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Trends in Food Science & Technology 22 (2011) S58–S66

TRENDS IN FOOD SCIENCE & TECHNOLOGY

The role of protective and probiotic cultures in food and feed and their impact in food safety

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In order to meet the increasing demand for food quality and safety, the control of pathogenic microorganisms from "farm to fork" is a continuous challenge. This challenge has become more important due to changes in animal production, product processing and distribution, new food habits, higher numbers of consumers at risk for infection and increased awareness. This review is focused on the use of protective and probiotic cultures as "natural" intervention measures to control and prevent the transmission of pathogens along the food chain and on the most used technologies to produce these cultures at the large scale.

Introduction

The quality and safety of foods, both of animal and plant origin, is of outmost importance for governments, industries and consumers. It is well recognized that pathogens, such as *Campylobacter*, *Salmonella*, *Escherichia coli* and *Listeria*, can be transmitted along the food chain and be a source of human illness; they can contaminate foods and multiply under suitable conditions. Today illness resulting from food-borne pathogens has become one of the most widespread public health problems in the world (Mor-Mur & Yuste, 2010). As a result, there is a need to look for solutions to break the transmission of harmful bacteria along the food chain.

Beneficial bacteria, mainly lactic acid bacteria (LAB) and bifidobacteria, may be a useful and effective strategy to prevent or reduce the incidence of pathogens, thus improving food safety and consumer health (Callaway *et al*., 2008; Gaggìa, Mattarelli, & Biavati, 2010). In addition, these microorganisms may possess anti-microbial activity against spoilage microorganisms, which sensibly reduce the shelf-life of a product. Such bacteria can be used either as protective cultures (live microorganisms that, once added to food products, inhibit pathogens and/or prolong the shelf-life) or as probiotic cultures (live microorganisms that, once ingested by animals at farm level, can help to improve animal health).

This review will deal with the current state of the art of biological prevention strategies for food-borne pathogens: the application of protective cultures as a tool for the preservation of foods and the use of probiotic cultures as feed additives for food-producing animals to control the shedding of pathogens at farm level. Moreover, the requirements and the most used technologies for large-scale industrial production will be examined.

The protective culture approach

Biological preservation refers to the extension of the shelf-life of food products and improvement of their microbial safety by using two different approaches: i) the inoculation of the food matrix with target microorganisms, defined as protective cultures, with consequent *in situ* production of inhibitory molecules and/or a competitive effect against pathogen and spoilage bacteria and ii) the use of microbial metabolites in purified form, in particular bacteriocins (Galvez, Abriouel, Benomar, & Lucas, 2010; Garcıa, Rodrıguez, Rodrıguez, & Martınez, 2010; Schillinger, Geisen, & Holzapfel, 1996). The application of pure bacteriocins in food has several drawbacks, the major of which is the reduced efficacy determined by the binding to food components (fat or protein particles) and food additives (Settanni & Corsetti, 2008). On the contrary, the use of microorganisms as protective cultures may have several advantages, as microorganisms can not only be the source of anti-microbial peptides but also of a wide

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0924-2244/$ - see front matter
doi:10.1016/j.tifs.2011.03.003

spectrum of molecules, such as organic acids, carbon dioxide, ethanol, hydrogen peroxide and diacetyl, whose antimicrobial action is well known (Vandenbergh, 1993). Competition of protective cultures with potential pathogens is another way to restrict the growth of undesired organisms. Moreover, these microorganisms may have additional functional properties and, in some circumstances, they can be beneficial for the consumers. Last but not least, they can contribute to the flavor, texture and nutritional value of the product. Therefore, the concept of "protective cultures" is a broad one and it is not strictly related to the production of bacteriocins.

LAB represent the microbial group most commonly used as protective cultures, as they are present in all fermented foods and have a long history of safe use (Franz, Cho, Holzapfel, & Gálvez, 2010; Mattila-Sandholm, Matto, & Saarela, 1999; Schillinger *et al*., 1996; Wessels *et al*., 2004). Safety for the consumers is an aspect of great importance, in particular for foods which are not cooked before consumptions, but also for other types of foods since cross-contaminations, both at the retail and consumer level, are possible. The absence of pathogenic traits should be demonstrated for cultures suggested for use in foods (Maragkoudakis *et al*., 2009). Beside safety, protective cultures should guarantee the absence of detrimental effects on the target food; since LAB may contribute to spoilage in several types of foods, it is essential to study their effect on food texture and quality, with particular emphasis on the nutritional value of the product (Castellano, González, Carduza, & Vignolo, 2010). Furthermore, the capability of surviving to industrial processing conditions is of great importance for industries producing protective cultures at the large scale (Santini *et al*., 2010). LAB have historically been used as preserving agents in a number of fermented foods, as reviewed by many Authors (Caplice & Fitzgerald, 1999; Giraffa, Chanishvili, & Widyastuti, 2010; Settanni & Moschetti, 2010). However, the role of LAB as protective cultures has also been evidenced in several non-fermented foods. This section will focus on recent applications of protective cultures to non-fermented foods, including meat, plant and seafood products, aimed at the increase of microbial safety and quality, as summarized in Table 1.

Meat products

Meat and meat products are excellent substrates for microorganisms growth (Galvez *et al*., 2010). Refrigeration is the technology of choice to extend the shelf-life of retailed meat, often applied in combination with vacuum-packaging. Refrigeration can contribute to the selection of spoilage psycotrophic bacteria, mainly Enterobacteriaceae, *Pseudomonas* spp. and *Brochothrix thermosphacta* (Katikou, Ambrosiadis, Georgantelis, Koidis, & Georgakis, 2005). Moreover, some mesophilic species, such as *Salmonella* spp. and pathogenic *E. coli*, are capable of growing in slightly temperature-abused refrigerated foods and seriously compromise the safety of the meat. In addition, *Listeria monocytogenes* has caused several outbreaks in recent years linked to meat products. Although several studies have focused on the *in vitro* selection and characterization of LAB strains to be

Table 1. Recent applications of live protective cultures in non-fermented foods aimed at increasing food safety.

