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International Journal of Food Microbiology 88 (2003) 223–233
INTERNATIONAL JOURNAL OF Food Microbiology
www.elsevier.com/locate/ijfoodmicro

Review

Functionalty of enterococci in meat products

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Accepted 26 February 2003

Abstract

The presence of enterococci in meat fermentation is a constant as reported in the literature. Despite the concern about pathogenicity of enterococci, recent studies point out that food and meat enterococci, especially *Enterococcus faecium* have a much lower pathogenicity potential than clinical strains. Enterococci possess a competitive advantage over other microbiota in meat fermentations, and many enterococci isolated from sausages have the ability to produce enterocins harbouring antimicrobial activity against pathogens and spoilage microorganisms of meat concern. The application of enterocins producing enterococci or their purified metabolites, as extra hurdles for preservation in sausage fermentation and in sliced-vacuum packed cooked meat products can be beneficial, preventing the outgrowth of *Listeria monocytogenes* and slime-producing lactic acid bacteria. Enterocins and bacteriocinogenic enterococci hold considerable promise as alternatives to traditional chemical preservatives and they could be exploited for the control of emergent pathogens in meat products. Their inhibitory effect can be increased when used in conjuction with particular physical and chemical processes, but current regulation is hampering the application of purified bacteriocins.

Keywords: Enterococci; Enterocins; Meat products; Bioprotection

1. Introduction

Enterococci have been used in many different applications as starters or adjunct cultures such as silage inoculants (Seale, 1986), dairy starter cultures (Giraffa et al., 1997) and probiotics (Fuller, 1989). In foods, they seem to have a major role in improving flavour development and quality of cheese (Centeno et al., 1996; Wessels et al., 1990) while as probiotics they may contribute to the improvement of microbial balance and to treat gastroenteritis in humans and animals (Lewenstein et al., 1979; Bellono et al., 1980; Franz et al., 1999). Additionally, enterococci harbour some useful biotechnological traits, such as the production of bacteriocins with anti-*Listeria* activity (Giraffa, 1995; Hugas, 1998).

Enterococci colonise raw foods of animal origin (meat and milk) by intestinal or environmental colonisation and can survive and even multiply during fermentation (Giraffa, 2002), especially in those products where no competitive starter cultures are used. Enterococci, like many other lactic acid bacteria, have been described as spoilage microorganisms in cooked processed meats, either by surviving pasteurization due to their heat resistance (Houben, 1982; Gordon and Amad, 1991) or by cross-contamination at the final stages of processing such as slicing and packaging. Enterococci in fermented foods might also

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doi:10.1016/S0168-1605(03)00184-3

reflect a given level of contamination or a poor curing process. Some authors consider them as technologically unacceptable in dry fermented sausages (Holley et al., 1988), and despite belonging to LAB (Lactic Acid Bacteria), there is controversy over considering them as GRAS (Generally Recognized As Safe) microorganisms (Giraffa et al., 1997). Some strains, like *Enterococcus faecium* K77D, are approved as acceptable for use as starters in dairy products by the UK Advisory Committee on Novel Foods and Processes (ACNFP, 1996) and they are widely used as starters or probiotics organisms by the International Dairy Federation.

2. Enterococci in meat fermentations

The role of enterococci in meat fermented products has not been thoroughly studied. In meat fermented products, the dominant microbiota is constituted by several species LAB, mainly of the genus *Lactobacillus*, e.g. *Lactobacillus sakei*, *Lactobacillus curvatus* and *Lactobacillus plantarum* and coagulase-negative staphylococci. Enterococci, *E. faecium* and *Ent. faecalis*, but especially *E. faecium* represent one of the LAB species that can be found in relatively high numbers during meat fermentation. They may contribute, together with lactobacilli, to the fermentation.

