Applications of Bacteriocins in Livestock

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Introduction

Antibiotics have been used as therapeutic and prophylactic treatments to control a variety of bacterial infections in livestock for more than 50 years. Different types of antibiotics have also been fed at sub-therapeutic levels to cattle, poultry and swine to increase productivity and feed efficiency (McDermott *et al*., 2002). The emergence of antibiotic-resistance in many bacteria relevant for animal and public health stresses the importance of decreasing the use of antibiotics in animal production. The reduction of antibiotic application in livestock can only be achieved if alternative antimicrobial strategies are available. Among those interventions that have been investigated and applied are: immunization, diet modification, sanitation, feed additives, and probiotic bacteria (Callaway *et al*., 2004; Gillor *et al*., 2004).

A variety of probiotic bacteria have been tested to control animal and foodborne pathogenic bacteria in livestock, but in many of them the beneficial effects have not been fully elucidated (Fuller, 1999). The mechanism of inhibition of pathogenic bacteria for several of those probiotic microorganisms is mediated by the production of bacteriocins. The application of bacteriocins such as colicins in livestock has been largely achieved by feeding bacteriocin-producing strains. Feeding purified bacteriocins to humans for control of diarrhea was reported in a few publications during the 1900's (Papavassiliou, 1961), but there is very little evidence that administering bacteriocins alone to livestock has ever been done. The absence of that type of studies could be due to the likely rapid degradation of these proteinaceous compounds in the digestive tract of mammals. Because of this lack of evidence, our discussion on the use of bacteriocins in livestock will be largely based on those studies that reported feeding or applying bacteriocin-producing bacteria (BPB).

Rationale for utilization of bacteriocins in livestock

Compared to antibiotics, most bacteriocins are relatively specific and can only affect a limited number of bacterial species. Bacteriocins of lactic acid bacteria can be inhibitory to many Gram-positive organisms, but they have little effect on Gram-negative species due to the protective effect of the outer membrane. Among the different types of bacteriocins, colicins probably have the greatest specificity because many of them only affect strains within the same species (i.e. *E. coli* colicins). The specificity

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of bacteriocins can be particularly advantageous for applications in which a single bacterial strain or species is targeted without disrupting other microbial populations. In the case of pathogens as target organisms that colonize the gastrointestinal tract of poultry, cattle and swine, the use of bacteriocin-producing strains would have little effect on most beneficial intestinal bacteria.

One of the potential benefits of using BPB in livestock is the stimulation of animal productivity. However, due to the specificity of bacteriocins it is very unlikely that their growth enhancement would be similar to the effect of antibiotics. In recent years, several reports have indicated that ruminal microorganisms are capable of producing a variety of bacteriocins and some of these organisms have been isolated for an eventual application to manipulate the rumen environment (Russell and Mantovani, 2002). The application of BPB for improvements in productivity has not been limited to cattle, as several researchers have explored the use of probiotic strains capable of producing bacteriocins to increase the growth rate of swine (Rodriguez *et al*., 2003). In poultry, the use of BPB has been mainly targeted for the control *Salmonella*.

The potential improvement of productivity in animals mediated by the utilization of BPB could be based on the inhibition of specific groups of organisms (Russell and Mantovani, 2002). BPB capable of producing inhibitory bacteriocins against methanogenic bacteria could improve feed efficiency by reducing the amount of carbon lost in the form of methane (Lee *et al*., 2002). Bacteriocins could help cellulolytic bacteria to become predominant in the rumen and increase cellulose degradation (Kalmokoff and Teather, 1997). *Streptococcus bovis* is one of the bacteria responsible for acidosis when cattle consumes grain-based diets and BPB capable of inhibit that organism may promote rumen homeostasis (Morovsky *et al*., 1998). Another rumen metabolic activity that could be inhibited to improve productivity is the reduction in amino acid degradation (Rychlik and Russell, 2002a).