Food products	Target microorganisms (pathogens or spoilage)	Protective culture employed	References
Meat			
Chicken meat	*S. enteriditis, L. monocytogenes*	*E. faecium* PCD71 *L. fermentum* ACA-DC179	Maragkoudakis *et al.* (2009)
Beef meat	Enterobacteriaceae, *Pseudomonas* spp., *B. thermosphacta*	*L. sakei* CETC 4808	Katikou *et al.* (2005)
	Spoilage LAB, *B. thermosphacta, Listeria* spp.	*L. curvatus* CRL705	Castellano *et al.* (2010)
Ham	Spoilage bacteria	*L. sakei* 10A	Vermeiren *et al.* (2006)
Fruit and vegetables			
Iceberg lettuce	*S. enteriditis* Typhimurium, *S. aureus, L. innocua.*	*Pseudomonas putida* LTH 5878	Wei *et al.* (2006)
	S. enteriditis Typhimurium, *E. coli, L. monocytogenes*	*L. mesenteroides* CM135, CM160, PM249	Trias *et al.* (2008)
Golden delicious apples	*S. enteriditis* Typhimurium, *E. coli, L. monocytogenes*	*L. mesenteroides* CM135, CM160, PM249	Trias *et al.* (2008)
Non-fermented pickles	*L. monocytogenes*	*L. curvatus* LR55	Reina *et al.* (2005)
Seafood			
Cold-smoked salmon	*L. monocytogenes* *L. innocua*	*C. divergens* V41 *L. casei* T3, *L. plantarum* Pe_2, *C. piscicola* Sal_3	Brillet *et al.* (2005) Vescovo *et al.* (2006)
Cooked and fresh peeled shrimp	*Vibrio* spp., *Salmonella* spp. *C. botulinum, S. aureus; Shewanella putrefaciens, Photobacterium phosphoreum, Aeromonas* spp. *Pseudomonas* spp.	*Leuconostoc gelidum* EU2247, *Lactococcus piscium* EU2441	Matamoros *et al.* (2009)

used as protective cultures on meat, there has been relatively little application on meat products, which mainly regards chicken meat, beef meat and ham. The work of Maragkoudakis *et al*. (2009) is the first successful application of live protective LAB on chicken meat. Two strains (*Enterococcus faecium* PCD71 and *Lactobacillus fermentum* ACA-DC179; Table 1) were applied to raw chicken meat, resulting in a reduced growth rate of *L. monocytogenes* and *Salmonella enteriditis*. Interestingly, the selection of the strains was performed among 600 LAB of food origin, with regards to desirable functional properties such as anti-microbial activity against the target pathogens and spoilage microorganisms, survival to food processing and gastrointestinal tract conditions and basic safety properties. Moreover, no spoilage effect and reduction of the nutritional values were evidenced. The bacteriostatic effect against *Listeria* has been ascribed to the action of bacteriocins; however, the Authors concluded that a complex array of factors not yet completely elucidated could be involved in the anti-microbial action. Protective cultures have a long description of application on sliced beef meat mainly against spoilage bacteria (Galvez *et al*., 2010). *Lactobacillus sakei* and *Lactobacillus curvatus* of meat origin are the most common applied strains (Castellano *et al*., 2010; Table 1). *L. sakei* CETC 4808, known to produce bacteriocin-like molecules, was successfully applied against spoilage bacteria on the surface of vacuum-packaged sliced beef without affecting chemical and sensory quality (Katikou *et al*., 2005). *L. curvatus* CRL705 strain was inoculated on the surface of vacuum-packaged refrigerated beef steaks stored for 60 days; the strain became the dominant population and was able to control the growth of spoilage microorganisms naturally present on the meat (Castellano *et al*., 2010; Table 1). Tissue degradation was delayed with respect to non-inoculated samples and sensory alterations could not be appreciated. In addition, the same strain was potentially active against *Listeria* spp. strains due to the action of a specific bacteriocin. Protective cultures have also been used for shelf-life prolongation of cooked meat products such as ham. *L. sakei* 10A, isolated from turkey meat, possessed antagonistic activity against *Leuconostoc mesenteroides* and *B. thermosphacta* (Vermeiren, Devlieghere, & Debevere, 2006).

Vegetables and fruits

The increasing importance of minimally processed vegetables and fruits, such as pre-washed and pre-cut salads, and prepared fruit salads, has initiated many studies for microbial safety of these products, which are sold in a ready-to-use form and do not generally contain preservatives (Trias, Badosa, Montesinos, & Baneras, 2008). However, the high humidity as well as the high number of cut surfaces with a resultant release of nutrients can provide ideal conditions for microbial growth, including pathogens listed in Table 1. Classical treatments, employing chlorine or ozone, very often fail to remove pathogens (Trias *et al*., 2008). An indigenous *Pseudomonas putida* strain was found to possess relevant pathogen antagonistic efficacy as well as a favorable effect on the quality of the inoculated, packaged and stored lettuce (Wei, Wolf, & Hammes, 2006). The strain possessed no risk potential; it can be applied post-harvest or at a process step in the production line anteceding the final washing. However, the majority of the strains applied as protective cultures in vegetable and fruit are LAB. Three *L. mesenteroides* strains, isolated from fresh fruit and vegetables (Trias *et al*., 2008; Trias, Baneras, Badosa, & Montesinos, 2008), have been applied as bioprotective cultures in wounded Golden Delicious apples and Iceberg lettuce leaf cuts and found to reduce the amount of *Salmonella enterica* serovar *Typhimurium* and *E. coli* and to completely inhibit the growth of *L. monocytogenes* without sensory or visual modifications of the product. A *L. curvatus* strain possessing anti-microbial activity against *L. monocytogenes* was isolated from non-fermented and not heat processed refrigerated pickles and used as biopreservative agent (Reina, Breidt, Henry, Fleming, & Kathariou, 2005).

