Chapter 5

The Role of Lactic Acid Bacteria in Safety and Flavour Development of Meat and Meat Products

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Additional information is available at the end of the chapter

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1. Introduction

Lactic acid bacteria (LAB) are widespread in nature and commonly occur on all kind of plant materials, on mucous membranes, in saliva and, in feces. Consequently and unavoidably they are part of the contamination flora of fresh meats after slaughter. Under certain conditions, e.g. in packaged refrigerated meats or raw sausage meats, they are able to compete efficiently with accompanying microorganisms for nutrients and may reach substantial viable counts. Their metabolic activities may ultimately result in either a desired preservative effect due to the repression of pathogenic and spoilage microorganisms, a desired tasty meat product, such as raw fermented sausage, or in meat spoilage through undesired transformations of raw and cooked meats. Heterofermentative LAB of the *Carnobacterium*, *Leuconostoc* and *Weissella* genera are usually more involved in meat spoilage than the homofermentative *Lactobacillus* and *Pediococcus* genera. Therefore, commercially available meat starter cultures for dry-fermented sausage production exclusively belong to the latter two. Homofermentative LAB produce almost exclusively lactic acid from fermentable carbohydrates present in meats, which is relatively mild and palatable, while heterofermentative species produce significant amounts of less desirable fermentation end products, such as CO_2 gas, ethanol, acetic acid, butanoic acid and acetoin. However, under certain conditions *Lactobacillus* spp. may also produce significant amounts of acetic acid, ropy slime and, discolouration (greening) of meats [1,2].

In food industry starter and protective cultures are currently used in a number of products to safeguard the microbial and sensory quality. Lactic acid bacteria (LAB) are the main players in the natural transformation of agricultural primary products into safe, delicious and shelf stable foods for human consumption. In meat products there are three basic fields of application for the targeted use of such cultures: raw fermented sausages, raw cured

hams, and pasteurised, sliced prepackaged meats (cold cuts) [3-8]. The use of protective cultures in prepackaged, refrigerated sliced Bologna-type sausage and cooked ham against pathogenic listeria is a much discussed, sustainable technology for improving the microbial safety and quality of these products. It helps to avoid chemical preservatives, such as sodium lactate/potassium acetate additives, or repasteurisation in package after slicing and packaging, which both have a negative impact on sensory product quality leaving a numb mouthfeel or warmed-over flavour, resp. [9,10].

2. Meat and meat products

2.1. Raw fermented sausages

The importance of starter and protective cultures for the manufacturing of safe and high-quality fermented sausages has been known for a long time and, lactobacilli play an important role in their production [11,5]. *Lb. sakei* and *Lb. curvatus* are quite often the predominant LAB in dry-fermented sausage while other lactobacilli, such as *Lb. versmoldensis*, *Lb. plantarum*, *Lb. brevis*, *Lb. farciminis*, *Lb. alimentarius*, *Weissella* species, pediococci, and leuconostocs, usually occur in significantly lower numbers [12]. This has recently been also shown for different traditional salamis from North Italy [13-15]. However, other recipes and ripening conditions may promote other LAB as well. LAB isolated from dry spontaneously fermented sausages from 15 different producers in Spain included mainly *Lb. sakei* (66%), *Lb. curvatus* (26%), and *Lb. plantarum* (8%) [16]. For dry fermented Spanish 'chorizo' sausage *Lb. sakei* (69%), *Lb. curvatus* (16%) and *Pediococcus* (9%) have been reported [17]. From naturally fermented Greek dry salami about 50% of the isolates belonged to *Lb. sakei/curvatus*, 30% to the *Weissella* genus, 10% to *Lb. plantarum* and 3% each to *Lb. farciminis* and *Enterococcus (Ec.) faecium* [18]. In "Alheira", a fermented sausage produced in Portugal, *Lb. plantarum* and *Ec. faecalis* prevailed while other LAB, such as *Lb. paraplantarum*, *Lb. brevis*, *Lb. rhamnosus*, *Lb. sakei*, *Lb. zeae*, *Lb. paracasei*, *Leuconostoc (Leuc.) mesenteroides*, *Pediococcus (Pc.) pentosaceus*, *Pc. acidilactici*, *Weissella (Ws.) cibaria*, *Ws. viridescens* and *Ec. faecium*, occurred in lower numbers [19].