The utilization of BPB as a pre-harvest food safety strategy is considered as one of the most viable interventions for reducing the gastrointestinal colonization of livestock by foodborne pathogens (Callaway *et al*., 2004; Gillor *et al*., 2004; Renter and Sargeant, 2002; Timmerman *et al*., 2004). The BPB can easily be administered to animals by mixing dried or wet cultures with feed or drinking water, and depending on the ability of the particular probiotic strain to colonize the gastrointestinal tract they could 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 *Escherichia coli* O157:H7, and long-term colonization with BPB would prevent further re-introduction of the pathogenic bacteria.
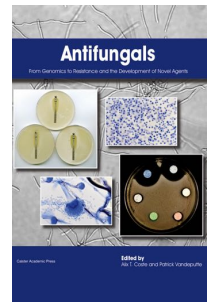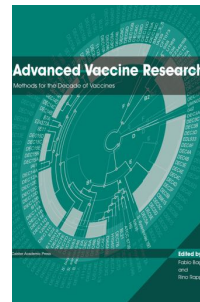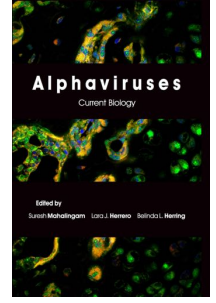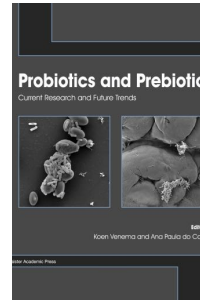
Despite the enormous potential of BPB to increase animal productivity and to reduce the likelihood of foodborne disease, there are relatively few studies that have investigated the factors influencing their applicability.
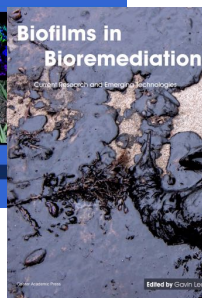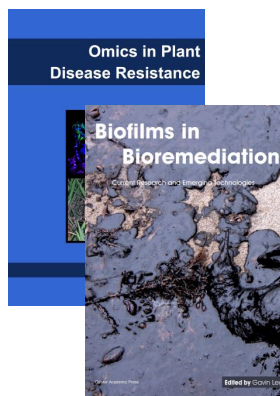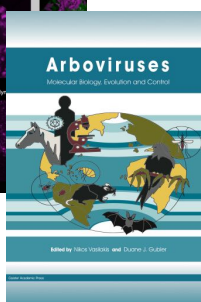
Further Reading

Even in those studies that have shown some degree of effectiveness, there are many questions that remain to be answered related to the long-term effect, the development of resistance, the survival and colonization of the probiotic strains and the effect of the environment. The lack of those studies has been partly due to the inherent limitation of our current microbiological techniques. For example, we know that in laboratory conditions BPB produce bacteriocins, but so far we have not been able to answer the question whether they are actually produced in the intestine because we cannot detect the bacteriocin in such a complex environment. In the coming years it will be critical to conduct research that would be focused to elucidate those important conditions that will help us understand the ecology of BPB and better assess their effectiveness.

Types of bacteriocins and bacteriocin-producing bacteria used in livestock

Colicins

Colicins are probably the group of bacteriocins that have been most thoroughly characterized. Colicins are antimicrobial proteins produced by *Escherichia coli* strains against other *E. coli* or enterobacteria, and at least 30% of all *E. coli* isolates are capable of producing at least one type of colicin (Riley and Gordon, 1996). Colicins are typically produced under stress conditions and more than 25 different colicins have been characterized and these can be classified according to their mode of action and their import pathways. Colicins can form pores, hydrolyze DNA, cleave rRNA, and inhibit protein or peptidoglycan synthesis (Lazdunski *et al*., 1998).

The ability for bacteriocin production in *E. coli* was known since the early 1900's and the application of colicinogenic *E. coli* (CEC) to inhibit undesirable strains was since envisioned (Nissle, 1959). At that time, several strains of CEC such as the Nissle 1917 strain were commercialized for the treatment of infantile diarrhea with relative success (Hardy, 1975). Due to the potential effect of some colicins on mammalian cells its use was discontinued, but products such as Mutaflor have continued to be marketed as human probiotics in some European countries. In recent years an increased interest on the Nissle 1917 strain has been driven by promising results to treat inflammatory bowel syndrome and Crohn's disease (Hamilton- Miller, 2001).

In 1961, one of the first reports that described the use of CEC as probiotic for piglets was published (Tadd and Hurst, 1961). In that study Tadd and Hurst fed weaned pigs with a CEC to reduce the incidence of diarrhea. However, the probiotic strain had no effect on the number of *E. coli* strains from the GI tract and did not reduce the incidence of disease in weaned pigs. Despite the lack of effect observed, their research indicated that the CEC was capable of colonizing the large intestine. The type of colicin that the CEC strain produced was unfortunately not elucidated.