Seafoods

Biopreservation is of extreme interest to ensure safety and quality of minimally processed seafood, whose demand has sensibly increased in recent years (Calo-Mata *et al*., 2008). Salt or sugar are often added to reduce the water activity (a_w) and a mild processing, such as cold-smoking, is frequently applied. Nevertheless, spoilage or pathogenic microorganisms can grow on these foods. The major microbial risks associated with seafood are *Clostridium botulinum* type E and *L. monocytogenes*. Whereas *C. botulinum* type E can be adequately controlled by the combination of salt and low temperatures, *L. monocytogenes* can grow at 0 °C and tolerate low a_w usually lethal for bacteria. Safety and spoilage control of seafood can be improved by applying protective cultures, mainly LAB, as reviewed by Leroi (2010). Some LAB strains are known to secrete active bacteriocins also at high salt concentration and low temperatures, both in aerobic and anaerobic atmospheres (Tomé, Pereira, Lopes, Gibbs, & Teixeira, 2008). In spite of the high number of *in vitro* studies, very few commercial applications have appeared in seafood products, as the organoleptic and nutritional quality of the food is often compromised and several bacteria that gave *in vitro* promising results proved to be ineffective in products (Leroi, 2010). *Carnobacterium divergens* V41 strain was applied to sterile cold-smoked salmon co-inoculated with a mixture of *L. monocytogenes* strains (Brillet, Pilet, Prevost, Cardinal, & Leroi, 2005). In samples possessing a high initial natural microbiota ($>10^4$–10^5 CFU/g), inoculated and autochthonous LAB quickly became dominant over potentially spoilage and pathogenic bacteria. The anti-listerial activity of 3 LAB strains used individually or as co-cultures (Table 1) was assayed on cold-smoked salmon artificially contaminated with *L. innocua* and stored under vacuum at 4 °C (Vescovo, Scolari, & Zacconi, 2006). The association of *Lactobacillus casei* T3 and *Lactobacillus plantarum* PE_2

was the most effective, probably due to a competition mechanism against the pathogen. Ready-to-eat seafoods such as cooked and peeled shrimps are highly susceptible to the colonization of pathogens and spoilage bacteria (Matamoros *et al*., 2009; Table 1). The growth of these microorganisms can be contrasted by psycotrophic LAB, which are capable of delaying the sensory spoilage of the products, beside inhibiting the growth of *L. monocytogenes* and *Staphylococcus aureus*.

The probiotic approach

Zoonoses cause very important losses to livestock, pork and poultry productivity with impact on the whole food-producing chain and, consequently, on human health. Zoonotic pathogens can contaminate meat or milk products during slaughter or at milking and be transferred to human once a contaminated food, not properly processed, reaches the consumer.

"Traditional" pathogens such as *Salmonella*, *Campylobacter, E. coli*, *Clostridium*, *Yersinia enterocolitica*, *S. aureus* and *Bacillus cereus* can be associated with the food-producing animal environment (Sofos, 2008). Increasing concerns are also related to "emerging" pathogens which have now been associated with food-borne transmission: *Campylobacter jejuni*, *S. enterica* serovar *Typhimurium* DT104, *E. coli* O157:H7, other enterohemorrhagic *E. coli* (EHEC), *L. monocytogenes*, *Arcobacter butzleri*, *Mycobacterium avium* subsp. *paratuberculosis*, *Aeromonas hydrophila*, and prions (Mor-Mur & Yuste, 2010). Evidences suggest that good animal husbandry practices and the application of food safety intervention measures make meat and dairy products safer and/or of better quality; whereas stressing, dietary change, therapeutic antibiotics may strongly disturb the composition of the gastrointestinal microbiota, thus increasing the susceptibility to infection and the shedding of food-borne pathogens (Oliver, Patel, Callaway, & Torrence, 2009). In support to specific intervention measures (e.g. animal husbandry, hygienic practices, feeding and transport prior slaughter), feeding probiotic supplements could be an integrated approach to improve food safety from the initial step of the "farm to fork" food chain, starting from the maintenance of a healthy intestinal ecosystem. Considering that many of the pathogens are asymptomatic for animal harboring and shedding them, it is imperative to minimise their presence at farm level, in particular at intestinal level where they can silently multiply and spread. Although a large number of benefits have also been ascribed to heat-killed probiotic microbes (Ishikawa *et al*., 2010; Lin, Yu, Lin, Hwang, & Tsen, 2007; Ouwehand & Salminen, 1998), probiotics are currently defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO, 2001). The use of probiotics in animals is well controlled and is regulated by Regulation (EC) no. 1831/2003. Among the mechanisms by which probiotics exert health effects, the reduction of luminal pH, competition with pathogens for adhesion sites and nutritional sources, secretion of anti-microbial substances, toxin inactivation, and immune stimulation are the most known (Salminen *et al*., 2010). LAB and bifidobacteria are widely used as probiotic feed supplement, owing to their recognition as members of the indigenous microbiota of both humans and animals, their history of safe use, and the general body of evidence that supports their positive roles. Their presence in high numbers is associated with good health status of the host and their number is strongly affected during stressful periods (Burkholder, Thompson, Einstein, Applegate, & Patterson, 2008). Microorganisms belonging to the genera *Bacillus* and *Clostridium* have also been fed to a wide range of farm animals, mainly pigs and poultry. Moreover, yeasts, in particular *Saccharomyces cerevisiae* and *Aspergillus oryzae*, are used in cattle (Diez-Gonzalez & Shamberger, 2006). Certain microorganisms may be problematic, particularly the enterococci, which may harbor transmissible antibiotic resistance determinants and *B. cereus* strains, which are known to produce enterotoxins and an emetic toxin (Anadón, Martínez-Larrañaga, & Martínez, 2006).

In this section the more significant applications of probiotic cultures on pigs, poultry and ruminants will be reported focusing on their positive effect on the gut microbiota, as summarized in Table 2.

The crucial event in the development of the probiotic approach was the finding that newly hatched chickens could be protected against Salmonella colonization of the gut by dosing it with a suspension of gut contents prepared from healthy adult chickens (Nurmi & Rantala, 1973). The phenomenon, known as competitive exclusion (CE), leads to the development of numerous products with large application to control Salmonella in chicks (Schneitz, 2005). Following the basis of CE, administration of well-characterized probiotics, not necessarily of animal origin, is currently carried out on target farm animals (Wiemann, 2003). Irrespective of the strategy applied and the mechanism involved, the re-establishment of the gut microbiota by probiotic treatment seems to be promising in view of the reduction of pathogen load both in monogastric and polygastric animals (Gaggìa *et al*., 2010). When probiotics are applied to newborns with a nearly sterile intestinal tract, results tend to be more consistent (Callaway *et al*., 2008).

Pigs, poultry and ruminants

In commercial swine production, the target period for probiotic administration is weaning/post-weaning, which represents a critical period leading to growth depression, infections, occurrence of diarrhea and eventually death (Mountzouris, 2007). Successful applications have been achieved to reduce diarrhea in post-weaning piglets following challenge with enterotoxigenic *E. coli* K88 or *Salmonella* spp. (Casey *et al*., 2007; Zhang *et al*., 2010). In a recent study (Giang, Viet, Ogle, & Lindberg, 2010), supplementation with a mixture of LAB increased significantly

Table 2. Overview of probiotics as feed supplement in different animals and beneficial effects.

