BACTERIOCINOGENIC LACTIC ACID BACTERIA FOR THE BIOPRESERVATION OF MEAT AND MEAT PRODUCTS

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ABSTRACT

The consumer demands for less preserved foods and the development of new food systems to fulfill these demands, urges new hurdles for pathogen growth. The strategies for pathogen reduction are not selective for pathogenic microorganism and therefore the non-spoilage microorganisms may become also inactivated, from this situation a question of concern about a freer way for pathogen growth is arised. Biopreservation refers to the extended storage life and enhanced safety of foods using their natural or controlled microflora and (or) their antibacterial products. In meats, lactic acid bacteria constitute a part of the initial microflora which develops easily after meat is processed. LAB growth in meat can cause microbial interference to spoilage and pathogenic bacteria through several mechanisms, specially bacteriocins. The paper deals with the description of meat-borne bacteriocins and their application in meat and meat products either to extend the shelf life or to inhibit meat pathogens. The application of bacteriocinogenic LAB together with new technological hurdles is discussed.

INTRODUCTION

Despite the recent progress in food biotechnology with the introduction of modern technologies and safety concepts (e.g. HACCP), the problem of food safety and security remains to be solved. The reported number of food-borne illnesses and intoxications is still increasing, the largest outbreaks of food-borne diseases (salmonellosis, listeriosis and haemorragic colitis) occurred in the last 12-13 years (Jay, 1996).

On the other hand, consumer trends lead to a loss of intrinsic preservation and to a potential loss of protection from processing, since consumers prefer more stable and safer products with a longer shelf life and without chemical preservatives, as well as mild and light products with a low acid, sugar, salt or fat content (Gould, 1992).

The manufacturers strategies to increase the safety of meat and meat products consist of developping new technologies to reduce the number of microorganisms in meat, and new products minimally processed which could represent new hazards for health because of the pathogen growth. The strategies for pathogen reduction are not selective for pathogenic microorganism and therefore the non-spoilage microorganisms may become also inactivated. From this situation a question of concern about a freer way for pathogen growth is arised.

The consumer demands for less preserved foods and the development of new food systems to fulfill these demands, urges the development of new hurdles for pathogen growth. The hurdle technology (Leistner, 1978, 1985; Leistner and Gorris, 1995) refers to the deliberate combination of existing (temperature, a_w, pH, Eh, preservatives etc.) and novel preservation techniques (gas packaging, bioconservation, bacteriocins, ultrahigh pressure treatment etc.) in order to stablish a series of more selective preservative factors (hurdles) that the spoilage and pathogenic microorganisms should not be able to overcome.

The hurdles established in a certain food could consistently control microbial spoilage and food poisoning leaving the bioprotective cultures unaffected. It is then, a selective method to control microorganisms.

Since the beginning of the microbiology in the last century, the concept of microbial interference has been known, reviewed by Jay in 1996 and described as the antagonism displayed by one microorganism towards another, a concept linking to lactic acid bacteria and the protective cultures and the bioprotection concepts of Holzapfel et al (1995) and Stiles (1996). According to the latter author, biopreservation refers to extended storage life and enhanced safety of foods using their natural or controlled microflora and (or) their antibacterial products.

Biopreservation can be applied in food and meat systems by four basic methods (Stiles, 1996; Gorris, 1997):

Adding a pure culture of the viable bacteriocin-producing LAB. This way offers an indirect way to incorporate bacteriocins in a food product, its success depends on the ability of the culture to grow and produce bacteriocin in the food

under the environmental and technological conditions (temperature, pH, additives etc..). As meat cannot be pasteurized prior to the addition of a LAB culture, the LAB cultures for biopreservation or fermentation of meat must be able to compete with the natural microflora.

Adding a crude bacteriocin-preparation, the fermentation liquor or concentrates obtained by growing the bacteriocin-producing LAB on a complex substrate. This mode avoids the use of a purified compound.