Enterohemorrhagic Escherichia coli

As a pre-harvest food safety strategy, CEC have been actively investigated to inhibit *Escherichia coli* O157: H7 in cattle populations in recent years. *E. coli* O157: H7 has been a major public health concern due to the frequent occurrence of outbreaks which have mostly been caused by foods of bovine origin and by water and foods contaminated with cattle manure (Rangel *et al*., 2005). Based on the epidemiological evidence and the fact that multiple studies have shown that this pathogen is widely distributed in cattle, it is now well established that cattle are its natural reservoir (Borczyk *et al*., 1987; Elder *et al*., 2000; Smith *et al*., 2001). The eradication of this pathogenic bacterium will require interventions that will strongly reduce its intestinal carriage by cattle.

The susceptibility of *E. coli* O157:H7 to colicins was first reported by Bradley and colleagues who observed that colicins E2 and V were capable of inhibiting 12 and 18 strains out of a total of 20, respectively (Bradley *et al*., 1990). In another study that tested several previously characterized colicins against *E. coli* O157:H7, as many as 13 different colicins that included colicins E2, E8, E7 among others were reported to inhibit 11 pathogenic strains (Murinda *et al*., 1996). Work conducted at our laboratory, however, indicated that only colicin E7 consistently inhibited all 22 pathogenic strains tested and follow up studies confirmed that this colicin could inhibit as many as 96 *E. coli* O157:H7 strains (Schamberger and Diez-Gonzalez, 2002; Schamberger and Diez-Gonzalez, 2005).

A summary of the cattle trials using CEC against *E. coli* O157:H7 and other enterohemorrhagic serotypes is presented in Table 1 and they will be discussed in the following paragraphs. In the first study that used CEC against *E. coli* O157:H7, several *E. coli* strains were identified on the basis of its ability to inhibit *E. coli* O157:H7 (Zhao *et al*., 1998). The authors isolated 1,200 bacterial isolates from intestinal tissue of cattle and they tested them for *in vitro* inhibitory activity against serotype O157: H7. Eighteen isolates, seventeen *E. coli* and one *Proteus mirabilis*, were capable of inhibiting five strains of *E. coli* O157:H7. This group of isolates was orally inoculated into two calves and 27 days after inoculation four of these *E. coli* strains were consistently recovered. This result suggested that these were capable of colonizing the cattle gastrointestinal tract and were selected for further experimentation.

In the first trial of calves artificially inoculated with *E. coli* O157:H7 and treated with CEC strains, six animals were fed cultures of the four selected anti-O157:H7 *E. coli*. After two days, the probiotic fed calves and nine control calves were inoculated with a mixture of *E. coli* O157:H7 strains. Eighteen days later, six out of the six calves in the treatment group no longer shed serotype O157:H7 in their feces. After 30 days, the pathogenic bacterium was isolated from the feces of all the calves of the control group, but was only detected in one CEC-treated animal. Three *E. coli* (strains 271, 786, 797) were part of the first anti-O157:H7 patent that described CEC and they have been recently licensed for commercial application (Doyle *et al*., 1999).

The three patented CEC strains were used in a follow-up trial that used adult cattle, but this study has yet to be published (Doyle, 2001). In that experiment, 20 steers were orally inoculated with 10^{10} cells of five O157:H7

Animal	Target Serotype	Colicinogenic *E. coli* strains	Dose	Major Findings	Ref.
Calves	O157:H7		Single dose 10^{10} CFU/calf	Reduction of FS and colonization of O157:H7	(Zhao *et al*., 1998)
Steers	O157:H7	271, 786 and 797	Two doses 10^{10} CFU/calf	Reduction of FS	(Doyle, 2001)
Calves	O157:H7, O26, O111	271, 786 and 797	Single dose 10^{10} CFU/calf	Fecal shedding (FS) reduction of O157:H7 and O111 after 8 days	(Tkalcic *et al*., 2003)
Neonatal calves	O157:H7, O26, O111	271, 786 and 797	Single dose 10^{10} CFU/calf	Reduction of FS of O26 and O111	(Zhao *et al*., 2003)
Calves	O157:H7	Mixture of six colicin-E7-producing strains	Daily dose 10^7–10^8 CFU/calf	Reduction of FS and colonization of O157:H7	(Schamberger *et al*., 2004)

Table 1 Summary of animal trials focused to test the effect of probiotic bacteria against enterohemorrhagic E. coli (EHEC)

strains and they were treated with two oral doses of 10^{10} cells of a mixture of the CEC strains 48 and 72 hours later. The effect of the CEC on the fecal shedding of pathogenic strains was monitored for a month. *E. coli* O157:H7 was no longer detected in fecal samples of the treatment steer group after only 12 days. In contrast, most control animals still had significant fecal counts after a total of 30 days. This work suggested that this CEC mixture could also be effective in reducing enterohemorrhagic *E. coli* (EHEC) in adult cattle.

