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Review

Utilization of microbes to process and preserve meat

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Abstract

This paper discusses how, and to what extent, the addition of microorganisms to meats helps to meet the needs of consumers and industry. Lactic acid bacteria adapted to meats improve the safety of fermented sausages by means of acid formation. Using selected strains, the safety of certain non-fermented, perishable meat products may be improved without affecting their shelf life. Certain bacteriocin-forming cultures may reduce the levels of *Listeria monocytogenes* in some meat products significantly, but their effect on the overall safety of meats is limited by the resistance of Gram-negative bacteria. Data on the effect of microorganisms on the sensory properties of fermented meats are summarized. For bacteria to have a probiotic effect, they need to attain high numbers during fermentation and/or storage of meats. Genetic engineering of cultures may improve certain properties of the strains but benefits to consumers and industry are too small to make them acceptable by consumers and regulatory bodies in the near future.

1. Introduction

Addition of desirable microorganisms to meats may have four different purposes, namely (1) to improve safety (inactivation of pathogens), (2) to improve stability (extension of shelf life by inhibiting undesirable changes brought about by spoilage microorganisms or abiotic reactions), (3) to provide diversity (modification of the raw material to obtain new sensory properties), and (4) to provide health benefits (through positive effects on the intestinal flora). ''Starter cultures'' are, by definition, used in order to change the sensory properties of the food. In meat fermentations, lactic acid bacteria generally serve purposes 1–3 while other microorganisms, namely, catalase-positive cocci (*Staphylococcus, Kocuria*), yeasts (*Debaryomyces*) and moulds (*Penicillium*) normally bring about and stabilize the desired sensory properties (purpose 3). A list of the species present in commercial cultures was provided recently by Hammes and Hertel (1998) and Lücke (1998a,b). The level to be added to meats depends on the growth potential of the organisms in the product: In most European-style sausage fermentations, for example, about 10^6 cells of lactic acid bacteria are added per gram whereas very high levels (ca. 10^8 g^{-1}) of *Lactococcus lactis* had to be added to give desired effects (Coffey, Ryan, Rose, Hill, Arendt & Schwarz, 1998). Irrespective of concentration, a homogenous distribution of added microorganisms is important for their effect (Katsaras & Leistner, 1991).

Antagonistic cultures that are only added to inhibit pathogens and/or to extend the shelf life (purposes 1 and 2), while changing the sensory properties of the product as little as possible, are termed ''protective cultures''. Using them (or their metabolic products, namely bacteriocins or enzymes) is often designated as ''biopreservation''. ''Probiotic'' cultures are, by definition, cultures that, after ingestion in sufficient numbers, exert health benefits beyond inherent basic nutrition (Lactic Acid Bacteria Industrial Platform Workshop on Probiotics, 1995; cited after Hammes & Haller, 1998). Today, probiotic strains are rarely used outside the dairy industry but this is likely to change in the future.

This paper will discuss the prospects and limitations of the utilization of microbial cultures in meat preservation and meat fermentations, in context with the requirements of consumers and industry.

2. Demands of the consumers and requirements of industry

Consumers, obviously, want their meats ''safe to eat''. Data from interviews indicate that in Germany, consumers have more doubts about meat safety than about the safety of other foods (see Lücke, 1998a). They doubt that animal production is environmentally sound and

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that animals are treated appropriately during rearing and transport, and they think rightly that mistreated animals are more likely to become sick, and products from sick animals are not safe. In addition, meat is prone to contamination by pathogens that are carried by animals, and provides a good growth substrate for these. Hence, Purpose 1 (safety) is very important both for consumers and industry, and an integrated, transparent approach involving the whole chain "from farm to fork" is needed.

Stability (Purpose 2) is important, obviously, for those consumers without easy, continuous access to meats and to refrigeration capacities. In countries like today's Germany, many consumers state that they want their foods "fresh", even though, in practice, many of them shop only once a week, and the "best-before date" is of major importance in their buying decision. However, industry also has a strong interest in saving distribution costs.

Food fermentations lead to an enormous product diversity. This is in the interest of both consumers and industry. Starter cultures may aid in developing and maintaining this diversity, but raw materials and other processing factors are more important.

To reduce costs, industry has a strong interest to standardize the properties and shelf life of the product, to improve control of microbial processes, and to shorten the time-consuming ageing processes required for flavour formation. These issues are probably the main incentives for the meat industry to use starter cultures. However, within a given brand of product, many consumers also expect a high level of uniformity.