Adding purified or semi-purified antagonistic substances. By using this method the dosage of bacteriocin is more accurate and thus more predictable. However, application is limited according to national regulations concerning food additives.

Adding mesophilic LAB as a "fail-safe" protection against temperature abuse. In this case the bioprotective strain will be kept at initial concentration in chilling conditions. Under temperature-abuse conditions, the strain will grow competitively in front of pathogenic bacteria avoiding health hazards.

LACTIC ACID BACTERIA IN MEAT AND MEAT PRODUCTS

Meat is highly sensitive to microbial spoilage because of its ecological properties (a_w, pH and nutrients). In meats, lactic acid bacteria (LAB) constitute a part of the initial microflora which develops easily after meat is processed to fermented sausages, chill stored or packed under vacuum or modified atmosphere. The generally considered as natural strains of LAB in meats and meat products are: *Carnobacterium piscicola* and *C.divergens*; *Lactobacillus sakei, Lb. curvatus* and *Lb. plantarum, Le. mesenteroides* subsp. *mesenteroides, Le. gelidum* and *Le. carnosum.*

LAB have been playing an important role in food fermentations causing flavour and texture changes together with a preservative effect resulting in an increase in the shelf life of the transformed product. The chill-storage under modified atmosphere of red meats has an effect on the meat microflora triggering a change from putrefactive gram negative bacilli to fermentative LAB. This change produces a dramatic effect on the shelf life extension (Dainty and Mackey, 1992). LAB in fresh meat bring about a mild fermentation process without producing any changes in the sensorial characteristics because of the low carbohydrate content and the strong buffering capacity of meat. In the same way the growth of LAB in naturally fermented meats after the addition of sugar transform the products through the production of lactic acid by the LAB, the subsequent decrease in pH denatures the meat proteins favouring the decrease of a_w which ends up in a microbial stabilisation of the transformed product.

Although *Pediococcus acidilactici* and *Pediococcus pentosaceus* constitute the preferred starter microorganisms in the U.S.A. for fermented sausages, do not have an important role in indigenous meat fermentation processes. In European fermented products specially in Southern mediterranean countries the most important organisms in indigenous fermentation processes and thereby the most used organisms as starter cultures for meat fermentation are *Lb.sakei, Lb.curvatus* and *Lb.plantarum.*

The metabolic products of LAB and the bacteria itself have a role in the preservation of foods, although the uncontrolled growth of some species of LAB can cause spoilage in meats and meat products. *Leuconostoc* and *Lb.sakei* have been described as slime-producing organisms in processed meats (Korkeală and Makelä, 1992), suplhide-producing strains of *Lb.sakei* have been described as spoiling vacuum-packaged meat (Egan et al, 1989), the growth of heterofermentative LAB can also cause off-odours and holes after the production of CO_2.

ANTIMICROBIAL MECHANISMS OF LAB

LAB growth in meat can cause microbial interference to spoilage and pathogenic bacteria through several mechanisms like nutrient and oxygen competition (Ha et al, 1994), competition for attachment/adhesion sites (Chan et al, 1985) and production of a wide range of inhibitory substances primarily lactic acid or lactic and acetic acids, acetoin, diacetyl, hydrogen peroxide, reuterin and bacteriocins.

Bacteriocins are ribosomally-produced antimicrobial polypeptides or proteins that produce, in their mature form, an antibacterial effect against a narrow spectrum of closely related bacteria (Jack et al, 1995). Bacteriocins due to their proteinaceous nature are probably inactivated by proteases in the gastrointestinal tract. Most of the bacteriocins known so far are cationic molecules up to 60 aminoacids residues and thermostable.

