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UTILIZATION OF MICROBES TO PROCESS AND TO PRESERVE MEAT

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Abstract

This paper discusses how and to which extent the addition of microorganisms to meats help 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. The effect of bacteriocin-forming starters on the overall safety of meat products is limited, in particular because Gram-negative bacteria such as *Escherichia coli* are insensitive to these types of bacteriocins. However, such strains may aid in the inactivation of *Listeria monocytogenes* in fermented sausages and some pasteurized, perishable meat products if recontamination by listeriae cannot be excluded. By inhibiting other recontaminants leading to undesired aroma and taste, some psychrotrophic lactic acid bacteria have also been found to extent the shelf life of such products, but again, this is best achieved by aseptic slicing and packaging. Microorganisms other than lactic acid bacteria may improve the sensory properties of meat products mainly by consuming oxygen and by metabolizing compounds arising from changes in lipids and proteins brought about by meat enzymes and autoxidation processes. Adding probiotic cultures to meats is a promising option but there is a need of strains attaining high numbers during fermentation and/or storage. 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.

Introduction

Addition of desirable microorganisms to meats may have four different purposes:

Purpose 1: „Safety", mainly inactivation of pathogens

Purpose 2: „Stability", i.e. extension of shelf life by inhibiting undesirable changes brought about by spoilage microorganisms or abiotic reactions (e.g. lipid oxidation)

Purpose 3: „Diversity", i.e.„fermentation" of the raw material to induce desirable changes of the sensory properties

Purpose 4: „Health benefits" through beneficial effects on the intestinal flora.

„Starter cultures" are 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 recently provided by Hammes & Hertel (1998) and Lücke (1998). Antagonistic cultures that are only added to inhibit pathogens and/or to extent 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 (Anonymous, 1995). 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.

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 (WBA-IPSOS, 1996). They doubt that animal production is environmentally sound and that animals are treated appropriately during rearing and transport, and they rightly think that mistreated animals are more likely to get sick, and products from sick animals are not safe. Deficiencies in crisis management by industry and authorities (as in the BSE crisis) and reports in the media amplify this scepticism. In addition, meat is prone to contamination of pathogens with reservoirs in 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.

A long shelf life (Purpose 2) is obviously important 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, there is also a strong interest in industry to save distribution costs.

Food fermentations lead to an enormous product variety (e.g. of wines and cheeses but also of meat products). Thus, Purpose 3 is in the interest of both consumers and industry. Manufacturers may increase their market share or establish themselves in market niches. Starter cultures may aid in developing and maintaining this diversity but raw materials and other processing factors are more important.

Moreover, industry is on considerable pressure to cut costs. Hence, there is much interest to standardize the properties and shelf life of the product, to better control 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 creating problems in providing safe and stable products:

- Consumers tend to live in smaller, urban households and to have less knowledge on raw materials and cooking. Hence, they cannot be relied upon too much with respect to proper treatment of meats in the household
- Consumers prefer „mild" foods, i.e. with less salt and acid, and low-fat foods. Hence, foods tend to have higher pH and water activity (a_w) values
- 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 keywords „Minimal processing" and „Hurdle technology" (Leistner & Gorris, 1995).

Mechanisms of antagonism

Microorganisms may inhibit competitors by withdrawing nutrients or by forming inhibitory metabolites. Particularly in solid substrates such as meats, a homogenous distribution of added microorganisms is important for their antagonistic effect (Katsaras & Leistner, 1991). Because meat is rich in nutrients (including iron), and lactic acid bacteria require more pre-formed nutrients than their undesired competitors, it is unlikely that competition for nutrients is of major importance in microbial antagonism in meats.

Formation of organic acids

In most food fermentations, organic acids produced by lactic acid bacteria and the concomitant decrease of pH are responsible for the preservation effect. Lactic and acetic acids are the major preservative acids produced by meat starter cultures. Because the specific antimicrobial effect of an acid depends largely on the concentration and lipophilicity of its undissociated form, acetic acid has a stronger antimicrobial effect than lactic acid at the same concentration and the same pH in the range of 3 -7. Propionic acid is even more effective but its producers, propionic acid bacteria, are not competitive at all in meats. Other acids such as formic acid are formed only in low amounts and are unlikely to contribute much to meat preservation. Sensitivity to organic 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 such as listeriae.