The meat industry faces trends that create problems in providing safe and stable products: consumers tend to live in smaller, urban households and to have less knowledge of raw materials and cooking than in the past. Hence, they cannot be entirely relied upon to store and prepare meats in the best way in the household. In addition, many consumers prefer "mild", "low-fat" foods with, as a consequence, higher pH and water activity (a_w) values. Moreover, consumers state that they want "fresh", "natural" food with no preservatives added. Hence, there is a growing interest in foods that are both "fresh" and "convenient". A skillful combination of "hurdles" including the "competitive flora" may lead to such foods. This approach is described by the phrases "Minimal processing" and "Hurdle technology" (Leistner, 1999; Leistner & Gorris, 1995).

3. Mechanisms of antagonism by microorganisms

The main mechanisms by which lactic acid bacteria suppress their competitors are the formation of lactic acid, acetic acid and possibly bacteriocins. Some other metabolites of lactic acid bacteria have been shown to inhibit Gram-negative bacteria in vitro (see Helander, von Wright & Mattila-Sandholm, 1997; Lücke & Earnshaw, 1991; Niku-Paavola, Laitila, Mattila-Sandholm & Haikara, 1999) but it is unlikely that they will be exploited to improve the safety and stability of meats: Some are not formed in sufficient amounts (e.g. reuterin), some interfere with the sensory properties (e.g. diacetyl, hydrogen peroxide), and some raise regulatory concern (e.g. benzoic acid). Antagonism as a result of competition for nutrients is unlikely in nutrient-rich substrates such as meats. Microorganisms that form antibiotics are unacceptable as starter or protective cultures.

3.1. Lactic and acetic acids

In most food fermentations, lactic and acetic acids produced by lactic acid bacteria and the resulting decrease in pH are responsible for the preservation effect. In meats, the main organic acid formed is lactic acid, and only low concentrations of acetic acid are acceptable from a sensory point of view. However, the antimicrobial effect of acetic acid in meats should not be neglected because, at the same concentration and pH, it is more effective than lactic acid. Sensitivity to these acids varies between different bacteria and also depends on the simultaneous action of other factors such as a_w and nitrite. This is why, under conditions prevailing in many meat products, even small differences in acid concentrations have a major effect on acid-sensitive microorganisms.

3.2. Bacteriocins

Bacteriocins are antimicrobial peptides or proteins that are destroyed by proteases in the upper intestinal tract and hence raise few safety concerns. They are produced by strains of all genera of lactic acid bacteria of relevance to meats and act against bacteria closely related to the producer organisms. Many bacteriocins formed by lactic acid bacteria also inhibit *Listeria* but only few are effective against *Bacillus, Clostridium* and *Staphylococcus*. For more detailed reviews on bacteriocins and their effects in meats, see Hugas (1998), Schillinger, Geisen and Holzapfel (1996), Stiles (1996), and Abee, Kröckel and Hill (1995).

It is generally accepted that bacteriocins exert their inhibitory action by formation of pores in the cytoplasmic membrane of sensitive cells. Different Gram-positive bacteria differ in their sensitivity to a bacteriocin, mainly because of differences in membrane composition and fluidity (Bennik, Verheul, Abee, Naaktgeboren-Stoffels, Gorris & Smid, 1997). Bacteriocin resistant mutants occur frequently even in populations of sensitive bacteria. For instance, the development of nisin-resistant mutants of *Listeria monocytogenes* has been reported to occur at a frequency of 10^{-6}–10^{-8} (Harris, Fleming &

Klaenhammer, 1991), and even higher rates were observed with some class II bacteriocins such as sakacin A (Lücke and Schillinger, unpublished). The exact mode of action differs somewhat between class I bacteriocins (“lantibiotics”, e.g. nisin) and class II bacteriocins (e.g. sakacin A; Abee, 1998; Abee et al., 1995). Thus, nisin-resistant variants of *List. monocytogenes* remained sensitive to in situ formed sakacin A (Schillinger, Chung, Keppler & Holzapfel, 1998), and such combinations may be more efficient against listeriae than single bacteriocins.

