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Functional meat starter cultures for improved sausage fermentation

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Abstract

Starter cultures that initiate rapid acidification of the raw meat batter and that lead to a desirable sensory quality of the end-product are used for the production of fermented sausages. Recently, the use of new, functional starter cultures with an industrially or nutritionally important functionality is being explored. Functional starter cultures offer an additional functionality compared to classical starter cultures and represent a way of improving and optimising the sausage fermentation process and achieving tastier, safer, and healthier products. Examples include microorganisms that generate aroma compounds, health-promoting molecules, bacteriocins or other antimicrobials, contribute to cured meat colour, possess probiotic qualities, or lack negative properties such as the production of biogenic amines and toxic compounds. The vast quantity of artisan fermented sausages from different origins represents a treasure chest of biodiversity that can be exploited to create such functional starter cultures.

Keywords: Fermented sausage; Meat; Starter cultures; Lactic acid bacteria; Staphylococcus; Kocuria; Moulds; Yeasts; Flavour; Aroma; Food safety; Bacteriocins; Probiotics; Biogenic amines; Conjugated linoleic acid; Vitamins

1. Introduction

Fermented sausage is prepared from seasoned, raw meat that is stuffed in casings and is allowed to ferment and mature (Campbell-Platt and Cook, 1995; Lücke, 1998). Inoculation of the sausage batter with a starter culture composed of selected lactic acid bacteria (LAB), i.e. homofermentative lactobacilli and/or pediococci, and Gram-positive, catalase-positive cocci (GCC), i.e. nonpathogenic, coagulase-negative staphylococci and/or kocuriae, improves the quality and safety of the final product and standardizes the production process (Campbell-Platt and Cook, 1995; Hugas and Monfort, 1997; Lücke, 1998, 2000). Nonetheless, small manufacturers continue to use the traditional method of spontaneous fermentation without added starter culture. In the latter case, the required microorganisms originate from the meat itself or from the environment and constitute a part of the so-called 'house flora' (Santos et al., 1998). Back-slopping is also used, if material from a successful previous batch is added to facilitate the initiation of a new fermentation process (Alley et al., 1992; Campbell-Platt and Cook, 1995). Such artisan fermented sausages are often of superior quality compared to controlled fermentations inoculated with industrial starters and possess distinctive qualities, partly due to the properties of the raw material and the characteristics of the technology used (Moretti et al., 2004), but also to the specific composition of the house flora. The flavour-generating, metabolic activity of GCC in artisan chorizo, for instance, has been shown to vary with the manufacturing location (García-Varona et al., 2000).

It has been suggested that commercial starter cultures in Europe, mainly produced in Northern European countries, are not always able to compete well with the house flora colonizing Southern European meat plants, so that their use often results in losses of desirable sensory characteristics (Samelis et al., 1998). The fitness of commercial meat starter cultures when applied to a particular type of salami is questionable since a culture that performs well in one type of fermented sausage is not necessarily efficient in another type. Appropriate cultures have to be selected according to the specific formulation of the batter and technology of fermentation since environmental factors will interact to select a limited number of strains that are competitive enough to

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dominate the process (Rebecchi et al., 1998). *Pediococcus acidilactici*, *Pediococcus pentosaceus*, *Lactobacillus pentosus* and *Lactobacillus plantarum*, species sometimes found in commercial starter cultures for meats, are rarely detected in large amounts in spontaneously fermented sausages because of their inferior competitiveness compared to, for instance, *Lactobacillus sakei* or *Lactobacillus curvatus* (Doßmann et al., 1998; Coppola et al., 2000). They initiate the acidification of the meat batter well, but are not always able to prevent spontaneous outgrowth of non-starter LAB with undesirable effects on the end-product (Coventry and Hickey, 1991; Hugas and Monfort, 1997). *Lb. plantarum* may also give rise to a product with overacidity, which is not well perceived by the consumer (Garriga et al., 1996).

Even if the rapid acidification initiated by the starter culture reduces microbial risks in fermented sausages, not all concerns have been solved, mainly so in slightly fermented or ripened varieties. Whereas pseudomonads, *Enterobacteriaceae*, and aerobic sporeformers are usually not of concern (Samelis et al., 1998; Aymerich et al., 2003), the pathogens *Staphylococcus aureus*, *Escherichia coli*, *Salmonella*, and *Listeria monocytogenes* are posing risks to food safety. Given the appropriate conditions, *Staph. aureus* has been responsible for food poisoning because of its ability to multiply and produce enterotoxin during the initial stage of raw meat fermentation (Lücke, 1998; Sameshima et al., 1998). Problems with *E. coli* and *Salmonella* have been reported, mainly in short ripened, semi-dry sausages (Sauer et al., 1997; Incze, 1998; Lücke, 1998; Ammon et al., 1999; Castaño et al., 2002; Normanno et al., 2002). *L. monocytogenes* is of concern because of its occasional presence in the end-product (Farber et al., 1993; Encinas et al., 1999; Jay, 2000; Aymerich et al., 2003). The latter bacterium is often associated with meat and the meat environment (Nesbakken et al., 1996; Lücke, 1998; Samelis and Metaxopoulos, 1999). For instance, it was present in more than 60% of the raw meat investigated for Greek-style fermented sausage manufacture (Samelis et al., 1998). Although listeriosis has not yet been associated with the consumption of dry fermented sausage, sausages that are slightly fermented and relatively moist, such as Mettwurst have been connected to listeriosis (Jay, 2000; Loncarevic et al., 1997). Listeriae represent a latent safety problem considering the high mortality rate of the disease, in particular among individuals with compromised immunity (McLauchlin, 1996; Schlech, 1996). Moreover, several countries including the United States have adopted a zero tolerance policy, i.e. the absence of the pathogen in two 25 g samples (Shank et al., 1996). This policy has direct consequences for food companies that operate in or export to such countries.

Besides the above-mentioned shortcomings concerning sensory quality in industrial fermented meat products and food safety, there seems to be a trend for more healthy meat products (Jiménez-Colmenero et al., 2001). The latter puts pressure on the producer to look for improvement of the nutritional image of their products. As a possible way to tackle all of the above, the use of starter cultures of a new generation has been suggested, the so-called 'functional starter cultures' (De Vuyst, 2000; Leroy and De Vuyst, 2003, 2004). Functional starter cultures contribute to microbial safety or offer one or more organoleptic, technological, nutritional, or health advantages.

