 and Lacticin 481. *Applied Environmental Microbiology, 68*, 3681–3685.

Ovchinnikova, T. V., Balandin, S. V., Aleshina, G. M., Tagaev, A. A., Leonova, Y. F., Krasnodembsky, E. D., et al. (2006). Aurelin, a novel antimicrobial peptide from jellyfish *Aurelia aurita* with structural features of defensins and channel-blocking toxins. *Biochemical and Biophysical Research Communications, 348*, 514–523.

Pao, S., Randolph, S. P., Westbrook, W., & Shen, H. (2004). Use of bacteriophages to control *Salmonella* in experimentally contaminated sprout seeds. *Journal of Food Science, 69*, 127–130.

Patel, J. R., Sanglay, G. C., Sharma, M., & Solomon, M. B. (2007). Combining antimicrobials and hydrodynamic pressure processing for control of *Listeria monocytogenes* in frankfurters. *Journal of Muscle Foods, 18*, 1—18.

Podda, E., Benincasa, M., Pacor, S., Micali, F., Mattiuzzo, M., Gennaro, R., et al. (2006). Dual mode of action of Bac7, a proline-rich antibacterial peptide. *Biochimica Et Biophysica Acta, 1760*, 1732–1740.

Podolak, R. K., Zayas, J. F., Kastner, C. L., & Fung, D. Y. C. (1996). Inhibition of *Listeria monocytogenes* and *Escherichia coli* O157:H7 on beef by application of organic acids. *Journal of Food Protection, 59*, 370–373.

Potter, A. A., Klashinsky, S., Li, Y., Frey, E., Townsend, H., Rogan, D., et al. (2004). Decreased shedding of *Escherichia coli* O157:H7 by cattle following vaccination with type III secreted proteins. *Vaccine, 22*, 362–369.

Prema, P., Bharathy, S., Palavesam, A., Sivasubramanian, M., & Immanuel, G. (2006). Detection, purification and efficacy of warnerin produced by *Staphylococcus warneri. World Journal of Microbiology & Biotechnology, 22*, 865–872.

Quiberoni, A., Suarez, V. B., & Reinheimer, J. A. (1999). Inactivation of *Lactobacillus helveticus* bacteriophages by thermal and chemical treatments. *Journal of Food Protection, 62*, 894–898.

Quintavalla, S., & Vicini, L. (2002). Antimicrobial food packaging in meat industry. *Meat Science, 62*, 373–380.

Raya, R. R., Varey, P., Oot, R. A., Dyen, M. R., Callaway, T. R., Edrington, T. S., et al. (2006). Isolation and characterization of a new T-even bacteriophage, CEV1, and determination of its potential to reduce *Escherichia coli* O157:H7 levels in sheep. *Applied Environmental Microbiology, 72*, 6405–6410.

Rice, B. E., Rollins, D. M., Mallinson, E. T., Carr, L., & Joseph, S. W. (1997). *Campylobacter jejuni* in broiler chickens: Colonization and humoral immunity following oral vaccination and experimental infection. *Vaccine, 15*, 1922–1932.

Riley, M. A., & Wertz, J. E. (2002). Bacteriocins: Evolution, ecology and application. *Annual Review of Microbiology, 56*, 117–137.

Rodriguez, E. J. L. A., Nunez, M., Gaya, P., & Medina, M. (2005). Combined effect of high-pressure treatments and bacteriocin-producing lactic acid bacteria on inactivation of *Escherichia coli* O157:H7 in raw-milk cheese. *Applied And Environmental Microbiology, 71*, 3399–3404.

Roesler, U., Marg, H., Schroder, I., Mauer, S., Arnold, T., Lehmann, J., et al. (2004). Oral vaccination of pigs with an invasive *gyrA-cpxA-rpoB Salmonella* Typhimurium mutant. *Vaccine, 23*, 595–603.

Russell, J. B., and Mantovan, H. C. (2002). The bacteriocins of ruminal bacteria and their potential as an alternative to antibiotics. *Journal of Molecular Microbiology and Biotechnology, 4*, 347–355.

Samelis, J., Sofos, J. N., Kendall, P. A., 7 Smith, G. C. (2002). Effect of acid adaptation on survival of *Escherichia coli* O157:H7 in meat decontamination washing fluids and potential effects of organic acid interventions on the microbial ecology of the meat plant environment. *Journal of food protection, 65*, 33–40.