The main role of LAB is to convert fermentable sugars in the sausage batter to lactic acid, thereby contributing to product safety by creating unfavourable conditions for pathogens and spoilage organisms. The production of lactic acid has also a direct impact on sensory product quality by providing a mild acidic taste, and by supporting the drying process which requires a sufficient decline in pH. Furthermore, LAB influence the sensory characteristics of the fermented sausages by the production of small amounts of acetic acid, ethanol, acetoin, pyruvic acid, carbon dioxide, and their ability to initiate the production of aromatic substances from proteinaceous precursors [20-22]. The selection criteria for lactic acid bacteria to be used in the production of fermented sausage include (i) fast production of lactic acid (ii) good growth at different temperatures, (iii) homofermentative metabolism, (iv) persistence over the whole fermentation and ripening process, (v) nitrate reduction, (vi) ability to express catalase, (vii) no fermentation of lactose, (viii) formation of flavour, (ix) no formation of peroxide, (x) no formation of

biogenic amines, (xi) no formation of ropy slime, (xii) tolerance or even synergy to other microbial components of the starter, (xiii) antagonism against pathogens, (xiv) antagonism against technologically undesirable microorganisms, (xv) improvement of the nutritional value of the sausage and, (xvi) economic factors [23]. Many homofermentative LAB associated with cured meat products are quite resistant to nitrite up to 200 ppm [24]. A new starter culture for raw sausages, 'BITEC Advance LD-20' from Frutarom Savory Solutions, containing *Lb. sakei* and *S. carnosus* is marketed as consistently providing a 'pleasant mild taste' while rapidly deminishing the pH value of the sausage batter. Rapid acidification is important for product safety while a high competitiveness against the spontaneous lactic flora is important for product quality. The culture can be used for firm and fresh raw sausages as well as sausage spreads.

The use of homofermentative lactic acid bacteria is desirable because acetic acid has an unpleasant taste as compared with lactic acid [25].

It must be kept in mind, however, that, although lactic acid production and pH reduction by LAB provide quite unfavorable conditions for pathogenic bacteria thereby preventing them from growing and contributing to their reduction, several pathogenic microorganisms are able to survive in fermented sausages under certain conditions for extended periods, especially during refrigerated storage of sparsely dried sausages. Pathogenic strains of *Escherichia (E.) coli*, *Listeria (Li.) monocytogenes* and *Yersinia (Y.) enterocolitica* are inactivated better after the initial fermentation and ripening stage if stored at ambient rather than at refrigeration temperature. Inclusion of a maturation period above refrigeration temperatures before distribution may increase the safety of these products [26-29].

2.2. Dry-cured hams

Currently there are only a few publications which clearly substantiate the advantages of starter and protective cultures during raw cured ham production. On the other hand, starter cultures have been more and more implemented by meat industry into the production of dry-cured hams since the early 1980s [6,30]. These cultures are expected to be active under the harsh manufacturing conditions (low temperatures, high salt, lack of oxygen, presence of nitrite). LAB contribute to a moderate pH decrease which promotes the microbial stability as well as product texture, reduce stickiness and pH variations of the raw material. As an example, FSC-111 Bactoferm^{R} from Chr.-Hansen A/S contains, besides a staphylococcal strain, also a strain of *Lb. sakei*.

