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Selection criteria for lactic acid bacteria to be used as functional starter cultures in dry sausage production: An update

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Abstract

Lactic acid bacteria (LAB) have long been used as starter cultures in the production of fermented dry sausages and other meat-derived commodities. These cultures are generally designed to meet food safety, shelf-life, technological effectiveness and economic feasibility criteria. Besides all these traditional properties, novel starter cultures should take into account the risks posed by the formation of biogenic amines in food, and the development and spreading of bacterial resistance to antibiotics. Further, 'functional starters' could protect consumers from harmful bacteria either by a rapid acidification or by the production of antimicrobials (bacteriocins). Specially-selected cultures may also provide probiotic benefits, and, if properly modified, they may even be endorsed with nutraceutical traits. The present review discusses the technological and new selection criteria that should be taken into account when selecting LAB starter cultures for the production of fermented dry sausages.

Keywords: Dry sausage; Lactic acid bacterial; Functional starter cultures; Probiotics; Selection criteria

1. Introduction

Fermented dry sausage is defined as a mixture of comminuted fat and lean meat, salt, nitrate and/or nitrite, sugar and spices (mostly oregano and black pepper), which is stuffed into casings, subjected to fermentation and then allowed to dry (Hugas & Monfort, 1997). The quality of the final product is closely related to the ripening that takes place during drying. This process, which confers on the product its particular slice ability, firmness, color and flavor, is characterized by a complex interaction of chemical and physical reactions associated with the microbiological development of the batter flora (Ordóñez, Hierro, Bruna, & de la Hoz, 1999).

The spontaneous fermentation of dry sausages involves the participation of Lactic Acid Bacteria (LAB), Coagulase Negative Cocci (CNC; mostly *Staphylococcus* and *Kocuria* species), and, less importantly, yeasts and moulds. Most of the commercially available meat starter cultures contain mixtures of LAB and CNC. According to the definition of Hammes, Bantleon, and Min (1990), meat starter cultures are preparations that contain active or dormant microorganisms that develop the desired metabolic activity in the meat. They are, by definition, used to change the sensory properties of the food. LAB originating from fermented meats are particularly well adapted to the ecological niche of meat fermentation (Hugas & Monfort, 1997) and thus should be considered for selection as starter cultures.

Phenotypic methods relying on physiological or biochemical criteria have been widely used for LAB identification (Montel, Talon, Fournaud, & Champomier, 1991). In order to overcome the tediousness, ambiguousness, and time consumed by these methods, molecular methods such as rRNA hybridization probes (Nissen & Dainty, 1995), species-specific PCR (Aymerich et al., 2006; Kwon, Yang, Yeon, Kang, & Kim, 2004), PCR-denaturing gel

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electrophoresis (Cocolin et al., 2004), real-time PCR (Furet, Quenee, & Tailliez, 2004) have been developed for LAB species identification. Randomly amplified polymorphic DNA (RAPD)-PCR analysis has been used to estimate the biodiversity among LAB (Aymerich et al., 2006).

The main LAB genera isolated from dry sausages are *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Weissella* and *Enterococcus* (Ammor et al., 2005; Aymerich et al., 2006; Huerta, Jordano, Medina, & López, 2004; Martín, Garriga, Hugas, & Aymerich, 2005; Santos et al., 2005). The dominant species are usually members of *Lactobacillus*, although in certain slightly acidified sausages *Lactobacillus* and *Enterococcus* populations reach similar sizes (Ammor et al., 2005; Martín et al., 2005). Although *Lactobacillus curvatus* and *Lactobacillus plantarum* are commonly isolated from fermented sausages, *Lactobacillus sakei* remains the predominant LAB in southern European sausages (Montel, 1999; Rantsiou & Cocolin, 2006). Under the conditions prevailing in fermented sausages, *L. sakei* is more competitive than other lactobacilli, showing a shorter lag phase, a higher maximum growth rate, and a higher final cell density (Dossmann, Vogel, & Hammes, 1996).

L. sakei, *L. curvatus*, *L. plantarum*, *Lactobacillus pentosus*, *Lactobacillus casei*, *Pediococcus pentosaceus* and *Pediococcus acidilactici* are the species most used as commercial meat LAB starter cultures (Hammes & Hertel, 1998; Hugas & Monfort, 1997). The first stage in designing a starter culture for a meat commodity is to characterize the LAB strains isolated from the meat product in question and then select those best suited. In meat fermentations, the main function of LAB is to obtain a rapid pH drop of the batter, which in turn favours (i) product safety by inactivating pathogens, (ii) product stability and shelf life by inhibiting undesirable changes caused by spoilage microorganisms or abiotic reactions, and (iii) creates the biochemical conditions to attain the new sensory properties of the ripe products through modification of the raw materials (Lücke, 2000). Currently the use of starters as functional flora is gaining importance; designed starter cultures have properties additional to those of the more classic type, helping to optimize the sausage fermentation process, and to produce tastier, safer, and healthier products. The aim of the present review is to provide an update on the criteria that ought to be taken into account when selecting new LAB strains for use as starter cultures in dry sausage production.

2. Technological features

2.1. Rapid and adequate production of lactic acid

The production of organic acids – mainly lactic acid – from carbohydrates is the major role of LAB in sausage fermentation. This depends on several chemical, physical and microbiological reactions. While acidifying the batter, LAB participate in the coagulation of muscle proteins, resulting in the increased slice stability, firmness and cohesiveness of the final product (Hugas & Monfort, 1997; Ordóñez et al., 1999). They also enhance the spontaneous reduction of nitrites to nitric oxide, which reacts with the myoglobin to form nitrosomyoglobin, the compound responsible for the typical pink color of cured sausage (Hugas & Monfort, 1997). Moreover, they contribute to the flavor of the final product through the formation of noticeable acidic and vinegary (acetic acid) tastes. Acidic conditions are also thought to increase the activity of cathepsin D, which is responsible for muscle proteolysis (Molly et al., 1997). The production of organic acids is undoubtedly the determining factor on which the shelf life and the safety of the final product depends. The inhibition of pathogenic and spoilage flora is also dependent on a rapid and adequate formation of these organic acids. Finally, it has been reported that a rapid decrease in pH caused by amine-negative starter cultures can largely prevent biogenic amine (BA) accumulation in sausages (Maijala, Eerola, Aho, & Hirn, 1993).