One of the main challenges is to explore the biodiversity of artisan products and to introduce qualities obtained with wild-type strains in standardized, industrial fermentations. In contrast to ill-adapted industrial starters, wild-type strains that naturally dominate traditional fermentations tend to have higher metabolic capacities which can beneficially affect product quality, for instance with regard to aroma formation or food safety. Natural selection is likely to have forced such strains to be more competitive by endowing them with ecological advantages, i.e. making them less auxotrophic (Ayad et al., 2000) and more able to produce antimicrobials (Maldonado et al., 2002).

The present review outlines potential functionalities of new starter cultures that could lead to improved sausage fermentation (with respect to flavour, safety, processing, technology, or health). It attempts to include the most recent findings, a multitude of functionalities for LAB, GCC, as well as for yeasts and moulds, and to clarify the relation with the raw material, sausage technology, and the quality of the end-product.

2. Microorganisms involved in sausage fermentation

The microorganisms that are primarily involved in sausage fermentation include species of LAB, GCC, moulds, and yeasts.

In spontaneously fermented European sausages, facultative homofermentative lactobacilli constitute the predominant flora throughout ripening. *Lb. sakei* and/or *Lb. curvatus* generally dominate the fermentation process (Hugas et al., 1993; Coppola et al., 1998, 2000; Rebecchi et al., 1998; Samelis et al., 1998; Santos et al., 1998; Andrighetto et al., 2001; Cocolin et al., 2001; Parente et al., 2001; Aymerich et al., 2003; Papamanoli et al., 2003; Rantsiou et al., 2004, 2005). *Lb. sakei* appears to be the most competitive of both strains, frequently representing half to two thirds of all LAB isolates from spontaneously fermented sausage, whereas *Lb. curvatus* is frequently found in amounts up to one fourth of all LAB isolates. Other lactobacilli that may be found, albeit generally at minor levels, include *Lb. plantarum*, *Lactobacillus bavaricus* (now reclassified as *Lb. sakei* or *Lb. curvatus*), *Lactobacillus brevis*, *Lactobacillus buchneri*, and *Lactobacillus paracasei* (Hugas et al., 1993; Rebecchi et al., 1998; Aymerich et al., 2003; Papamanoli et al., 2003). Recently, the new species *Lactobacillus versmoldensis* has been isolated from German, quick-ripened, salami-style sausages where it was present in numbers of up to 10^8 cfu g^{-1} (Kröckel et al., 2003).

Pediococci are less frequently isolated from European fermented sausages but occasionally occur in small percentages (Santos et al., 1998; Papamanoli et al., 2003). They are more common in fermented sausages from the United States where they are deliberately added as starter cultures to accelerate acidification of the meat batter.

Enterococci are sometimes associated with fermented meat products, in particular artisan products from Southern Europe, where they increase during early fermentation stages and can be detected in the end-product at levels of 10^2–10^5 cfu g^{-1} (Rebecchi et al., 1998; Samelis et al., 1998; Aymerich et al., 2003; Franz et al., 2003; Papamanoli et al., 2003). They are ubiquitous in food processing establishments and their presence in the gastrointestinal tract of animals leads to a high potential for contamination of meat at the time of slaughter (Franz et al., 1999). Opinions about their significance vary, as they may enhance food flavour but also compromise safety if opportunistic pathogenic strains proliferate or antibiotic resistance is spread (Franz et al., 1999, 2001, 2003; Vancanneyt et al., 2002; Cocconcelli et al., 2003; De Vuyst et al., 2003).

Coagulase-negative staphylococci and kocuriae are GCC that participate in desirable reactions during ripening of dry fermented sausages, such as colour stabilisation, decomposition of peroxides, proteolysis, and lipolysis (see Section 3.3). They are poorly competitive in the presence of actively growing aciduric bacteria, often not growing more than one log cfu g^{-1} during ripening (Samelis et al., 1998). The nonpathogenic, coagulase-negative staphylococci are dominated by *Staphylococcus xylosus*, *Staphylococcus carnosus*, and *Staphylococcus saprophyticus*, but other species occur too (Arkoudelos et al., 1997; Coppola et al., 1997; Rebecchi et al., 1998; García-Varona et al., 2000; Papamanoli et al., 2002; Gardini et al., 2003; Blaiotta et al., 2004; Mauriello et al., 2004). In addition to staphylococci, *Kocuria varians*, formerly known as *Micrococcus varians*, or other kocuriae are sometimes isolated in small quantities from naturally fermented sausage (Coppola et al., 1997; Papamanoli et al., 2002; Gardini et al., 2003).

Moulds, usually *Penicillium nalgiovense* and *Penicillium chrysogenum*, are used in mould-ripened sausages, particularly in Southern Europe (López-Díaz et al., 2001; Sunesen and Stahnke, 2003). A yeast population, dominated by *Debaryomyces hansenii*, may also be found on the sausage surface and originates from the house flora or is sometimes added as starter culture (Samelis et al., 1994b; Coppola et al., 2000; Encinas et al., 2000; Olesen and Stahnke, 2000).

3. Functional starter cultures for a more tasty product

3.1. Bacteria versus meat enzymes

The flavour of fermented sausage is influenced by several factors, primarily the source, quantity and type of ingredients (e.g. meat, salt, and spices), but also the temperature, processing time, smoking, and choice of starter culture. Basic flavour results from the interaction of taste (mainly determined by lactic acid production and the pattern of peptides and free amino acids resulting from tissue-generated proteolysis) and aroma (mainly determined by volatile components derived from bacterial metabolism and lipid autoxidation) (Ordóñez et al., 1999; Stahnke, 1999a,b; Beriain et al., 2000; Stahnke et al., 2002; Claeys et al., 2004). In Northern Europe, smoking is important for flavour and lactic acid is believed to be the main taste component. However, acetic acid is also present and is actually needed in small amounts for full dry sausage flavour (Erkkilä et al., 2001a). Too high concentrations of acetic acid, however, produce a prickly, astringent flavour (Mateo and Zumalacárregui, 1996). In Southern European sausage, predominant acidity is not sought for and may be rejected. In the latter type of sausage, where acidity is mild and smoking is rare, flavour is primarily generated by proteolytic and lipolytic activities from tissue enzymes, but the starter culture influences flavour too because of its aroma-generating metabolic activities. For instance, flavour variability between chorizo manufacturers has been related to ester formation, attributed to different starter cultures employed, followed by the effects of smoking (phenols) and spices (terpenes) if applied (Ansorena et al., 2001). Usually, Mediterranean sausages are also inoculated on the surface with moulds or yeasts that contribute to the sensory properties as well.