Gram-negative bacteria are protected by their outer membrane, which prevents bacteriocins (and most other compounds of molecular weight above 600) from reaching the cytoplasmic membrane (Abee et al., 1995). Strains of lactic acid bacteria have been constructed that form and export colicin V, a bacteriocin formed by some strains of *Escherichia coli* (McCormick, Klaenhammer & Stiles, 1999). However, the inhibitory spectra of bacteriocins formed by *E. coli* (Murinda, Roberts & Wilson, 1996) and other Gram-negative bacteria are so narrow that they are unlikely to be exploitable for meat preservation. Gram-negative bacteria such as *Salmonella* may be sensitized to bacteriocins by treatment with chelating agents that permeabilize the outer membrane (Stevens, Sheldon, Klapes & Klaenhammer, 1991), by sublethal heating or freezing (Kalchayanand, Hanlin & Ray, 1992) or by ultra-high pressure (> 3000 bar; Kalchayanand, Sikes, Dunne & Ray, 1994). However, it remains to be shown whether or not such combinations work in actual meat systems.

It is observed commonly that bacteriocins are less effective in solid foods than in liquid media. Amongst others, bacteriocin activity may be reduced by the binding of bacteriocin molecules to food components, by the destabilizing action of proteases and other enzymes and by an uneven distribution in the food matrix (Schillinger et al., 1996).

4. Effect of cultures on the safety and shelf life of meat products

4.1. Fermented sausages

Microbial antagonism is used empirically in sausage fermentations where lactic acid bacteria accumulate lactic acid to levels that inhibit meat-borne pathogenic bacteria and coagulate soluble meat proteins, thereby reducing water binding capacity and facilitating drying of the product. The dominance of lactic acid bacteria is favoured by anaerobic conditions, added curing salt and sugars, and by the low initial pH of the mix (< 5.8). Formulations (initial a_w and pH, addition of curing agents and sugars) and fermentation conditions preventing growth of pathogens in various types of fermented sausages have been defined (see Lücke, 1998b, for a recent review). A rapid pH drop to below 5.3 proved to be important for the inhibition of salmonellae and *Staphylococcus aureus* if the products are fermented at temperatures above 18°C (Schillinger & Lücke, 1989). This can be assured by adding a starter culture that is active enough at the fermentation temperature selected. In products such as spreadable or Italian-type, unsmoked raw sausages that should not taste sour, formation of lactic acid should be restricted to the rate and extent necessary to inhibit pathogens and other unwanted bacteria. This is achieved by limiting the amount of added sugar or by drying the product to water activity below 0.91 and thereby preventing post-process acidification.

Listeria monocytogenes is regularly found in raw meat. Its growth potential during commercial sausage fermentation is low (Farber, Daley, Holley & Usborne, 1993), there is no epidemiological evidence for the involvement of fermented sausages in outbreaks of listeriosis, and the International Commission on Microbiological Specifications for Foods (ICMSF) recommends to tolerate up to 100 cells of *List. monocytogenes* per gram of such meats [unless served to highly susceptible individuals; International Commission on Microbiological Specifications for Food, (ICMSF), 1994]. However, *List. monocytogenes* is inactivated only slowly during sausage fermentation, and it is desirable to eliminate this organism from raw ready-to-eat meat products. By using lactic acid bacteria that produce bacteriocins active against *List. monocytogenes,* the levels of this pathogen in fermented sausages could be reduced further by about one or two log cycles compared with a control to which non-bacteriocinogenic cultures with similar souring activity had been added. This applies to various types of fermented sausages (Table 1). However, a reduction of listeriae by 5 or more log cycles was never observed. Insufficient stability of the bacteriocins due to their interaction with membrane material of meat cells may be the main reason for their limited effect in meat (Schillinger, Kaya & Lücke, 1991).

The presence of enterohaemorrhagic *Escherichia coli* (EHEC) is of great concern because the ingestion of only a few cells of these bacteria may cause severe infections, as illustrated by recent outbreaks (see, for example, Tilden et al., 1996). It is highly desirable, therefore, to eliminate this organism during sausage fermentation. Lactic acid bacteria may accelerate the destruction of EHEC by lowering the pH and thus facilitating drying, but even at low pH and a_w and prolonged ageing time of the sausages, it proved to be very difficult to attain a 5-log reduction of EHEC during fermentation and ageing without severely affecting the sensory properties of the product (Incze, 1998).

Bacteriocin formation in situ may also contribute to the dominance of the producing strains over other lactic acid bacteria during sausage fermentation (Vogel, Pohle, Tichaczek & Hammes, 1993). With appropriately

Table 1
Effect of various bacteriocinogenic lactobacilli on *Listeria*[a] in fermented sausages

Type of sausage	Bacteriocinogenic strain used (*Lb., Lactobacillus, Ped., Pediococcus*)	Decimal reduction of *Listeria* count compared with bac$^-$ control strain	Reference[b]
"Fresh" (undried, low-acid)	*Lb. sakei* Lb706	1 (regrowth at pH >6.0)	(1)
Dried, German-style	Various *Lb. sakei* and *Lb. curvatus* strains	0–2	(2), (3), (4)
Spanish style	Various *Lb. sakei* and *Lb. curvatus* strains	0–2	(2), (5)
Italian style	*Lb. plantarum* MCS	Slight effect on survival	(6)
US-style	*Ped. acidilactici*	0.5–2.5	(7), (8), (9), (10)

[a] *Listeria monocytogenes* (References 1, 3, 4, 6–10 see below), *Listeria innocua* (Ref. 2, 5 see below).