The numbers of viable enterococci in contaminated poultry, pork and beef are usually in the range of 10^2–10^4 cfu g^{-1} (Teuber et al., 1996). During fermentation, the contaminating enterococci may survive and multiply. In natural fermented sausages, Metaxopoulos et al. (2001) found 10^2–10^3 cfu g^{-1} of enterococci contaminating the sausage batter in three different manufacturing plants in Greece. In two out of the three plants, enterococci grew during fermentation and ripening to 10^4 cfu g^{-1} after 28 days of processing while in the third plant no growth was observed during the first week and no recovery was possible until after the end of the process.

In commercial fermented sausages manufactured with competitive starter cultures, like *L. sakei* and *L. curvatus*, a complete outgrowth of these microorganisms over the endogenous microbiota can be observed. However, in German and Italian fermented sausages, Marchesino et al. (1992) reported concentrations of enterococci from 10^3 to 10^5 cfu g^{-1} at the end of the ripening process in sausages manufactured both with and without starter cultures.

In Mediterranean countries, many traditional and artisanal meat fermented products are manufactured mostly by small companies, farms or local butchers. Usually, they are low-acid products with a pH higher than standard sausages (>5.3). In these types of products, the fermentation is carried out without the use of starter cultures relying on the endogenous flora to keep the traditional organoleptic qualities. As a consequence, the natural microbiota is constituted by a mixture of species of lactic acid bacteria including enterococci and lactobacilli as well as coagulase-negative staphylococci and yeasts. These products have a long history of safe consumption by humans. In a survey of 31 naturally fermented sausages, purchased at several supermarkets in the north–east of Spain (Martín et al., 2001), the number of enterococci ranged from 1.3 to 4.48 log cfu g^{-1}, the population of total lactic acid bacteria being from 7.12 to 9.07 log cfu g^{-1}.

The number of enterococci during fermentation in natural fermented sausages of high pH, varied during ripening in the same manufacturing plant with the same formulation and technology. Four batches manufactured with raw meat from different suppliers in two succesive weeks and on different days of the same week showed differences. The initial counts of enterococci varied from 2.18 to 3.26 log cfu g^{-1}. The batch with the lowest enterococci counts (1.9 log cfu g^{-1}) showed a further inhibition during fermentation (<10 cfu g^{-1}) while in the batch with the highest initial counts (3.26 log cfu g^{-1}), enterococci persisted during fermentation with a 1 log cycle increase after 3 days and 3.70 log cfu g^{-1} by the end of the process (Fig. 1). The use of a competitive starter culture such as *L. curvatus* CTC371 gave lower and significant different counts of enterococci along fermentation when compared to a non-inoculated batch (Bover-Cid et al., 2000). These results indicate that the hygienic quality of the raw meat may be essential as a source of enterococci in meat fermentation but also to provide the right physical and biochemical conditions allowing the growth of enterococci during fermentation.

The persistence of enterococci during ripening can be attributed to their wide range of growth temperatures and their high tolerance to salt. It seems that in sausages, no hurdles are found for their inhibition, allowing them to coexist with lactobacilli as the dom-

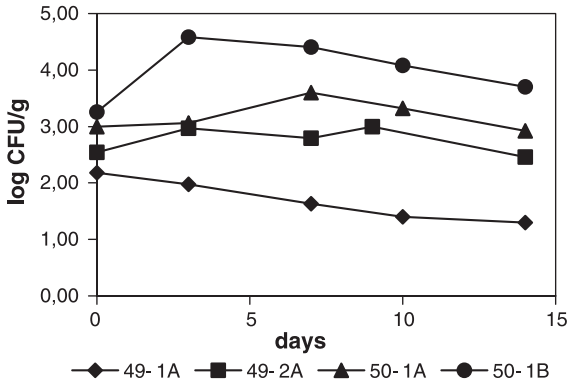

Fig. 1. Enterococci counts in several batches of traditional fermented sausages of low pH (>5.3) along processing. The four batches were manufactured in the same manufacturing plant with the same formulation and technology with raw meat from different suppliers in two successive weeks and on different days of the same week.

inant population. The growth of lactobacilli in meat can provide an ecological niche advantageous for enterococci. Enterococci are poor acidifiers in milk (Sarantinopoulos et al., 2001) and in meat (Aymerich et al., 2000a), therefore in sausages with a high percentage of fermentable sugars in the formulation they might not be as competitive as lactobacilli and their growth might become more difficult due to a hostile environment. But, in artisanal sausages of high pH, they may find better conditions for survival and growth. In fact, most data reported in the literature are about the presence of enterococci in sausages where pH is not below 5.0.