Proteolytic enzymes, principally those endogenous to the meat, are of major importance for flavour. Meat proteases, particularly cathepsin D-like enzymes, seem to be responsible for proteolysis and peptide formation during fermentation (Hierro et al., 1999; Molly et al., 1997), while microbial enzymes rather act on the released oligopeptides during the later stages of ripening (Hugas and Monfort, 1997; Lizaso et al., 1999; Sanz et al., 1999; Hughes et al., 2002). Microbial proteolytic activity against meat proteins is low under conditions found in fermented sausages (Kenneally et al., 1999), but a certain activity, albeit to a minor extent and in a strain dependent manner, may partly contribute to initial protein breakdown (Molly et al., 1997; Sanz et al., 1999; Fadda et al., 1999a,b, 2002b). More importantly, the peptides generated by muscle proteolysis can be taken up by bacteria that further split them intracellularly into amino acids and may convert them to aroma components (see below).

Lipolysis is believed to play a central role in aroma formation. It leads to the release of free fatty acids and is mainly due to tissue lipases (Kenneally et al., 1998b; Galgano et al., 2003), although bacterial lipolytic activity has been described too, in particular by staphylococci (Hierro et al., 1997; Hugas and Monfort, 1997; Montel et al., 1998). Short chain fatty acids ($C<6$) lead to strong cheesy odours, whereas medium and long chain fatty acids can act as precursors (Ansorena et al., 2001). Lipolysis is only the first step in the process and is followed by further oxidative degradation of the liberated fatty acids into alkanes, alkenes, alcohols, aldehydes, ketones, and furanic cycles (Viallon et al., 1996; Chizzolini et al., 1998). The bacterial flora plays a role in the oxidation of free fatty acids although non-enzymatic reactions occur too (Molly et al., 1996; Lizaso et al., 1999).

With the exception of post mortem glycolysis, carbohydrate catabolism is mainly of bacterial origin (both LAB and GCC) and compounds such as lactic acid, acetic acid, ethanol, acetoin, and diacetyl, all may play a role in the complexity of sausage flavour (Viallon et al., 1996).

The use of selected strains that produce interesting aroma components as functional starter cultures could lead to more tasty sausages as well as to a reduction of the ripening time.

Still, one has to bear in mind that the raw material (e.g. type of meat and spices) and the sausage technology (e.g. fermentation temperature, salting, and ripening time) will interact with the metabolism of the starter culture (Selgas et al., 1995; Masson et al., 1999; Stahnke, 1999b; Olesen and Stahnke, 2003, 2004). Developing of functional starters for improved flavour needs thus to take into account knowledge about raw materials, technology, and sensory quality, and should be targeted to specific applications. Moreover, starters should not possess undesirable characteristics, such as the formation of toxic compounds (see Section 7.3) or too high quantities of acetic acid or acetoin. Acetoin production, stimulated by low pH and low sugar availability at the end of the ripening period, may lead to fermented sausages with a dairy product odour (Montel et al., 1996; García-Varona et al., 2000).

3.2. Lactic acid bacteria (LAB)

The primary contribution of LAB to flavour generation is ascribed to the production of large amounts of lactic acid and some acetic acid, although they also produce volatiles through fermentation of carbohydrates (Molly et al., 1996). They usually do not possess strong proteolytic or lipolytic properties, although a degree of peptidase and lipase activity has been observed for some meat strains.

Exopeptidases from meat lactobacilli contribute, in conjunction with muscle aminopeptidases, to the generation of free amino acids, contributing to flavour (Demeyer et al., 2000). LAB isolated from Greek sausages exhibited high in vitro leucine and valine aminopeptidase activities (Papamanoli et al., 2003). However, lactobacilli and pediococci display low catabolism of branched-chain amino acids, and hence do not play a major role in the formation of typical sausage aroma compounds such as 3-methyl butanal, as it is the case for staphylococci (Larrouture et al., 2000).

Little information is available about the lipolytic activity of lactobacilli during sausage fermentation, but some in vitro activity has been documented for *Lb. sakei*, *Lb. curvatus*, and *Lb. plantarum* (Hugas and Monfort, 1997; Lopes et al., 1999; Papamanoli et al., 2003). However, lipases from lactobacilli often display little or no activity under conditions found in fermented sausages (Kenneally et al., 1998a; Demeyer et al., 2000), although for some the production of lipase appears to be significant under conditions relevant for sausage ripening (Lopes et al., 1999).

In addition to lactobacilli, other LAB may be added to the sausage batter to influence flavour. Enterococci display several metabolic activities such as proteolytic, lipolytic, and esterolytic activities (Sarantinopoulos et al., 2001). They play an important role in the ripening and the development of flavour in several traditional Mediterranean cheeses (Centeno et al., 1996; Suzzi et al., 2000; Franz et al., 2003; Giraffa, 2003), and it is speculated that they may also be of importance in traditional fermented sausages (Franz et al., 2003; Hugas et al., 2003). Also, the addition of *Lactococcus lactis* subsp. *cremoris* could result in a higher amount of free amino acids (Herranz et al., 2004). Finally, a strain of *Carnobacterium piscicola* has been suggested as a new starter culture because of its high formation of aroma compounds derived from leucine metabolism, similar to the ones formed by staphylococci (see Section 3.3) (Larrouture-Thiveyrat and Montel, 2003). Besides the necessary amino acid converting enzymes, strains need also to be able to possess specific peptide uptake mechanisms to transfer the required amino acids intracellularly, since peptides, rather than free amino acids, are the preferred substrate for lactic acid bacteria. Indeed, no relation could be found between volatile products of amino acid degradation and free amino acid concentration (Bolumar et al., 2001).

The major contribution of LAB to flavour seems, however, to be limited to their carbohydrate catabolism, mainly the production of organic acids, whereas GCC appear to be more appropriate for the generation of specific aroma compounds.

3.3. Gram-positive, catalase-positive cocci (GCC)

To ensure sensory quality of fermented sausages, the contribution of GCC is needed (Hugas and Monfort, 1997). Staphylococci, in particular *Staph. xylosus* and *Staph. carnosus*, modulate the aroma through the conversion of amino acids (particularly the branched-chain amino acids leucine, isoleucine, and valine) and free fatty acids (Stahnke et al., 2002; Beck et al., 2004; Olesen et al., 2004; Tjener et al., 2004a,b). Aroma generation depends however on sausage technology and variety. For fast-ripened sausages, increasing inoculum levels of staphylococci may increase methyl-branched aldehyde production, whereas in slow-ripened sausages the situation is more complex (Tjener et al., 2004b). In the latter case, aroma production is particularly pronounced (Tjener et al., 2004a) and high inoculation levels favour the formation of methyl-branched acids and sulphites, whereas low levels favour diacetyl and ethyl ester production (Tjener et al., 2004b). It is thus possible to modify sausage aroma profiles by changing the inoculation level of the *Staphylococcus* starter culture. In addition, additives such as nitrate, nitrite, or ascorbate, precultivation parameters, and environmental factors clearly influence the generation of aroma compounds (Olesen and Stahnke, 2003, 2004; Olesen et al., 2004).

