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Competing Microbiota and its Products on Fresh and Processed Meat

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SUMMARY

The application of packaging technology with good refrigeration has given meat processors the option to use protective cultures as antimicrobial hurdles to improve the storage life and safety of meats. Lactic acid bacteria produce a number of antimicrobial substances that can be exploited to control the microbiota on meat and meat products. This presentation will focus on the use of lactic acid bacteria and their bacteriocins to control the development of microbial populations in meats and will include an overview of the impact of biopreservative cultures on the sensory quality of the products. Research on biopreservative cultures in meat products has shown that the choice of culture needs to be product specific to ensure an extended storage life. As an example, in fresh meat products, **Leuconostoc gelidum** has been shown to be an effective biopreservative; whereas, in processed meat products, **Carnobacterium piscicola** would be the preferred biopreservative culture. In addition to giving a predictable storage life, bacteriocin-producing cultures also enhance the safety of fresh and processed meat products by inhibiting the growth of potential pathogens, in particular **Listeria monocytogenes**. Environmental conditions have been shown to have an impact on the production and activity of bacteriocins. The interaction of bacteriocins with salt, curing additives, temperature and other food components will be discussed. The presentation will include an overview of research to enhance the antimicrobial activity of a competitive microbiota through the use of genetically modified lactic acid bacteria that produce multiple bacteriocins and the potential for the use of combinations of bacteriocins with other antimicrobial agents or processes.

INTRODUCTION

In today's meat distribution and marketing system, most fresh and processed meat products are packaged in an atmosphere that encourages the development of a microflora consisting of lactic acid bacteria. The growth of a competitive lactic acid bacteria microflora interferes with the growth of spoilage and pathogenic bacteria on meats. Under refrigeration conditions, the development of a lactic acid bacteria microflora dramatically increases the storage life of the products (Dainty and McKay, 1992).

Use of starter cultures has a long history in the dairy industry but has only been applied in the meat industry for the production of fermented meat products. Other products, such as packaged fresh and processed meats could also benefit from the use of "starter" or "protective" cultures. These cultures could be used to ensure the development of a predictable lactic acid bacteria microflora that will give the expected sensory properties and product performance in terms of storage life with the potential for added protection for food safety.

This paper will discuss the prospects of the use of lactic acid bacteria starter cultures in preservation of fresh meats with a focus on the use of bacteriocin-producing lactic acid bacteria and their impact on the quality and safety of meat products.

Keywords

competing microbiota, fresh meat, processed meat, lactic acid bacteria, antimicrobial products, GMO

Lactic Acid Bacteria of Meats and their Antimicrobial Products

The lactic acid bacteria that are typically found on meat products include **Carnobacterium**, **Lactobacillus**, **Leuconostoc** and **Weisella** spp. (Stiles and Holzapfel, 1997). Although **Pedicococcus** spp. are used as starter cultures for meat fermentations, they are not typically found as part of the adventitious lactic acid bacteria microbiota of meats.

Although lactic acid bacteria are generally considered to have a limited impact on the sensory quality of packaged meat products until sometime after they reach maximum numbers, there are documented examples where the adventitious lactic acid bacteria microflora will cause early and overt spoilage of the product (Leisner et al., 1996; Shay and Egan, 1981). An understanding of the impact of lactic acid bacteria on the sensory properties of meat products is critical to their successful use as protective cultures.

Lactic acid bacteria are able to prevail in packaged meat and meat products stored at refrigeration temperatures through several mechanisms including the production of a wide variety of antimicrobial substances, including organic acids (lactic and acetic acids), hydrogen peroxide, diacetyl, reuterin and bacteriocins.

The production of lactic or acetic acids may reduce the pH of some meat products to a level where spoilage and pathogenic bacteria are inhibited. This is highly desirable in fermented meat products; however, the low concentration of carbohydrate and the strong buffering capacity of fresh meats limits the impact of these acids on the competitive microbiota. In processed meats, the formation of organic acids can result in off-flavours (Huis in't Veld, 1996). Hydrogen peroxide is only produced by lactic acid bacteria growing under oxygen rich conditions and is usually detrimental to the sensory quality of meats. Diacetyl has an intense buttery aroma that would be deleterious to the sensory quality of meat products. Reuterin is produced by **L. reuteri**. It has broad spectrum antimicrobial activity including an ability to inhibit the growth of gram positive and gram negative bacteria as well as yeasts, fungi, and protozoa (Axelsson et al., 1989). El-Ziney et al. (1999) demonstrated that reuterin is able to reduce the viability of **Listeria monocytogenes** and **Escherichia coli** O157: H7 on pork surfaces and in raw ground ham at an elevated refrigeration temperature (7°C).