Using the same combination of CEC, Zhao *et al*. (Zhao *et al*., 2003) have conducted additional cattle experiments to determine their ability to prevent cattle colonization by *E. coli* O157:H7 and other EHEC strains O26:H11 and O111:NM in neonatal calves. Less than a week old claves were orally fed the CEC strains two days before they were inoculated with mixtures of either serotype O26, O111 or O157. The fecal counts of serotypes O26 and O111 were significantly reduced in CEC-treated animals by day 7, in comparison with control calves. However, for serotype O157 the fecal shedding of both treatment and control groups declined at the same rate. Based on this latter result, it was speculated that *E. coli* O157 was not fit for colonizing the gastrointestinal tract of milk-fed calves. The effect of those three CEC strains on EHEC was confirmed in a recent cattle trial with weaned calves (Tkalcic *et al*., 2003). In this latter case the probiotic *E. coli* was again capable to reduce the fecal shedding of serotype O157:H7. The count of serotype O111 were also significantly reduced, but in only 25% of the sampling days.

In our laboratory we have identified and characterized a collection of 14 CEC strains that are highly inhibitory against *E. coli* O157:H7 (Schamberger and Diez-Gonzalez, 2002; Schamberger and Diez-Gonzalez, 2004; Schamberger and Diez-Gonzalez, 2005; Schamberger *et al*., 2004). This set of potentially probiotic *E. coli* were originally identified among a collection of 540 *E. coli* strains isolated from humans and 9 different animal species (cats, cattle, chickens, deer, dogs, ducks, horses, pigs, sheep). The selection of those 14 CEC was achieved after eliminating those CEC strains that were not capable of inhibiting all 96 strains of *E. coli* O157:H7 tested, those that encoded virulence factors or that were antibiotic resistant (Schamberger and Diez-Gonzalez, 2004). Further characterization of those 14 isolates indicated that they could also inhibit other pathogenic *E. coli* strains and most of them produced more than two colicins. The colicins that this set of CEC strains encoded

were B, E1, E2/E7, E7, Ia/Ib, K, and M. In a follow-up study, the potential for resistance development in EHEC strains against these CEC isolates was determined to be relatively unlikely for those strains that encoded more than one colicin (Schamberger and Diez-Gonzalez, 2005).

Based on our initial observation that colicin E7 was the only colicin capable of consistently inhibiting *E. coli* O157:H7, we conducted a cattle trial in which 8 CEC encoding colicin E7 or E2/E7 hybrids were used to treat calves artificially inoculated with pathogenic strains (Schamberger *et al*., 2004). Using a modified crossover experiment, in the initial period a group of calves that were daily fed 10^7 CFU/g of feed of a the mixture of the colicin-E7 CEC strains for 24 days had consistently smaller fecal counts of *E. coli* O157:H7 as compared to the control group, but this difference was only significant on two of the 9 sampling days. In the final period of the experiment the control group was switched to receive daily CEC doses of 10^8 CFU/g of feed. When the results of the same group of animals that had first been control and then treatment were compared, differences between 1 to 1.8 log CFU/g of feces were determined and in this case they were all statistically significant (Figure 1). *E. coli* O157:H7 strains were 50% more likely to be detected in the intestinal tissue of the treatment group at the end of the experiment compared to the control animals. This result suggested that the colicin E7-encoding CEC reduced the colonization of EHEC in cattle.

A similar approach to those described above has recently been reported by Etcheverria and coworkers (Etcheverria *et al*., 2006). In that study, more than 2000 isolates were recovered from the intestinal tissue of cattle that had tested negative for the presence of shiga toxin producing bacteria and screened for their ability to inhibit *E. coli* O157:H7. A total of 13 *E. coli* strains were identified to inhibit the growth of indicator *E. coli* O157:H7 strains tested. Those strains were characterized for the presence of virulence factors and it was determined that three of them produced low molecular weight bacteriocins.