The immediate and rapid formation of acid at the beginning of the fermentation process, and the production of sufficient amounts of organic acids allowing a pH below 5.1 to be reached, are therefore essential requirements of meat LAB starters. Excessive acid formation, however, is often associated with color defects (due to the inhibition of the CNC) and sometimes with gas formation – one of the most important problems in sausage fermentation (Buckenhüskes, 1993).

2.2. Growth rate at different temperatures, salt concentrations and pHs

The ability of the starter culture to compete with the natural microbiota of the raw material and to undertake the metabolic activities expected is conditioned by its growth rate and survival in the conditions prevailing in the sausage, i.e., an anaerobic atmosphere, rather high salt concentrations, low temperatures and low pH. The salt concentration is about 2% ($a_w = 0.94$–0.98) in the batter and can reach 15% ($a_w = 0.85$–0.86) in the final product (Lücke & Hechelmann, 1987; Montel, 1999). The manufacturing temperature ranges from 4 to 7 °C when preparing the batter (Baracco, Durand, Frentz, Jacquet, & Zert, 1990), from 18 to 24 °C during the fermentation period (Montel, 1999), and from 12 to 15 °C during the drying and ripening period (Montel, 1999). The initial pH of the batter, which is generally around 6.0 decreases during fermentation and reaches values between 4.6 and 5.1. Thereafter, yeasts, mostly *Debaryomyces hansenii*, increase the pH of the product (Cook, 1995), achieving final values which range from 5.1 to 5.5.

Thus, the growth rate at different temperatures (2–4 to 24 °C), the tolerance of salt concentrations of 2–10 (max 15)%, and of pHs in the range 4.2–6.0 are limiting factors affecting the persistence and competitiveness of the starter culture over the entire fermentation and ripening process. *L. sakei* can grow at 4 °C, in the presence of 6.5% NaCl, and at pH 4.2 (Ammor, Dufour, Zagorec, Chaillou, & Chevallier, 2005). At 15 °C and in the presence of 2% NaCl, this

meat-borne LAB shows growth rates which allow 0.55 generations to be produced per hour (Ammor et al., 2005). Its psychrotrophic character and salt tolerance may be due to its ability to efficiently accumulate osmo- and cryoprotective solutes such as betaine and carnitine, and to its cold stress response: *L. sakei* has more putative cold-stress genes than any other lactobacilli (Chaillou et al., 2005). A combination of mechanisms, including modification of carbohydrate metabolism (downregulation of glycolysis) and stimulation of oxidative stress may also increase its resilience to cold (Marceau, Zagorec, Chaillou, Mera, & Champomier-Verges, 2004).

2.3. Gas production from carbohydrates

Heterofermentative LAB are not suitable for sausage production because the formation of large amounts of carbon dioxide leads to holes of different sizes in the product (Buckenhüskes, 1993). In addition, these LAB produce concentrations of acetic acid that cause a pungent off-flavor.

2.4. Catalase activity and hydrolysis of hydrogen peroxide

Most lactobacilli are able to form hydrogen peroxide by oxidizing lactate. Hydrogen peroxide can interfere with the organoleptic properties of fermented meat products by increasing rancidity and the discoloration of the final product. Catalase hydrolyses hydrogen peroxide. Some LAB strains involved in meat fermentation, such as *L. sakei*, *L. plantarum*, *L. pentosus* and *P. acidilactici*, possess heme-dependent catalase activity which is active in meat products since these substrates contain heamin in abundance (Abriouel et al., 2004; Ammor et al., 2005; Mares, Neyts, & Debevere, 1994). Noonpakdee, Sitthimonchai, Panyim, and Lertsiri (2004) showed that the expression of the *katA* gene of *L. sakei* in the catalase-deficient species *L. plantarum* (isolated from sausage) resulted in catalase activity approximately three times higher than that seen in the original strain. The level of lipid oxidation in fermented meat products seeded with this catalase-modified starter culture was significantly lower than that seen in the original catalase-deficient strain. Thus, although weak compared to the constitutive catalase of CNC, the inducible catalase activity is a desirable property of meat LAB starter strains.

2.5. Nitrate and nitrite reduction

As mentioned above, while decreasing the batter pH, LAB participate in the formation of the typical pink color through the spontaneous reduction of nitrites to nitric oxide. Some meat LAB have also been reported to possess nitrate reductases and heme-dependent and heme-independent nitrite reductases (Hammes et al., 1990; Wolf, Arendt, Pfahler, & Hammes, 1990). These are directly involved in the mechanisms of nitrosomyoglobin formation. Thus, screening for nitrate and nitrite reductases is desirable, even though LAB undertake these activities at much lower intensities than CNC.

2.6. Proteolytic and lipolytic enzyme activities

LAB have only weak proteolytic action on myofibrillar proteins (Hammes et al., 1990; Molly et al., 1997; Sanz et al., 1999a). However, some *L. casei*, *L. plantarum*, *L. curvatus* and *L. sakei* strains actively contribute to the hydrolysis of the sarcoplasmic proteins (Fadda et al., 1999a, 1999b; Sanz et al., 1999b) and to the subsequent decomposition of peptides into amino acids. Several peptidase activities have been reported in *L. sakei*, *L. curvatus* and *L. plantarum* isolated from sausages (Fadda et al., 1999a, 1999b). Further, some *L. sakei*, *L. curvatus* and *L. plantarum* strains possess leucine and valine amino-peptidases, which contribute to the catabolism of proteins and peptides generating free amino acids, precursors of flavor compounds in the final product (Ammor et al., 2005; Papamanoli, Tzanetakis, Litopoulou-Tzanetaki, & Kotzekidou, 2003). Screening for proteinases, peptidases, and aminopeptidases activities is therefore recommended.

2.7. Tolerance to or even synergy with other microbial components of the starter

As mentioned earlier, meat starter cultures are mainly mixtures of LAB and CNC. Thus, to perform their expected functions, LAB starters must be able to tolerate or even show synergy with CNC starter components. Hammes et al. (1990) showed some *L. sakei* and *L. curvatus* strains to inhibit others used in meat starter cultures (in addition to unwanted flora), such as *Kocuria varians*. Therefore, the strains intended for use in any starter mixture should be checked for antagonism.