Bacteriocins are a group of ribosomally synthesized antibacterial peptides or proteins that are primarily active against bacteria that are closely related to the producer organism (Klaenhammer, 1993). According to DeVuyst and Vandamme (1994), bacteriocin production has been described for all genera of lactic acid bacteria. The production of bacteriocins is not limited to lactic acid bacteria, as bacteriocins are produced by both gram negative and gram positive bacteria. It was bacteriocin production by **Escherichia coli** that received initial attention with the discovery of colicin V (Jack et al., 1995).

Three distinct classes of bacteriocins have been defined. Class I includes lantibiotics, which are small, heat stable peptides that are differentiated from other bacteriocins by extensive posttranslational modification and the presence of dehydroamino acids: dehydroalanine and dehyrobutyrine. Typically, lantibiotics have a spectrum of inhibitory activity that includes other lactic acid bacteria, **Listeria** spp. and **Clostridium** spp. The classical example of a lantibiotic is nisin A. Class II bacteriocins are small, heat stable peptides that undergo minimal postranslational modification. Class II bacteriocins have been divided into different subclasses either on the basis of chemical structure or how they are secreted from the cell. Klaenhammer (1993) initially divided the Class II bacteriocins into Class IIa, single peptide bacteriocins which includes the pediocin-like group, Class IIb, two-peptide bacteriocins and Class IIc thiol activated peptides. Nes et al. (1996) defined Class IIc as the group of bacteriocins secreted from the cell via the general secretory pathway. However, more recently, a classification based strictly on the chemical structure of the bacteriocin was proposed for the Class II bacteriocins (van Belkum and Stiles, 2000). Class II bacteriocins can have a wide range of inhibitory activities, ranging from a very restrictive antibacterial spectrum including only one species of lactic acid bacterium (Stiles, unpublished data) or having a relatively broad spectrum of activity, similar to that of nisin. An example of a Class II bacteriocin with an antibacterial spectrum similar to that of nisin is brochocin-C (McCormick et al., 1998). Class III bacteriocins are large, heat labile peptides which are relatively uncommon among lactic acid bacteria.

The focus on use of bacteriocin-producing strains in meats has been on those organisms that produce Class I and Class II bacteriocins. The reader is referred to reviews by Holzapfel et al. (1995) and Hugas (1998) for a summary of the different bacteriocin-producing lactic acid bacteria that have been isolated from meats and the application of bacteriocin-producers in meat products. Since that time, there has been continued interest in the discovery and application of bacteriocin-producing lactics in meats. A search of the ISI Web of Science Database using the keywords "bacteriocin" and "meat" yielded a total of 43 scientific publications since January 1999. Of the publications cited, 16 focused on the specific application of bacteriocin-producing lactic acid bacteria to control the growth of **Listeria monocytogenes** in meats; 11 articles described the isolation of new bacteriocin-producing strains from meats; 9 focused on the general application of bacteriocins in meats; and 7 articles included other aspects of bacteriocin research related to the detection of bacteriocins in meats, growth characteristics of the producer organisms and bacteriocin resistance.

Application of a Competitive Microbiota for Preservation of Fresh and Processed Meats

The use of a controlled microbiota can offer the meat industry many new advantages that are currently not exploited. This review will focus on the use of a competitive microbiota in non-traditional meat environments.

One advantage to always having a consistent and predictable microflora develop in a packaged meat product is consistent product quality that allows the processor to more accurately predict the storage life of packaged meats. Bacteriocin-producing protective cultures can prevent the growth of lactic acid bacteria that may spoil meat products. **L. sakei** 1218 has been documented to cause distinct sulphur odors in vacuum packaged meat stored at 2°C (Leisner et al, 1996). However, co-inoculation of meat with a bacteriocinogenic strain of **Leuc. gelidum** delays the growth of **L. sakei** and inhibits the spoilage of meat for up to 8 weeks of storage (Leisner et al., 1996).