Formation of other compounds of low molecular mass

From the list of microbial products of low molecular weight, some compounds can be deleted because at inhibitory concentrations, they cause sensory defects. This applies to carbon dioxide (pore formation) and hydrogen peroxide which, if allowed to accumulate, causes discolourations of cured meats and deterioration of fats. Other primary metabolites of lactic acid bacteria such as diacetyl (see Helander *et al.*, 1997), benzoic acid and some heterocyclic organic compounds (Niku-Paavola *et al.*, 1999) have been shown to inhibit other microorganisms, but only at concentrations far exceeding the levels in fermented meats and the thresholds of sensory acceptability. However, in combination with other antimicrobial factors, such results may explain why, in culture media, some strains are observed to be more inhibitory against Gram-negative bacteria than others. Other antimicrobial compounds (e.g. 3-hydroxypropionaldehyde, „reuterin") are not formed by microorganisms capable of growth in salted or chilled meats.

Bacteriocins

Bacteriocins have been studied most extensively during the last years because they may inactivate undesired bacteria without altering the sensory properties of the products. Bacteriocins are peptides or proteins that are destroyed by proteases in the upper intestinal tract and hence raise less safety concerns than the presence of antibiotics in food. 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 Abee *et al.* (1995), Stiles (1996), Schillinger *et al.* (1996) and Hugas (1998).

It is generally accepted that bacteriocins exert their inhibitory action by formation of pores in the cytoplasmic membrane of sensitive cells. Gram-positive bacteria vary in their sensitivity to a bacteriocin, mainly because of differences in membrane composition and fluidity (Bennik *et al.*, 1998). 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 *et al.*, 1991), and even higher rates were observed with some class II bacteriocins such as sakacin A (Lücke & 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 *et al.*, 1995; Abee, 1998). Hence, nisin-resistant variants of *List. monocytogenes* were found to be still sensitive to *in situ* formed sakacin A (Schillinger *et al.*, 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). It has been shown that Gram-negative bacteria such as *Salmonella* are rendered sensitive to bacteriocins by treatment with chelating agents which permeabilize the outer membrane (Stevens *et al.*, 1991) or by sublethal heating or freezing (Kalchayanand *et al.*, 1992). However, it remains to be shown whether or not such combinations work in actual meat systems.

It is commonly observed 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 the uneven distribution in the food matrix (Schillinger *et al.*, 1996).

Effect of cultures on the safety and shelf life of meats

Microbial antagonism is empirically used 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). Their activity, however, results in a product with sensory characteristics quite different from the original mix. In the last few years, evidence accumulated that it may be possible to use microbial antagonism for improving the safety and/or extending the shelf life of certain other meats where a major pH drop is not acceptable or (due to lack of sugars) not possible. Products that could be preserved 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.

Fermented sausages

Formulations and fermentation conditions preventing growth of pathogens in various types of fermented sausages have been defined (see Lücke, 1998, for a recent review). This statement also applies to products such as spreadable or Italian-type, unsmoked raw sausages that should not taste sour, and in which formation of lactic acid should be restricted to the rate and extent necessary to inhibit pathogens and other undesired bacteria. 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 in the temperature range around 20°C (e.g. *Lactobacillus sakei* strains). An atypical sour taste could be avoided by limiting the amount of added sugar or by drying the product to water activity below 0.91 and thereby preventing post-process acidification.

List. monocytogenes is regularly found in raw meat. Growth potential of listeriae during commercial sausage fermentation is low (Farber *et al.*,1993), and there is no epidemiological evidence for the involvement of fermented sausages in outbreaks of listeriosis. 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, even though food inspectors in Germany, following an official recommendation (Anonymous, 1991), usually tolerate up to 100 cells of *List. monocytogenes* per gram of such meats. 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 European-style (Schillinger *et al.*, 1991; Campanini *et al.*, 1993; Hugas *et al.*, 1995, 1996; Kröckel, 1996, 1998) and US-style sausages (Berry *et al.*, 1990; Foegeding *et al.*, 1991; Luchansky *et al.*, 1992; Baccus-Taylor *et al.*, 1993). 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 may be the main reason for their limited effect in meat (Schillinger *et al.*, 1991).

It is highly desirable to eliminate enterohaemorrhagic *Escherichia coli* (EHEC) during sausage fermentation. Reduction is highest when sausages fermented to pH below 5.2 are dried to water activities below 0.93 and aged for 3 or more weeks (see Incze, 1998; Kofoth *et al.*, 1998; Stiebing *et al.*, 1998). Lactic acid bacteria may accelerate the destruction of EHEC by lowering the pH and thus facilitating drying, but there is no evidence so far of other mechanisms being involved. 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 *et al.*, 1993). With appropriately selected producer strains, it may thus be possible to better control the sensory properties of the fermentation and to minimize the formation of biogenic amines.

None of the strains of lactic acid bacteria active in sausage fermentation has been found to efficiently inhibit *St. 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} which may easily be prevented by conventional methods. Therefore no additional safety factors appear to be necessary for inactivation of this organism during normal sausage fermentation. Pathogenic clostridia and bacilli do not grow during sausage fermentation (see Lücke, 1998); hence, there is no need for bacteriocinogenic starters inactivating them.