The LAB induced acidification is usually more pronounced with injected or compound meats than with dry-salted ones. Modern turkey hams are produced by squeezing turkey breast over the screw of an extruder in the presence of (g/kg) nitrite curing salt (35), diphosphate (2,5), dextrose (2), water (100), starter culture and a spice compound, and subsequent tumbling until protein release. This mixture is then stuffed into fiber casings and left for 5 days at 2°C. This is followed by a fermentation step of around 16 hours at 22°C and 92-94% relative humidity until a pH below 5.4 is reached. Finally, the product is heated in a cabinet at 47 °C to a core temperature of 40°C. The desired result is a fresh looking product

with a slightly hyaline appearance with an optimum safety against undesired and pathogenic microorganisms [30].

2.3. Fresh meats

In chilled vacuum-packaged beef, even close to the freezing point, psychrotrophic LAB are able to attain high population densities. At -1.5 °C LAB grew to 8-9 $\log_{10}$ cfu ml^{-1} drip in 16 weeks with maximum doubling times of around 2-4 days [31]. In this study, *Cb. divergens*, *Leuc. mesenteroides* and *Lb. delbruckii* dominated the LAB flora after 4, 8-12 and 16 weeks, respectively. At 2 °C other workers have reported *Lb. sakei, Lb. curvatus, Carnobacterium (Cb.) divergens, Cb. maltaromaticum, Leuconostoc spp.* and *Lactococcus raffinolactis* as relevant LAB with t_d of around 19 hours and less [32]. After 25 days maximum LAB numbers of around 7-8 $\log_{10}$ cfu cm^{-2} were reached and after 8 weeks the the meat odour immediately after opening the bags was regarded "definitely off" ("slightly off" between 4-6 weeks).

LAB may be useful as protective cultures during the ripening of vacuum-packaged raw beef and, bioprotective cultures may also help to reduce *E. coli* O157:H7 in frozen ground-beef patties [33,34]. Peptides generated by LAB have been suggested as sensorial and hygienic biomarkers in meat conditioning and fermentation [35].

Today, meat industry is forced to produce meats with a shelf life long enough to fulfill logistic, retail sale and consumer demands. Besides general hygienic considerations, including appropriate temperature control modified-atmosphere packaging (MAP) with 30-40% CO_2 is used to prevent early spoilage. While Gram-negative spoilage bacteria are suppressed, psychrotrophic LAB are not [36-38].

2.4. Cooked meats

Cooked, sliced and prepackaged meat products are popular convenience foods. They are retailed under refrigeration with varying shelf lifes, e.g. at 5 to 7 °C for 14 to 28 days. During slicing and packaging the slices may be contaminated with microorganisms from the production environment. Especially certain psychrotrophic LAB may then attain high cell counts during cold storage and impair the sensory quality of the products [39-42]. More than 2/3 of the refrigerated sliced cooked meats from the German retail market contained LAB counts above 7 $\log_{10}$ cfu g^{-1} one week past the indicated shelf life (Figure 1) [43]. The LAB flora on Bologna-type sausage is mostly dominated by the *Lb. sakei/curvatus* cluster while *Leuc. carnosum* frequently dominates on cooked ham. Occasionally, also *Ws. viridescens*, *Cb. maltaromaticum* and *Leuc. mesenteroides* ssp. *mesenteroides* may occur in higher numbers. Independent from dominant occurrence, eight LAB species have been identified in German retail samples. The number of samples (n) out of 50 in which these species occurred were *Lb. sakei* (40), *Leuc. carnosum* (22), *Lb. curvatus* (18), *Ws. viridescens* (11), *Leuc. mesenteroides* ssp. *mesenteroides* (8), *Cb. maltaromaticum* (4), *Lactobacillus* sp. (4), *Lactococcus* sp. (4), *Cb. divergens* (2), *Leuc. gelidum* (1), *Leuconostoc* sp. (1) (Figure 2) [43].