The biochemical activities of enterococci in the sausage matrix have not been studied. They might contribute to sausage aromatisation by their glycolytic, proteolytic and lipolytic activities; enterococci from dairy origin are lipolytic and produce volatile compounds (Sarantinopoulos et al., 2001). Metmyoglobin reduction activity has been described for meat enterococci (Ariharia et al., 1994), with a possible role of maintaining the red colour of fresh meat. Some authors have also reported the production of biogenic amines (BA) by enterococci.

3. Biogenic amines production

Biogenic amines (BA) are basic organic compounds which occur in different kinds of food, such as cheese, wine, beer, dry sausages and other fermented foods (Halàsz et al., 1994). Several toxicological problems resulting from the ingestion of food containing relatively high levels of BA have been reviewed (Mariné-Font et al., 1995). In foods, BA are mainly generated by decarboxylation of the corresponding amino acids through substrate-specific enzymes of the microorganisms present in the food.

The prolific growth of enterococci of a dairy origin in milk and milk products leading to formation of significant levels of biogenic amines (BA) has been observed (Garg and Mital, 1991). During meat fermentation, the microbial growth, the acidification and the proteolysis offer favourable conditions for BA production. Bover-Cid et al. (2001) reported that all strains of enterococci isolated from fermented pork sausages produced biogenic amines. Tyramine (419–4334 mg l^{-1} broth) was the major amine yielded by these bacteria followed by phenylethylamine (40–585 mg l^{-1} broth). However, they did not produce tryptamine, putrescine or cadaverine. The activity of amino acid-decarboxylase activity in a synthetic broth depends not only on the availability of amino acids, but also on the phase of microbial growth and the microbial strain. The factors that may influence the ability of microorganisms to form BA during the manufacture of a fermented sausage (such as pH, water activity, nutrients, other food components, and technological conditions) should be further studied in order to select those conditions favouring a proper fermentation process but a reduced BA accumulation.

In commercial artisanal fermented meat products of high pH, the BA content ranged from 45 to 240 mg kg^{-1} fresh weight of tyramine and from non-detectable to 125 mg kg^{-1} of putrescine (Miguélez-Arrizado et al., in preparation), however, no direct relationship of tyramine content to the number of enterococci was found.

4. Concern about meat enterococci as nosocomial pathogens

For a long time, enterococci were considered unimportant medically, but in the last decades, they have emerged as important nosocomial pathogens of concern, causing a variety of infections (Jett et al., 1994) in immunosuppressed and intensive care unit

patients (Moellering, 1992). The most specific cause of concern about these strains is their resistance to a wide variety of antibiotics (Simjee and Gill, 1997; Robredo et al., 1999), specially to vancomycin (Edwards, 2000).

With respect to antibiotic resistance and contrary to clinical strains, meat enterococcal strains are mostly susceptible to clinically relevant antibiotics (Teuber et al., 1999). In Europe there have been numerous reports of vancomycin-resistant enterococci (VRE) isolated from food animals and food products (Van Den Braak et al., 1998; Wegener et al., 1999; Robredo et al., 1999). In the United States, Knudtson and Hartman (1993) studied the prevalence of antimicrobial resistance in enterococci from water, pork and clinical isolates; no VRE was detected from pork carcasses or fresh or spoiled pork products. Bodnaruk et al. (2001) examined enterococci isolated from packaging areas of meat-processing facilitites for high-level vancomycin resistance. High-level vancomycin resistance was not demonstrated in any enterococci out of 406 strains isolated from 12 meat-processing plants producing ready-to-eat meat products. These data suggest that enterococci with VanA resistance phenotype are uncommon in US meat processing facilities.