3. Safety

As aforementioned, one of the main roles of starter cultures is to improve safety by inactivating pathogens and spoilage microorganisms via acid production and bacteriocins. Furthermore, it is essential that starter cultures show no pathogenic or toxic activities. This general requirement has been widely reviewed (Hammes & Hertel, 1998; Hugas & Monfort, 1997). Two other issues are addressed here: the need to prevent antibiotic resistance and the production of biogenic amines.

3.1. Biopreservation

3.1.1. Acid production

As mentioned above, one of the main roles of meat LAB starter cultures is the rapid production of organics acids; this inhibits the growth of unwanted flora and enhances product safety and shelf-life. The antimicrobial effect of organic acids lies in the reduction of pH, and in the action of undissociated acid molecules (Podolak,

Zayas, Kastner, & Fung, 1996). It has been proposed that low external pH causes acidification of the cytoplasm. The lipophilic nature of the undissociated acid allows it to diffuse across the cell membrane (Kashket, 1987) collapsing the electrochemical proton gradient. Alternatively, cell membrane permeability may be affected, disrupting substrate transport systems (Snijders, van Logtestijn, Mossel, & Smulders, 1985).

The types and levels of organic acids produced during the fermentation process depend on the LAB strains present, the culture composition, and the growth conditions (Lindgren & Dobrogosz, 1990). L(+) lactic acid is more inhibitory than its D(−) counterpart (Benthin & Villadsen, 1995). Since the D(−) isomer is not hydrolyzed by human lactate dehydrogenase and may cause health problems, only strains producing mainly L(+) lactic acid should be selected (Buckenhüskes, 1993).

3.1.2. Bacteriocin production

During the last decade, interest in the bacteriocins produced by meat LAB increased dramatically, reflecting their growing importance with respect to the functional properties of starter cultures (Abee, Krockel, & Hill, 1995). A number of bacteriocins are produced by most LAB species involved in sausage fermentation, including *L. sakei* (Tichaczek, Nissen-Meyer, Nes, Vogel, & Hammes, 1992), *L. curvatus* (Tichaczek et al., 1992), *L. plantarum* (Enan, El-Essawy, Uyttendaele, & Debevere, 1996), and *P. acidilactici* (Foegeding, Thomas, Pilkington, & Klaenhammer, 1992).

Meat-borne LAB produce a range of bacteriocins that are generally active towards other LAB (contributing to the competitiveness of the producing strain) and food borne Gram–positive pathogens such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium perfringens* and *Bacillus cereus* (Aymerich, Garriga, Monfort, Nes, & Hugas, 2000; Enan et al., 1996; Messi, Bondi, Sabia, Battini, & Manicardi, 2001; Noonpakdee, Santivarangkna, Jumriangrit, Sonomoto, & Panyim, 2003). Bacteriocins exert their inhibitory action via the formation of pores in the cytoplasmic membrane of sensitive cells. Gram-negative bacteria are protected by their outer membrane, which prevents bacteriocins (and most other compounds of molecular weight above 600 Da) from reaching the plasma membrane (Abee et al., 1995).

It is generally accepted that bacteriocin activity is less effective in sausage than in *in vitro* systems. Activity may be reduced by the binding of the bacteriocin molecules to food components (mainly the fat matrix), and by the destabilizing action of proteases and other enzymes. Further limitations of bacteriocin effectiveness are uneven distribution in the food matrix and their inhibition by salt and curing agents (Leroy & de Vuyst, 1999; Schillinger, Geisen, & Holzapfel, 1996). Even so, several authors report that certain bacteriocinogenic meat LAB could be used as bioprotective cultures to prevent the growth of pathogens in sausage. Indeed, the use of bacteriocin-producing *L. sakei* as a starter culture decreases the numbers of *Listeria* in fermented sausage (De Martinis & Franco, 1998; Hugas, Garriga, Aymerich, & Monfort, 1995; Hugas, Neumeyer, Pages, Garriga, & Hammes, 1996; Schillinger, Kaya, & Lücke, 1991). Antilisterial effects have also been demonstrated with bacteriocinogenic *L. curvatus* (Dicks, Mellett, & Hoffman, 2004; Hugas et al., 1996), *L. plantarum* (Campanini, Pedrazzoni, Barbuti, & Baldini, 1993; Dicks et al., 2004) and *P. acidilactici* (Luchansky et al., 1992). The production of bacteriocins with a broad inhibition range, especially towards food-borne pathogens is therefore highly desirable since this would ensure the competitiveness of the starter strain while reducing the numbers of harmful flora.

3.2. Antibiotic resistance

Antibiotic resistance is a worldwide public health problem that continues to grow. Limiting the transmission of antibiotic resistance genes to unrelated pathogenic or opportunistic bacteria is essential. The food chain has been recognized as one of the main routes for the transmission of antibiotic resistant bacteria between animal and human populations (Witte, 2000). European Authorities have recently concluded that some bacteria used for or in feed production might pose a risk to human and animal health because of harboring strains with transferable resistance genes (European Commission, 2005). Fermented meats that are not heat-treated before consumption provide a vehicle for such bacteria and can act as a direct link between the indigenous microflora of animals and the human GIT. Recently, food-associated bacteria such as *L. sakei*, *L. curvatus*, *Leuconostoc mesenteroides*, and *P. pentosaceus* have been isolated from human feces, suggesting their ability to survive passage through the human gastrointestinal tract (GIT) (Walter et al., 2001).

Several studies have reported antibiotic resistance in LAB from meats and meat products; a few strains involved in sausage fermentation such as *L. sakei*, *L. curvatus* and *L. plantarum* have been found to show such resistance (Gevers, Danielsen, Huys, & Swings, 2003; Holley & Blaszyk, 1997; Teuber & Perreten, 2000). Although most of these resistances have been characterized as intrinsic, some genetic determinants such as chloramphenicol acetyltransferase (*cat*-TC), erythromycin [*erm*(B)] and tetracycline [*tet*(M), *tet*(S)] resistance genes have been identified, suggesting that horizontal gene transfer may have occurred (Ahn, Collins-Thompson, Duncan, & Stiles, 1992; Gevers et al., 2003; Lin, Fung, Wu, & Chung, 1996; Tannock et al., 1994) (Table 1). A recent study has shown that seven out of 62 lactobacilli strains might harbor transferable resistance genes on the basis of their resistance levels to chloramphenicol, erythromycin/clindamycin, tetracycline and oxacillin (Danielsen & Wind, 2003). Therefore, before launching a starter culture or probiotic product, it is important to verify that the bacterial strains involved do not content transferable resistance genes.