On a scientific basis four defined classes of bacteriocins in LAB have been established (Klaenhammer, 1993): Class I for the lantibiotics (lanthionine-containing peptides with antibiotic activity) they are small peptides that have been differentiated from other bacteriocins by their content of dehydroamino acids and thioeter amino acids; Class II for the (<10 KDa) heat-stable non-lantibiotics divided into three subclasses on the basis of either their distinctive N-terminal sequence, their formation of bicomponent pores, or the presence of a functional sulfhydryl group; Class III for the large (>30 KDa) heat-labile bacteriocins which include many bacteriolytic extracellular enzymes (hemolysins and muramidases) that may mimic the physiological activities of bacteriocins and Class IV, complex bacteriocins that contain essential lipid or carbohydrates moieties in addition to protein. Class I and II bacteriocins are by far the most studied because they are the most abundant and the most prominent candidates for industrial applications. Class II bacteriocins are subdivided into three different subclasses: a, b and c. Class IIa comprises single peptide bacteriocins which includes the pediocin-like group; Class IIb, double-peptide bacteriocins and Class IIc, the sec-dependent secreted bacteriocins (Von Heijne, 1986, 1988).

BACTERIOCINS OF MEAT-BORNE LACTIC ACID BACTERIA

Meat-borne bacteriocinogenic LAB have been described in the literature, the bacteriocins produced by these strains belong to Class I and II.

Meat-borne bacteriocinogenic strains producing Class I bacteriocins

Lactococcus lactis BB24 isolated from fermented sausages secrete nisin (Rodriguez et al, 1995a). Nisin (Rogers, 1928; Harris et al, 1992) is the most studied bacteriocin at practical, biochemical and genetic levels. In dairy products it shows a strong antibotulinical, antilisterial and anti-staphylococcal activity when used at a reasonable level of 3.75-12.5 mg Kg^{-1} in the finished product. Nisin is licensed for use as a food additive in more than 45 countries (Delves-Broughton, 1990).

Lb.sakei L45 isolated from Norwegian dry sausages, *Lb.sakei* 148 and *Lb.sakei* V18 isolated from Spanish fermented sausages secrete lactocin S (Mortvedt and Nes, 1990, 1991; Sobrino et al, 1992; Rodriguez et al, 1995b; Cintas, 1995) which is also a lantibiotic. Its spectrum of activity comprises strains of the genera *Lactobacillus, Pediococcus, Leuconostoc* and *Clostridium.*

The abundance of strains producing lactocin S shows a special relevance of this substance in fermented meat products since it could be isolated from different climatic environments such as those of Spain and Norway.

Meat-borne bacteriocinogenic strains producing Class II bacteriocins

The pediocin-like bacteriocins are currently the major subgroup of bacteriocins with strong antilisterial activity. This subgroup has been named pediocin-family as pediocin was the first and most extensively studied bacteriocin. Strains producing these type of bacteriocins are found in many species of meat-borne LAB like *Pediococcus* (González and Kunka, 1987; Bhunia et al, 1989); *Leuconostoc* (Hastings et al, 1991); *Lactobacillus* (Schillinger and Lücke, 1989; Tichaczek et al, 1992; Hugas et al, 1995) and *Enterococcus* (Aymerich et al, 1996).

The antilisterial pediocin-like bacteriocins share strong amino acid sequence homology which is most pronounced in the N-terminal part of the peptide (Aymerich et al, 1996).

Pd.acidilactici PAC1.0, *Pd.acidilactici* JD, *Pd.acidilactici* H, *Pd.acidilactici* E, *Pd. acidilactici* F and *Pd. acidilactici* M isolated from American-style fermented sausages, *Pd.pentosaceus* Z102 isolated from Spanish-style fermented sausages produce the bacteriocin PA-1 (González and Kunka, 1987; Harris et al, 1989; Bhunia et al,1989,1991; Richter et al, 1989; Ray et al, 1989, 1992; Motlagh et al, 1992, 1994; Cintas, 1995). The bacteriocin produced by these strains was first named pediocin PA-1 in strain PAC1.0 and pediocin AcH in strain H. It is active against *Pediococcus, Lactobacillus, Leuconostoc, Listeria monocytogenes, Clostridium perfringens, Cl. botulinum* and *Staph. aureus.*