Enterococci are also known for their capacity to exchange genetic information by conjugation (Clewell, 1990). Teuber et al. (1999) reported successful transfer of antibiotic resistant determinants from several species of *Enterococcus* isolated from meat products (salami, pancetta and raw meat) into the collection strain *E. faecalis* JH2-2 as recipient at frequencies ranging from 10^{-4} to 10^{-7}.

Despite the low incidence of virulent traits in food and starter enterococcal strains, among them *E. faecium* strains, their ultimate presence is strain specific. So, for safety considerations, the strains to be used as starter, probiotic or bioprotective cultures should be tested for the presence of different virulence traits either phenotypically or genotypically, since there might be silent genes present (Eaton and Gasson, 2001). In this context, *E. faecium* CTC492 was screened for antimicrobial resistance, adhesin genes and BA formation; so far, this strain is quite sensitive to most antibiotics and lacks cell wall adhesins as shown by negative PCR amplification of *efa Afm* and *efa Afs* (Aymerich et al., unpublished).

5. Competitiveness of enterococci in the meat environment

In contrast with most fermentations of milk, meat fermentations do not start from a sterile raw material. Production of fermented sausages starts from raw lean meat and backfat which have been colonised through slaughtering and conditioning processes with multiple microorganisms ranging from pathogenic and spoilage to protective and technologically important strains (total aerobic count of 10^5 cfu g^{-1}) LAB being an important part of this microbiota (10^3–10^4 cfu g^{-1}).

During fermentation a fast growth of highly competitive starter cultures or endogenous lactobacilli comporting acidification, decrease of the redox potential and the water activity is essential for the prevention of spoilage and pathogenic bacterial growth. When used as starter cultures, meat-isolated enterococci appear to be highly competitive in the meat environment leading the fermentation over the endogenous microbiota. Using *E. faecium* CTC492 isolated from fermented sausages as a starter culture in sausage fermentation, the strain was able to grow and lead the fermentation, constituting 100% of LAB population by the end of the process (28 days) (Aymerich et al., 2000b). However, other enterococci isolated from several sources, such as *E. faecium* RZS isolated from cheese (Vlaemynck et al., 1994), *E. faecium* CCM4231 isolated from calf rumen (Lauková et al., 1993) and *E. faecalis* AS48 isolated from a human wound exudate (Gálvez et al., 1986) also proved to be competitive in the meat environment (Callewaert et al., 2000; Ananou et al., 2002) at lower percentages. During sausage fermentation, *E. faecium* strains RZS and CCM 4231 constituted 50% of the total LAB population. Upon further ripening, the *Enterococcus* starters gradually disappeared from the microflora, and after 21 days no colony could be isolated and identified as being the original *Enterococcus* starter culture (Callewaert et al., 2000). All these strains produce antimicrobial compounds which could confer on them a major advantage in the competition (Vogel et al., 1993). However, the production of a bacteriocin from a given strain is not always expressed in complex matrixes such as fermented sausages. *E. faecium* CTC492 is able to lead the fermentation but not to produce enterocins in the meat environment because of the inhibitory effect of salt, pepper and the low pH

(Aymerich et al., 2000b), but *E. faecalis* AS48, despite not being isolated from food, was able to grow and produce bacteriocin in the meat batter (Ananou et al., 2002). *E. faecium* F688 is a commercial strain sold as a probiotic (European Patent Application 0508701A2, 1991) in meat fermented products. This strain is also very competitive in sausages reaching 10^7 cfu g^{-1} at the end of the ripening (Quest Int. Bussum, The Netherlands).