Table 1
Overview of antibiotic resistances reported in meat LAB

Species/strain name or number	Origin	Resistance gene(s)	Database accession number	References
Lactobacillus alimentarius				
DG500, DG499, DG498	Fermented dry sausage	*tet*(M)	AY149587, AY149586, AY149585	Gevers et al. (2003)
Lactobacillus curvatus				
DG524, DG484 DG142, DG143, G048	Fermented dry sausage	*tet*(M)	AY149595, AY149580, AY149576, AY149577 AY149575	Gevers et al. (2003)
Lactobacillus plantarum				
DG507	Fermented dry sausage	*tet*(M), *erm*(B)	AY149588	Gevers et al. (2003)
DG533, DG522, DG520, DG515, DG512, DG509, DG013	Fermented dry sausage	*tet*(M)	AY149597, AY149594, AY149593, AY149591, AY149590, AY149589, AY149574	Gevers et al. (2003)
caTC2R	Pork, raw and ground	*cat*-TC	–	Ahn et al. (1992)
Lactobacillus reuteri				
100-63	Poultry	*erm*(T)	M64090	Tannock et al. (1994)
G4	Poultry	*cat*-TC	U75299	Lin et al. (1996)
Lactobacillus sakei				
DG516, DG489, DG488, DG485, DG483, DG165	Fermented dry sausage	*tet*(M)	AY149592, AY149583, AY149582, AY149581, AY149579, AY149578	Gevers et al. (2003)
Leuconostoc citreum				
–	Sausage process line	*tet*(S)	–	Gevers et al. (2003)

Key of resistance gene designation: *cat*, chloramphenicol acetyltransferase; *erm*, erythromycin resistant gene; *tet*, tetracycline resistant gene.

3.3. Biogenic amines

Biogenic amines (BA) – organic bases with aliphatic, aromatic or heterocyclic structures – are found in several foods, and are mainly produced by the microbial decarboxylation of amino acids (with the exception of physiological polyamines) (Silla Santos, 1996). Histamine, tryptamine, tyramine, cadaverine, putrescine and phenylethylamine are regarded as undesirable because of their toxic effects (Silla Santos, 1996). Excessive consumption of these amines could cause nervous, gastric, intestinal, and blood pressure problems (Suzzi & Gardini, 2003). Nowadays, increasing attention is given to BA because of the growing number of consumers who are sensitive to them; in such people the action of amine oxidases, the enzymes involved in the detoxification of these substances, is deficient (Suzzi & Gardini, 2003).

The accumulation of BA in foods requires the presence of precursors (i.e., amino acids), microorganisms with amino acid decarboxylase activity, and favourable conditions for growth and decarboxylation (ten Brink, Damink, Joosten, & Huis in't Veld, 1990). Such requirements are met in dry sausages. The large quantities of protein present and the proteolytic activity seen during ripening provide the precursors for later decarboxylase reactions performed by both starter cultures and wild microbiota (Suzzi & Gardini, 2003). Many LAB from meat and meat products can decarboxylate amino acids (Bover-Cid & Holzapfel, 1999). Most strains of*L. curvatus*, one of the main species used as a starter in sausage production, are associated with high BA production (Bover-Cid & Holzapfel, 1999; Pereira, Crespo, & Romao, 2001).

A key requirement in the selection of LAB starter cultures for sausage production is, therefore, that they show no amino decarboxylase activity. Rapid growth and acid production will further prevent the development of wild amine-producing microflora. Several papers have reported on the ability of selected *L. sakei* to greatly reduce BA accumulation in fermented sausages (Bover-Cid, Izquierdo-Pulido, & Vidal-Carou, 2001; González-Fernández, Santos, Jaime, & Rovira, 2003). The introduction of starters with amine oxidase activity might be a way of further reducing the amount of biogenic amines produced *in situ*. Such activity has already been described in some LAB involved in sausage fermentation, such as *L. plantarum* and *L. casei* (Fadda, Vignolo, & Oliver, 2001).

4. Probiotic features

Probiotic organisms are defined as "non-pathogenic microorganisms that, when ingested in certain numbers exert a positive influence on host physiology and health beyond inherent general nutrition" (Ouwehand, Salminen, & Isolauri, 2002). Large numbers of these bacteria are consumed to ensure a healthy microbial balance in the intestine, to increase the returns of their beneficial activities, and/or to counteract the action of harmful populations (Ouwehand et al., 2002). Meat products are usually not heated, so they are thought to be adequate for the carriage of probiotics (GIT) (Hugas & Monfort, 1997; Incze, 1998). Further, probiotic meat starter cultures which do not alter the technological and sensory properties of the products have recently been proposed and used in the manufacturing of dry fermented sausages (Erkkilä et al., 2001; Erkkilä, Suihko, Eerola, Petäjä, & Mattila-Sandholm, 2001).

The most critical characteristics of probiotic strains are acid and bile salt resistance; without these they could not reach the human intestine, where they are expected to exert their health

promoting effects. The capacity of the strains to adhere to the intestinal mucosa is crucial if they are to promote changes in intestinal microecology (Erkkilä & Petäjä, 2000).

4.1. Tolerance to low pH

The survival of bacteria in the gastric juice depends on their ability to tolerate low pH. The transit time can be from <1 h to 3–4 h depending on the individual, the diet and other reigning conditions. Several LAB isolated from sausages, e.g., *L. sakei*, *L. plantarum*, *L. pentosus*, *P. acidilactici* and *P. pentosaceus*, can tolerate such acidic conditions (Erkkilä & Petäjä, 2000; Klingberg, Axelsson, Naterstad, Elsser, & Budde, 2006; Pennacchia et al., 2004). Therefore, some authors propose that strains intended for probiotic purposes should be screened for tolerance to pH 2.5 in an HCl-acidified culture medium for 4 h (Klingberg et al., 2006; Pennacchia et al., 2004).