Lb.sakei Lb706, *Lb.curvatus* LTH1174 and *Lb.sakei* CTC494 isolated from fermented sausages produce the same bacteriocin named sakacin A, curvacin A and sakacin K respectively (Schillinger and Lücke,1989; Holck et al, 1992; Hammes et al, 1990; Tichaczek et al, 1992; Vogel et al, 1993; Hugas et al, 1995, 1996 and Remiger, 1996). Their activity spectrum comprises other *Lactobacillus* species, *L.monocytogenes* and *Ent. faecalis.*

Lb.sakei LTH673 and *Lb.sakei* Lb674 isolated from meat and *Lb.bavaricus* MI404 isolated from sourdough produce the same bacteriocins, respectively named sakacin P, sakacin 674 and bavaricin A (Tichaczek et al, 1994; Holck et al, 1994a and Larsen et al, 1993). The bacteriocin is active against *Lactobacillus, L.monocytogenes* and *Ent.faecium* but not against *Staph.aureus* and Gram-negative bacteria. *Lb.sakei* MN (formerly *Lb.bavaricus* MN) produces bavaricin MN (Lewus et al, 1991) inhibits *L.monocytogenes, Staphylococcus spp., Cl. perfringens* and Cl. botulinum spores. The bacteriocin is produced at either at refrigeration and abuse temperatures and there is an enhancement of antibotulinal activities by 3 and 4% NaCl in synthetic media (Okereke and Montville, 1991).

Ent.faecium CTC492 isolated from Spanish slightly fermented sausages (Aymerich et al, 1996) and *Ent.faecium* DCP1146 isolated from Irish dairy products (Parente and Hill, 1992b; O'Keefe et al, 1996) produce the same bacteriocin, named enterocin A and enterocin 1146 respectively. Enterocin A inhibits other *Lactobacillus, L.monocytogenes, C.divergens, Ent.faecalis* and *C.perfringens*. recently it has been shown that *E.faecium* T136 produces enterocin A and B (Casaus et al, 1997), while *E.faecium* P13 produces enterocin P (Cintas et al, 1997) and *E.faecium* L50 produces two novel enterocins L50A and L50B (Cintas et al, 1998), all of them with a wide antimicrobial spectrum.

Leu.gelidum A-UAL187 (Harding and Shaw, 1990) and *Leu.mesenteroides* TA33a (Papathanasopulos et al, 1995) isolated from chill stored vacuum-packaged meat produce a bacteriocin named leucocin A-UAL and leucocin TA33a respectively. Its spectrum of action comprises LAB, *L.monocytogenes* and *Ent.faecium*. *Leu.carnosum* B-TA11a isolated from vacuum-packaged meat produces the bacteriocin leucocin B-Ta11a which is very similar to leucocin A-UAL (Felix et al, 1994). It may be considered as a natural variant of leucocin A-UAL as described for nisin A and nisin Z (Mulders et al, 1993).

Several members of the genus *Carnobacterium*, a group of LAB which have been isolated in large numbers in chilled meat products, have been found to produce bacteriocins which would give them a favourable competitive advantage over psychrotrophic meatborne pathogens and spoilage organisms. The bactericidal range is restricted to closely related LAB although inhibition of *A.hydrophila* and *L. monocytogenes* has also been reported.

C.piscicola (C.maltaromicus) KLV17B isolated from vacuum-packaged meat produces two bacteriocins named carnobacteriocin B1 and B2. Carnobacteriocin B1 is also produced by a multibacteriocinogenic strain of *C.piscicola* V1 isolated from fish (Bhugaloo-Vidal et al, 1996). These bacteriocins are active against other carnobacteria, *Lactobacillus, Pediococcus, Listeria* and *Enterococcus*. A cassete gene including carnobacteriocin B2, leucocin A and brochocin-C is being constructed to be assayed in raw and cured meat (Stiles, 1996).