A very novel strategy to control *E. coli* O157:H7 has been proposed by using colicin cloned into cattle feed. Based on the observation that colicin E7 was one of the most inhibitory bacteriocins against *E. coli* O157:H7 (Schamberger and Diez-Gonzalez, 2004) transgenic corn plants capable of expressing colicin E7 were recently developed in our laboratory (Jacobs *et al*., 2005). The colicin E7 gene (*cea*) naturally encoded in the ColE7-K317 plasmid was cloned into plasmid pIBT210, a
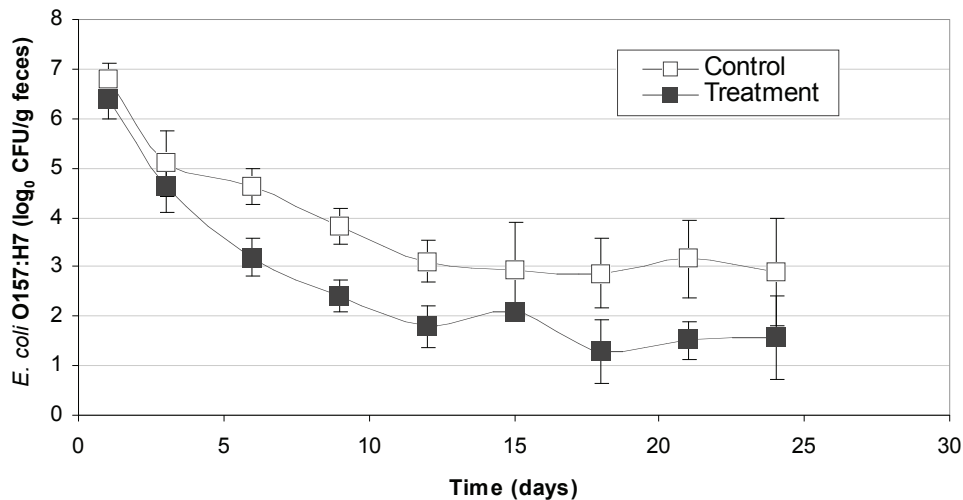
Figure 1 The effect of feeding colicin E7-producing *Escherichia coli* on the fecal shedding of *E. coli* O157:H7 in calves. The same group of calves was first inoculated with 10^{10} CFU per animal with a mixture of six *E. coli* O157:H7 without any other treatment and the fecal count was determined for 24 days. At day 25 the calves received daily doses of 10^8 CFU/g of feed for two weeks. The calves were then re-inoculated with the EHEC dose and the count was monitored for the following 24 days. At the end of the experiment, the calves were euthanized.

transformation vector for maize, and this vector was bombarded into highly embryogenic, friable, type II callus. The presence of the *cea* gene and the synthesis of mRNA was confirmed in callus tissue and new transformed callus tissue was regenerated into maize plants. The presence, expression and activity of colicin E7 in maize leaves was confirmed by PCR, Southern, reverse-transcriptase PCR, Western analysis and inhibitory assays. These results provide the first evidence that colicins could be cloned into plants for antimicrobial applications. Further work is needed to evaluate the effect of this novel approach for the control of enterohemorrhagic *E. coli*.

Neonatal calf diarrhea

A significant fraction of neonatal calf diarrhea is caused by enterotoxigenic *E. coli* strains such as serotype K99. In the most recent study that utilized CEC strains in cattle, the Nissle 1917 strain was used to treat calf diarrhea (von Buenau *et al*., 2005). As mentioned above, this strain has been marketed as a human probiotic and recent renewed interest have attracted several research groups (Hamilton- Miller, 2001; Hardy, 1975; Nissle, 1959). The Nissle 1917 strain was reported to produce colicine X, but little has been published about its inhibition mechanism (Papavassiliou, 1961). In two separate cattle trials, that included 162 and 173 newborn calves the incidence of diarrhea was reduced after daily feeding of Nissle 1917 strain for 12 days from approximately 65% to 26 and 12%, respectively, as compared to the control groups. In Germany, the Nissle 1917 strain has been approved as a probiotic preparation for calves since 2001 and is currently commercialized with the brand of "Ponsocol."

Swine diarrhea

Colicins are typically produced by *E. coli* and related Gram-negative enterobacteria, but McCormick *et al*. (McCormick *et al*., 1999) demonstrated that expression of a colicin was possible in Gram-positive bacteria. In that landmark report, the cloning and modification of colicin V is described for its production by a *Lactoccoccus lactis*

strain. The expression of colicin V in *Lac. lactis* was achieved by replacing the leader peptide by a signal peptide from divergicin A. Colicin V has been further cloned into *Lactobacillus fermentum* that was fed to pigs in a study, yet to be published (Stiles *et al*., 2005). The colicin V-producing *L. fermentum* appeared to reduce the incidence of diarrhea in swine, but it had a negative impact on gain weight.