4.2. Tolerance to bile salts

Bacteria that survive the acidic conditions of the stomach must then face the detergent-like function of the bile salts released into the duodenum after the ingestion of fatty meals. Microorganisms can reduce the emulsifying effect of the bile salts by hydrolyzing them with bile salt hydrolase enzymes (BSHs), thus decreasing their solubility (Erkkilä & Petäjä, 2000). BSH activity has been described in some intestinal lactobacilli such as *L. acidophilus*, *L. casei,* and *L. plantarum* (Gilliland & Speck, 1977). Furthermore, some LAB strains from sausages, such as *L. sakei*, *L. plantarum*, *L. pentosus*, and *P. acidilactici*, have been shown to resist bile salts (0.3% (w/v) oxgall) (Erkkilä & Petäjä, 2000; Klingberg et al., 2006; Pennacchia et al., 2004).

4.3. Adherence to human intestinal cells

Probiotic bacterial cells need to adhere to the mucosa and, at least temporarily, colonise the ileum, where probiotics are thought to exert their beneficial effects, which are dependent on the bacteria adhering to the mucosa for sufficient time (Goldin & Gorbach, 1992; Ouwehand et al., 2002). The ability of potential probiotic meat LAB strains to colonise the human GIT has been studied *in vitro* using Caco-2 cells. *P. pentosaceus*, *L. pentosus* and *L. plantarum* have shown high adhesion capacities (13–21%) compared to *L. rhamnosus* GG (15.7 ± 8.8%) (frequently used as a control in such experiments) (Klingberg et al., 2006). This last strain has been successfully used as a starter for production of dry fermented sausages (Erkkilä et al., 2001). Therefore, probiotic starter strains should be screened for adherence and persistence in the human GIT.

4.4. Antimicrobial activity

The antagonism shown by potentially probiotic meat LAB strains towards pathogenic microorganisms has to be functional under anaerobic conditions. Some *L. pentosus* and *L. plantarum* strains inhibit the growth of *L. monocytogenes* strains, as well as enterohaemorrhagic *Escherichia coli*, and strains of *Salmonella typhimurium*, *B. cereus*, *Shigella flexneri*, and *Yersinia enterocolitica* (Klingberg et al., 2006).

4.5. Nutraceutical properties

LAB are ideal cellular factories for the production of nutraceutical compounds (Hugenholtz & Smid, 2002). Recent progress in the engineering of LAB, especially in *Lactococcus lactis*, underscores the possibility of developing meat LAB starter cultures for the *in situ* production of vitamins via the overexpression and/or disruption of relevant metabolic genes (Burgess, O'Connell-Motherway, Sybesma, Hugenholtz, & van Sinderen, 2004; Sybesma, Starrenburg, Tijsseling, Hoefnagel, & Hugenholtz, 2003; Sybesma, Burgess, Starrenburg, van Sinderen, & Hugenholtz, 2004). Some species involved in sausage fermentation, such as *L. plantarum*, have been engineered to produce excess of folate (vitamin B_{11}) (Sybesma et al., 2003). This is thought to reduce fetal neural tube defects and to help maintain normal plasma homocysteine levels and cognitive functions, as well as to provide protection against certain forms of cancer, notably colon cancer (Jägerstad, Jastrebova, & Svensson, 2004). This affords the possibility of fortifying meat products with vitamins and other essential compounds, thus producing healthier meat products.

5. Conclusions

Over the past two decades, interest in the physiology and genetics of meat LAB species has increased greatly, reflecting the growing importance of these bacteria as starters and their increasing potential in the market of probiotics. LAB possess a myriad of desirable properties that could be of value in the production of fermented products such as dry sausages, and they have added value in terms of their promotion of end-product safety, improved sensorial characteristics, and health-related benefits. Careful screening of meat LAB should take into consideration as many of the above-mentioned selection criteria as possible; this would contribute towards improving the quality and safety of fermented dry sausages.

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References

Abee, T., Krockel, L., & Hill, C. (1995). Bacteriocins: modes of action and potentials in food preservation and control of food poisoning. *International Journal of Food Microbiology, 28*, 169–185.

Abriouel, H., Herrmann, A., Starke, J., Yousif, N. M., Wijaya, A., Tauscher, B., et al. (2004). Cloning and heterologous expression of hematin-dependent catalase produced by *Lactobacillus plantarum* CNRZ 1228. *Applied and Environmental Microbiology, 70*, 603–606.

Ahn, C., Collins-Thompson, D., Duncan, C., & Stiles, M. E. (1992). Mobilization and location of the genetic determinant of chloramphenicol resistance from *Lactobacillus plantarum* caTC2R. *Plasmid, 27*, 169–176.

Ammor, S., Dufour, E., Zagorec, M., Chaillou, S., & Chevallier, I. (2005). Characterization and selection of *Lactobacillus sakei* strains isolated from traditional dry sausage for their potential use as starter cultures. *Food Microbiology, 22*, 529–538.

Ammor, S., Rachman, C., Chaillou, S., Prevost, H., Dousset, X., Zagorec, M., et al. (2005). Phenotypic and genotypic identification of lactic acid bacteria isolated from a small-scale facility producing traditional dry sausages. *Food Microbiology, 22*, 373–382.

Aymerich, M. T., Garriga, M., Monfort, J. M., Nes, I., & Hugas, M. (2000). Bacteriocin-producing lactobacilli in Spanish-style fermented sausages: characterization of bacteriocins. *Food Microbiology, 17*, 33–45.

Aymerich, T., Martín, B., Garriga, M., Vidal-Carou, M. C., Bover-Cid, S., & Hugas, M. (2006). Safety properties and molecular strain typing of lactic acid bacteria from slightly fermented sausages. *Journal of Applied Microbiology, 100*, 40–49.

Baracco, P., Durand, P., Frentz, J. C., Jacquet, B., Zert, P., et al. (1990). *Encyclopédie de la charcuterie*. Paris: Soussana.

Benthin, S., & Villadsen, J. (1995). Different inhibition of *Lactobacillus delbrueckii* subsp. bulgaricus by D- and L-lactic acid: effects on lag phase, growth rate and cell yield. *Journal of Applied Bacteriology, 78*, 647–654.

Bover-Cid, S., & Holzapfel, W. H. (1999). Improved screening procedure for biogenic amine production by lactic acid bacteria. *International Journal of Food Microbiology, 53*, 33–41.

Bover-Cid, S., Izquierdo-Pulido, M., & Vidal-Carou, M. C. (2001). Effectiveness of a *Lactobacillus sakei* starter culture in the reduction of biogenic amine accumulation as a function of the raw material quality. *Journal of Food Protection, 64*, 367–373.