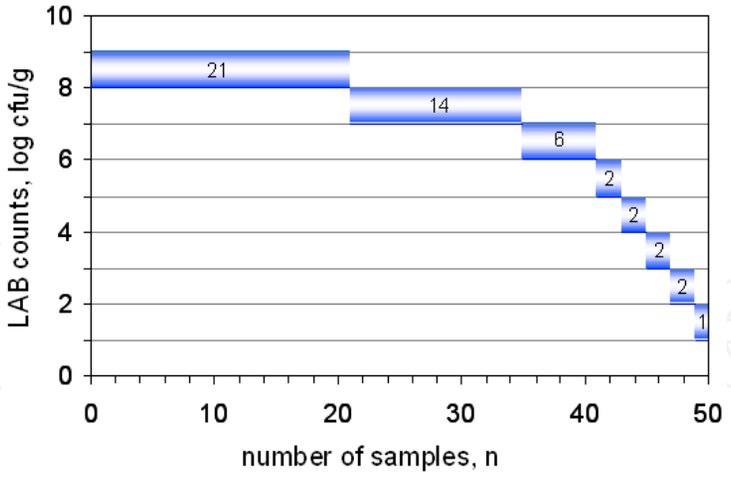

Figure 1. Distribution of samples of refrigerated sliced cooked meats from the German retail market with respect to different LAB counts one week past the indicated shelf life [43].

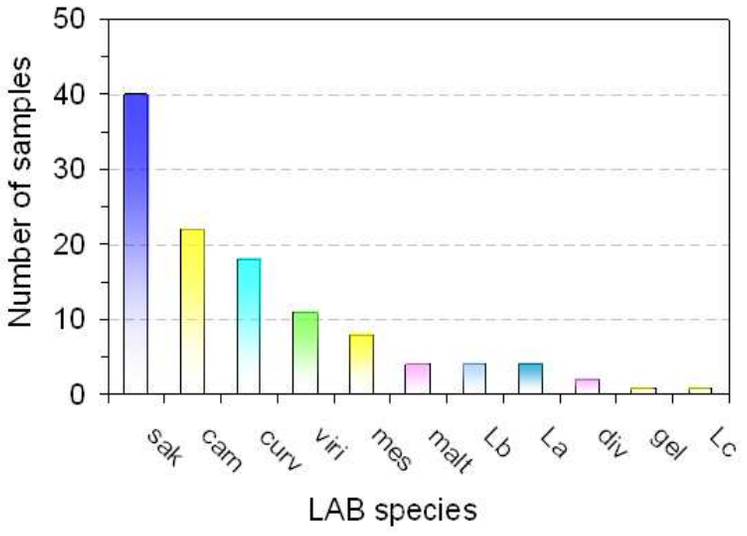

Figure 2. Abundancy of different LAB species in refrigerated sliced cooked meats from German retail (n=50). sak, *Lb. sakei*; carn, *Leuc. carnosum*; curv, *Lb. curvatus*; viri, *Ws. viridescens*; mes, *Leuc. mesenteroides*; malt, *Cb. maltaromaticum*; Lb, *Lactobacillus* sp.; La, *Lactococcus* sp.; div, *Cb. divergens*; gel, *Leuc. gelidum*; Lc, *Leuconostoc* sp. [43].

3. Biopreservation

Biopreservation of meats refers to the control of pathogenic and spoilage microorganisms by a competitive microflora of desired indigenous microorganisms or so-called starter and protective cultures. The development of starter cultures for meats is tightly coupled with the industrialisation of the traditional artisanal processes. The production of safe and tasty fermented sausages by traditional technologies requires expert knowledge and continous attention to guide the fermentation into the desired direction, i.e. to promote the development of the desired microorganisms and to suppress the development of undesired microorganisms. Mistakes are heavily paid for by dangerous and/or low quality outcomes.

Starter cultures, added at the beginning of fermentation, allow a standardization of the product quality and considerably reduce the risk of product defects. However, it should be kept in mind that starter cultures can not replace good manufacturing practice which besides the selection of the appropriate raw materials with acceptable hygienic parameters also includes the implementation and control of appropriate processing conditions. This is especially true with respect to the health risks associated with enterohaemorrhagic *E. coli*. Because of its increased acid tolerance and low infective dose for human infection, additional hurdles besides starter cultures have become very important for the production of safe raw fermented sausages. The hurdles principle for controlling undesired microorganisms in raw sausage fermentation has been illustrated by LEISTNER [27,44,45] and, in the meantime the implementation of HACCP (hazard analyis critical control point) concepts have become mandatory in food production [46].