Microcins

The production of colicins by *Escherichia coli* strains was well known during the first half of the 1900's, but it was not until 1976 that researchers realized that some strains had the capability of producing antimicrobial peptides that were significantly smaller than colicins (Asensio and Perez-Diaz, 1976). This group of antimicrobial peptides has been referred as microcins (Baquero and Moreno, 1984). The molecular size of microcins is typically smaller than 10 kDa and they have several differences with colicins. Some of these differences include: mechanism of induction, some of them are chromosome encoded, and have different secretion pathways. The reader is encouraged to check Gillor and Riley (Gillor *et al*., 2004) for a thorough comparison of colicins and microcins.

The spectrum of inhibitory activity of microcins appears to be broader than colicins and some of the microcins produced by *E. coli* are capable of inhibiting *Salmonella* species. *Salmonella* is probably the most important foodborne pathogenic bacteria based on the total number of human infections and deaths (Mead *et al*., 1999). Many of the salmonellosis cases are related to the consumption of contaminated poultry and eggs. *Salmonella* serovar Typhimurium frequently colonizes the gastrointestinal tract of poultry and *S.* serovar Enteriditis is often found in the cecum of layer hens. The prevention of salmonellosis cases will require the reduction of prevalence of this pathogenic group of bacteria in poultry populations. The use of probiotic bacteria and competitive exclusion cultures are considered two of the potential control measures for *Salmonella* in poultry.

Among the approximately 10 different antimicrobial peptides produced by *Escherichia coli* strains, microcin 24 has been applied as a control method for *Salmonella* in chicken. A microcin 24-producing transformant *E. coli* capable of inhibiting *Salmonella* and *E. coli* O157: H7 *in vitro* was produced and its use in chicken was patented (Wooley *et al*., 1999; Wooley and Shotts Jr., 2000). The microcin 24-producing strain (AvGOB18) was developed by transformation of a poultry *E. coli* isolate with a plasmid encoding microcin 24. Microcin 24 was first identified in an uropathogenic *E. coli* and cloned into a pBR322 plasmid vector (O'Brien and Mahanty, 1994). In animal experiments that used chicks to investigate the control of *Salmonella,* a treatment group was orally inoculated with individual doses of strain AvGOB18, but after four weeks no reduction in the counts of *Salmonella* Typhimurium was observed as compared to the control group. However, when the microcin 24-producing strain was offered in the drinking water at a concentration of approximately 10^6 cells/ml the count of *S.* Typhimurium was no longer detected after 3 weeks (Wooley *et al*., 1999). A patent as a pre-harvest intervention in livestock to control foodborne pathogens has been issued for this strain AvGOB18 (Wooley and Shotts Jr., 2000), but little is known if this technology has been licensed.

Microcin 24 was also used to transform an *E. coli* K12 strain and the resulting mutant, strain GOB18 was used to treat pigs against *Salmonella* Typhimurium colonization (Frana *et al*., 2004). In that study, during 18 days there was no difference in the extent of fecal shedding of *Salmonella* between the groups that received daily doses of 10^8 CFU of strain GOB18 and of the parent strain. This lack of effect could have been due to the fact that strain K12 is a laboratory strain that was isolated from a person long time ago and might not have the capability of colonizing the gastrointestinal tract of swine.

Lantibiotics

Many species of lactic acid bacteria (LAB) genera such as *Lactobacillus, Lactococcus*, *Leuconostoc, Streptococcus, Carnobacterium* among others are capable of producing small peptides that can inhibit a broad range of Gram-positive bacteria (Cleveland *et al*., 2001). Most LAB bacteriocins inhibit bacteria by forming pores in the cell membrane and dissipating the proton motive force. Gram-negative bacteria are protected from the lethal effect of LAB bacteriocins by the outer membrane. Many different types of LAB bacteriocins have been studied and characterized, but the most widely known are: nisin, lacticin, enterocin, pediocin, and plantaricin (Ray, 2003). These have been extensively studied for their application in foods, but just a few of them have been used in livestock.

Lantibiotics are bacteriocins produced by LAB that contain lanthionine rings in their molecule and are typically classified as Class I bacteriocins. There are several LAB species capable of producing lantibiotics (McAuliffe *et al*., 2001). The types of lantibiotics that have been more frequently identified and characterized are nisin and lacticin. Nisin is typically produced by *Lactococcus lactis* strains and lacticin can be produced by *Lac. lactis* and *Lac. sake* (Ray, 2003). These antimicrobial peptides have between 23 to 25 amino acid residues. Nisin is probably the best characterized LAB bacteriocin and is the only antimicrobial peptide approved for use in foods.