6. Production of enterocins by meat enterococci

Enterococcal strains have long been shown to be prominent bacteriocin producers and may play an important role in the natural preservation of meat products by controlling the growth of some pathogens. Bacteriocin-producing *Enterococcus* strains with strong anti-listeria activity have been isolated from numerous and diverse environments including silage (Kato et al., 1994), dairy products (Parente and Hill, 1992; Olasupo et al., 1994; Torri-Tarelli et al., 1994; Vlaemynck et al., 1994; Maisnier-Patin et al., 1996; Nuñez et al., 1997; O'Keeffe et al., 1999), fish (Ben Embarek et al., 1984), vegetables (Villani et al., 1993; Franz et al., 1996; Bennik et al., 1998; Floriano et al., 1998) and fermented sausages (Lyon et al., 1995; Aymerich et al., 1996; Cintas et al., 1997, 1998; Casaus et al., 1997). Enterococcal bacteriocins (enterocins A, B, P, L50, Q and 1071) proved to be strong inhibitors of foodborne pathogens such as *Listeria monocytogenes*, *Clostridium tyrobutiricum* and *Staphylococcus aureus* (Aymerich et al., 1996; Casaus et al., 1997; Cintas et al., 1997, 1998, 2000; Balla et al., 2000). Therefore, bacteriocinogenic enterococci can be used to enhance preservation in meat products.

Most of the enterococcal bacteriocins are identical to enterocin A or enterocin B originally described from *E. faecium* CTC492 and *E. faecium* T136 (Aymerich et al., 1996; Casaus et al., 1997) isolated from fermented sausages: *E. faecium* WHE 81 (Ennahar et al., 2001), *E. faecium* DPC1146 (O'Keeffe et al., 1999) both from dairy origin and from *E. faecium* BFE 900 isolated from black olives (Franz et al., 1999). These bacteriocins appear to be, like nisin and pediocin PA-1, among the most common LAB bacteriocins. Moreover, enterocins A and B act in synergistic and complementary ways: survivors from exposure to one bacteriocin could not resist the antibacterial action of the other (Casaus et al., 1997) and their genetic determinants are located in the chromosome, suggesting the stability of the character during long-term use.

The application of bacteriocins produced by enterococci and of bacteriocin-producing *Enterococcus* starters or protective cultures is well documented in cheese manufacture (Giraffa, 1995). In the manufacturing of meat products the use of enterococci as bacteriocinogenic starter or bioprotective cultures and/or their bacteriocins can enhance preservation by natural means.

7. Application of enterococci and enterocins for the bioprotection of meat products

The implementation of modern technologies and safety concepts like HACCP have not been able to overcome food outbreaks due to classical and emergent pathogens. The role of LAB and their bacteriocins as food biopreservatives may increase in the future as a result of consumer awareness of the potential risks derived, not only from food-borne pathogens, but also from the chemical preservatives currently used to control them (Martínez et al., 2000).

The application of bacteriocins in food biocontrol is mainly oriented in two alternative directions: (i) the use of bacteriocin-producing LAB or (ii) the direct addition of bacteriocin preparations, either synthetic or purified from the supernatant culture of the producer strains. The *in situ* production of bacteriocins may increase the competitiveness of the producer strain in the food matrix and contribute to the prevention of spoilage. However, the endogenous microbiota, the formula and the technology may influence the performance of the bacteriocinogenic cultures. The main factors affecting the efficacy and production of bacteriocins in meat are: inadequate environment for growth and/or bacteriocin production, loss of bacteriocin-production ability, antagonism by other microflora, development of bacteriocin-resistant organisms and formation of non-active complexes between bacteriocins and macromolecules. The antimicrobial efficiency of a bacteriocin may be enhanced by combining it with other bacteriocins (Hanlin et al., 1993; Schillinger et al., 1996; Mulet-Powell et al.,

1998) or by GRAS strains with heterologous production of several bacteriocins. Martínez et al. (2000) reported the production of enterocin A and pediocin PA-1 in *Lactococcus lactis* IL1403.

During sausage fermentation, the major microbial hazards to be controlled are *Salmonella*, enterohemorragic *E. coli*, *L. monocytogenes* and *S. aureus*. *E. faecium* CTC492 producing enterocins A and B is a competitive strain in the meat environment and could be used as a starter/bioprotective culture in meat fermentations to prevent the survival of *L. monocytogenes* and other pathogenic bacteria.