Animals	Probiotic microorganisms	Reported effects	References
Pigs			
Weaned piglets	*Lactobacillus* spp., *Pediococcus* spp.	Reduced incidence of diarrhea	Casey *et al.* (2007)
	L. rhamnosus (LGG)	Reduced incidence of diarrhea	Zhang *et al.* (2010)
	E. faecium 6H2, *L. acidophilus* C3, *P. pentosaceus* D7, *L. plantarum* 1K8 *and L. plantarum* 3K2.	Reduced incidence of diarrhea, increased LAB counts	Giang *et al.* (2010)
	P. acidilactici NRRL B-5627, *L. lactis* subsp. *lactis* CECT 539, *L. casei* subsp. *casei* CECT 4043, *E. faecium* CECT 410	Reduced coliform counts	Guerra *et al.* (2007)
	B. animalis subsp. *lactis*	Increased ratio bifidobacteria/*E. coli*	Modesto *et al.*, (2009)
Poultry			
Chickens	*L. johnsonii* F19185	Reduced *E. coli* O78K80 and *C. perfringens* colonization	La Ragione *et al.* (2004)
	Spores of *B. cereus* var. *toyoi*	Reduced *S. enteritidis* colonization	Vila *et al.* (2009)
	L. acidophilus, L. casei, E. faecium, B. thermophilus.	Reduced *C. jejuni* counts	Willis and Reid (2008)
	B. longum PCB 133	Reduced *C. jejuni counts*	Santini *et al.* (2010)
Ruminants			
Cattle	*L. acidophilus* NP51	Decrease fecal shedding of *E. coli* O157:H7	Brashears *et al.* (2003)
Beef cattle	*L. acidophilus* NP51	Reduced *E. coli* O157:H7 prevalence in fecal and hides samples	Younts-Dahl *et al.*, (2004)
	L. acidophilus NP51	Reduced fecal shedding of *E. coli* O157:H7	Peterson *et al.* (2007)
	L. acidophilus NP51	Reduced fecal shedding of *E. coli* O157:H7	Tabe *et al.* (2008)
Sheep	*Lactobacillus* spp.	Reduced *E. coli* O157:H7 fecal counts	Lema *et al.* (2001)
Goats	*L. plantarum* PCA 236 (ACA-DC 201)	Reduced *Clostridium* spp. fecal counts, increased LAB counts	Maragkoudakis *et al.* (2010)

the LAB counts and piglets were less affected by diarrhea. A significant decrease in the viable coliform counts was observed in weaned piglets fed separately *Pediococcus acidilactici*, *E. faecium*, *Lactobacillus lactis* and *L. casei* (Guerra, Fajardo, Méndez, Cachaldora, & Pastrana, 2007). The daily administration of *Bifidobacterium animalis* subsp. *lactis* to weaned piglets, at a dose of 10^{10} CFU, affected positively the ratio of bifidobacteria to *E. coli* in the gut (Modesto *et al.*, 2009).

In poultry, the post-hatching period is undoubtedly a critical one because of several stressors such as feed changes or imbalances, transportation, processing at the hatchery and high stocking densities. *C. jejuni* is reported as the most common gastrointestinal bacterial pathogen in humans, and broiler meat and raw milk as the most important food vehicles in food-borne Campylobacter outbreaks (EFSA, 2010). Moreover, in commercial poultry flocks, young chicks are extremely susceptible also to *S. enterica* and *Clostridium perfringens* infections.

The supplementation of well-characterized probiotics, aimed to the reduction of pathogen load in poultry gut, has been carried out in several field trials (Table 2). *Lactobacillus johnsonii* F19185 was successful in reducing significantly the colonization of *E. coli* O78K80 and *C. perfringens* (La Ragione, Narbad, Gasson, & Woodward, 2004). Vila *et al.* (2009) reported a reduction of *S. enteritidis* colonization and invasion by continuously feeding spores of the probiotic strain *B. cereus* var. *toyoi* in broiler chickens as well as in white leghorn chickens. LAB and *Bifidobacterium thermophilus* were used to reduce the presence of *C. jejuni* in broiler chickens (Willis & Reid, 2008). A strain of *Bifidobacterium longum*, selected for its capability of inhibiting *C. jejuni* growth, was administered to 20 days old broiler chickens. Results evidenced the persistence of the probiotic strain in chicken fecal samples and a significant reduction of *C. jejuni* counts compared to the untreated group (Santini *et al.*, 2010).

Ruminants, especially young animals, are implicated as the principal reservoir of *E. coli* O157:H7, with undercooked ground meat being the major vehicle of food-borne outbreaks (Zhao *et al.*, 1998). Mostly, applications have been addressed to calves and cows targeting both the incidence/severity of diarrhea and the reduction of the shedding of pathogenic *E. coli* O157:H7. Interestingly, as reported in Table 2, evidence of efficacy came from different Authors dealing with the same probiotic strain, *Lactobacillus acidophilus* NP51 (Brashears, Galyean, Lonergan, Mann, & Killinger-Mann, 2003; Younts-Dahl, *et al.*, 2004). Besides, a field trial, lasting two years, clearly showed that *E. coli* O157:H7 fecal shedding decreased (35%) in beef cattle, following daily administration of *L. acidophilus* strain NP51 (Peterson *et al.*, 2007). In the same way, Tabe *et al.* (2008) reported a 32% reduction in fecal shedding of *E. coli* O157:H7. The probiotic approach in ruminants has been extensively investigated in beef cattle compared to other ruminants such as sheep and goats where only a few data on the control of pathogens are available. In sheep, Lema, Williams, and Rao (2001) showed that an

E. faecium strain and a mixture of LAB were able to reduce *E. coli* 0157:H7 faecal counts and improve meat quality parameters. In dairy goats, the *L. plantarum* PCA 236 (ACA-DC 201) strain was used by Maragkoudakis, Chingwaru, Gradisnik, Tsakalidou, and Cencic (2010) to reduce pathogen load in dairy goats. Prior to application, an *in vitro* screening revealed that the strain possessed interesting properties: a protective effect against viral disruption of goat intestinal epithelium cell lines (Maragkoudakis *et al*., 2010), anti-microbial activity against *C. jejuni* (Santini *et al*., 2010) and *Mycobacterium avium* subsp. *paratuberculosis* (Gaggìa, Nielsen, Biavati, & Siegumfeldt, 2010). Overall, the *in vivo* study indicated that the *L. plantarum* PCA 236 strain beneficially modulated the goat fecal microbiota by a significant increase in LAB coupled to a significant decrease in fecal clostridia populations. Interestingly, the probiotic feed inclusion improved also the milk fatty acid composition.