Nisin

The use of nisin in foods has been approved for cheeses, but there is an enormous amount of information about its application to inhibit a variety of pathogenic and spoilage bacteria in many food products (Delves-Broughton *et al*., 1996). One of the most promising applications of nisin is on the control of *Listeria monocytogenes* in ready-to-eat meats (Ariyapitipun *et al*., 2000). Despite the widespread use of nisin, its application in livestock has been largely limited. One of the few uses that this bacteriocin has been investigated for is as part of a germicidal preparation for cows teats (Sears *et al*., 1992). That germicidal preparation was capable of reducing the population of *Staphylococcus aureus* by more than 3 log CFU/g and has been commercialized (Ross *et al*., 1999).

Monensin is an ionophore that has been widely used in cattle production as a growth promoter because of its multiple beneficial effects on rumen fermentation. Monensin was originally developed as a coccidiostat, but monensin is largely used in cattle to increase productivity by inhibiting methane-producing bacteria, amino acid fermenters and lactic acid bacteria (Russell and Mantovani, 2002). Nisin has been tested as a potential replacement for monensin. The effect of nisin on rumen fermentation was investigated and experiments conducted *in vitro* indicated that its action was similar to monensin (Callaway *et al*., 1997). The effect of nisin on rumen fermentation was compared to monensin using an artificial rumen system (Jalc and Laukova, 2002). In this latter report, nisin increased the degradation of hemicellulose and the production of acetate and butyrate, but had no effect on cellulose degradation, methane production and microbial synthesis efficiency. The limited effect of nisin on rumen microorganisms was apparently due to its degradation (Russell and Mantovani, 2002). These results suggested that nisin was not a feasible replacement for monensin.

Other lantibiotics

LAB strains that produce lacticin 3147 are considered as generally recognized as safe and can be used for food production, but approval to the purified bacteriocin preparation has not been granted (Ross *et al*., 1999). Significant evidence has shown that lacticin is capable of inhibiting a variety of Gram-positive bacteria for food applications, but similar to nisin its application in live domestic animals has been rather scarce. Because of its ability to inhibit *Staphylococcus aureus* and *Strepococcus dysgalactiae,* lacticin 3147 has also been used to disinfect cow's teats and to treat mastitis with relative success (Twomey *et al*., 2000). Incorporation of lacticin 3147 into a teat seal preparation reduced approximately 10-fold the prevalence of mastitis-causing bacteria in animals that were inoculated with *S. dysagalactiae* (Ross *et al*., 1999).

A few lantabiotics have been identified in microorganisms isolated from the rumen and a number of researchers have suggested that those BPB or their bacteriocins could be used to modify the rumen fermentation. At least three different bacteriocins have

been identified in *Streptococcus bovis*, but only one of them was characterized as a lantibiotic (Lee *et al*., 2002; Whitford *et al*., 2001; Xiao *et al*., 2004). *S. bovis* is an important ruminal LAB that is predominant when cattle are fed starch-based materials and is largely responsible for rumen acidosis (Hungate, 1966). Bovicin HJ50 was identified in *S. bovis* strain isolated from milk, and this lantibiotic was capable of inhibiting a wide spectrum of Gram-positive strains. The potential application of this particular bovicin on rumen fermentation, however, remains to be elucidated.

Lantibiotics can also be produced by the ruminal anaerobe Gram-positive *Butyrivibrio fibrisolvens* (Kalmokoff *et al*., 1999). *B. fibrisolvens* is one of the predominant rumen bacteria capable of degrading fiber compounds and many species appear to be capable of producing bacteriocins (Kalmokoff *et al*., 1996). Buryrivibriocin OR79A has been the only lantibiotic characterized from *B. fibrisolvens* and it had broad inhibitory activity against rumen Gram-positive isolates. Despite the promising characteristics of this lantibiotic, no application has been reported.