[b] (1) Schillinger et al., 1991; (2) Hugas, Neumeyer, Pagés, Garriga and Hammes, 1996; (3) Kröckel, 1996; (4), Kröckel, 1998; (5) Hugas, Garriga, Aymerich & Monfort, 1995; (6) Campanini, Pedrazzoni, Barbuti and Baldini, 1993; (7) Berry, Liewen, Mandigo and Hutkins, 1990; (8) Foegeding, Thomas, Pilkington and Klaenhammer, 1992; (9) Luchansky et al., 1992; (10) Baccus-Taylor, Glass, Luchansky and Maurer, 1993.

selected producer strains, it may thus be possible to better control the sensory properties of the fermentation and to minimize the formation of histamine and other unwanted biogenic amines that are formed by some strains of lactic acid bacteria common in meats (Maijala, Eerola, Lievonen, Hill & Hirvi, 1995).

None of the strains of lactic acid bacteria active in sausage fermentation has been found to inhibit efficiently *Staphylococcus aureus* in meats by means of bacteriocins or lytic enzymes. Hence, such strains would probably have to be constructed by genetic engineering, with subsequent problems related to safety, licensing and public acceptance. Moreover, such cultures would inhibit staphylococci that are commonly used for sausage fermentations. Lastly, *St. aureus* constitutes a health hazard only after growth in a food to levels of about 10^7 g^{-1}. Such growth may easily be prevented by conventional methods. Therefore, no additional safety factors are necessary for inactivation of this organism during normal sausage fermentation. Pathogenic clostridia and bacilli do not grow during sausage fermentation (see Lücke, 1998b); hence, there is no need for bacteriocinogenic starters to inactivate them.

Some selected mould cultures can be regarded as protective cultures because when inoculated onto the surface of meats, they suppress growth of mycotoxin producers (Leistner, Geisen & Fink-Gremmels, 1989).

4.2. Raw hams, ready-to-eat

Raw cured hams and comparable whole-meat products owe their microbiological stability and sensory properties to salt, curing agents and the action of tissue enzymes, with little if any contribution from microorganisms. For the manufacture of these products, it is very important to select cuts of normal pH ($\leqslant 5.8$), particularly for large pieces; otherwise, salting proceeds slowly, and the risk of growth of pathogens during the salting process increases unless special precautions are taken. Injection of psychrotrophic lactic acid bacteria together with fermentable sugar has been suggested to render meat of high pH suitable for curing (Hammes, 1986).

4.3. General remarks on protective cultures for non-fermented meats

Decontamination processes inactivating the spoilage flora do not necessarily make a food safer (Jay, 1996), and, in principle, all non-pathogenic organisms that overgrow pathogens in meats and, by spoiling the product, warn the consumer against eating it, may be called "protective cultures". Meats that could be made safer with the aid of protective cultures include raw, unsalted or semi-processed meats, and certain pasteurized, perishable products such as vacuum-packed sliced Bologna-type sausages. There are three approaches to develop "protective cultures" for these meats. The first approach involves selection of psychrotrophic lactic acid bacteria producing bacteriocins active against *Listeria monocytogenes* and other undesirable Gram-positive bacteria. Table 2 shows the effect of such strains on listeriae in meat systems. The second approach involves selection of psychrotrophic lactic acid bacteria that, during chill storage of the products, produce enough lactic acid to affect the growth of other psychrotrophic bacteria but do not form compounds with low sensory threshold. To date, published results have been obtained with a commercial psychrotrophic, non-bacteriocinogenic *Lactobacillus sakei* strain (originally designated as "FloraCarn L-2" and identified as *Lactobacillus alimentarius*) and they are summarized in Table 3. Lastly, one may add mesophilic lactic acid bacteria that become active rapidly if the product is temperature-abused.