Industrial perspectives

One of the major challenges in the commercialization of protective and probiotic cultures is the technology used to produce them at the large scale, taking into account all the critical aspects, such as preservation, storage, and oral administration. Hence, the development of suitable methods to guarantee both storage for long time and maintenance of the viability and efficacy of the initial population is imperative to industries.

Drying techniques

Freeze-drying is historically the preferred technology by the microbial culture-producing industry, allowing easy and inexpensive shipping and handling of the freeze dried material. However it could bring about undesirable side effects mainly due to the reduced bacterial survival during the freezing process and the high costs and energy consumption (Morgan, Herman, White, & Vesey, 2006). Microorganism initial concentration, growth and drying medium, rehydration and storage conditions, as well as microbial intrinsic features, have a great incidence on the bacterial survival during freeze-drying and storage (Carvalho *et al*., 2004). The incorporation of cryoprotectants enables higher survival of microorganisms and research efforts have been devoted to the choice of the best agents, generally carbohydrates (Leslie, Israeli, Lighthart, Crowe, & Crowe, 1995). In conclusion, several aspects need to be considered in order to meet all the quality requirements and to make the process cost-effective. Alternative drying processes aimed at reducing the costs are spray drying, fluidized bed drying and vacuum drying (Santivarangkna, Kulozik, & Foerst, 2007). In spray drying, the liquid culture is atomized at high velocity and the resulting droplets are dried for a short time into a flow of hot hair (150–200 °C). The major parameter affecting viability of spray-dried cultures is the temperature at which the product leaves the drying chamber; the capability of the strains to tolerate high temperatures, although for short times, is therefore crucial (Santini *et al*., 2010). Regarding the application of this technology to protective cultures, Silva, Carvalho, Teixeira, and Gibbs (2002) stressed the importance of evaluating the capability of spray-dried cultures of maintaining their anti-microbial activity. Although bacteriocin production by several spray-dried LAB was strain dependent, the Authors concluded that spray drying is a useful process for large-scale production of dried viable organisms with antagonistic activity against pathogens. Fluidised bed drying is a method of drying solid particles with a stream of hair blowing through them thus creating a fluid-like behavior. Particles are dehydrated by rapid exchange of heat and mass with hair. The technique allows gentle drying of sensitive compounds and consumes less time and energy of freeze-drying; it has been successfully applied to the production of commercially dried yeasts but it has not yet received widespread application in bacteria (Santivarangkna *et al*., 2007). Strasser, Neureiter, Geppl, Braun, and Danner (2009) compared freeze-drying and fluidised bed drying on the viability of *E. faecium* and *L. plantarum*. Results evidenced that *L. plantarum* was more sensitive to both drying methods than *E. faecium*. Moreover, without the addition of a protectant, cells of both strains suffered higher losses during fluidized bed drying. Similarly to freeze-drying, several factors can influence viability and stability of the microorganisms. Therefore, as previously reported, during the selection of probiotic or protective cultures, it is of great importance to choose strains having intrinsic tolerance against harsh conditions such as heat or osmotic stresses as well as the induction of known defense system at genetic level (e.g. heat shock proteins) which allow them to withstand severe conditions (Prasad, McJarrow, & Gopal, 2003). Finally, vacuum drying is an effective tool to dry heat-sensitive materials. As drying operates under vacuum, moisture can be removed at low temperatures and, in addition, oxidation reactions can be minimized for oxygen-sensitive bacteria. The main drawback of this technique is the long drying time compared to spray and fluidized bed drying (Santivarangkna *et al*., 2007).

Microencapsulation

Another important requirement to be considered for a successful application of probiotics is the maintenance of their viability and functionality until they reach their destination in the gut. The extreme acidic environment of stomach can seriously decrease the number of living cells reaching the intestine. In this regard microencapsulation can enhance the survival of probiotics during gastric transit (Heidebach, Först, & Kulozik, 2009). Microencapsulation is a way of packaging materials in miniature, sealed capsules that can release their contents at controlled rates under specific conditions (Anal & Singh, 2007). Various coating polymers and different technologies can be applied to obtain microencapsulated cells. Spray drying is one of the most used techniques to perform microencapsulation

both of LAB and bifidobacteria (Calo-Mata *et al*., 2008; Simpson, Stanton, Fitzgerald, & Ross, 2005). Moreover, during fluidised bed drying, a spray can be incorporated within the fluidised bed dryer in order to add a coating to the granulated product (Morgan *et al*., 2006). The advantages of microencapsulation can be summarized as follows: i) cryo- and osmo-protective components can be incorporated into the matrix, enhancing the survival of cells during processing and storage; ii) once the microcapsules have been dried, a further surface coating can be applied to improve the sensory properties of the product and also provide an extra level of protection; and iii) the coating layer can have desirable dissolution properties, thus permitting delayed release of the cells or release in particular condition, such as, for example, upon a change in pH after gastric transit (Anal & Singh, 2007). Microcapsule, using various biopolymers, is very easy to prepare on a lab-scale; however, the scaling up of the process may have troubles and processing costs can be too high to guarantee feasibility at the large scale.

Conclusion and future perspectives

Food safety is of fundamental importance to the consumer, food industry and economy. Moreover, increasing consumer awareness and desire for natural products and processes have given emphasis to the finding of natural alternatives to traditional techniques, thus preventing the use of chemical additives and hurdle technologies for conserved foods and antibiotics to reduce disease spreading at the primary production levels.

The increasing number of studies in the field of protective cultures confirms this trend and more and more applications are expected to appear in the future years, both for prolonging the shelf-life of products which have to be cooked, such as meat, and for ready-to-eat foods, which are widely appreciated by the modern consumer. Research in the future years will have to focus in a better understanding of the mechanism of action of the inhibiting activity of the used strains and to define the most suitable technologies to preserve this activity when the microorganisms are produced on the large scale by industries. Synergistic action by academic research and industry is therefore envisaged. In addition, it has to be pointed out that application of protective cultures on chicken meat against *C. jejuni* is lacking in the literature, although Campylobacteriosis incidence has the wider incidence in Europe and, potentially, several microorganisms can be used for this purpose (Chaveerach, Lipman, & van Knapen, 2004; Santini *et al*., 2010). In addition, more research is foreseen in the field of psycotrophic LAB which possess interesting perspectives for the expansion of biopreservation technology in ready-to-eat products which are stored at low temperatures.