The use of well-selected strains that generate high amounts of aroma components could permit to achieve improved sensory qualities and/or to accelerate the meat fermentation process. Selection of appropriate staphylococci in view of the application will be crucial. Strains of *Staph. xylosus*, for instance, predominate in Southern European salamis, which are characterised by a rounded aroma and a less acidic taste, and have been recommended when production of very aromatic sausage is intended (Samelis et al., 1998). The species has been shown to produce, amongst others, 3-methyl-1-butanol, diacetyl, 2-butanone, acetoin, benzaldehyde, acetophenone, and methyl-branched ketones (Stahnke, 1999a; Søndergaard and Stahnke, 2002). In comparison, *Staph. carnosus* is believed to direct more of the branched-chain amino acids into methyl-branched aldehydes (e.g. 3-methyl-butanal, 2-methyl-butanal, and 2-methyl-propanal) and their acids (Masson et al., 1999; Stahnke, 1999a; Søndergaard and Stahnke, 2002; Larrouture-

Thiveyrat and Montel, 2003). The compounds 3-methyl-butanal and 3-methyl-butanoic acid, derived from leucine (Fig. 1), have been linked with sausage aroma (Stahnke, 1995; Montel et al., 1996). Moreover, they can further be converted into 'fruity' esters (e.g. ethyl 2- or 3-methyl-butanoate), which are believed to occur mainly in products with high contents of acids and alcohols, and where microbial esterase activity is low (Montel et al., 1996).

The presence of an incomplete β-oxidation pathway in staphylococci explains the formation of methyl ketones (2-pentanone and 2-heptanone) as being derived from intermediates of this pathway (Montel et al., 1996; Stahnke, 1999a; Engelvin et al., 2000; Fadda et al., 2002a). β-Ketoacyl-CoA esters are deacylated into β-ketoacids by a thioesterase and then decarboxylated to the methyl ketone.

Besides contributing to flavour, GCC also prevent the formation of off-flavours and can be used to control the oxidation of unsaturated fatty acids, due to their nitrate reductase and antioxidant activities (Montel et al., 1998; Barrière et al., 2001a,b).

In conclusion, selected *Staph. carnosus* or *Staph. xylosus* strains, with specific peptide uptake systems and branched-chain amino acid converting and fatty acid oxidising activities, could be used as functional starter cultures to obtain a tastier end-product. For instance, it has been shown that sausages with *Staph. carnosus* 833 mature more than 2 weeks faster than control sausages (Stahnke et al., 2002). Maturity correlates significantly with higher amounts of branched-chain aldehydes and alcohols arising from the breakdown of branched-chain amino acids, and branched- and straight-chain methyl ketones in turn derived from microbial β-oxidation of fatty acids.

3.4. Moulds and yeasts

Moulded sausages are very common in the Mediterranean area. It has been shown that the superficial inoculation of the sausage with atoxigenic moulds, e.g. *Penicillium* or *Mucor* species, contributes to sensory quality (Bruna et al., 2000, 2001, 2003; García et al., 2001). This contribution is mediated by lactate oxidation, proteolysis, degradation of amino acids, lipolysis, lipoxidation, the delay of rancidity, and reduced water loss due to slower evaporation (Sunesen and Stahnke, 2003; Benito et al., 2004; Sunesen et al., 2004). Moreover, moulds contribute to the overall attractiveness of the end-product due to their characteristic white or greyish appearance, to the stabilisation of colour through catalase activity, oxygen consumption and protection against light, and to easy skin peeling. A characteristic popcorn odour in mould-fermented sausages has been ascribed to 2-acetyl-1-pyrroline, which may be caused by conversion of proline, often found in sausage collagen casings, by the moulds (Sunesen and Stahnke, 2003). However, as with bacterial starter cultures, the selection of mould starter strains should be done carefully since the proteolytic and lipolytic capabilities, and hence the effect on the end-product, can significantly differ between strains and depend on the applied technology (Selgas et al., 1995, 1999; Sunesen and Stahnke, 2003).

The role of yeasts in sausage flavour formation is not well characterized. In model sausage minces, Olesen and Stahnke (2000) have observed that *Candida utilis* is able to produce several volatile compounds, in particular esters and alcohols, of which many are probably derived from branched-chain amino acids, whereas *D. hansenii* has very little effect on the production of volatile compounds. In other studies, *Debaryomyces* spp. affects proteolysis (Durá et al., 2004) and volatile generation (Flores et al., 2004). Appropriate amounts of Debaryomyces influence volatile production by inhibiting lipid oxidation due to its antioxidative effect and by promoting the generation of ethyl esters. However, too large amounts of Debaryomyces may generate high amounts of acids (e.g. 2-methyl-propanoic and 2- and 3-methyl-butanoic acid) that mask the positive effect. Also, due to the fungistatic effect of garlic or the presence of other starter cultures, yeast cultures may die out before the ripening process ends, and fail to improve sensory quality (Olesen and Stahnke, 2000).

4. Functional starter cultures for a safer product

4.1. Bacteriocin production

The main antimicrobial effect responsible for safety is evidently the rate of acidification of the raw meat (Lücke, 2000). Nevertheless, certain antimicrobials such as bacteriocins may also play a role, in particular in slightly acidified products or to eliminate undesirable microorganisms that display acid tolerance (e.g. *L. monocytogenes*).