C.piscicola JG126 isolated from ham produces a bacteriocin named piscicolin 126 showing a strong antilisterial activity like the other pediocin-like bacteriocins.

The next bacteriocins share physicochemical characteristics with the pediocin group, though they do not have a strong antilisterial activity and they have completely different amino acid sequences than the pediocin-like group.

L.curvatus FS47 isolated from minced beef produces curvaticin FS47 (Garver and Murinana, 1994) active against *Lactobacillus, Pediococcus, Enterococcus* and *Bacillus*.

Lb. brevis SB27 isolated from fermented sausages secretes the bacteriocin brevicin 27 in the medium (Benoit et al, 1996). Active against other lactobacilli and pediococci.

Ped. acidilactici L50 isolated from Spanish-fermented sausages secretes a new bacteriocin in the medium named pediocin L50 with a wide antibacterial spectrum: *Lactobacillus, Pediococcus, Leuconostoc, Lactococcus, Enterococcus, Propionibacteria, C.perfringens, C.botulinum, Listeria* and *Staph.aureus* (Cintas et al, 1995). Recently, it has been specified that the antimicrobial activity initially adscribed to pediocin L50 corresponds in fact to enterocins L50A and L50B (Cintas et al, 1998).

C.divergens 750 (Holck et al, 1996) isolated from vacuum-packaged meat produces divergicin 750 a new bacteriocin active against *Carnobacterium, Enterococcus, L.monocytogenes* and *C.perfringens*.

C.piscicola mutant LV17A (Worobo et al, 1994) and *C.piscicola* LV61 (Holck et al, 1994b) secrete other non-pediocin like bacteriocins named carnobacteriocin A in the former strain and piscicolin 61 in the latter.

APPLICATION OF BACTERIOCINS IN MEAT AND MEAT PRODUCTS

Meat-borne lactic acid bacteria have been described as bacteriocin producers by many reports in the last years, however only a few have been studied as biopreservatives in food and specially in meat systems. The most studied bacteriocins in meat and meat products include nisin A, A,P and K and leucocin A but specially pediocin PA-1/AcH.

The production of a certain bacteriocin in laboratory media does not imply its effectiveness in a food system. When evaluating a bacteriocin-producing culture for sausage fermentation or biopreservation, one must bear in mind that meat and meat products are complex systems with a number of factors influencing microbial growth and metabolite production. Therefore, the influence of formula and fermentation technology on the performance of bacteriocin-producing cultures needs to be assayed.

The first studies on the biopreservation of meat were carried out by Schillinger and Lücke in 1987 in chill-stored vacuum-packaged raw meat and since then many different studies have been performed either in raw meats, cooked and fermented meat products. Most of the studies on the application of bacteriocinogenic cultures and/or their bacteriocins have been carried out by using their potential to control *L.monocytogenes,* a common contaminant of raw and processed meats (Johnson et al, 1990) and other studies have been lead to the extension of the shelf life of the product.

The data available on the use of nisin in cured and fermented meat are equivocal (Vandenberg et al, 1993). Compared to dairy products, nisin use in meat products has not been very succesful because of its low solubility, uneven distribution and lack of stability. Moreover the required dose to be effective is uneconomical and exceeding the acceptable daily intake (CEC, 1992) for a consumption of 100 g day^{-1} and an average weight of 60 Kg.

Sprayed nisin (Ambicin^{R}, 5,000 AU ml^{-1}) has been effective for the decontamination of meat surfaces (Cutter and Siragusa, 1994) and a combination of nitrite and 1,000-10,000 IU g^{-1} of nisin was effective against *Clostridium* and some other Gram-positive pathogens such as *Listeria* and *Staphylococcus* in frankfurters, pork slurries and raw meat (Caserio et al, 1979; Rayman et al, 1983 and Chung et al, 1989). Nisin producers isolated from fermented meat products seem to have some ecological adaptations that may improve their effectiveness in these products (Rodriguez et al, 1995b), although further studies are needed.