The efficacy of probiotics to reduce pathogen load at the primary production level has been demonstrated for pigs, poultry and ruminants; therefore, probiotics represent an acquired and real intervention strategy alternative to the use of antibiotics to prevent zoonotic disease from occurring. In addition, new investigation techniques, the use of functional cell model of the gut and advanced molecular methods will allow scientists to acquire more information on the strains with particular regard to their interaction with pathogens and the host. In this way, the selection of new probiotic strains, targeting selected food-borne pathogens and suitable to the host, will be more effective. Nevertheless, research efforts are needed on the way of administrating them to animals, in order to maintain both viability and functionality. Encapsulated strains to enhance microorganisms storage time in the feed and for controlled release in the gut after the gastric barrier represent a promising way of administering probiotics to animals. Our group is currently involved in the development of proper strategies, including microencapsulation, to administer probiotic strains in animal feeding.

Acknowledgements

This work was supported by grant from the EU-FP6 project PathogenCombat-FOOD-CT-2005-007081.

References

Anadón, A., Martínez-Larrañaga, M. R., & Martínez, M. A. (2006). Probiotics for animal nutrition in the European Union. Regulation and safety assessment. *Regulatory Toxicology and Pharmacology, 45*, 91–95.

Anal, A. K., & Singh, H. (2007). Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. *Trends in Food Science & Technology, 18*, 240–251.

Brashears, M. M., Galyean, M. L., Lonergan, G. H., Mann, J. E., & Killinger-Mann, K. (2003). Prevalence of *Escherichia coli* O157:H7 and performance by beef feedlot cattle given *Lactobacillus* direct-fed microbials. *Journal of Food Protection, 66*, 748–754.

Brillet, A., Pilet, M. F., Prevost, H., Cardinal, M., & Leroi, F. (2005). Effect of inoculation of *Carnobacterium divergens* V41, a biopreservative strain against *Listeria monocytogenes* risk, on the microbiological, chemical and sensory quality of cold-smoked salmon. *International Journal of Food Microbiology, 104*, 309–324.

Burkholder, K. M., Thompson, K. L., Einstein, M. E., Applegate, T. J., & Patterson, J. A. (2008). Influence of stressors on normal intestinal microbiota, intestinal morphology, and susceptibility to *Salmonella enteritidis* colonization in broilers. *Poultry Science, 87*, 1734–1741.

Callaway, T. R., Edrington, T. S., Anderson, R. C., Harvey, R. B., Genovese, K. J., Kennedy, C. N., et al. (2008). Probiotics, prebiotics and competitive exclusion for prophylaxis against bacterial disease. *Animal Health Research Reviews, 9*, 217–225.

Calo-Mata, P., Arlindo, S., Boehme, K., de Miguel, T., Pascoal, A., & Barros-Velazquez, J. (2008). Current applications and future trends of lactic acid bacteria and their bacteriocins for the biopreservation of aquatic food products. *Food Bioprocess Technology, 1*, 43–63.

Caplice, E., & Fitzgerald, G. F. (1999). Food fermentations: role of microorganisms in food production and preservation. *International Journal of Food Microbiology, 50*, 131–149.

Carvalho, A. S., Silva, J., Ho, P., Teixeira, P., Malcata, F. X., & Gibbs, P. (2004). Relevant factors for the preparation of freeze-dried lactic acid bacteria. *International Dairy Journal, 14*, 835–847.

Casey, P. G., Gardiner, G. E., Casey, G., Bradshaw, B., Lawlor, P. G., Lynch, P. B., et al. (2007). A five-strain probiotic combination reduces pathogen shedding and alleviates disease signs in pigs