Appropriately selected mould cultures inoculated onto the surface of meats help in suppressing growth of mycotoxin producers (Leistner *et al.*, 1989) and may therefore also be regarded as protective cultures. They are discussed below, in context with their effects on the sensory properties of meats.

Raw hams, ready-to-eat

Raw 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 micro-organisms. For the manufacture of raw hams and comparable products, it is very important to select cuts of normal pH (≤ 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 along with fermentable sugar has been suggested to render meat of high pH suitable for curing (Hammes, 1986).

Raw, unsalted meat

Raw meat stored aerobically under chilled conditions is spoiled by Gram-negative bacteria, predominantly pseudomonads, and lactic acid bacteria compete poorly 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 *List. monocytogenes* on meat of pH 5.6 - 5.8 (Gouet *et al.*, 1978; Kaya & Schmidt, 1989). By inoculating a bacteriocinogenic *Lb. sakei* strain on raw meat, Hugas *et al.* (1998) obtained a further 1 - 2 log reduction of listeriae.

If meat is packed in films of low oxygen permeability under vacuum or modified atmosphere (elevated CO_2 level) and subsequently stored under proper refrigeration, pseudomonads are inhibited, the growth potential of facultatively anaerobic pathogens is reduced, and a microflora mainly consisting of lactic acid bacteria develops (Grau, 1981). However, meat of pH >6.0 contains only low levels of fermentable sugar and lactic acid and restricts growth of lactic acid bacteria, and psychrotrophic acid-sensitive bacteria such as *Brochothrix thermosphacta* and *List. monocytogenes* may successfully compete with them.

The composition of the lactic flora on vacuum-packed chilled meat may be influenced by inoculating selected strains of psychrotrophic lactic acid bacteria (Schillinger & Lücke, 1987). By this way, it is possible to suppress other lactic acid bacteria that degrade amino acids to undesired compounds such as sulfides (Schillinger & Lücke, unpublished observations; Leisner *et al.*, 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 *et al.*, 1995, 1996). Juven *et al.* (1998) observed an about 2 log reduction of *List. monocytogenes* by a psychrotrophic, non-bacteriocinogenic *Lb. sakei* strain (designated as „FloraCarn L-2™" and originally identified as *Lb. alimentarius*) during storage of vacuum-packaged ground beef.

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 meats such as bacon and fresh sausages (see e.g. Andersen, 1997). It appears that acid formation under these conditions is sufficient to affect the growth of other psychrotrophic bacteria but insufficient to cause major organoleptic deterioration. Protection from detrimental effects of oxygen may also play a role and may be the reason why one manufacturer includes yeasts in his preparation. Andersen (1995) was also able to partially inhibit growth of *List. monocytogenes* on chill-stored bacon cubes packed under 10 % CO_2/ 90 % N_2 by adding 10^7 cells g^{-1} of *Lb. sakei* „FloraCarn L-2™".

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 *et al.* (1985a,b) 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. The process has been approved by the United States Department of Agriculture. However, one should take into account that (i) clostridial spores usually show long lag times before they start developing in foods, thus leaving enough time for the pediococci (1 - 2 days at 27°C) to lower the pH to inhibitory levels, (ii) frying the bacon in the usual way will probably conceal any sensory deviations due to slow souring of the product during refrigerated storage, and (iii) 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 far sufficient to destroy any botulinum toxin present (Hauschild, 1982).

Ped. acidilactici was also reported to inhibit *List. monocytogenes* on temperature-abused wieners (Degnan *et al.*, 1992).

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 minimum (Nielsen & Zeuthen, 1985). In particular, *List. 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 (Schwartz *et al.*, 1988; Salvat *et al.*, 1995). In the absence of lactic acid bacteria adapted to chill-stored meats, the growth potential of *List. monocytogenes* on pasteurized sliced sausages is higher, whereas products sliced and stored unpackaged in butcher shops or retail outlets are normally recontaminated with considerable numbers of meat lactobacilli (e.g. from fermented sausages); 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. This culture also somewhat extended 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 *Lb. sakei* CTC494 was effective against slime formation by *Lb. sakei* but not by *Leuconostoc mesenteroides* (Garriga *et al.*, 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 *L. sakei* (Kröckel & Schmidt, 1994; Hugas *et al.*, 1998; Kröckel, 1998). At low initial numbers (about 10^3 cells g^{-1}) of *Lb. sakei* Lb 706, Kaya *et al.* (unpublished results, cited by Geisen *et al.*, 1992) observed an antilisterial effect whereas Buncic *et al.* (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 only minimally change 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 adding 0.1 % sodium acetate (Schmidt, 1995) or 0.25 - 0.5 % glucono-δ-lactone (Qvist *et al.*, 1994) to the formulation of the sausage, is much more effective than applying protective cultures.