On the literature there are extensive studies on the use of *Ped. acidilactici* (Bac+) and pediocin in raw meat, cooked and fermented products. Pediocin AcH and PA/1 are more suitable to be used in meat and meat products than nisin. However, *Ped. acidilactici* is not an indigenous meat strain and is not able to grow and thus to produce bacteriocin at refrigeration temperatures.

In *in situ* assays with fresh meat and fermented sausages, the bacteriocin pediocin has been able to reduce or at least stabilize the indicator strains (Nielsen et al, 1990; Foegeding et al, 1992). In chilled stored and vacuum packed beef, Pediocin PA-1/AcH, nisin and Microgard$^{®}$(were added as biopreservatives and compared to the addition of 2% sodium lactate (Rozbeh et al, 1993). Pediocin and nisin inhibited *Leu. mesenteroides* isolated from spoiled beef. Sodium lactate was the most and Microgard the least effective in controlling the bacterial population over eight weeks at 3ºC.

In wiener sausages inoculated on the surface with *L.monocytogenes* and *P.acidilactici* (Luchansky et al, 1992) and stored at 4ºC and 25ºC, it was shown that at 4ºC the bioprotective cultures were not able to inhibit the pathogen but at 25ºC *Ped. acidilactici* inhibited *Listeria;* showing a bioprotective effect in conditions of temperature-abuse. Similar results were obtained with turkey summer sausages fermented with *Ped. acidilactici* (Bac+).

In fermented american-style sausages (Foegeding et al, 1992) the pediocin production in sausages prevented *L.monocytogenes* growth in absence of acidity. The activity of pediocin PA-1 was not affected by fat or proteins present in foods, while a synergistic action was noted between the effect of the bacteiocin and lactic acid. The Wisonsin process, use of a pediocin-producing strain of *P.acidilactici* with a carbohydrate, has been approved by the USDA for use in reduced nitrite bacon to aid in the prevention of botulinum toxin production by outgrowth of *C.botulinum* (Tanaka et al, 1980). This process has also been adapted for use in low-acid refrigerated foods such as chicken salad at pH 5.1 (Hutton et al, 1991).

The bacteriocinogenic LAB that are psychrotrophs have a good potential to be used for the bioprotection of meat and meat products. The bacteriocins produced by these strains such as sakacin, curvacin, bavaricin, leucocin and carnobac-

teriocin with the exception of enterocin A and lactocin S do not have an antibacterial spectra equivalent to nisin or pediocinPA-1/AcH, but most of them are active are active against *Listeria*.

In ground pork the bacteriocinogenic *Lb. sakei* Lb706 was tested to suppress *L.monocytogenes* growth. In normal pH meat *Listeria* did not grow but survive. In high pH pork (DFD meat) the bacteriocinogenic strain reduced the counts and delayed the growth of *Listeria* than a bacteriocinogenic negative variant (Schillinger and Lücke, 1989; Schillinger et al, 1991).

Lb.sakei Lb706, *Lb.curvatus* LTH1174 and *Lb.sakei* CTC494 producing the same bacteriocin (sakacin A, curvacin A and sakacin K respectively) have been assayed in *in situ* experiments and their activity against *Listeria* have been demonstrated. In fermented sausages *Lb.sakei* CTC494, *Lb. curvatus* LTH1174 and *Lb.sakei* Lb706 were subsequently able to reduce the number of *Listeria* by 1.8, 2 and 0.5 log when compared to a standard starter culture (Hugas et al, 1997a). The objective of the study was to show the influence of formula and fermentation technology on the effectiveness of bacteriocin producing cultures in controlling listeria growth by assaying two different technologies, (A) nitrate-nitrite curing with abundant addition of ingredients, particularly glucose (7 g Kg^{-1}), as usual in Spain and (B) nitrate curing with 3 g Kg^{-1} glucose, the first treatment was more effective in reducing listeria growth than B. The extra components added in series A have surely a considerable effect on the growth of the microorganisms, as well as on the efficacy of the produced bacteriocins (Gänzle et al, 1996). The study probed that bacteriocin producing competitive starter strains surpass the antilisterial effect of conventional, non bacteriocinogenic cultures.