The production of enterocins A and B is co-induced in vitro by several parameters (salt, pepper, nitrite, tween and carbohydrates). The growth of *E. faecium* CTC492 is not affected by the concentration of the ingredients used in sausage manufacture, although sodium chloride, sodium nitrite and pepper lowered bacteriocin production by 50%. The combination of sodium chloride and pepper, which are important ingredients for the salt-spice taste, and for the technology of this kind of sausage, is detrimental to bacteriocin production, lowering its production 16-fold (Aymerich et al., 2000b). The sodium ions of the salt and the manganese content of pepper might compete with enterocin F, the induction factor (Nilsen et al., 1998), for the binding sites of the sensor protein, blocking the production of enterocins A and B.

Enterocin production in vitro by *E. faecium* CTC492 was maximal at pH 6.0 while at pH 5.0 both production and growth were strongly inhibited in accord with the results of dairy enterococcal strains also producing enterocins A and B (Parente and Ricciardi, 1994). Thus, enterocins production will not be favoured in fermentations when pH falls below 5.5.

The results obtained in vitro conditions were validated in real sausage fermentations spiked with listeria and with CTC492 as starter culture being able to lead the fermentation but it was uncapable of inhibiting the pathogen significantly compared to non-starter sausages. Nonetheless, and more importantly, the addition of enterocins significantly diminished *Listeria* counts compared with the control batch or with a standard starter culture (CTC371), keeping the number of listeria from 3 log cfu g^{-1} to 6 MPN g^{-1} from the third day of fermentation until the end of the drying period (Fig. 2). The above facts may warn of an important constraint in the use of *E. faecium* CTC492 as an anti-listerial factor in cured and fermented meat products, and suggest the need to optimize the production of enterocins in vitro as GRAS antimicrobial metabolites to be included in the sausage batter.

Other bacteriocinogenic strains of *E. faecium* (RZS C13 and CCM4231) and *E. faecalis* AS48 were assayed as anti-listerial starter cultures in real or model sausage fermentation (Callewaert et al., 2000; Ananou et al., 2002). All *Enterococcus* strains inhibited *Listeria* further than the positive control with a decrease of 3 log cycles by the end of ripening.

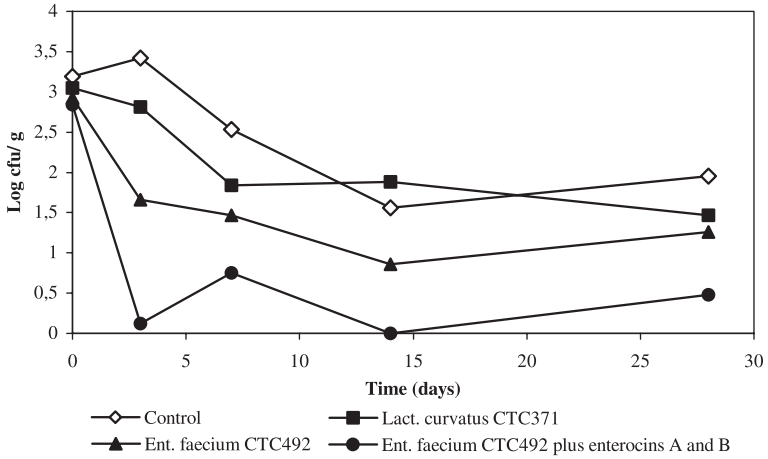

Fig. 2. Inhibition of artificially spiked *Listeria innocua* by *E. faecium* CTC492 in fermented sausages.