Bacteriocins produced by LAB are antibacterial peptides or proteins that kill or inhibit the growth of other Gram-positive bacteria (De Vuyst and Vandamme, 1994; Cintas et al., 2001; Cleveland et al., 2001; Diep and Nes, 2002). They often have narrow inhibitory spectra and are most active towards closely related bacteria likely to occur in the same ecological niche (Eijsink et al., 2002). LAB produce a diversity of bacteriocins that are generally active towards other LAB, contributing to the competitiveness of the producer, but also towards foodborne pathogens such as *L. monocytogenes*. The so-called class IIa

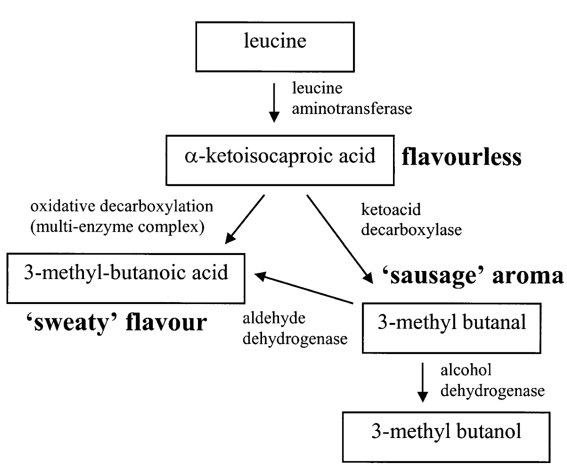

Fig. 1. Hypothetical pathway for the conversion of leucine into aroma compounds (after Stahnke, 1995, 1999a,b; Masson et al., 1999; Schmidt and Berger, 1998).

bacteriocins are particularly active towards *Listeria* (Ennahar et al., 1999). The application of bacteriocin-producing LAB in the meat industry offers therefore a way of natural food preservation (Stiles and Hastings, 1991; McMullen and Stiles, 1996; Hugas and Monfort, 1997; Hugas, 1998; De Martinis et al., 2002).

Lactobacilli sausage isolates frequently produce bacteriocins or bacteriocin-like compounds, as has been shown for *Lb. sakei* (Sobrino et al., 1991; Tichaczek et al., 1992; Garriga et al., 1993; Samelis et al., 1994a; De Martinis and Franco, 1998; Aymerich et al., 2000b; Rosa et al., 2002; Tantillo et al., 2002), *Lb. curvatus* (Tichaczek et al., 1992; Sudirman et al., 1993; Mataragas et al., 2002), *Lb. plantarum* (Garriga et al., 1993; Rekhif et al., 1995; Enan et al., 1996; Aymerich et al., 2000b; Messi et al., 2001), *Lb. brevis* (Benoit et al., 1994), and *Lb. casei* (Vignolo et al., 1993).

The use of bacteriocin-producing *Lb. sakei* as starter cultures permits to decrease *Listeria* levels in fermented sausage (Schillinger et al., 1991; Hugas et al., 1995; Hugas et al., 1996; De Martinis and Franco, 1998). Antilisterial effects have also been demonstrated with bacteriocinogenic *Lb. curvatus* (Hugas et al., 1996; Dicks et al., 2004) and *Lb. plantarum* (Campanini et al., 1993; Dicks et al., 2004) sausage starter cultures.

In certain products, e.g. in American-style sausages fermented at elevated temperatures (27–38 ° C versus 20–25 °C), bacteriocin-producing pediococci rather than lactobacilli are used (Berry et al., 1990; Foegeding et al., 1992; Lahti et al., 2001).

Many enterococci from food origin are known to produce bacteriocins with high antilisterial activity (Franz et al., 1999; De Vuyst et al., 2003), including enterococci isolated from fermented sausage (Aymerich et al., 1996; Casaus et al., 1997; Cintas et al., 1997, 1998, 2000; Herranz et al., 1999, 2001; Sabia et al., 2002). The use of bacteriocin-producing enterococci is well documented in cheese manufacture (Giraffa et al., 1995; Sarantinopoulos et al., 2002; Foulquié Moreno et al., 2003), but information on their potential as bioprotective cultures in the meat industry is scarce (Hugas et al., 2003; Leroy et al., 2003a). Bacteriocin-producing *Enterococcus faecium* strains have been included into the starter culture in Spanish-style dry fermented sausage to effectively inhibit *Listeria* (Callewaert et al., 2000). The enterococci were partially competitive in the early stages of the fermentation, reducing listerial counts, but disappeared during further ripening. In addition, kinetic studies have indicated that bacteriocin production by *Ent. faecium* RZS C5 occurs in the very early growth phase (Leroy and De Vuyst, 2002; Leroy et al., 2003b). These observations suggest that bacteriocin-producing enterococcal strains may be used as co-cultures that are effective during the first stages of the fermentation process. Also, the bacteriocin-producing *Enterococcus casseliflavus* IM 416K1 has been applied in the production of cacciatore sausages, the habitat from which the strain was isolated, leading to the elimination of *L. monocytogenes* (Sabia et al., 2003). As an alternative to using enterococci, application of enterococcal bacteriocins as additives to the sausage mixture may reduce *Listeria* counts (Lauková et al., 1999; Sabia et al., 2003). The addition of enterocins A and B significantly reduced *L. innocua* counts, while the application of the producer strain *Ent. faecium* CTC 492 did not, probably due to an insufficient enterocin production (Aymerich et al., 2000a).

Bacteriocin-producing lactococci (Rodríguez et al., 1995; Noonpakdee et al., 2003) and a bacteriocin-producing *Leuconostoc mesenteroides* (Mataragas et al., 2003) have been isolated from fermented sausage too. Bacteriocin-producing *L. lactis* strains have been used as new, functional starters for fermented sausage manufacture, despite the fact that they are not particularly adapted to sausage technology, e.g. displaying sensitivity to nitrite (Coffey et al., 1998; Scannell et al., 2001; Benkerroum et al., 2003).

Based on the above, the use of bacteriocin producers as new starter cultures may offer considerable food safety advantages, without risk for human health due to toxicological side effects (Cleveland et al., 2001). It represents a safe way of ‘natural’ food preservation that, most likely, has always occurred in fermented foods for centuries. Moreover, in situ bacteriocin production does generally not lead to organoleptic or flavour imperfections, as has been shown through taste panels with different strains (Hugas et al., 1995; Coffey et al., 1998). Still, some disadvantages have to be considered related to the fact that bacteriocin activity in situ is lower than may be expected from in vitro experiments (Schillinger et al., 1991; Campanini et al., 1993). Activity may be less effective in the sausage due to low production, genetic instability, the inability to uniformly distribute bacteriocin throughout the product, low solubility of the bacteriocin, inactivation by meat proteases, resistance of the target strain, and interference by meat components, in particular adsorption to fat and meat particles (Knorr, 1998; Ennahar et al., 1999; Cleveland et al., 2001; Aasen et al., 2003; Dicks et al., 2004). It is recommended to use strains that are well adapted to the sausage environment, preferably sausage isolates, for optimal performance and bacteriocin production (Leroy et al., 2002b). For instance, the results obtained during sausage fermentation with *Lb. plantarum* (Campanini et al., 1993) are less convincing than the results that are generally obtained with *Lb. sakei* or *Lb. curvatus*. In this context, it has been shown kinetically that the sausage isolates *Lb. sakei* CTC 494 (Leroy and De Vuyst, 1999a), *Lb. curvatus* LTH 1174 (Messens et al., 2003), and *Lb. curvatus* L442 (Mataragas et al., 2003) optimally produce bacteriocin under conditions of pH and temperature that prevail during European sausage fermentation. Also, appropriate cultures are to be selected according to the specific formulation and the technology used (Hugas and Monfort, 1997). For instance, pediococci are more suitable than lactobacilli when a fast fermentation at a high temperature is desired. Also, the performance of bacteriocinogenic LAB as starter or co-cultures depends on the sausage ingredients and the recipe used. For instance, performance is different if a nitrate or a nitrate/nitrite recipe is followed (Hugas et al., 1996) and depends on the use of additives such as sodium chloride and pepper (Leroy and De Vuyst, 1999b; Hugas et al., 2002; Verluyten et al., 2003, 2004).