Use of a protective culture of **Leuc. gelidum** UAL187 in coarsely ground beef stored in vacuum for 35 days at 2°C prior to grinding and packaging for retail sale resulted in stabilization of the red color of the meat longer during retail display as compared to uninoculated control samples (Worobo et al., 1996). Lactic acid bacteria have been demonstrated to have metmyoglobin reducing ability (Arihara et al., 1993), which may explain the improved color stability of ground beef noted by Worobo et al. (1996). In addition to improving the color stability of ground beef, **Leuc. gelidum** UAL187 also reduced the incidence of putrid odors in vacuum packaged ground beef stored at 4°C for up to 21 days (McMullen et al., 1997).

One of the concerns with the addition of a competitive microflora to meat products is that the initial numbers of bacteria are higher than that found in naturally contaminated meats and that the addition of the competitive microflora may cause flavour changes in the product faster than the adventitious population. In our research with **Leuc. gelidum** UAL187 in ground beef, a descriptive sensory panel found no differences between the flavour profiles of vacuum packaged ground beef that were inoculated with a protective culture of **Leuc. gelidum** UAL187 and the uninoculated control samples (McMullen et al., 1996).

Other studies on the impact of bacteriocin-producing lactic acid bacteria on the sensory quality of meats have demonstrated that care must be taken in the choice of protective culture in relation to the characteristics of the product. Although **Leuc. gelidum** UAL187 was the culture of choice for vacuum packaged ground beef, when this culture was added to ground meat containing a spice unit, overt spoilage occurred, significantly reducing consumer acceptability of the product. In contrast, a protective culture of **Carnobacterium piscicola** LV17 resulted in a spiced ground beef product that was similar in consumer acceptability to the uninoculated control (McMullen, unpublished data). In processed meat products, a protective culture of **Leuc. gelidum** would be unacceptable due to the potential for slime production and the development of off-odors during refrigerated storage (McMullen, unpublished data). A protective culture of **C. piscicola** is more suitable for use in processed meats and has limited impact on the sensory quality of frankfurters (McMullen, unpublished data). This work clearly demonstrates the need for a careful assessment to match the product characteristics with those of the protective culture.

One significant potential advantage to the application of bacteriocin-producing organisms as protective cultures is their ability to inhibit the growth or kill strains of **L. monocytogenes**. As mentioned previously, this has been the focus of much of the research on the applications of bacteriocin-producing lactic acid bacteria in meats. Results for the inhibition of **L. monocytogenes** with bacteriocin-producing cultures in fresh and processed meats has been variable. Results obtained with fresh meat products have shown good inhibition of the growth of **L. monocytogenes** (Hugas et al., 1998; Juven et al., 1998; Panayach, 1998). However, with processed meats, storage temperature seems to play a role in the ability of some cultures to inhibit the growth of **L. monocytogenes**. Degnan et al. (1992) found that **Pediococcus acidilactici** JBL1095 was able to inhibit the growth of **L. monocytogenes** when vacuum packaged wieners were stored at 25°C but at 4°C the protective culture was ineffective. Katla et al (2002) found 10^4 CFU/g of **L. sakei** LB790 which produces sakacin P, was not able to inhibit the growth of **L. monocytogenes** on chicken cold cuts stored at 4°C but inhibition was detected when the product was stored at 10°C. This illustrates the potential for bacteriocin-producing lactic acid bacteria to have a protective effect in conditions of temperature abuse. However, we have found that strains of **C. piscicola** are able to inhibit the growth and significantly reduce the numbers of different strains of **L. monocytogenes** on vacuum packaged wieners stored at refrigeration temperatures (Stiles and McMullen, unpublished data). It is possible that the inhibition of **L. monocytogenes** in chilled stored meats is strain dependent, illustrating the need for discovery of new bacteriocin-producing cultures that have potential for use under the conditions of product storage.