challenged with *Salmonella enterica* Serovar *Typhimurium*. *Applied Environmental Microbiology*, *414*, 1858–1863.
Castellano, P., González, C., Carduza, F., & Vignolo, G. (2010). Protective action of *Lactobacillus curvatus* CRL705 on vacuum-packaged raw beef. Effect on sensory and structural characteristics. *Meat Science*, *85*, 394–401.
Chaveerach, P., Lipman, L. J., & van Knapen, F. (2004). Antagonistic activities of several bacteria on *in vitro* growth of 10 strains of *Campylobacter jejuni/coli*. *International Journal of Food Microbiology*, *90*, 43–50.
Diez-Gonzalez, F., & Shamberger, G. P. (2006). Use of probiotics in preharvest food safety applications. In I. Goktepe, V. K. Juneja, & M. Ahmedna (Eds.), *Probiotics in food safety and human health* (pp. 251–267). Boca Raton: Taylor & Francis Group.
EFSA (2010). The Community Summary Report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in the European Union in 2008. *EFSA Journal*, *8*(1), 1496.
Franz, C., Cho, G.-S., Holzapfel, W. H., & Galvez, A. (2010). Safety of lactic acid bacteria. In F. Mozzi, R. R. Raya, & G. M. Vignolo (Eds.), *Biotechnology of lactic acid bacteria. Novel applications* (pp. 341–360). Iowa, USA: Wiley-Blackwell.
Gaggìa, F., Mattarelli, P., & Biavati, B. (2010). Probiotics and prebiotics in animal feeding for safe food products. *International Journal of Food Microbiology*, *141*, S15–S28.
Gaggìa, F., Nielsen, D. S., Biavati, B., & Siegumfeldt, H. (2010). Intracellular pH of *Mycobacterium avium* subsp. *paratuberculosis*, following exposure to antimicrobial compounds monitored at the single cell level. *International Journal of Food Microbiology*, *141*, S188–S192.
Galvez, A., Abriouel, H., Benomar, N., & Lucas, R. (2010). Microbial antagonists to food-borne pathogens and biocontrol. *Current Opinion in Biotechnology*, *21*, 142–148.
Garcıa, P., Rodrıguez, L., Rodrıguez, A., & Martınez, B. (2010). Food biopreservation: promising strategies using bacteriocins, bacteriophages and endolysins. *Trends in Food Science & Technology*, *21*, 373–382.
Giang, H. H., Viet, T. Q., Ogle, B., & Lindberg, J. E. (2010). Growth performance, digestibility, gut environment and health status in weaned piglets fed a diet supplemented with potentially probiotic complexes of lactic acid bacteria. *Livestock Science*, *129*, 95–103.
Giraffa, G., Chanishvili, N., & Widyastuti, Y. (2010). Importance of lactobacilli in food and feed biotechnology. *Research in Microbiology*, *161*, 480–487.
Guerra, N. P., Fajardo, P., Méndez, J., Cachaldora, P., & Pastrana, L. (2007). Production of four potentially probiotic lactic acid bacteria and their evaluation as feed additives for weaned piglets. *Animal Feed Science and Technology*, *134*, 89–107.
Heidebach, T., Först, P., & Kulozik, U. (2009). Microencapsulation of probiotic cells by means of rennet-gelation of milk proteins. *Food Hydrocolloids*, *23*, 1670–1677.
Ishikawa, H., Kutsukake, E., Fukui, T., Sato, I., Shirai, T., Kurihara, T., et al. (2010). Oral administration of heat-killed *Lactobacillus plantarum* strains b240 protected mice against *Salmonella enterica* Serovar *typhymurium*. *Bioscience, Biotechnology and Biochemistry*, *74*, 1338–1342.
Joint FAO/WHO Expert Consultation (2001). Evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Available from: http://www.who.int/foodsafety/publications/fs_management/en/probiotics.pdf
Katikou, P., Ambrosiadis, I., Georgantelis, D., Koidis, P., & Georgakis, S. A. (2005). Effect of *Lactobacillus*-protective cultures with bacteriocin-like inhibitory substances-producing ability on microbiological, chemical and sensory changes during storage of refrigerated vacuum-packaged sliced beef. *Journal of Applied Microbiology*, *99*, 1303–1313.
La Ragione, R. M., Narbad, A., Gasson, M. J., & Woodward, M. J. (2004). In vivo characterization of *Lactobacillus johnsonii* FI9785 for use as a defined competitive exclusion agent against bacterial pathogens in poultry. *Letters in Applied Microbiology*, *38*, 197–205.
Lema, M., Williams, L., & Rao, D. R. (2001). Reduction of fecal shedding of enterohemorrhagic *Escherichia coli* O157:H7 in lambs by feeding microbial feed supplement. *Small Ruminant Research*, *39*, 31–39.
Leroi, F. (2010). Occurrence and role of lactic acid bacteria in seafood products. *Food Microbiology*, *27*, 698–709.
Leslie, S. B., Israeli, E., Lighthart, B., Crowe, J. H., & Crowe, L. M. (1995). Trehalose and sucrose protect both membranes and proteins in intact bacteria during drying. *Applied Environmental Microbiology*, *61*, 3592–3597.
Lin, W.-H., Yu, B., Lin, C.-K., Hwang, W.-Z., & Tsen, H.-Y. (2007). Immune effect of heat-killed multistrain of *Lactobacillus acidophilus* against *Salmonella typhymurium* invasion to mice. *Journal of Applied Microbiology*, *102*, 22–31.
Maragkoudakis, P. A., Mountzouris, K. C., Psyrras, D., Cremonese, S., Fischer, J., Cantor, M. D., et al. (2009). Functional properties of novel protective lactic acid bacteria and application in raw chicken meat against *Listeria monocytogenes* and *Salmonella enteritidis*. *International Journal of Food Microbiology*, *130*, 219–226.
Maragkoudakis, P. A., Mountzouris, K. C., Rosu, C., Zoumpopoulou, G., Papadimitriou, K., Dalaka, E., et al. (2010). Feed supplementation of *Lactobacillus plantarum* PCA 236 modulates gut microbiota and milk fatty acid composition in dairy goats — A preliminary study. *International Journal of Food Microbiology*, *141*, S109–S116.
Maragkoudakis, P. A., Chingwaru, W., Gradisnik, L., Tsakalidou, E., & Cencic, A. (2010). Lactic acid bacteria efficiently protect human and animal intestinal epithelial and immune cells from enteric virus infection. *International Journal of Food Microbiology*, *141*, S91–S97.
Matamoros, S., Leroi, F., Cardinal, M., Gigout, F., Kasbi Chadli, F., Cornet, J., et al. (2009). Psychrotrophic lactic acid bacteria used to improve the safety and quality of vacuum-packaged cooked and peeled tropical shrimp and cold-smoked salmon. *Journal of Food Protection*, *72*, 365–374.
Mattila-Sandholm, T., Matto, J., & Saarela, M. (1999). Lactic acid bacteria with health claims- interactions and interference with gastrointestinal flora. *International Dairy Journal*, *9*, 25–35.
Modesto, M., D'Aimmo, M. R., Stefanini, I., Trevisi, P., De Filippi, S., Casini, L., et al. (2009). A novel strategy to select *Bifidobacterium* strains and prebiotics as natural growth promoters in newly weaned pigs. *Livestock Science*, *122*, 248–258.
Morgan, C. A., Herman, N., White, P. A., & Vesey, G. (2006). Preservation of micro-organisms by drying; A review. *Journal of Microbiological Methods*, *66*, 183–193.
Mor-Mur, M., & Yuste, J. (2010). Emerging bacterial pathogens in meat and poultry: an overview. *Food Bioprocess Technology*, *3*, 24–35.
Mountzouris, K. C. (2007). Assessment of the efficacy of probiotics, prebiotics and synbiotics in swine nutrition: a review. *Food Science and Technology Bulletin: Functional Foods*, *3*, 51–57.
Nurmi, E., & Rantala, M. (1973). New aspects of Salmonella infection in broiler production. *Nature*, *241*, 210–211.
Oliver, S. P., Patel, D. A., Callaway, T. R., & Torrente, M. E. (2009). ASAS centennial paper: development and future outlook for preharvest food safety. *Journal of Animal Science*, *87*, 419–437.
Ouwehand, A. C., & Salminen, S. J. (1998). The health effects of viable and non-viable cultured milks. *International Dairy Journal*, *8*, 749–758.
Peterson, R. E., Klopfenstein, T. J., Erickson, G. E., Folmer, J., Hinkley, S., Moxley, R. A., et al. (2007). Effect of *Lactobacillus acidophilus* strain NP51 on *Escherichia coli* O157:H7 fecal shedding and finishing performance in beef feedlot cattle. *Journal of Food Protection*, *70*, 287–291.

Prasad, J., McJarrow, P., & Gopal, P. (2003). Heat and osmotic stress responses of probiotic *Lactobacillus rhamnosus* HN001 (DR20) in relation to viability after drying. *Applied Environmental Microbiology*, *69*, 917–925.