The development of heterologous expression systems for bacteriocins may offer advantages over native systems, such as facilitating the control of bacteriocin gene expression, achieving higher production levels, producing multiple bacteriocins, or acquisition of antimicrobial properties by industrial strains (Rodríguez et al., 2002). Also, in some cases the engineering of bacteriocin molecules could lead to more active compounds (Miller et al., 1998) or increase their stability (Johnsen et al., 2000). However, such techniques are not easily acceptable by the consumer.

Evidently, bacteriocins are not meant to be used as the sole means of food preservation, but should be appropriately integrated in a multihurdle preservation system, at all times respecting good manufacturing practice.

4.2. Other antimicrobials

As an alternative to bacteriocin-producing starter cultures, strains that produce other specific antimicrobial compounds may be proposed.

The introduction of the lysostaphin gene of *Staphylococcus simulans* biovar. *staphylolyticus* into meat starter lactobacilli (Gaier et al., 1992; Cavadini et al., 1996, 1998) or *P. nalgiovense* (Geisen et al., 1990) can be used to prevent the growth of *Staph. aureus*. Lysostaphin is an endopeptidase that specifically cleaves the glycine–glycine bonds unique to the interpeptide cross-bridge of the *Staph. aureus* cell wall.

Another point of interest to improve food safety could be the use of *Lactobacillus reuteri*-containing starter cultures that produce reuterin or reutericyclin (Ross et al., 2002; Gänzle and Vogel, 2003). Reuterin is a mixture of monomeric, hydrated monomeric and cyclic dimeric forms of β-hydroxy-propionaldehyde that has a broad spectrum of activity, including fungi, protozoa and a wide range of Gram-positive and Gram-negative bacteria. Reutericyclin is a tetramic acid antibiotic that is active towards Gram-positive bacteria. Application of purified reuterin on the surface of Turkish-style beef sausage was able to inhibit growth of *L. monocytogenes* but not *Salmonella* (Kuleaşan and Çakmakçi, 2002).

New antimicrobials with application possibilities are still being discovered. For instance, strains of *Lb. plantarum* produce a number of interesting compounds, including a mixture of low-molecular-mass molecules that act synergistically with lactic acid (Niku-Paavola et al., 1999), 3-hydroxy fatty acids (Sjögren et al., 2003), antifungal cyclic peptides (Ström et al., 2002), phenyllactic acid, and 4-hydroxyphenyllactic acid (Ström et al., 2002; Valerio et al., 2004). Most of these compounds are active towards moulds and yeasts, but some of them also towards Gram-positive and Gram-negative bacteria, including *Listeria* and *Salmonella*, respectively.

Microbial inhibitory action is not always ascribed to specific metabolites and remains frequently unspecified. A commercial starter culture to which strains of *Lactobacillus acidophilus*, *Lb. paracasei*, or *Bifidobacterium lactis* from intestinal or dairy origin were added, increased the safety of Hungarian salami, because these cultures displayed strong inhibition of *L. monocytogenes* and *E. coli* O111 during sausage fermentation (Pidcock et al., 2002). The latter reduction was one to two log units higher than when the commercial starter was used alone, but the mechanism of action has not been studied. Also, staphylococci and lactobacilli isolated from spontaneously fermented Greek sausage exhibited unprecised activity towards *L. monocytogenes* and *Staph. aureus* (Papamanoli et al., 2002, 2003). Application of a *Staph. xylosus* sausage isolate that produces an antagonistic substance resulted in *Listeria*-free Italian sausage (Villani et al., 1997). The bioprotective cultures *Lactobacillus rhamnosus* E-97800, *Lb. rhamnosus* LC-705, and *Lb. plantarum* ALC01 displayed antilisterial activity in Northern-European type dry sausages (Työppönen et al., 2003a).

5. Functional starter cultures for a more reliable production process

The use of bacteriocin-producing starter cultures may not only contribute to food safety (see Section 4.1), but also to the prevention of food spoilage. In particular, the growth of LAB that produce hydrogen peroxide or cause sliminess, off-odours or off-flavours may be inhibited (Ennahar et al., 1999). Because bacteriocin producers are more competitive than non-producing variants, the application of such strains as new starter cultures may improve the competitiveness of the starter and lead to a more controlled and standardized fermentation process. *Lb. curvatus* LTH 1174, for instance, achieved a dominance of 97% after inoculation at 10^5 cfu g^{-1} in German-style fermented sausage (Vogel et al., 1993), whereas rep-PCR has confirmed the competitiveness of *Lb. sakei* CTC 494 and *Lb. curvatus* LTH 1174 in Belgian-type fermented sausage (Foulquié-Moreno M.R., Verluyten J., Vancanneyt M., Adriany T., Leroy F., Swings J., and De Vuyst L., unpublished results).

The use of strains with antioxidant properties due to catalase or superoxide dismutase, for instance *Staph. carnosus*, may help to inhibit lipid oxidation and prevent deterioration of colour and texture as well as the formation of toxic compounds (Barrière et al., 2001a).

6. Functional starter cultures with a technological advantage

The use of functional starter cultures may be useful to reduce levels of nitrite and nitrate, which are under discussion because of their contribution to the formation of health-affecting nitrosamines. Nitrite and nitrate are required in sausage fermentation technology as curing agents for microbial stability and colour formation (Campbell-Platt and Cook, 1995). Bacteriocin-producing strains may be used to replace part of the preservative action (see Section 4.1), whereas the use of strains that are able to generate nitrosylated derivatives of myoglobin, i.e. to convert brown metmyoglobin into red myoglobin derivatives, could be useful to partially take over the colouration function of the curing agents. The latter possibility has been demonstrated with strains of *Lactobacillus fermentum* in smoked sausages (Moller et al., 2003).