Sang, Y., Teresa Ortega, M., Rune, K., Xiau, W., Zhang, G., Soulages, J. L., et al. (2007). Canine cathelicidin (K9CATH): Gene cloning, expression, and biochemical activity of a novel promyeloid antimicrobial peptide. *Developmental And Comparative Immunology,* In Press, Corrected Proof.

Scannell, A. G. M., Hill, C., Ross, R. P., Marx, S., Hartmeier, W., & Arendt, E. K. (2000). Development of bioactive food packaging materials using immobilised bacteriocins Lacticin 3147 and Nisaplin. *International journal of food microbiology, 60*, 241–249.

Schamberger, G. P., Phillips, R. L., Jacobs, J. L., & Diez-Gonzalez, F. (2004). Reduction of *Escherichia coli* O157:H7 populations in cattle by feeding colicin E7-producing *E. coli. Applied and Environmental Microbiology*, i, 6053–6060.

Schmieger, H., & Schicklmaier, P. (1999). Transduction of multiple drug resistance of *Salmonella enterica* serovar Typhimurium DT104. FEMS Microbiology Letters, *170*, 251–256.

Schneider, R., Fernandez, F. J., Aguilar, M. B., Guerrero-Legerreta, I., Alpuche-Solis, A., & Ponce-Alquicira, E. (2006). Partial characterization of a class IIa pediocin produced by *Pediococcus parvulus* 133 strain isolated from meat (Mexican 'chorizo'). *Food Control, 17*, 909–915.

Schneitz, C., & Hakkinen, M. (1998). Comparison of two different types of competitive exclusion products. *Letters in Applied Microbiology, 26*, 338–341.

Schoeni, J. L., & Wong, A. C. L. (1994). Inhibition of *Campylobacter jejuni* colonization in chicks by defined competitive exclusion bacteria. *Applied And Environmental Microbiology, 60*, 1191–1197.

Schoenis, J. L., & Doyle, M. P. (1992). Reduction of *Campylobacter jejuni* colonization of chicks by cecum-colonizing bacteria producing anti-*C. jejuni* metabolites. *Applied And Environmental Microbiology, 58*, 664–670.

Senne, M. M., & Gilliland, S. E. (2003). Antagonistic action of cells of *Lactobacillus delbrueckii* subsp. *lactis* against pathogenic and spoilage microorganisms in fresh meat systems. *Journal of food protection,*: 418–425.

Sheng, H., Knecht, H. J., Kudva, I. T., & Hovde, C. J. (2006). Application of bacteriophages to control intestinal *Escherichia coli* O157:H7 levels in ruminants. *Applied And Environmental Microbiology,*: 5359–5366.

Shin, K., Yamauchi, K., Teraguchi, S., Hayasawa, H., Tomita, M., Otsuka, Y., et al. (1998). Antibacterial activity of bovine lactoferrin and its peptides against enterohaemorrhagic *Escherichia coli* O157:H7. *Letters in Applied Microbiology,* 407–411.

Si, W., Gong, J., Tsao, R., Zhou, E., Yu, H., Poppe, C., et al. (2006). Antimicrobial activity of essential oils and structurally related synthetic food additives towards selected pathogenic and beneficial gut bacteria. *Journal of Applied Microbiology*, 296–305.

Siragusa, G. R., Cutter, C. N., & Willett, J. L. (1999). Incorporation of bacteriocin in plastic retains activity and inhibits surface growth of bacteria on meat. *Food Microbiology, 16*, 229–235.

Skandamis, P. N., & Nychas, G.-J. E. (2001). Effect of oregano essential oil on the microbiological and physico-chemical attributes of minced meat stored in air and modified atmospheres. . *Journal of Applied Microbiology, 91*, 1011.

Skandamis, P., Tsigarida, E., & Nychas, G.-J. E. (2002). The effect of oregano essential oil on survival/death of *Salmonella typhimurium* in meat stored at 5C under aerobic, VP/MAP conditions. *Food Microbiology, 19*, 97–103.

Sklar, I. B., & Joerger, R. D. (2001). Attempts to utilize bacteriophage to combat *Salmonella enterica* serovar Enteritidis infection in chickens. *J Food Safety, 21*, 15–29.