Reina, L. D., Breidt, F., Henry Jr., Fleming, P., & Kathariou, S. (2005). Isolation and selection of lactic acid bacteria as biocontrol agents for nonacidified, refrigerated pickles. *Journal of Food Science*, *70*, M7–M11.

Salminen, S. J., Nybom, S., Meriluoto, J., Collado, M. C., Vesterlund, S., & El-Nezami, H. (2010). Interaction of probiotics and pathogens—benefits to human health? *Current Opinion in Biotechnology*, *21*, 157–167.

Santini, C., Baffoni, L., Gaggìa, F., Granata, M., Gasbarri, R., Di Gioia, D., et al. (2010). Characterization of probiotic strains: an application as feed additives in poultry against *Campylobacter jejuni*. *International Journal of Food Microbiology*, *141*, S98–S108.

Santivarangkna, C., Kulozik, U., & Foerst, P. (2007). Alternative drying processes for the industrial preservation of lactic acid starter cultures. *Biotechnology Progress*, *23*, 302–315.

Schillinger, U., Geisen, R., & Holzapfel, W. H. (1996). Potential of antagonistic microorganisms and bacteriocins for the biological preservation of foods. *Trends in Food Science & Technology*, *71*, 58–64.

Schneitz, C. (2005). Competitive exclusion in poultry – 30 years of research. *Food Control*, *16*, 657–667.

Settanni, L., & Corsetti, A. (2008). Application of bacteriocins in vegetable food biopreservation. *International Journal of Food Microbiology*, *121*, 123–138.

Settanni, L., & Moschetti, G. (2010). Non-starter lactic acid bacteria used to improve cheese qualità. *Food Microbiology*, *27*, 691–697.

Silva, J., Carvalho, A. S., Teixeira, P., & Gibbs, P. A. (2002). Bacteriocin production by spray-dried lactic acid bacteria. *Letters in Applied Microbiology*, *34*, 77–81.

Simpson, P. J., Stanton, C., Fitzgerald, G. F., & Ross, R. P. (2005). Intrinsic tolerance of *Bifidobacterium* species to heat and oxygen and survival following spray drying and storage. *Journal of Applied Microbiology*, *99*, 493–501.

Sofos, J. N. (2008). Challenge to meat safety in the 21st century. *Meat Science*, *78*, 3–13.

Strasser, S., Neureiter, M., Geppl, M., Braun, R., & Danner, H. (2009). Influence of lyophilization, fluidized bed drying, addition of protectants, and storage on the viability of lactic acid bacteria. *Journal of Applied Microbiology*, *107*, 167–177.

Tabe, E. S., Oloya, J., Doetkott, D. K., Bauer, M. L., Gibbs, P. S., & Khaitsa, M. L. (2008). Comparative effect of direct-fed microbials on fecal shedding of *Escherichia coli* O157:H7 and *Salmonella* in naturally infected feedlot cattle. *Journal of Food Protection*, *71*, 539–544.

Tomé, E., Pereira, V. L., Lopes, C. I., Gibbs, P. A., & Teixeira, P. C. (2008). *In vitro* tests of suitability of bacteriocin-producing lactic acid bacteria, as potential biopreservation cultures in vacuum-packaged cold-smoked salmon. *Food Control*, *19*, 535–543.

Trias, R., Badosa, E., Montesinos, E., & Baneras, L. (2008). Bioprotective *Leuconostoc* strains against *Listeria monocytogenes* in fresh fruits and vegetables. *International Journal of Food Microbiology*, *127*, 91–98.

Trias, R., Baneras, L., Badosa, E., & Montesinos, E. (2008). Bioprotection of golden delicious apples and iceberg lettuce against foodborne bacterial pathogens by lactic acid bacteria. *International Journal of Food Microbiology*, *123*, 50–60.

Vandenbergh, P. A. (1993). Lactic acid bacteria, their metabolic products and interference with microbial growth. *FEMS Microbiology Review*, *1993*(12), 221–238.

Vermeiren, L., Devlieghere, F., & Debevere, J. (2006). Co-culture experiments demonstrate the usefulness of *Lactobacillus sakei* 10A to prolong the shelf-life of a model cooked ham. *International Journal of Food Microbiology*, *108*, 68–77.

Vescovo, M., Scolari, G., & Zacconi, C. (2006). Inhibition of *Listeria innocua* growth by antimicrobial-producing lactic acid cultures in vacuum-packed cold-smoked salmon. *Food Microbiology*, *23*, 689–693.

Vila, B., Fontgibell, A., Badiola, I., Esteve-Garcia, E., Jiménez, G., Castillo, M., et al. (2009). Reduction of *Salmonella enterica* var. *enteritidis* colonization and invasion by *Bacillus cereus* var. *toyoi* inclusion in poultry feeds. *Poultry Science*, *88*, 975–979.

Wei, H., Wolf, G., & Hammes, W. P. (2006). Indigenous microorganisms from iceberg lettuce with adherence and antagonistic potential for use as protective culture. *Innovative Food Science & Emerging Technologies*, *7*, 294–301.

Wessels, S., Axelsson, L., Hansen, E. B., De Vuyst, L., Laulund, S., Lähteenmäki, L., et al. (2004). The lactic acid bacteria, the food chain, and their regulation. *Trends in Food Science & Technology*, *15*, 498–505.

Wiemann, M. (2003). How do probiotic feed additives work? *International Poultry Producer*, *11*, 7–9.

Willis, W. L., & Reid, L. (2008). Investigating the effects of dietary probiotic feeding regimens on broiler chicken production and *Campylobacter jejuni* presence. *Poultry Science*, *87*, 606–611.

Younts-Dahl, S. M., Galyean, M. L., Lonergan, G. H., Elam, N. A., & Brashears, M. M. (2004). Dietary supplementation with *Lactobacillus-Propionibacterium*-based direct-fed with microbials and prevalence of *Escherichia coli* O157 in beef feedlot cattle and on hides at harvest. *Journal of Food Protection*, *67*, 889–893.

Zhang, L., Xu, Y. Q., Liu, H. Y., Lai, T., Maa, J. L., Wanga, J. F., et al. (2010). Evaluation of *Lactobacillus rhamnosus* GG using an *Escherichia coli* K88 model of piglet diarrhoea: effects on diarrhoea incidence, faecal microflora and immune responses. *Veterinary Microbiology*, *141*, 142–148.

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 by inoculation with probiotic bacteria. *Journal of Clinical Microbiology*, *36*, 641–647.