Also, the use of strains that lead to accelerated ripening of the sausage may yield technological advantages, resulting in a reduction of storage time and an increased profit margin and competitiveness of the end product (Fernández et al., 2000). Bacterial strains, such as lactococci, that display strong proteolytic activity or autolysis during maturation could be applied as new starter cultures (Blom et al., 1996; Hagen et al., 1996; Zambonelli et al., 2002; Herranz et al., 2004).

7. Functional starter cultures for a healthier product

7.1. Probiotic starter cultures

In view of the recent interest for healthier meat products (Jiménez-Colmenero et al., 2001), probiotic starter cultures seem promising. Probiotics consist of live microorganisms as food or feed supplement, which, when ingested in sufficient amounts and when applied to animal or man, beneficially affect the host by improving the properties of the indigenous microbiota in the intestinal tract (Holzapfel et al., 1998; Saarela et al., 2000). Some strains of *Lactobacillus* are good candidates as probiotic cultures because they are normal components of the gut flora and display health-promoting properties in vivo. Enterococci are also marketed as probiotic preparations in health care (Franz et al., 1999, 2003). The use of probiotic strains is already very common in the dairy industry. In the future, probiotic LAB strains may be used in functional, probiotic meat products too (Hammes and Hertel, 1998; Incze, 1998; Työppönen et al., 2003b). In some countries, however, meats do not have a reputation of being "health foods", which may compromise their marketing potential (Lücke, 2000).

A first strategy consists of checking existing commercial starter cultures (Erkkilä and Petäjä, 2000) or sausage isolates (Papamanoli et al., 2003; Pennacchia et al., 2004) for probiotic properties. For instance, the commercial meat starter strains *Lb. sakei* Lb3 and *P. acidilactici* PA-2 can be regarded as potentially probiotic starter cultures because of their survival capacities under simulated gastrointestinal conditions (Erkkilä and Petäjä, 2000).

Alternatively, it may be investigated if strains with probiotic properties, for instance intestinal isolates, perform well in a meat environment during sausage fermentation. Such strains should be strong competitors against the natural meat microbiota and grow to numbers that might have health-promoting effects. Several lactobacilli of intestinal origin have been shown to survive the sausage manufacturing process and can be detected in high numbers in the end-product (Arihara et al., 1998; Sameshima et al., 1998; Erkkilä et al., 2001a,b; Pidcock et al., 2002). It is certainly an asset if these new meat starter cultures also contribute to food safety. In this matter, the intestinal lactobacilli *Lb. rhamnosus* FERM P-15120 and *Lb. paracasei* subsp. *paracasei* FERM P-15121 have been shown to inhibit the growth and enterotoxin production of *Staph. aureus* to the same extent as a commercial *Lb. sakei* starter culture in sausages fermented at either 20 °C or 35 °C (Sameshima et al., 1998). On the other hand, the intestinal strain *Lb. acidophilus* FERM P-15119 could not satisfactorily decrease *Staph. aureus* numbers, indicating the importance of careful strain selection.

In all cases, it should be checked that the sensory properties are not negatively affected when strains from non-meat origin are used. The (potential) probiotic strains *Lb. rhamnosus* GG, *Lb. rhamnosus* LC-705, *Lb. rhamnosus* E-97800 and *Lb. plantarum* E-98098 have been tested as functional starter culture strains in Northern European sausage fermentation without negatively affecting the technological or sensory properties, with a (minor) exception for *Lb. rhamnosus* LC-705 (Erkkilä et al., 2001a,b). Similarly, the intestinal isolates *Lb. paracasei* L26 and *B. lactis* B94 had no negative impact on the sensory properties of the product when applied in conjunction with a traditional meat starter culture (Pidcock et al., 2002).

Ultimately, human studies should confirm the functionality of such probiotic fermented sausages. The daily consumption of 50 g of probiotic sausage by healthy volunteers, containing *Lb. paracasei* LTH 2579, during several weeks has been shown to modulate various aspects of host immunity but there was no significant influence on serum concentration of different cholesterol fractions and triacylglycerides (Jahreis et al., 2002). In faecal samples, there was a statistically significant increase in the numbers of *Lb. paracasei* LTH 2579, but not in the faeces of all volunteers.

7.2. Nutraceutical and micronutrient producers

The use of strains that produce micronutrients and nutraceuticals, e.g. vitamins and conjugated linoleic acid (CLA), as starter cultures may lead to health-promoting meat products. A nutraceutical is defined as any substance that may be considered a food or part of a food that provides medical or health benefits, including the prevention and treatment of disease (Andlauer and Fürst, 2002).

Most LAB have limited biosynthetic capacities for vitamin production. However, careful selection could reveal strains that produce vitamins in considerable amounts (Morishita et al., 1999; Lin and Young, 2000; Sybesma et al., 2003). Moreover, through metabolic engineering it could be possible to develop starter cultures for the in situ production of vitamins, such as folic acid and riboflavin (Hugenholtz et al., 2002).

Several health-promoting properties have been ascribed to CLA, including anti-atherogenic action, inhibition of carcinogenesis, anti-diabetic effects, enhancement of immunological function, and reduction of body fat (Belury, 2002). Generally, CLA is found in meat from ruminants because of the bacterial hydrogenation of dietary linoleic acid in the rumen. However, meat from ruminants is not so often used in fermented sausages as is pork, in particular in Europe and North America. In addition to rumen-derived bacteria, it has been shown that other bacterial species, including lactobacilli, bifidobacteria and propionibacteria, can produce CLA (Jiang et al., 1998; Alonso et al., 2003; Coakley et al., 2003). This opens perspectives to increase the nutritional value of fermented sausages when such strains are used as new starter cultures.

7.3. Reduction of undesirable compounds

It is important during strain selection that no undesirable compounds such as toxins, biogenic amines, or D(−)-lactic acid, that could aversely affect health, are formed.

If surface mould growth is desirable, it must be checked if the mould starter culture produces mycotoxins or antibiotics (López-Díaz et al., 2001; Holzapfel, 2002; Sunesen and Stahnke, 2003). The use of moulds that are free of mycotoxin production as starter cultures could be useful in outcompeting mycotoxin-producing strains from the house flora. Moulds may also produce green or dark spots that are not acceptable to most consumers or have a negative impact on flavour and taste (Sunesen and Stahnke, 2003).