4.4. Raw, unprocessed meat

Raw meat stored aerobically under chilled conditions is spoiled by Gram-negative bacteria, predominantly pseudomonads, and lactic acid bacteria compete poorly

Table 2
Effect of various bacteriocinogenic strains on *Listeria*[a] in perishable, unfermented meats

Meat product	Bacteriocinogenic strain used (*Lb., Lactobacillus, Ped., Pediococcus)*	Decimal reduction of *Listeria* count compared with bac$^-$ control strain	Refe-rence[b]
Cured pasteurized sliced vacuum-packed meats, chilled	*Lb. sakei* Lb706	1–3	(1)
	Lb. sakei Lb674	4	(2)
	Lb. sakei CTC494	1–1.5	(3)
Wieners, stored at 25°C	*Ped. acidilactici* JBL1095	1–3	(4)
Raw ground meat, chilled	*Lb. sakei* CTC494	1–2	(3)
Raw chicken breast, chilled	*Lb. sakei* CTC494	1.5–3	(3)
Cooked unsalted meat, chilled	*Lb. sakei* Lb706	1 (regrowth after 7 days)	(5)

[a] *Listeria monocytogenes* (Refs. 1, 2, 4, 5 see below), *Listeria innocua* (Ref. 3 see below).
[b] (1) Kröckel and Schmidt, 1994; (2) Kröckel, 1998; (3) Hugas, Pagés, Garriga and Monfort, 1998; (4) Degnan, Yousef and Luchansky, 1992; (5) Schillinger et al., 1991.

Table 3
Effect of a commercial protective culture (*Lactobacillus sakei* "FloraCarn L-2", 10^5–10^7 cells added/g) on microbial quality of some perishable meats

Product	Storage conditions	Target organism(s)	Effect (compared to control)	Reference[a]
Ground beef	Vacuum, 4°C	*Listeria monocytogenes*	2 log reduction	(1)
Bologna-type sausage	Vacuum, 9°C	*Listeria monocytogenes*	1–2 log reduction	(2)
Bacon cubes	10% CO_2, 90% N_2, 2 or 15°C	*Listeria monocytogenes*	0.5–2 log reduction	(2)
British fresh sausages	Uncooked, vacuum, 5°C	*Brochothrix thermosphacta*	1–1.5 log reduction	(3)
Cooked ham	Vacuum, 4°C	Spoilage flora	Extension of shelf life (7 days)	(4)
Frankfurter-type sausages	Vacuum, 6–8°C	Spoilage flora	Extension of shelf life (18 days)	(5)
Frankfurter-type sausages	Vacuum, 6°C	Slime-forming *Lactobacillus sakei*	No inhibition	(6)

[a] (1) Juven et al., 1998; (2) Andersen, 1995; (3) Andersen, 1997; (4) Kotzekidou and Bloukas, 1996; (5) Kotzekidou and Bloukas, 1998; (6) Björkroth and Korkeala, 1997.

under these conditions. Hence, very high inocula of lactic acid bacteria are required to observe an effect on shelf life of such meats (see Lücke & Earnshaw, 1991). Moreover, bacterial pathogens of most significance to the consumer of raw meat (salmonellae, *Campylobacter,* EHEC*, Yersinia enterocolitica*) are Gram-negative and thus insensitive to bacteriocins of Gram-positive bacteria. On the other hand, psychrotrophic lactic acid bacteria may contribute to the control of *Listeria monocytogenes* on meat of pH 5.6–5.8 (Gouet, Labadie & Serratore, 1978; Kaya & Schmidt, 1989). By inoculating a bacteriocinogenic *Lb. sakei* strain on raw meat, Hugas, Pagés, Garriga and Monfort (1998) obtained a further 1–2 log reduction of listeriae (Table 2).

The microflora on vacuum-packed chilled meat is dominated by Gram-positive psychrotrophs and may be influenced by inoculation of selected strains of psychrotrophic lactic acid bacteria (Schillinger and Lücke, 1987). By this method, it is possible to suppress other lactic acid bacteria that degrade amino acids to undesirable compounds such as sulfides (Schillinger and Lücke, unpublished observations; Leisner, Greer & Stiles, 1996) or biogenic amines. A bacteriocin-forming strain of *Leuconostoc gelidum* proved particularly suitable for this purpose — even under aerobic conditions — because it had little effect on the sensory properties of the meat (Leisner, Greer & Stiles, 1996; Leisner, Greer, Dilts & Stiles 1995). Juven, Barefoot, Pierson, McCaskill and Smith (1998) observed an approximately 2 log reduction of *Listeria monocytogenes* by *Lb. sakei* "FloraCarn L-2" during storage of vacuum-packaged ground beef.