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. Some are added to the sausage as such (salt, constituents of spices and smoke), others are formed during ripening. Abiotic reactions (in particular, lipid peroxidation), tissue enzymes (in particular, proteases and lipases) and microbial enzymes all contribute to the development of aroma and taste, as well as to the spoilage of the products. There is much commercial interest in accelerating ripening processes and extending shelf life (see Lücke *et al.*, 1990). A review on the flavour chemistry of fermented sausages has been published by Dainty & Blom (1995), and knowledge on the role of bacteria in flavour development has been recently summarized by Montel *et al.* (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 obviously 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.

The longer the ripening time, and the higher the activity of microorganisms other than lactic acid bacteria, the higher levels of volatile compounds with low sensory thresholds are found. Lipids and nitrogen-containing compounds are precursors of most of these substances. Tissue enzymes are the main agents of lipolysis (Dobbertin *et al.*, 1975; Garcia *et al.*, 1992; Molly *et al.*, 1996) and of proteolysis (Demeyer, 1992; AIR2 Report No., CT94 - 1517, 1997), 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 *et al.*, 1997), and Næs *et al.* (1995) reported that a sausage prepared with the addition of a proteinase isolated from the cell wall of *Lactobacillus paracasei* developed a „mature" flavour after already 2 weeks. However, this could also be due to an indirect effect, e.g. stimulation of microbial activity (Molly *et al.*, 1997).

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 (AIR2 Report No., CT94 - 1517, 1997). For example, Stahnke (1995) reported a positive correlation between the levels of 2-alkanones, salami odour and levels of *Staphylococcus xylosus,* and different staphylococci differ in their effects (Berdagué *et al.*, 1993). Furthermore, various esters (mainly ethyl esters) are found among the volatiles of fermented sausages (Dainty & Blom, 1995), and to contribute to salami odour. The data of Stahnke (1995) suggest staphylococci (e. g. *St. xylosus*) as main agents.

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. The intensity of flavour of such sausages was found to be proportional to the degree of proteolysis (Geisen *et al.*, 1992). 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. Otherwise, there is a risk of surface growth of undesired bacteria such as listeriae (Rödel *et al.*, 1993).

A suitable surface starter should

- rapidly colonize the surface in order to (i) suppress undesired moulds; (ii) protect the product from detrimental effects of oxygen; (iii) facilitate drying by „buffering" against fluctuations in humidity in the ripening chamber
- have a whitish or yellowish appearance, i.e. moulds should develop white conidia or no conidia at all during growth on the product
- degrade proteins and amino acids to bring about the desired aroma and taste of the product
- adhere to the sausage casings throughout ripening and storage
- not form mycotoxins 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 are not known to produce any of the known toxic secondary metabolites. *Pen. 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/or antibiotic formation (El-Banna *et al.*, 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 which could have a positive effect on bacon aroma (Hinrichsen & Andersen, 1994).

Probiotic meat products?

In the European Union, there is yet no official regulation as to the minimum content of probiotic microorganisms in a food labeled „probiotic", but experts generally recommend a level of 10^6 bacteria g^{-1} (see Holzapfel *et al.*, 1998). A „probiotic" fermented sausage manufactured with the addition of bifidobacteria has been marketed for some time in Germany, but bifidobacteria survive 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 *et al.* (1998). Although there is a vast difference between the habitats „sausage" and „intestine", 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 subsequently be tested 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.

Prospects and limitations of genetically engineering of meat 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 *et al.*,1995; Chikindas *et al.*, 1995; McCormick *et al.*, 1996) or lytic enzymes such as lysostaphin. The gene for the latter compound was expressed in *Pen. nalgiovense* (see Geisen, 1993) and in *Lb. curvatus* (Gaier *et al.*, 1992), and the latter strain was recently shown to produce lysostaphin in quantities sufficient to inactivate *St. aureus* during sausage fermentation (Cavadini *et al.*, 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 *List. monocytogenes* on such meats.

It may also become possible to engineer metabolic pathways to better control the rate and extent of the formation of lactic and acetic acids, and to eliminate undesired properties such as formation of biogenic amines. However, the latter purposes can more easily - and with better acceptance by the public and regulatory bodies - be achieved 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 a major role of gene technology in shortening ageing processes and improving sausage flavour.

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 *List. monocytogenes* on some perishable meat products. It appears to be possible to select strains that cause only little sensory deviation 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. Use of bacteriocin-producing lactic acid bacteria cannot be expected to significantly contribute 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 which would warn the consumer from eating a hazardous product, or which themselves 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 on 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.

Probiotic cultures may also find their way into meat products if strains become available which tolerate the conditions both in meat fermentations and in the intestinal tract. For successfully marketing these cultures, 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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