In previous experiments *Lb.sakei* CTC494 reduced the numbers of *Listeria* in 1.25 log (Hugas et al, 1995) in fermented sausages. Moreover, *Lb.sakei* CTC494 was able to reduce L.innocua (inoculated at 10^2 cfu g^{-1}) in refrigerated cooked ham, poultry and minced meat by 1.2 and 1.5 log when stored for seven days at 7ºC (Hugas et al, 1997b).

In fermented sausages, *Lb.sakei* LTH673 and *Lb.bavaricus* producing the same bacteriocin (sakacin P and bavaricin A) were not very effective as starter cultures since the endogenous flora was able to grow in the sausage and coexist with the starter cultures. These strains were able to reduce the number of listeria by 0.5 and 1 log when compared to the non-bacteriocinogenic standard starter strain (Hugas et al, 1997a).

Lb. sakei Lb674 producing also sakacin P has been assayed in vacuum-packed sliced bologna-type sausages (Kröckel, 1997). Inoculation of the bacteriocinogenic strain at low level offered no advantage in preventing *Listeria* growth and the direct application of bioprotective cultures at high levels should be most efficient. The bacteriocin alone added to the sausage could not prevent *L.monocytogenes* from reaching high numbers although sufficient active bacteriocin could be extracted from the sausage even after 28 days.

The ability of *Lb. bavaricus* MN to inhibit the growth of three *L.monocytogenes* strains was examined in heat treated, vacuum-sealed beef cubes stored at 4ºC and 10ºC. At 4ºC *Listeria* was inhibited or killed depending on the initial inoculum level of *Lb.bavaricus*. At 10ºC, a reduction of at least 10-fold occurred, except in the beef without gravy. At the lower refrigeration temperatures, the addition of glucose-containing gravy and a higher inoculum level of *Lb.bavaricus* were significantly more effective in reducing the number of *Listeria* (Winkowsky et al, 1993).

Ent. faecium CTC492 isolated from slightly fermented sausages produces enterocin A belonging to the pediocin-like family of bacteriocins (Aymerich et al, 1996). Enterocin A is a wide-spectrum bacteriocin inhibiting the pathogens *Listeria monocytogenes* and *Clostridium perfringens*. The production of enterocin A in sausages is inhibited by the additives salt and pepper. In *in situ* assays, enterocin A was able to inhibit the growth of *Listeria* in fermented sausages, minced raw pork, cooked ham, patè and bacon. When liquor concentrate of *Ent. faecium* CTC492 with an activity of 800 AU g^{-1} of enterocin A was added to the formulation of fermented sausages the initial number of *listeria* was reduced by 4 logs, 4 800 AU g^{-1} were necessary to completely inhibit the growth of the same organism in patè (Hugas et al., manupscript in preparation).

The application of bacteriocinogenic strains *(C.piscicola* LV17, *C.piscicola* UAL26, *Le. gelidum* UAL-187 and *Lb. sakei* Lb706) on sterile slices of lean beef stored anaerobically at 2ºC for 10 weeks with a subsequent aerobic storage at 7ºC for up to 10 days (Leisner et al, 1995) resulted in a good growth of all the strains but all strains except for *Le. gelidum* UAL187 caused off-odours and discoloration. In chilled fresh beef the bacteriocin (leucocin) was active for eight weeks against *Lb.sakei* 1218, which is responsible for the sulphur spoilage of meat (Leisner et al, 1996). In both studies *Le. gelidum* showed good potential to extend the storage life of beef.