4.5. Salted, semi-processed raw meats

Strains of lactic acid bacteria are commercially available that, according to their manufacturers, improve the shelf life and "freshness" of refrigerated semi-processed meats such as bacon and fresh sausages (see e.g. Andersen, 1997). The effects observed could be due to inhibition of spoilage microorganisms, but protection from detrimental effects of oxygen may also play a role. Yeasts could contribute to this effect, and this may be the reason why one manufacturer includes yeasts in his preparation.

The "Wisconsin process" of bacon manufacture employs the addition of sucrose and a mesophilic starter (*Pediococcus acidilactici*) to the injection brine. It has been shown by Tanaka, Meske, Doyle, Traisman, Thayer and Johnston (1985) that, under temperature

abuse, the pediococci start growing and forming acid, thus restricting the outgrowth of any *Clostridium botulinum* spores present while under proper refrigeration, their activity does not lead to organoleptic deterioration. However, there is little need to protect bacon from growth of *Cl. botulinum* in countries where gross temperature abuse is unlikely and where the product is normally fried to an extent more than sufficient to destroy any botulinum toxin present (Hauschild, 1982).

4.6. Pasteurized, ready-to-eat meats

The shelf life of most heat processed meats is limited by *Lactobacillus* and *Leuconostoc* strains that recontaminate the product during handling and slicing. Growth of these organisms, however, also tends to suppress the growth of pathogens at temperatures close to their growth minima (Nielsen & Zeuthen, 1985). In particular, *Listeria monocytogenes* may grow on many pasteurized meats during refrigerated storage, and, unlike fermented sausages, heat processed ready-to-eat chilled meats have been involved in outbreaks of human listeriosis (Anon., 1998; Salvat, Toquin, Michel & Colin, 1995; Schwartz et al., 1988). In the absence of lactic acid bacteria adapted to chill-stored meats, the growth potential of *List. monocytogenes* on pasteurized sliced sausages is higher than on products sliced and stored unpackaged in butcher shops or retail outlets, because these products are recontaminated normally with considerable numbers of meat lactobacilli. This restricts the growth of listeriae but also leads to rapid spoilage by souring (Schmidt & Leistner, 1993). On vacuum-packed Bologna-type sausage, growth of *List. monocytogenes* at 5 or 10°C was partially inhibited when about 10^6 cells g^{-1} of *Lb. sakei* "FloraCarn L-2" had been added (Andersen, 1995). The author did not observe a major pH drop and reported an extension of shelf life. Using this culture resulted in some extension of the refrigerated shelf life of Greek-type cooked ham and frankfurter sausage, probably by inhibition of *Brochothrix thermosphacta* (Kotzekidou & Bloukas, 1996, 1998). However, it was unable to suppress growth of a slime-producing *Lb. sakei* strain on frankfurter-type sausages (Björkroth & Korkeala, 1997) whereas the bacteriocinogenic strain *Lactobacillus sakei* CTC494 was effective against slime formation by *Lb. sakei* but not by *Leuconostoc mesenteroides* (Garriga, Aymerich, Costa, Gou, Monfort & Hugas, 1998). Growth of *List. monocytogenes* during storage of vacuum-packed Bologna-type sausage at 7°C could be prevented by adding about 10^7 cells g^{-1} of bacteriocin-forming strains of *Lb. sakei* (Table 2). At low initial numbers (about 10^3 cells g^{-1}) of *Lb. sakei* Lb 706, Kaya et al. (unpublished results, cited by Geisen, Lücke & Kröckel, 1992) observed an antilisterial effect whereas Buncic, Avery and Moorhead (1997) did not.

The available data indicate that certain strains of lactic acid bacteria may be used as protective cultures for pasteurized meats, provided that they cause only a minimal change in the desired sensory properties of the products while inhibiting listeriae and other lactic acid bacteria. However, the prevention of recontamination by listeriae during handling, slicing and packaging operations, possibly in conjunction with the addition of 0.1% sodium acetate (Schmidt, 1995) or 0.25–0.5% glucono-δ-lactone (Qvist, Sehested & Zeuthen, 1994) to the formulation of the sausage, is more effective than applying protective cultures.

5. Effect of cultures on sensory properties of fermented meats

A large variety of compounds are likely to contribute to the desired (and undesired) aroma and taste of fermented sausages (see Dainty and Blom, 1995, for review). Some are added to the sausage as such (salt, constituents of spices and smoke), others are formed by abiotic reactions, tissue or microbial enzymes during ripening. There is much commercial interest in accelerating ripening processes and extending shelf life (see Lücke, Brümmer, Buckenhüskes, Garrido Fernandez, Rodrigo & Smith, 1990).