Buckenhüskes, H. J. (1993). Selection criteria for lactic acid bacteria to be used as starter cultures for various food commodities. *FEMS Microbiology Reviews, 12*, 253–271.

Burgess, C., O'Connell-Motherway, M., Sybesma, W., Hugenholtz, J., & van Sinderen, D. (2004). Riboflavin production in *Lactococcus lactis*: potential for in situ production of vitamin-enriched foods. *Applied and Environmental Microbiology, 70*, 5769–5777.

Campanini, M., Pedrazzoni, I., Barbuti, S., & Baldini, P. (1993). Behaviour of *Listeria monocytogenes* during the maturation of naturally and artificially contaminated salami: effect of lactic-acid bacteria starter cultures. *International Journal of Food Microbiology, 20*, 169–175.

Chaillou, S., Champomier-Verges, M. C., Cornet, M., Crutz-Le Coq, A. M., Dudez, A. M., Martin, V., et al. (2005). The complete genome sequence of the meat-borne lactic acid bacterium *Lactobacillus sakei* 23K. *Nature Biotechnology, 23*, 1527–1533.

Cocolin, L., Rantsiou, K., Iacumin, L., Urso, R., Cantoni, C., & Comi, G. (2004). Study of the ecology of fresh sausages and characterization of populations of lactic acid bacteria by molecular methods. *Applied and Environment Microbiology, 70*, 1883–1894.

Cook, P. E. (1995). Fungal ripened meats and meat products. In G. Campbell-Platt & P. E. Cook (Eds.), *Fermented meats* (pp. 110–129). London: Chapman & Hall.

Danielsen, M., & Wind, A. (2003). Susceptibility of *Lactobacillus* spp. to antimicrobial agents. *International Journal of Food Microbiology, 82*, 1–11.

De Martinis, E. C., & Franco, B. D. (1998). Inhibition of *Listeria monocytogenes* in a pork product by a *Lactobacillus sakei* strain. *International Journal of Food Microbiology, 42*, 119–126.

Dicks, L. M. T., Mellett, F. D., & Hoffman, L. C. (2004). Use of bacteriocin-producing starter cultures of *Lactobacillus plantarum* and *Lactobacillus curvatus* in production of ostrich meat salami. *Meat Science, 66*, 703–708.

Dossmann, M. U., Vogel, R. F., & Hammes, W. P. (1996). Mathematical description of the growth of *Lactobacillus sakei* and *Lactobacillus pentosus* under conditions prevailing in fermented sausages. *Applied Microbiology and Biotechnology, 46*, 334–339.

Enan, G., El-Essawy, A. A., Uyttendaele, M., & Debevere, J. (1996). Antibacterial activity of *Lactobacillus plantarum* UG1 isolated from dry sausage: characterization, production and bactericidal action of plantaricin UG1. *International Journal of Food Microbiology, 30*, 189–215.

Erkkilä, S., & Petäjä, E. (2000). Screening of commercial meat starter cultures at low pH and in the presence of bile salts for potential probiotic use. *Meat Science, 55*, 297–300.

Erkkilä, S., Petäjä, E., Eerola, S., Lilleberg, L., Mattila-Sandholm, T., & Suihko, M. L. (2001). Flavor profiles of dry sausages fermented by selected novel meat starter cultures. *Meat Science, 58*, 111–116.

Erkkilä, S., Suihko, M. L., Eerola, S., Petäjä, E., & Mattila-Sandholm, T. (2001). Dry sausage fermented by *Lactobacillus rhamnosus* strains. *International Journal of Food Microbiology, 64*, 205–210.

European Commission (2005). *Opinion of the FEEDAP panel on the updating of the criteria used in the assessment of bacteria for resistance to antibiotics of human or veterinary importance*. EFSA J 223 (pp. 1–12).

Fadda, S., Sanz, Y., Vignolo, G., Aristoy, M., Oliver, G., & Toldrá, F. (1999a). Characterization of muscle sarcoplasmic and myofibrillar protein hydrolysis caused by *Lactobacillus plantarum*. *Applied and Environmental Microbiology, 65*, 3540–3546.

Fadda, S., Sanz, Y., Vignolo, G., Aristoy, M., Oliver, G., & Toldrá, F. (1999b). Hydrolysis of pork muscle sarcoplasmic proteins by *Lactobacillus curvatus* and *Lactobacillus sakei*. *Applied and Environmental Microbiology, 65*, 578–584.

Fadda, S., Vignolo, G., & Oliver, G. (2001). Tyramine degradation and tyramine/histamine production by lactic acid bacteria and *Kocuria* strains. *Biotechnology Letters, 23*, 2015–2019.

Foegeding, P. M., Thomas, A. B., Pilkington, D. H., & Klaenhammer, T. R. (1992). Enhanced control of *Listeria monocytogenes* by in situ-produced pediocin during dry fermented sausage production. *Applied and Environmental Microbiology, 58*, 884–890.

Furet, J. P., Quenee, P., & Tailliez, P. (2004). Molecular quantification of lactic acid bacteria in fermented milk products using real-time quantitative PCR. *International Journal of Food Microbiology, 97*, 197–207.

Gevers, D., Danielsen, M., Huys, G., & Swings, J. (2003). Molecular characterization of tet(M) genes in *Lactobacillus* isolates from different types of fermented dry sausage. *Applied and Environmental Microbiology, 69*, 1270–1275.

Gilliland, S. E., & Speck, M. L. (1977). Deconjugation of bile acids by intestinal lactobacilli. *Applied and Environmental Microbiology, 33*, 15–18.

Goldin, B., & Gorbach, S. (1992). Probiotics for humans. In R. Fuller (Ed.), *Probiotics: the scientific basis* (pp. 355–376). London: Chapman and Hall.

González-Fernández, C., Santos, E. M., Jaime, I., & Rovira, J. (2003). Influence of starter cultures and sugar concentrations on biogenic amine contents in chorizo dry sausage. *Food Microbiology, 20*, 275–284.

Hammes, W. P., Bantleon, A., & Min, S. (1990). Lactic acid bacteria in meat fermentation. *FEMS Microbiology Letters, 87*, 165–173.