Protective cultures may be distinquished from starter cultures by their lack of, or their reduced product transformation capabilities. Protective cultures my be used for a number of applications with the main focus on pathogen control, especially of *Li. monocytogenes*, but also of spoilage organisms such as LAB involved in the spoilage of deli meats, or of *Brochothrix thermosphacta* and *Clostridium estertheticum* in vacuum-packaged raw meats [47-50].

Of special interest are strains which excrete powerful anti-listerial bacteriocins *in situ* and, which at the same time have no or only a very weak spoilage potential [21, 51].

A strain of *Lactococcus (Lc.) lactis*, marketed as Bactoferm® Rubis by Chr.-Hansen A/S, is offered as a protective culture to be used instead of chemicals to preserve/stabilize the normal colour of vacuum packed or controlled atmosphere packaged, sliced, cured meat products [52].

The big retail chains and the official food control authorities look at high microbial counts in deli meats, regardless of the responsible microflora, usually with suspicion. The German Society for Hygiene and Microbiology (DGHM), e.g., recommends a maximum of $5x10^6$ cfu g^{-1} [53]. In reality, however, many of the prepackaged sliced cold cuts display 10-100 times higher counts at the the end of their indicated shelf lives without being recognized as spoiled by sensory panels. On the other hand, unpleasant tastes and smells (not fresh, sour) are often associated with high LAB counts [54]. But a high count *per se* does not tell how long the product has been exposed to this high count already. Protective LAB cultures are looked at with suspicion because they have to be added in high numbers and, if metabolically too active, may reduce shelf life. Some authors generally view psychrotrophic LAB as spoilage organisms, regardless of their generally moderate role in spoilage [55]. There is no doubt that cold-cuts with protective cultures will differ from products without protective culture. But, as long as this difference is only manifested in a minor sour taste this kind of sensory deviation may be a reasonable price to pay for an increased food safety, especially with respect to *Li. monocytogenes*, without chemical preservatives and the control of more striking spoilage organisms, e.g. such as *Brochothrix thermosphacta*. Food preferences are changing, and presently many consumers tend to prefer products which are as much as

possible free of chemical preservatives [8], processing aids and allergenic additives, and which are not overly treated by physical processes, such as heat, high pressure and irradiation. Nevertheless, many consumers also simply do not care, as long as the product is safe and affordable. Thus, protective cultures may be interesting for health and wellness-oriented consumers in countries with higher living standards. But less developed countries could also benefit, especially where cold-chain management is difficult and high-tech processing aids are not readily available. The challenge simply is to find the right LAB cultures for the particular product.

4. Sensory acceptance of bioprotective cultures on prepackaged cold cuts

As already mentioned, the application of bioprotective microbial cultures to prepackaged cold cuts is a much discussed innovative and sustainable technology for improving the microbiological safety and overall quality of these products. It could be an alternative to chemical preservatives or to a second pasteurisation step after packaging which both have a negative sensory impact. Although quite a number of lactic acid bacteria (LAB) have been suggested as protective cultures for sliced cooked meats, there is basically no information on consumer perception of products with added LAB. At the International Green Week Berlin 2010 the concept was introduced for the first time to a broader public and visitors were asked to participate in a sensory preference test [7].