The role of bacteria in flavour development has been summarized recently by Montel, Masson and Talon (1998). The main products of carbohydrate fermentation by lactic acid bacteria (lactic acid with small amounts of acetic acid) give the sausages an "acid" flavour which predominates in semi-dry products which are sold after less than about two week's ripening. The intensity of this flavour depends on the pH value, but at a given pH, a high proportion of acetic acid gives the product a less "pure" and more "sour" flavour. High levels of acetic acid accumulate if glucono-δ-lactone (GdL) is added as an acidulant because this compound is fermented by many lactobacilli to lactic and acetic acid. In undried products, acid formation is restricted in order to keep them spreadable, and they retain more of the aroma and taste of fresh meat.

Longer ripening times and greater activity of microorganisms other than lactic acid bacteria lead to higher levels of volatile compounds with low sensory thresholds. Lipids and nitrogen-containing compounds are precursors of most of these substances. Tissue enzymes are the main agents of lipolysis (Dobbertin, Siems & Sinell, 1975; Garcia, Selgas, Fernandez & Ordóñez, 1992; Molly, Demeyer, Civera & Verplaetse, 1996) and of proteolysis (Demeyer, 1992), at least in sausages with no surface mould. Cathepsin D is activated at pH values around 5.0 and produces peptides which are then further metabolized by the ripening flora. Later in ageing, bacterial enzymes may also play a role in the degradation of

peptides formed (Molly, Demeyer, Johansson, Raemaekers, Ghistelinck & Geenen, 1997), and Næs, Holck, Axelsson, Andersen and Blom (1995) reported that a sausage prepared with the addition of a proteinase preparation isolated from the cell wall of *Lactobacillus paracasei* developed a "mature" flavour already after 2 weeks. However, this might have been due to an indirect effect such as stimulation of microbial activity.

Microorganisms, in particular catalase-positive cocci, may affect the aroma and taste of fermented sausages by transforming compounds originating from (non-microbial) lipid and protein degradation into compounds which add to the desired aroma of the sausages. For example, the levels of 2-alkanones and esters were higher in salami prepared with a mixed starter than in the control (Schmidt & Berger, 1998), and the levels were positively correlated with salami odour and levels of *Staphylococcus xylosus* (Stahnke, 1995). Different staphylococci differ in their effects (Berdagué, Monteil, Montel & Talon, 1993).

Some fermented sausages and raw salted meats — particularly those produced in France, Spain and Italy — are allowed to develop a surface flora consisting of moulds and yeasts that contribute to the desired sensory properties of the product. Lactate oxidation and proteolysis lead to a taste markedly different from smoked sausages. Because mould growth elevates the surface pH of the sausage, it is important that it commences only after the pH and water activity of the sausages is low enough to prevent surface growth of undesired bacteria such as listeriae (Rödel, Stiebing & Kröckel, 1993).

A suitable surface starter should colonize rapidly and adhere to the sausage surface. This enables it to suppress undesired moulds, protect the product from detrimental effects of oxygen, facilitate drying by "buffering" against fluctuations in humidity in the ripening chamber, and bring about the desired changes in appearance (whitish or yellowish) and flavour. Of course, it must not form mycotoxins and/or antibiotics. Selected strains are now available as starters for these products. Most of them contain *Penicillium nalgiovense* (biotypes 2, 3, and 6), sometimes combined with the yeast *Debaryomyces hansenii* (or its imperfect form, *Candida famata*). These fungi have not been found to produce any of the known toxic secondary metabolites. *Penicillium chrysogenum* strains are closely related to *Pen. nalgiovense* according to morphological and molecular criteria (Geisen, 1993) and may also be suitable but must be carefully screened for the absence of roquefortin and antibiotic formation (El-Banna, Fink-Gremmels & Leistner, 1987).

A halophilic *Halomonas* strain from curing brines was shown to improve the sensory properties of hams, probably mainly by nitrate reduction (Meisel, 1988) and a German patent (no. DE 4035836 C2) has been awarded for its use (see Hammes & Hertel, 1998). Inoculation of bacon curing brines with a *Vibrio* strain led to higher levels of methylbutanals that could have a positive effect on bacon aroma (Hinrichsen & Andersen, 1994).

6. "Probiotic" meat products?