Hammes, W. P., & Hertel, C. (1998). New developments in meat starter cultures. *Meat Science, 49*, S125–S138.

Holley, R. A., & Blaszyk, M. (1997). Antibiotic challenge of meat starter cultures and effects upon fermentations. *Food Research International, 30*, 513–522.

Huerta, R., Jordano, R., Medina, L. M., & López, C. (2004). Population dynamics of the constitutive biota of French dry sausages in a pilot-scale ripening chamber. *Journal of Food Protection, 67*, 2306–2309.

Hugas, M., Garriga, M., Aymerich, M. T., & Monfort, J. M. (1995). Inhibition of Listeria in dry fermented sausages by the bateriocinogenic *Lactobacillus sakei* CTC494. *Journal of Applied Bacteriology, 79*, 322–330.

Hugas, M., & Monfort, J. M. (1997). Bacterial starter cultures for meat fermentation. *Food Chemistry, 59*, 547–554.

Hugas, M., Neumeyer, B., Pages, F., Garriga, M., & Hammes, W. P. (1996). Antimicrobial activity of bacteriocin-producing cultures in meat products: 2. Comparison of bacteriocin producing lactobacilli on Listeria growth in fermented sausages. *Fleischwirtschaft, 76*, 649–652.

Hugenholtz, J., & Smid, E. J. (2002). Nutraceutical production with food-grade microorganisms. *Current Opinions in Biotechnology, 13*, 497–507.

Incze, K. (1998). Dry fermented sausages. *Meat Science, 49*, S169–S177.

Jägerstad, M., Jastrebova, J., & Svensson, U. (2004). Folates in fermented vegetables: a pilot study. *Lebensmittel-Wissenschaft Und-Technologie, 37*, 603–611.

Kashket, E. R. (1987). Bioenergetics of lactic acid bacteria: cytoplasmic pH and osmotolerance. *FEMS Microbiology Reviews, 46*, 233–244.

Klingberg, T. D., Axelsson, L., Naterstad, K., Elsser, D., & Budde, B. B. (2006). Identification of potential probiotic starter cultures for Scandinavian-type fermented sausages. *International Journal of Food Microbiology, 105*, 419–431.

Kwon, H. S., Yang, E. H., Yeon, S. W., Kang, B. H., & Kim, T. Y. (2004). Rapid identification of probiotic *Lactobacillus* species by multiplex PCR using species-specific primers based on the region extending from 16S rRNA through 23S rRNA. *FEMS Microbiology Letters, 239*, 267–275.

Leroy, F., & de Vuyst, L. (1999). The presence of salt and a curing agent reduces bacteriocin production by *Lactobacillus sakei* CTC 494, a potential starter culture for sausage fermentation. *Applied and Environmental Microbiology, 65*, 5350–5356.

Lin, C. F., Fung, Z. F., Wu, C. L., & Chung, T. C. (1996). Molecular characterization of a plasmid-borne (pTC82) chloramphenicol resistance determinant (cat-TC) from Lactobacillus reuteri G4. *Plasmid, 36*, 116–124.

Lindgren, S. E., & Dobrogosz, W. J. (1990). Antagonistic activities of lactic acid bacteria in food and feed fermentations. *FEMS Microbiology Reviews, 7*, 149–163.

Luchansky, J. B., Glass, K. A., Harsono, K. D., Degnan, A. J., Faith, N. G., Cauvin, B., et al. (1992). Genomic analysis of *Pediococcus* starter cultures used to control *Listeria monocytogenes* in turkey summer sausage. *Applied and Environmental Microbiology, 58*, 3053–3059.

Lücke, F. K. (2000). Utilization of microbes to process and preserve meat. *Meat Science, 56*, 105–115.

Lücke, F. K., & Hechelmann, H. (1987). Starter cultures for dry sausages and raw ham composition and effect. *Fleischwirtschaft, 67*, 307–314.

Maijala, R., Eerola, S., Aho, M., & Hirn, J. (1993). The effect of GDL-induced pH decrease on the formation of biogenic amines in meat. *Journal of Food Protection, 56*, 125–129.

Marceau, A., Zagorec, M., Chaillou, S., Mera, T., & Champomier-Verges, M. C. (2004). Evidence for involvement of at least six proteins in adaptation of *Lactobacillus sakei* to cold temperatures and addition of NaCl. *Applied and Environmental Microbiology, 70*, 7260–7268.

Mares, A., Neyts, K., & Debevere, J. (1994). Influence of pH, salt and nitrite on the heme-dependent catalase activity of lactic acid bacteria. *International Journal of Food Microbiology, 24*, 191–198.

Martín, B., Garriga, M., Hugas, M., & Aymerich, T. (2005). Genetic diversity and safety aspects of enterococci from slightly fermented sausages. *Journal of Applied Microbiology, 98*, 1177–1190.

Messi, P., Bondi, M., Sabia, C., Battini, R., & Manicardi, G. (2001). Detection and preliminary characterization of a bacteriocin (plantaricin 35d) produced by a *Lactobacillus plantarum* strain. *International Journal of Food Microbiology, 64*, 193–198.

Molly, K., Demeyer, D., Johansson, G., Raemaekers, M., Ghistelinck, M., & Geenen, I. (1997). The importance of meat enzymes in ripening and flavor generation in dry fermented sausages. First results of a European project. *Food Chemistry, 59*, 539–545.

Montel, M. C. (1999). Fermented meat products. In C. A. Batt & P. D. Patel (Eds.), *Encyclopedia of food microbiology: fermented foods* (pp. 744–753). London: Academic Press.

Montel, M. C., Talon, R., Fournaud, J., & Champomier, M. C. (1991). A simplified key for identifying homofermentative *Lactobacillus* and *Carnobacterium* spp. from meat. *Journal of Applied Bacteriology, 70*, 469–472.

Nissen, H., & Dainty, R. (1995). Comparison of the use of rRNA probes and conventional methods in identifying strains of *Lactobacillus sakei* and *L. curvatus* isolated from meat. *International Journal of Food Microbiology, 25*, 311–315.

Noonpakdee, W., Santivarangkna, C., Jumriangrit, P., Sonomoto, K., & Panyim, S. (2003). Isolation of nisin-producing *Lactococcus lactis* WNC 20 strain from nham, a traditional Thai fermented sausage. *International Journal of Food Microbiology, 81*, 137–145.