Bologna-type sausages in 70 mm fiber casings were produced and stored at 2 °C until slicing. On the day of packaging the casings were removed and the sausages were briefely submersed in an aqueous suspension of a protective culture consisting of *Lb. sakei* strain Lb674 (sakacin P positive) and containing 8.5 $\log_{10}$ LAB ml^{-1}. Subsequently, the sausages were sliced, vacuum-packaged in polyethylene bags and kept refrigerated at 5°C until presentation to interested visitors. The consumers reacted predominantly positive on the possibility of safeguarding cold cuts with bioprotectants. Up to day 15 after packaging the inoculated samples reached a relative preference score (achieved points versus achievable points) of more than 45% (max. 60%) as compared to 60-70% for the freshly sliced samples without added LAB. Thereafter, the overall liking of the inoculated prepackaged sausage gradually decreased (Figure 3). The results indicate a potential market for more natural, microbiologically safe and sound cold cuts as a specialized segment of the convenience sector. As stated above, a mild acidic note may not be completely avoided when using protective cultures. But, this 'disadvantage' should be balanced against the risk of an uncontrolled growth of listeria on the one hand and the demand of many consumers for less chemical preservatives or thermal treatments on the other hand.

5. Probiotics

The steeply increasing business in the industrialised countries with health and wellness oriented foods in the 1990s, starting with probiotics in dairy products, has also raised interest in the development of probiotic meat products [56]. The concept of probiotics requires the intake of relevant amounts by the consumer of living probiotic microorgansims,

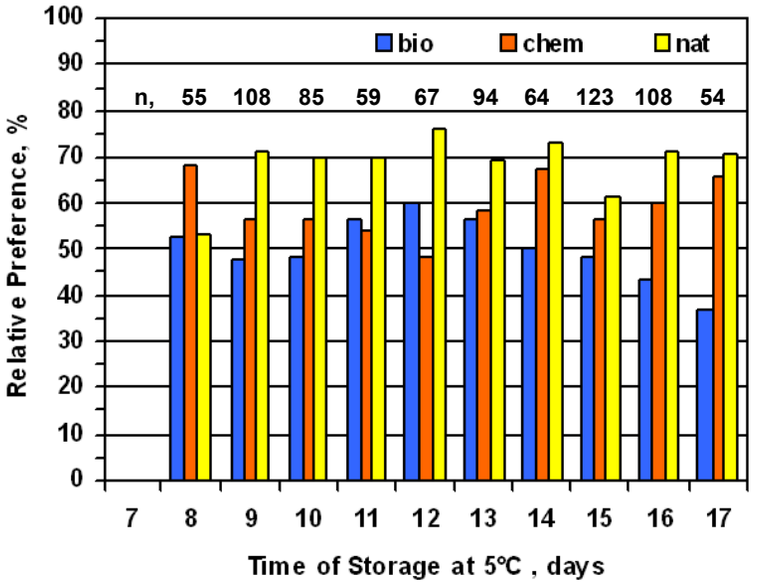

Figure 3. Consumer preference of vacuum-packaged Bologna-type sausage with *Lb. sakei* protective culture (bio) in comparison to non-packaged, sliced on-the-spot sausages without (nat) and with chemical (chem) preservatives presented at the International Green Week Berlin 2010. n, number of responses [7].