In Mediterranean countries, the consumption of traditional fermented sausages with a pH>5.3 is increasing. Most of them are produced without the use of starter cultures so as to keep their artisanal and traditional organoleptic qualities. However, the inhibition of some pathogenic bacteria contaminating raw meat at low numbers might not be achieved by the hurdles present in the product. In these kind of sausages, with the following physico-chemical characteristics: A_w (0.84–0.87), pH (6.0), fat percentage (40%), nitrate (150 ppm), nitrite (150 ppm), NaCl (2%), pepper (2%), if spiked with listerias they can grow from 1 to 4 log cycles during processing. When these sausages were treated with 648 AU g^{-1} of enterocins, *Listeria* numbers decreased immediately by three log cycles, compared to the control batch and no growth was observed until the end of the ripening time (Aymerich et al., 2000a). Thus, enterococcal bacteriocins may be considered as additional anti-listerial hurdles that could act cooperatively with other ingredients and additives and the global drying process typical in sausage manufacture to enhance salubrity.

The application of the fermentation product from *E. faecium* strains like enterocins as natural preservatives can also be of interest for cooked, ready-to-eat meat products. These products offer no protection against spoilage and pathogenic organisms when contamination occurs during post-processing, as for example during slicing and packaging. The pathogenic (e.g. *L. monocytogenes*) and spoilage bacteria (e.g. slime-producing LAB) will grow exponentially even at refrigeration temperatures reaching very high numbers within days due to the absence of competitive flora. In cooked ham, 4800 AU g^{-1} of enterocins could limit the growth of listeria by 7.98 log cycles compared to control. A similar growth was observed with pork liver patè; in this product, the addition of enterocins completely inhibited *Listeria* outgrowth (< 3 MPN g^{-1}) (Aymerich et al., 2000a).

In many countries, the formation of ropy slime on vacuum-packed cooked meat products is a common spoilage problem, with high financial losses. The slime is often formed before the sell-by date and consumers find the appearance of slimy products very offensive, although no off-odours are detected. Slime formation is due to the secretion of long-chain, high molecular mass, viscosifying or gelling exocellular polisaccharides into the environment by LAB. The application of *E. faecium* CTC492 as bioprotective culture in cooked ham after slicing and before packaging could inhibit the production of slime due to *L. sakei* CTC746 for up to 7 days of storage. However, enterocins A and B could prevent the slime formation for up 21 days of vacuum storage at 8 °C (Aymerich et al., 2002). Concerning the organoleptic analysis, samples with enterocins developed tart and floral odours which cause the panellists to give a low score to the samples. However, the odours observed could be avoided by improving the purification methods of the bacteriocins applied.

The possibility of adding bacteriocins to the meat before cooking due to their thermotolerance is of great interest; the microbial growth after processing may be prevented at the most critical step, slicing. The thermoresistance of bacteriocins in buffer and broths is well known, but their ability to resist pasteurization protocols in meat matrixes depends on the bacteriocins. As reported by Aymerich et al. (2002), the interaction with the soluble meat matrix, although not altering the activity of sakacin and nisin, affected the activity of enterocins A and B by a 2 $\log_2$ AU ml^{-1} decrease. This decrease in activity may be the result of adsorption to the meat particulates, but this might also represent an uneven homogeneity in the food system.

Several limitations may hamper the application of enterocins as with other bacteriocins in meat products, as well as in other types of foods. Bacteriocins do not have a broad host range, they are ineffective against spoilage and pathogenic Gram-negative bacteria, and insensitive variants appear rather frequently. Therefore from the point of view of safety, it is not possible to rely solely on the antimicrobial effect of bacteriocins to control all spoilage and pathogenic bacteria. But, synergistic approaches can overcome this, and one of the most interesting ideas is to influence the permeability of the cell envelope of Gram-negative bacteria by various means. In this context, the hurdle concept proposed by Leistner and Gorris (1995), could be useful for improving the efficiency of enterocins when used together with high pressure processing (HPP). Gram-positive and, more importantly, Gram-negative bacteria surviving pressurization will also become sublethally injured and sensitive to bacteriocins (Kalchayanand et al., 1998). Synergy of

HPP and bacteriocins on the inactivation of *E. coli* in milk has already been shown (García-Graells et al., 1999).