During the ripening of fermented sausages, biogenic amines such as tyramine, histamine, tryptamine, cadaverine, putrescine, and spermidine, may be formed by the action of microbial decarboxylases on amino acids that originate from meat proteolysis (Komprda et al., 2004). Microbial decarboxylation reactions may be ascribed to both the microorganisms that were introduced via the starter culture and the ones that constitute part of the natural population of the meat. In general, starter bacteria have limited tyrosine-decarboxylating activity, but contaminant non-starter LAB, in particular enterococci, are believed to be responsible for tyramine production (Ansorena et al., 2002). The use of decarboxylase-negative starter cultures that are highly competitive and fast acidifiers prevents the growth of biogenic amine producers and leads to end-products nearly free of biogenic amines (Bover-Cid et al., 2000a,b; González-Fernández et al., 2003; Suzzi and Gardini, 2003), as long as the raw material is of sufficient quality (Bover-Cid et al., 2001). Also, the introduction of starter strains that possess amine oxidase activity might be a way of further decreasing the amount of biogenic amines produced in situ (Leuschner and Hammes, 1998; Martuscelli et al., 2000; Fadda et al., 2001; Gardini et al., 2002; Suzzi and Gardini, 2003). Although it is known that the superficial inoculation with *P. camemberti* in cheeses increases the concentration of certain amines, the production of amines by moulds in fermented sausages does not appear significant but has not been fully studied yet (Bruna et al., 2003).

The nature of the lactic isomer produced by the LAB strains is of concern, since high levels of the D(−)-lactic acid isomer are not hydrolysed by lactate dehydrogenase in humans and are thus capable of causing acidosis. Therefore, strains producing L(+)-lactic acid should be preferably selected (Holzapfel, 2002).

8. Conclusion and future perspectives

Fermented sausages not only contribute to regional pride and culture identity, they are also highly appreciated specialities with gastronomic value. Examples in Europe include varieties of Italian salami, Spanish chorizo, French saucisson, Portuguese lingüiça, German Mettwurst, and many others. Products from different origins clearly posses different sensory qualities (Schmidt and Berger, 1998). Moreover, traditional fermented sausages are a rich source of interesting wild-type microorganisms. The deliberate use of such microorganisms in large-scale fermentations could help to introduce advantages of artisan specialities in industrial products. Also, a better understanding of the microbiota and population dynamics of artisan fermented sausages could improve the fermentation process and the safety and standardization of such products and hence reduce economic losses, in this way reinforcing their position in a market characterized by ever increasing globalization and industrialization. Useful information on the characterization of the microbiota and the study of population dynamics and strain dominance in meat products is to be expected from molecular techniques, e.g. denaturing gradient gel electrophoresis (DGGE) (Rebecchi et al., 1998; Zhang and Holley, 1999; Cocolin et al., 2001; Rantsiou et al., 2004, 2005).

Previous papers have aimed at applying mathematical modelling, biokinetics, and predictive microbiology, which are classically used to study 'negative' aspects related to food pathogens, toxin production, and food spoilage, to simulate and predict the behaviour of beneficial microorganisms, in casu functional starter cultures (Leroy et al., 2002a,b). For instance, the kinetics of *Lb. sakei* CTC 494 (Leroy and De Vuyst, 1999a,b) and *Lb. curvatus* LTH 1174 (Messens et al., 2003; Verluyten et al., 2003, 2004) have been studied in detail in function of the sausage environment. In the future, rather than studying the food ecology of a single population, research will have to focus on a synecological approach, not exclusively taking into account interactions with the food environment but also with other microbial populations.

Recent advances in the field of metabolic engineering, genomics, and bioinformatics are expected to contribute to the future development of new, functional starter cultures (Henriksen et al., 1999; Kuipers et al., 2000; de Vos, 2001; Hansen, 2002; Hugenholtz et al., 2002). Exploration studies of the natural diversity of wild strains occurring in traditional, artisan foods, including comparative genomics, microarray analysis, transcriptomics, proteomics, and metabolomics, may generate a framework leading to the generation of new, industrial starters with increased diversity, stability, and industrial performance. It will permit rapid, high-throughput screening of promising wild strains with interesting functional properties and lacking negative characteristics, as well as the construction of genetically modified starter cultures with a tailored functionality.

Bioinformatic tools can be used to search in genomes for essential components, for instance with regard to flavour development, such as peptidases, aminotransferases, enzymes for biosynthesis of amino acids, and transport systems for peptides and amino acids (van Kranenburg et al., 2002). Genetic engineering of starters can be performed for a variety of purposes where a suitable starter cannot easily be found in nature. However, it is currently difficult to predict in what direction the future regulatory requirements will influence innovation through biotechnology, in particular genetic modification (Hansen, 2002). One of the most promising applications is self-cloning, i.e. if the resulting strain does not contain DNA originating or derived from any other species than the host organism, which is currently exempted from the regulation on genetically modified organisms by the European Union (regulations 90/219 and 90/220) (Knorr, 1998). However, the use of genetically modified microorganisms in food and food processing remains controversial due to a lack of acceptance by consumers, especially in Europe (Grunert et al., 2000).

Besides the focus on functional characteristics of potential new starter cultures, negative aspects such as antibiotic resistance, virulence genes, and undesirable metabolite formation should not be overlooked. The control of antibiotic resistance, for instance, is a topic of importance since the high load of endogenous bacteria in meat raw material and the inoculation with starters may represent a problem concerning the spreading of antibiotic resistance (Cocconcelli et al., 2003; Gevers et al., 2003).

In addition, it should be emphasized that the performance of a selected starter culture should be seen in a context of the application, since functionality will depend on the type of sausage, the technology applied, the ripening time, and the ingredients and raw materials used (Leroy and De Vuyst, 1999a,b; Masson et al., 1999; Stahnke, 1999b; Hugas et al., 2002; Mataragas et al., 2003; Verluyten et al., 2003, 2004; Olesen and Stahnke, 2003, 2004; Olesen et al., 2004; Sunesen et al., 2004). For instance, interactions between animal variability in tissue enzymes and bacterial peptidase or oxidative activity should be investigated. Moreover, fermentation temperature and ripening time will affect microbial ecology and hence performance. Nevertheless, our knowledge in the fields of raw materials, technologies and quality characteristics of fermented meat products is rapidly expanding and industrial application possibilities of new, functional meat starter cultures are becoming more concrete.

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