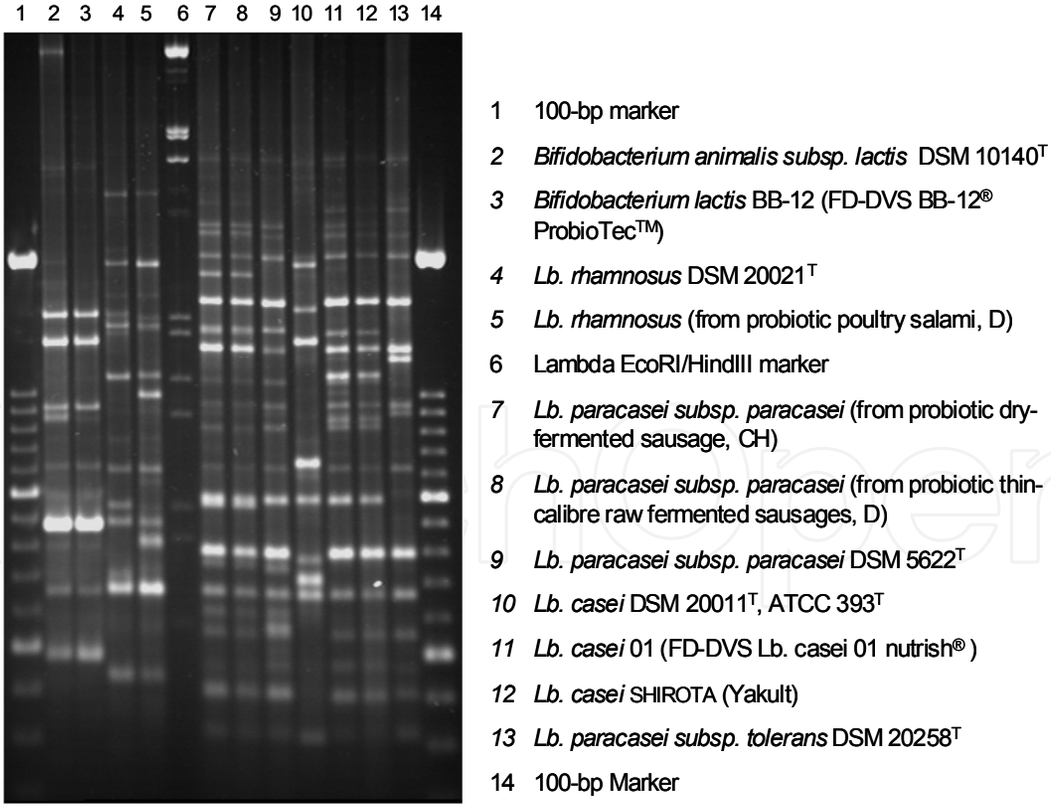

Figure 4. Genetic fingerprints of probiotic LAB and related reference strains using BOX-PCR [57].

and raw fermented sausages were considered as an appropriate vehicle for these probiotics. However, these environments are quite different from the human gastrointestinal (GI) tract,

and the strains under consideration have to cope with and survive in the presence of nitrite, sodium chloride, reduced pH and water activity, various processing steps and, eventually, long-term storage. Due to the manufacturing process raw fermented sausages contain high numbers of lactic acid bacteria which, however, are not regarded as probiotics. On the other hand, most of the known probiotic bacteria are unable to establish themselves in the raw sausage environment. Exceptions thereof are microbial cultures belonging to the *Lactobacillus plantarum group* and to the *Lactobacillus casei* group [57-59]. The use of protective and probiotic cultures may be a useful and effective strategy to prevent or reduce pathogens in the food chain, improve food safety and consumer health.

Within a project investigating the possibilities for manufacturing high quality and microbiologically sound products from meat of mother sheep, salami-type raw fermented sausages were produced with added conventional (*Lb. sakei*, *Lb. plantarum*) and probiotic lactic starter cultures (*Lb. paracasei*). The products were subjected to microbiological and sensory evaluation for up to nine months. All sausage batches with added cultures resulted in microbiological safe and sensory appealing products. The *Lb. sakei* culture survived during the whole storage period on a high level (> 10^8 cfu/g) while the two other cultures (*Lb. plantarum, Lb. paracasei*) partly reached the threshold of 10^6 cfu g^{-1} already after 3 months and were replaced by indigenous lactic acid bacteria of the *Lb. sakei* / *curvatus* group. For some batches, however, an acceptable number of probiotic bacteria could still be detected after nine months. Overall, *Lb. paracasei* showed a better survival in the ripened sausage than *Lb. plantarum* [7].

One problem for official authorities involved in consumer protection is to verify the presence of the indicated probiotics at sufficiently high levels. In the absence of simple and relyable identification procedures this may be a challenging task. In such cases genetic fingerprinting of isolates recovered on suitable agar media at relevant dilutions is the method of choice (Figure 4) [57, 60]. In the past, *Lb. rhamnosus* and *Lb. paracasei* ssp. *paracasei* have been used in fermented sausages, and labelling was quite confusing (Table 1). As can be seen, *Lb. paracasei* survived in relatively high numbers even in very dry salami. More recently, also other LAB species have been suggested as probiotics, and microencapsulation of strains has been used to overcome survival problems in the sausage environment. Still, human verification studies for probiotic administration are quite rare [61].