Impact of Environmental Conditions and Processing on the Inhibition by Protective Cultures

Strains of **Lactococcus lactis**, which produce nisin the most studied lantibiotic, are mesophillic organisms which limits their application as a protective culture in chill stored meats. However, nisin producing strains of **Lac. lactis** have been isolated from fermented meats (Rodriguez et al., 1995; Noonpakdee, 2003) and other lantibiotic-producing lactic acid bacteria have been applied to meat systems (Scannell et al., 2001a). Although lantibiotics may have some antimicrobial effects on meats, we recently showed that

nisin is inactivated in meats through an enzymatic reaction with glutathione (Rose et al., 1999, 2001). Glutathione molecules can attach to at least 3 of the dehydroresidues of nisin, resulting in the loss of antibacterial activity (Rose et al., 2003). Although this work was done exclusively with nisin, there is no reason to believe that glutathione, which is abundant in meats, could react with other lantibiotics and diminish their antibacterial activity.

A number of researchers have demonstrated the formulation of a product can influence the antibacterial ability of protective cultures. Salt, at the concentration found in a number of processed meat products, including fermented meats, can reduce the growth of protective cultures and thereby reduce the production of bacteriocins (Leroy and De Vuyst, 1999; Vignolo, et al., 1998). Addition of 3, 5 or 7% salt to a meat slurry resulted in the protection of **L. monocytogenes** from the action of lactocin 705 (Vignolo et al., 1998). Similar results were reported by Hugas et al. (2002) for **L. sakei** CTC494. However, the presence of other ingredients in sausage formulations help to overcome the reduced impact of bacteriocins on the growth of **L. monocytogenes** in the presence of salt. Black pepper, which contains relatively high levels of manganese, and manganese enhance the anti-listerial activity of **L. sakei** CTC494 and reduce the growth of **L. monocytogenes** in sausage (Hugas et al., 2002).

The addition of nitrite reduced the concentration of lactocin 705 required to inhibit the growth of **L. monocytogenes** (Vignolo et al., 1998). High concentrations of nitrite can have a negative impact on the growth of starter cultures and bacteriocin production in fermented salami (Scannell et al., 2001). There is potential for the use of bacteriocin-producing lactic acid bacteria to reduce the concentration of nitrite required in processed meats, such as the case with bacon produced by the Wisconsin Process (Tanaka et al., 1985) where **Pediococcus acidilactici,** a bacteriocin-producing culture, is used as a competitive microflora.

In addition to the effects of different ingredients on the activity of bacteriocins in meats, different processing steps may impact the efficacy of microbial control by bacteriocin-producing lactic acid bacteria. Although many processes may kill protective cultures, they may have some applications where the partially purified bacteriocin is used in a food. The combination of high pressure processing with bacteriocins enhanced antibacterial activity in a meat model system (Kalchayanand, et al., 1998; Garriga et al., 2002).

Genetically Modified Organisms as Protective Cultures

The application of genetically modified bacteria as protective cultures in meats has promise as a technology in that it will allow targeted applications with the appropriate bacterial host for a given product. For example, if a very broad spectrum bacteriocin is discovered in organism X, but that organism causes overt spoilage of meats, it would be unwise to use organism X as a protective culture in meats. However, we have the molecular tools to move the genetic elements required for the production of the "new" broad spectrum bacteriocin in a host that is suitable for application in meats. There are a number of examples where this has been done. Molecular biology has given us the tools to develop a protective culture that expresses bacteriocins targeting gram negative pathogens, such as **Escherichia coli** O157:H7 and **Salmonella** spp. This would require the insertion of a colicin gene into a vector that is transformed into a suitable lactic acid bacterial strain for application in meats. McCormick et al. (1999) developed a number of **C. piscicola** strains that were able to express colicin V. Although the activity spectrum of colicin V is limited, the system may serve as a model for the expression of other bacteriocins. This approach could be used to expand the antibacterial spectrum of a protective culture by incorporating the genetics for production of multiple bacteriocins into one strain. One of the hurdles to the application of genetically modified organisms to control either spoilage or pathogenic bacteria in meats is consumer acceptability. The time may come when consumers can be convinced that the benefit from genetically modified cultures is sufficient to warrant their use in foods.

CONCLUSIONS

The use of protective lactic acid bacteria cultures has the potential to allow the meat industry to better predict the storage life of their products and provides an additional barrier to the growth of foodborne pathogens. However, along with other processing hurdles, the use of protective cultures should only be considered as an additional process that complements Good Manufacturing Practices. As consumers continue to demand products that are minimally processed and preserved, the use of protective cultures may become more common as a means of "natural" preservation of foods.

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