Information on probiotic bacteria has recently been summarized by Holzapfel, Haberer, Snel, Schillinger and Huis in't Veld (1998). As Hammes and Haller (1998) pointed out, significant metabolic activity of probiotic strains in the large intestine would require a daily update of 10^8 or more cells. From this, one may conclude that a food labelled "probiotic" should contain not less than 10^6 probiotic bacteria g^{-1} unless the manufacturer provides evidence that the health claims on the label do not mislead the consumer. A "probiotic" fermented sausage manufactured with the addition of bifidobacteria has been marketed for some time in Germany, but the strain used survived only poorly during sausage ripening, and a very high inoculum had to be added in order to attain at least 10^6 bifidobacteria g^{-1} after fermentation. Mesophilic lactobacilli are much better candidates for use as probiotic meat cultures, as recently shown by Sameshima, Magome, Takeshita, Arihara, Itoh and Kondo (1998) and Andersen (1998). Screening programs may lead to strains that survive both meat fermentations and the passage through the stomach and small intestine (Hammes & Haller, 1998) and that may be tested subsequently for their benefits to health. In Germany, it is not easy to market probiotic meats because, unlike sourmilks, meats do not have a reputation of being a "health food", but this may be different in other countries.

7. Prospects and limitations of the use of genetically engineered cultures

The tools are available to modify the genome of various microorganisms suitable as starters or protective cultures for meats. Research has focused on transfer of defined, single genes encoding for bacteriocins (see e.g. Allison, Ahn, Stiles & Klaenhammer, 1995; Chikindas, Venema, Ledeboer, Venema & Kok, 1995; McCormick, Klaenhammer & Stiles, 1999; McCormick, Worobo & Stiles, 1996) or lytic enzymes such as lysostaphin. The gene for the latter compound was expressed in *Penicillium nalgiovense* (see Geisen, 1993) and in *Lactobacillus curvatus* (Gaier, Vogel & Hammes, 1992). This lactobacillus was subsequently shown to produce lysostaphin in quantities sufficient to inactivate *Staphylococcus aureus* during sausage fermentation (Cavadini, Hertel & Hammes, 1998). By transfer of genes encoding antagonistic proteins, and by increasing the expression rate of such genes, one may be able to increase the biocontrol

potential of a suitable host strain even at low cell densities. However, as outlined above, the possible benefit of this approach is very limited and most probably restricted to improved control of *Listeria monocytogenes* on such meats.

It may also become possible to engineer metabolic pathways to give better control the rate and extent of the formation of lactic and acetic acids, and to eliminate unwanted properties such as formation of biogenic amines. However, the latter purposes can — with better acceptance by the public and regulatory bodies — be achieved more easily by selecting strains from nature. Likewise, the aroma and taste of fermented sausages are affected by so many factors that it appears unrealistic to expect that there is a major role for gene technology in shortening the ageing processes and improving the flavour of sausage.

8. Conclusions

Use of appropriately selected psychrotrophic lactic acid bacteria may reduce the risk of growth of salmonellae and other vegetative pathogens during sausage fermentation, and may contribute to the inhibition of *Listeria monocytogenes* on some perishable meat products. It appears to be possible to select strains that cause only little sensory alteration in the product. The most important mechanism of action of protective cultures is the formation of lactic acid; the effect of bacteriocins is diminished by their inactivation in the meat, the possibility of resistance development in target organisms, and, in particular, the resistance of Gram-negative pathogens to them. The use of bacteriocin-producing lactic acid bacteria cannot be expected to contribute significantly to the prevention of meat-borne enteric diseases (including EHEC infections) caused by Gram-negative bacteria, and to the extension of shelf life of aerobically stored meat.

Protective cultures only improve the safety of meats if they do not destroy organisms that would warn the consumer against eating a hazardous product, or that would suppress pathogens. In view of their limited effects, the use of protective cultures cannot compensate for poor control of the manufacturing processes, and culture manufacturers are well advised not to make unrealistic claims of the ability of their cultures to inactivate pathogens or spoilage organisms.

Starter cultures may affect the aroma and taste of fermented sausages and, possibly, brine-cured raw meats. The effects appear to be related to microbial transformations of compounds generated by meat enzymes and abiotic reactions involving molecular oxygen. However, the scientific data are still puzzling, and it is difficult to predict effects from laboratory data. The experience of the meat processor will continue to be crucial for the selection of cultures and for obtaining the desired sensory properties of the product.

Probiotic cultures may also find their way into meat products if strains become available that tolerate the conditions both in meat fermentations and in the intestinal tract. If these cultures are to be marketed, evidence of their health benefit should be provided.

In the near future, benefits from genetically engineered cultures are so small that it will be difficult to convince the consumers and regulatory bodies of their technological necessity.

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