Smith, H. W., Huggins, M. B., & Shaw, K. M. (1987). Factors influencing the survival and multiplication of bacteriophages in calves and in their environment. *Journal of General Microbiology, 133*, 1127–1135.

Smulders, F. J., & Greer, G. G. (1998). Integrating microbial decontamination with organic acids in HACCP programmes for muscle foods: Prospects and controversies. *International Journal of Food Microbiology, 44*, 149–169.

Stergiou, V. A., Thomas, L. V., & Adams, M. R. (2006). Interactions of nisin with glutathione in a model protein system and meat. *Journal of Food Protection, 69*, 951–956.

Stern, N. J., Svetoch, E. A., Eruslanov, B. V., Kovalev, Y. N., Volodina, L. I., Perelygin, V. V., et al. (2005). *Paenibacillus polymyxa* purified bacteriocin to control *Campylobacter jejuni* in chickens. *Journal of Food Protection, 68*, 1450–1453.

Svetoch, E. A., Stern, N. J., Eruslanov, B. V., Kovalev, Y. N., Volodina, L. I., Perelygin, V. V., et al. (2005). Isolation of *Bacillus circulans* and *Paenibacillus polymyxa* strains inhibitory to *Campylobacter jejuni* and characterization of associated bacteriocins. *Journal of Food Protection, 68*, 11–17.

Tanji, Y., Shimada, T., Yoichi, M., Miyanaga, K., Hori, K., & Unno, H. (2004). Toward rational control of *Escherichia coli* O157:H7 by a phage cocktail. *Applied Microbiology and Biotechnology, 64*, 270–274.

Tassou, C. C., Drosinos, E. H., & Nychas, G. J. (1995). Effects of essential oil from mint (*Mentha piperita*) on *Salmonella enteritidis* and *Listeria monocytogenes* in model food systems at 4 degrees and 10 degrees C. *The Journal of Applied Bacteriology, 78*, 593–600.

Tsigarida, E., Skandamis, P., & Nychas, G.-J. E. (2000). Behaviour of *Listeria monocytogenes* and autochthonous flora on meat stored under aerobic, vacuum and modified atmosphere packaging conditions with or without the presence of oregano essential oil at 5C. *Journal of Applied Microbiology, 89*, 901–909.

United States Department of Agriculture, Economic Resource Service (2001). Economics of foodborne disease: Estimating the benefits of reducing foodborne disease. United States Department of Agriculture, E.R.S. (ed.) Washington DC.

United States Department of Agriculture-Food Safety and Inspection Service (1996). Notice of policy change; achieving the zero tolerance performances stard for beef carcasses by knife trimming and vacuuming with hot water or steam; use of acceptable carcass interventions for reducing carcass contamination without prior agency approval. In *Fed. Reg. 61:15024–15027*. United States Department of Agriculture, F.S.a.I.S. (ed.).

van Diemen, P. M., Dziva, F., Abu-Median, A., Wallis, T. S., van den Bosch, H., Dougan, G., et al. (2007). Subunit vaccines based on intimin and Efa-1 polypeptides induce humoral immunity in cattle but do not protect against intestinal colonisation by enterohaemorrhagic *Escherichia coli* O157:H7 or O26:H. *Veterinary Immunology and Immunopathology, 116*, 47–58.

Van Donkersgoed, J., Hancock, D., Rogan, D., & Potter, A. A. (2005). *Escherichia coli* O157:H7 vaccine field trial in 9 feedlots in Alberta and Saskatchewan. *The Canadian Veterinary Journal, 46*, 724–728.

Vignolo, G., Palacios, J., Farias, M. E., Sesma, F., Schillinger, U., Holzapfel, W. H., et al. (2000). Combined effect of bacteriocins on the survival of various *Listeria* species in broth and meat system. *Current Microbiology, 41*, 410–416.

Vrinda Menon, K., & Garg, S. R. (2001). Inhibitory effect of clove oil on *Listeria monocytogenes* in meat and cheese. *Food Microbiology, 18*, 647–650.

Waddell, T., Mazzocco, A., Johnson, R. P., Pacan, J., Campbell, S., Perets, A., et al. (2000). Control of *Escherichia coli* O157:H7 infection in calves by bacteriophages. *Presentation, 4th International Symposium and Workshop on Shiga toxin (Verocytotoxin)- Producing Escherichia coli Infections*, Kyoto, Japan.