In a cooked meat model system, the synergy of enterocins and nisin with HPP on the inhibition of spoilage and pathogenic bacteria was assessed during shelf life (Garriga et al., in press). Slime producing LAB (*L. sakei* CTC746 and *Lc. carnosum* CTC747), *S. aureus* CTC1008, *Salmonella enterica* subsp. *enterica* ser London CTC1003, *L. monocytogenes* CTC1010 and *E. coli* CTC1023 isolated from meat were spiked in a cooked meat model containing enterocins or nisin and compared to control after pressurization and during chilled storage. *Staphylococcus* was the genus least sensitive to pressurization. Slime-producing strains of LAB were sensitive to pressurization and recorded 6 log cycles reduction after pressurization, but a re-growth was observed during storage. In the lots spiked with *L. sakei* CTC746 (slime^{+}) and treated with either nisin or enterocins, the population after pressurization was kept stable throughout the storage. The control of *L.monocytogenes* in pressurized samples was only achieved with enterocins during storage (Fig. 3).

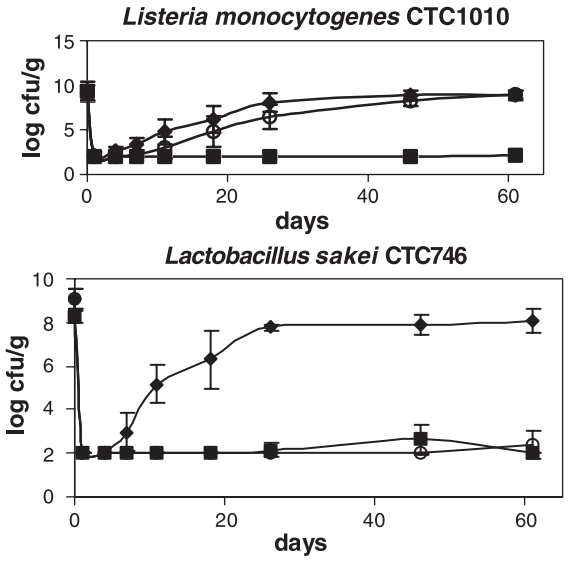

Fig. 3. Behaviour of several bacterial strains after high pressure treatment (400 MPa, 10 min, 17 °C) in a meat model system during storage at 4 °C; (○) nisin A, (□) enterocins, (♦)control. Values are mean ± standard deviation. The minimum level of detection was 2 $\log_{10}$ cfu g^{-1}.

However, the sensitization of Gram-negative bacteria to bacteriocins was only observed with nisin.

The use of bacteriocins and moderately high pressure (400 MPa) increases the lethality of some pathogenic and spoilage bacteria enabling the extension of the shelf life of the product, but the performance of the combined system needs to be assayed in every meat product and with different strains and bacteriocins.

8. Regulatory and scientific aspects that must be met before approval of a certain bacteriocin

In the last decade, knowledge of LAB bacteriocins has been dramatically improved, but the applied aspects have not deserved the same attention. There are several reasons that might explain this fact. Bacteriocins do not have a broad host range, thus their effect on the improvement of the overall acceptabiliy and shelf life extension is not so evident in many cases, compared to other chemical preservatives. In every food system there are many factors affecting the effectivity of bacteriocins like the food composition, additives, physico-chemical conditions, etc., and last but not least legislation on food additives is very restrictive and has not been modified.

Food additives have to fulfil the criteria laid down in Directive 89/107 EC. Before proposing the permission for any new use of an additive, the Commission would have to verify that these criteria are fulfilled by consulting the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food of the European Food Safety Authority to ensure the substance is safe for human health. Safety information should be provided according to specific guidelines.

As alternatives to the traditional chemical preservatives, the bacteriocins and especially enterocins, hold considerable promise. Most bacteriocins may be exploited for the control of pathogens such as *L. monocytogenes* and spoilage bacteria like slime-producing LAB. In addition, when used in conjuction with particular physical and chemical parameters and processes, the inhibitory effect of many bacteriocins can be increased.

In conclusion, bacteriocinogenic enterococci could be used to solve some hygienic and spoilage problems still occurring in meat products despite the implemen-

tation of HACCP, but current regulation is hampering their application.

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