Other bacteriocins

Two of the bacteriocins isolated from *Streptococcus bovis* strains have been proposed as a potential feed additive to inhibit indigenous ruminal *S. bovis* and prevent rumen acidosis (Mantovani *et al*., 2002; Whitford *et al*., 2001). Bovicin HC5 was identified in a rumen isolate and was found that was capable of inhibiting most Gram-positive ruminal organisms tested. This bacteriocin was characterized as a novel type of bacteriocin because it had 4 amino acid residues not previously reported. Bovicin HC5 was capable of reducing approximately 50% the methane production when added to mixed ruminal cultures as semi-purified preparations (Lee *et al*., 2002). In addition, methanogenic bacteria did not appear to be capable of developing resistance to bovicin HC5. The inhibitory activity of the same bacteriocin has recently been tested against *Listeria monocytogenes* strains as a potential method to prevent the proliferation of this pathogen in silages (Mantovani and Russell, 2003). Further work needs to be conducted to confirm the feasibility of bovicins to enhance animal productivity.

In addition to the lantibiotic compound produced by *B. fibrisolvens* described above, there are at least two other bacteriocins reported in the literature (Kalmokoff and Teather, 1997; Rychlik and Russell, 2002b). Kalmokoff and Teather characterized butyrivibriocin AR10, the first bacteriocin identified in a ruminal anaerobic bacteria (Kalmokoff and Teather, 1997). More recently a bacteriocin was detected in *B. fibrisolvens* strain JL5 and this compound could inhibit several Gram-positive rumen microorganisms (Rychlik and Russell, 2002b). Because this latter bacteriocin was capable of inhibiting *Clostridium aminophilum*, an amino acid fermenting rumen bacterium, it was hypothesized that it could have a role in preventing ammonia production in the rumen and eventually improving feed efficiency. The potential utilization of this bacteriocin was, however, not supported by additional studies that showed that *C. aminophilum* was capable of developing resistance against it (Rychlik and Russell,

2002a). These results suggest that novel bacteriocins need to be identified that would have a significant effect on modifying rumen fermentation.

Commercial applications of bacteriocins

Several of the bacteriocin-producing bacteria described in this chapter have been patented, but to the end of 2005 none of them were at the commercialization stage (Doyle *et al*., 1999; Wooley and Shotts Jr., 2000). A mixture of *Lactobacillus* strains has shown promising results to reduce the fecal shedding of *E. coli* O157:H7 in cattle and is currently being marketed as a probiotic with the name of Bovamine® (Brashears *et al*., 2003). These lactobacilli were originally selected on the basis of its *in vitro* inhibitory activity against pathogenic *E. coli*, but there was little evidence that they produced a bacteriocin (Brashears *et al*., 2003). The most recent patent of bacteriocins for livestock utilization was a really broad invention of a mixture of sorbic acid with bacteriocins or bacteriocin-producing bacteria to be included in feed rations (Raczek, 2004). This patent listed more than 50 bacteria and more than 30 different bacteriocins as possible ingredients of this mixture. Based on the website of the company that patented this invention, Nutrinova, it did not appear that this product is yet in the market and there was almost no information about its effectiveness.

Future trends

The utilization of bacteriocins or bacteriocin-producing bacteria in livestock is a field with enormous possibilities for both research and commercialization. We can easily say that there has been very limited research in this area, but in recent years the number of investigators has dramatically increased. As more countries develop antibiotic-limiting policies, the need for alternative antimicrobial will probably be the main driving force to continue identifying novel bacteriocins and testing existing ones. Because of the relative specificity of bacteriocins as compared with antibiotics, it can be anticipated that the identification of broader spectrum bacteriocins will be an active research endeavor. Similarly, it is foreseen that researchers will likely utilize combinations of bacteriocins to obtain a broader spectrum for target organisms.

The second major area of opportunity for bacteriocin application into animal production is the pre-harvest control of foodborne pathogens. The seminal publications discussed above have provided the proof of concept that this is an approach with great potential. The two bacteria of interest that will likely continue to be the main focus of future work will be *Salmonella* in different animals and enterohemorrhagic *E. coli* in cattle. In the work conducted in our laboratory, we showed that the use of a single type of colicinogenic *E. coli* could reduce the fecal sheding of EHEC (Schamberger *et al*., 2004), but a mixture of strains capable of producing different colicins would likely accomplish a greater reduction (Schamberger and Diez-Gonzalez, 2004). For *Salmonella* control it is likely that a similar strategy will be used.

Finally, the use of bacteriocins as growth promoters remains also a largely unexplored field with very interesting possibilities. Specifically, significant efforts would be devoted to find bacteriocins capable of replacing

ionophores such as monensin and lasolacid. For all these future areas of active research on bacteriocins, it will be critical that studies that assess the potential development of resistance will be incorporated early in the scientific process as well as risk assessment studies that will indicate any potential collateral effect.

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