Wagenaar, J. A., Mevius, D. J., & Havelaar, A. H. (2006). *Campylobacter* in primary animal production and control strategies to reduce the burden of human campylobacteriosis. *Revue Science et Technique, 25*, 581–594.

Wagenaar, J. A., Van Bergen, M. A., Mueller, M. A., Wassenaar, T. M., & Carlton, R. M. (2005). Phage therapy reduces *Campylobacter jejuni* colonization in broilers. *Veterinary Microbiology, 109*, 275–283.

Wagner, P. L., and Waldor, M. K. (2002). Bacteriophage control of bacterial virulence. *Infection and Immunity, 70*, 3985–3993.

Wagner, R. D. (2006). Efficacy and food safety consideration of poultry competitive exclusion products. *Molecular Nutrition & Food Research, 50*, 1061–1071.

Whichard, J. M., Sriranganathan, N., & Pierson, F. W. (2003). Suppression of *Salmonella* growth by wild-type and large-plaque variants of bacteriophage Felix O1 in liquid culture and on chicken frankfurters. *Journal of Food Protection*, 220–225.

Widders, P. R., Thomas, L. M., Long, K. A., Tokhi, M. A., Panaccio, M., & Apos, E. (1998). The specificity of antibody in chickens immunised to reduce intestinal colonisation with *Campylobacter jejuni*. *Veterinary Microbiology, 64*, 39–50.

Woodward, M. J., Gettinby, G., Breslin, M. F., Corkish, J. D., & Houghton, S. (2002). The efficacy of Salenvac, a *Salmonella enterica* subsp. Enterica serotype Enteritidis iron-restricted bacterin vaccine, in laying chickens. *Avian Pathology, 31*, 383–392.

Yaron, S., Rydlo, T., Shachar, D., & Mor, A. (2003). Activity of dermaseptin K4-S4 against foodborne pathogens. *Peptides, 24*, 1815–1821.

Yin, L. J., Wu, C. W., & Jiang, S. T. (2003). Bacteriocins from *Pediococcus pentosaceus* L and S from pork meat. *Journal of Agricultural and Food Chemistry, 51*, 1071–7076.

Yuste, J., Pla, R., Capellas, M., & Mor-Mur, M. (2002). Application of high-pressure processing and nisin to mechanically recovered poultry meat for microbial decontamination. *Food Control, 13*, 451–455.

Zasloff, M. (2002). Antimicrobial peptides of multicellular organisms. *Nature, 415*, 389–395.

Zhang, G., & Mustapha, A. (1999). Reduction of *Listeria monocytogenes* and *Escherichia coli* O157:H7 numbers on vacuum-packaged fresh beef treated with nisin or nisin combined with EDTA. *Journal of Food Protection, 62*, 1123–1127.

Zhang, G., Ma, L., & Doyle, M. P. (2007a). Salmonellae reduction in poultry by competitive exclusion bacteria *Lactobacillus salivarius* and *Streptococcus cristatus*. *Journal of Food Protection, 70*, 874–878.

Zhang, G., Ma, L., & Doyle, M. P. (2007b). Potential competitive exclusion bacteria from poultry inhibitory to *Campylobacter jejuni* and *Salmonella. Journal of Food Protection, 70*, 867–873.

Zhao, T., Doyle, M. P., Harmon, B. G., Brown, C. A., Mueller, P. O. E., & Parks, A. H. (1998). Reduction of carriage of Enterohemorrhagic *Escherichia coli* O157:H7 in cattle in inoculation with probiotic bacteria. *Journal of Clinical Microbiology*, 36: 641–647.

Zhao, T., Tkalcic, S., Doyle, M. P., Harmon, B. G., Brown, C. A., & Zhao, P. (2003). Pathogenicity of Enterohaemorrhagic *Escherichia coli* in neonatal calves and evaluation of fecal shedding by treatment with probiotic *Escherichia coli. Journal of Food Protection, 66*, 924–30.

Ziprin, R. L., Hume, M. E., Young, C. R., & Harvey, R. B. (2002). Inoculation of chicks with viable non-colonizing strains of *Campylobacter jejuni*: Evaluation of protection against a colonizing strain. *Current microbiolog*y. *44*, 221–223.

Zuckerman, H., & Abraham, R. B. (2002). Quality improvement of kosher chilled poultry. *Poultry science, 81*, 1751–1757.