In *in situ* experiments, *C.piscicola* KLV17B did not cause off-odours for 10 weeks when inoculated at low concentrations under vacuum (Leisner et al, 1995). The addition of piscicolin JG126 to a devilled ham paste test food system inhibited the growth of *L.monocytogenes* for at least 14 days, performing better than ALTA 2341 (a commercial shelf-life extender with antilisterial activity) and nisin preparations (Jack et al, 1996).

DEVELOPMENTS NEEDED BEFORE APPLICATION AND REGULATORY STATUS OF BACTERIOCINS

The narrow host range of bacteriocins that are only effective to closely related bacteria is a handicap for their use as biopreservatives in meat, however the bacteriocinogenic cultures can have a positive effect to the Gram-negative population by indirect ways like establishing a dominant microflora and displacing the gram-negative psycrotrophs.

Different treatments have been assayed in order to make Gram-negative bacteria sensitive to bacteriocins, specially nisin. Use of nisin with a chelating agent (EDTA, Tween, Triton-X 100) expands the antibacterial spectrum of nisin to include Gram-negative bacteria (U.S patent 4,980,163). An osmotic shock (high salt) has also been assayed as a means to sensitize Gram negative bacteria to nisin.

The hurdle concept of food preservation (two or more antimicrobial agents at suboptimal levels are more effective than one at optimal level without affecting the acceptance quality of a food) can have an important role in sensitizing Gram-negative bacteria to bacteriocins, because of the synergistic effect of different hurdles. Several studies carried out by the group of Dr. Ray at the University of Wyoming have shown that antibacterial peptides or bacteriocins of LAB are bactericidal to subletally injured Gram-positive and Gram-negative bacteria (Kalchayanand et al, 1992). The use of ultrahigh hydrostatic pressure (UHP) and pulsed electric field (PEF) caused viability loss and sublethal injury to cells of *L.monocytogenes* Scott A, *E.coli* 0157:H7,and *S.typhimurium* M1 (Kalchayanand et al, 1994). Because of the sensitivity of injured cells to bacteriocins, an increase in cell death occurs when UHP or PEF is given in the presence of a bacteriocin (nisin or pediocin).

The application of non-thermal treatments such as UHP and PEF, in combination with biopreservatives, such as bacteriocins of LAB, can be used to increase bactericidal efficiency and enhance the safety and shelf life of foods.

Up to now nisin is the only approved bacteriocin in more than 46 countries for the inhibition of clostridia in cheese and canned foods. Two other commercial compounds that have been licensed for addition to foods, Microgard and Alta 2341 are ferments of food grade bacteria conferring antibacterial properties to foods.

According to Fields (1996) if the substances are considered GRAS by qualified experts they could be exempted from premarket approval. If the substances are not granted GRAS status, they will require premarket approval as food additive. According to Gorris, (1997), the general conception should be that the introduction of bacteriocins in foods at levels analogous to those capable of being produced by starter cultures, should be as safe as the consumption of the cultured products themselves.

In a future, it has to be considered the possibility to use safe genetically engineered LAB with multiple bacteriocin gene cassettes. According to Stiles (1996) it may also be possible to extend this strategy to target Gram-negative bacteria, for example by incorporating genes for bacteriocins from appropiate Gram-negative bacteria into the gene cassettes.

CONCLUSIONS

The research trends towards food preservation focus on mild, physical preservation techiques and the use of natural antimicrobial compounds because of consumer attitude in the last years towards chemical, unnatural preservatives and the demand for "natural" and fresher foods. The production of competitive and bacteriocinogenic lactic acid bacteria may well provide an additional hurdle to improve meat preservation by natural means. However, the application of biopreservation technology to meat and foods in general, should be considered only as an additional measure or hurdle to good manufacturing, processing and distribution practices.

The implementation of biopreservation in a certain food system will depend on the influence of formula and technology on the performance of bacteriocin-producing cultures as well as the adaptation of the culture to the specific ecological habitat of the food.

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