Noonpakdee, W., Sitthimonchai, S., Panyim, S., & Lertsiri, S. (2004). Expression of the catalase gene katA in starter culture *Lactobacillus plantarum* TISTR850 tolerates oxidative stress and reduces lipid oxidation in fermented meat product. *International Journal of Food Microbiology, 95*, 127–135.

Ordóñez, J. A., Hierro, E. M., Bruna, J. M., & de la Hoz, L. (1999). Changes in the components of dry-fermented sausages during ripening. *Critical Reviews in Food Science and Nutrition, 39*, 329–367.

Ouwehand, A. C., Salminen, S., & Isolauri, E. (2002). Probiotics: an overview of beneficial effects. *Antonie Van Leeuwenhoek, 82*, 279–289.

Papamanoli, E., Tzanetakis, N., Litopoulou-Tzanetaki, E., & Kotzekidou, P. (2003). Characterization of lactic acid bacteria isolated from a Greek dry-fermented sausage in respect of their technological and probiotic properties. *Meat Science, 65*, 859–867.

Pennacchia, C., Ercolini, D., Blaiotta, G., Pepe, O., Mauriello, G., & Villani, F. (2004). Selection of *Lactobacillus* strains from fermented sausages for their potential use as probiotics. *Meat Science, 67*, 309–317.

Pereira, C. I., Crespo, M. T., & Romao, M. V. (2001). Evidence for proteolytic activity and biogenic amines production in *Lactobacillus curvatus* and *Lactobacillus homohiochii*. *International Journal of Food Microbiology, 68*, 211–216.

Podolak, P. K., Zayas, J. F., Kastner, C. L., & Fung, D. Y. C. (1996). Inhibition of *Listeria monocytogenes* and *Escherichia coli* O157:H7 on beef by application of organic acids. *Journal of Food Protection, 59*, 370–373.

Rantsiou, K., & Cocolin, L. (2006). New developments in the study of the microbiota of naturally fermented sausages as determined by molecular methods: a review. *International Journal of Food Microbiology, 108*, 255–267.

Santos, E. M., Jaime, I., Rovira, J., Lyhs, U., Korkeala, H., & Bjorkroth, J. (2005). Characterization and identification of lactic acid bacteria in "morcilla de Burgos". *International Journal of Food Microbiology, 97*, 285–296.

Sanz, Y., Fadda, S., Vignolo, G., Aristoy, M. C., Oliver, G., & Toldrá, F. (1999a). Hydrolytic action of *Lactobacillus casei* CRL 705 on pork muscle sarcoplasmic and myofibrillar proteins. *Journal of Agricultural and Food Chemistry, 47*, 3441–3448.

Sanz, Y., Fadda, S., Vignolo, G., Aristoy, M. C., Oliver, G., & Toldrá, F. (1999b). Hydrolysis of muscle myofibrillar proteins by *Lactobacillus curvatus* and *Lactobacillus sakei*. *International Journal of Food Microbiology, 53*, 115–125.

Schillinger, U., Geisen, R., & Holzapfel, W. H. (1996). Potential of antagonistic microorganisms and bacteriocins for the biological preservation of foods. *Trends in Food Science Technology, 7*, 158–164.

Schillinger, U., Kaya, M., & Lücke, F. K. (1991). Behaviour of *Listeria monocytogenes* in meat and its control by a bacteriocin-producing strain of *Lactobacillus sakei*. *Journal of Applied Bacteriology, 70*, 473–478.

Silla Santos, M. H. (1996). Biogenic amines: their importance in foods. *International Journal of Food Microbiology, 29*, 213–231.

Snijders, J. M., van Logtestijn, J. G., Mossel, D. A., & Smulders, F. J. (1985). Lactic acid as a decontaminant in slaughter and processing procedures. *Veterinary Quarterly, 7*, 277–282.

Suzzi, G., & Gardini, F. (2003). Biogenic amines in dry fermented sausages: a review. *International Journal of Food Microbiology, 88*, 41–54.

Sybesma, W., Burgess, C., Starrenburg, M., van Sinderen, D., & Hugenholtz, J. (2004). Multivitamin production in *Lactococcus lactis* using metabolic engineering. *Metabolic Engineering, 6*, 109–115.

Sybesma, W., Starrenburg, M., Tijsseling, L., Hoefnagel, M. H., & Hugenholtz, J. (2003). Effects of cultivation conditions on folate production by lactic acid bacteria. *Applied and Environmental Microbiology, 69*, 4542–4548.

Tannock, G. W., Luchansky, J. B., Miller, L., Connell, H., Thode-Andersen, S., Mercer, A. A., et al. (1994). Molecular characterization of a plasmid-borne (pGT633) erythromycin resistance determinant (ermGT) from *Lactobacillus reuteri* 100–63. *Plasmid, 31*, 60–71.

ten Brink, B., Damink, C., Joosten, H. M., & Huis in't Veld, J. H. (1990). Occurrence and formation of biologically active amines in foods. *International Journal of Food Microbiology, 11*, 73–84.

Teuber, M., & Perreten, V. (2000). Role of milk and meat products as vehicles for antibiotic-resistant bacteria. *Acta Veterinaria Scandinavica, Suppl. 93*, 75–87.

Tichaczek, P. S., Nissen-Meyer, J., Nes, I. F., Vogel, R. F., & Hammes, W. P. (1992). Characterization of the bacteriocins curvacin A from *Lactobacillus curvatus* LTH 1174 and sakacin P from *Lactobacillus sakei* LTH 673. *Systematic and Applied Microbiology, 15*, 460–468.

Walter, J., Hertel, C., Tannock, G. W., Lis, C. M., Munro, K., & Hammes, W. P. (2001). Detection of *Lactobacillus*, *Pediococcus*, *Leuconostoc*, and *Weissella* species in human feces by using group-specific PCR primers and denaturing gradient gel electrophoresis. *Applied and Environmental Microbiology, 67*, 2578–2585.

Witte, W. (2000). Selective pressure by antibiotic use in livestock. *International Journal of Antimicrobial Agents, 16*, S19–S24.

Wolf, G., Arendt, E. K., Pfahler, U., & Hammes, W. P. (1990). Heme-dependent and heme-independent nitrite reduction by lactic acid bacteria results in different N-containing products. *International Journal of Food Microbiology, 10*, 323–329.