6. Functional starter cultures

In fermented sausage production classical starter cultures are usually also protective cultures, especially with respect to the acid-sensitive microflora. Modern cultures may provide additional protective action, e.g. by producing bacteriocins inhibitory to listeria and/or undesired LAB, or they may possess an additional probiotic functionality. Strains combining these traits have been termed also 'functional starter cultures' [62].

7. Bacteriocin production

Strains from many LAB species excrete anti-listerial bacteriocins, of which nisin produced by *Lc. lactis* and pediocin produced by *Pc. acidilactici* are the most wellknown. Besides,

Sausage no.	1	2	3	4
Type	soft, quickly ripened, smoked salami	very hard, air-dried salami	quickly ripened, thin-calibre, smoked sausage	smoked, dry-fermented salami with 30% weightloss
Characteristics	pH 4.7; a_w 0.954	pH 5.6	pH 4.9	n. d.
Origin	D	CH	D	D
Claims	'probiotic poultry salami with three probiotic cultures (*Bifidus, Lb. casei, Lb. acidophilus*)'	‚probiotic', with beef and pork	'probiotic culture in high numbers ($5x10^8$ cfu/g)', with beef and pork	'Probiotic !!!, naturally ripened', with beef and vegetable fat (no lard)
advertised culture detectable	no	not labeled	yes	not labeled
detected potentially probiotic culture	*Lb. rhamnosus*	*Lb. paracasei* subsp. *paracasei*	*Lb. paracasei* subsp. *paracasei*	*Lb. paracasei* subsp. *paracasei*
Viable counts (cfu/g) of probiotic culture	1-6 x 10^7	4-9 x 10^7	3 x 10^7	1 x 10^7

n. d., not determined; D, Germany; CH, Switzerland.

Table 1. Detection of probiotic cultures in probiotic raw fermented sausages from retail [57].

bacteriocin-producing LAB with anti-listerial activity naturally occur on a wide range of ready-to-eat foods, including meats [63-65]. From a meat point of view the sakacins of *Lb. sakei* are the most interesting because of the high competitivity of this species in the meat environment [22,49]. The pediocin producer *Pc. acidilactici* is commonly used by the Spanish meat industry as a starter culture [66].

8. Hydrogen peroxide production

The demonstration of hydrogen peroxide formation by meat-borne lactic acid bacteria is of considerable importance for the characterization of individual strains, the selection of suitable starter and protective cultures for various applications for meat and meat products as well as for the search of potential microbiological causes for undesired sensory deviations (discolourations/'greening', rancidity). Many LAB are able to form hydrogen peroxide as a by-product of O_2-dependent metabolic pathways. Dependent on the environment, this trait may be desired or undesired [1,23,67,69,104].

In foods and feed it may contribute to the inhibition of an undesired accompanying microbiota [67]. The H_2O_2 formed by LAB acts bacteriostatic on GRAM-positive bacteria and bactericidal on Gram-negatives [12,68].

In a recent study a novel agar medium ('Prussian Blue' (PB) agar) was applied for the first time to lactic acid bacteria relevant to meat and meat products [69]. The PB agar detects H_2O_2 through the formation of Prussian Blue (Figure 5). It principally delivers similar results

as the traditional manganese dioxide agar. However, it is more sensitive and, it is also more easily prepared and delivers results more quickly. A representative number of strains was used in the evaluation of the new medium (Table 2).

As to the production of H_2O_2, the study revealed large differences within the *Lb. sakei/curvatus* group. The bacteriocin producers frequently seemed to be relatively weak peroxide producers, while many commercial starter cultures were recognized as more or less strong peroxide producers. More recent field isolates of *Lb. sakei/curvatus* from prepackaged sliced Bologna-type sausage gave an essentially similar picture. In this case, however, only one of ten isolates of *Lb. curvatus* gave rise to a positive reaction.

Species	strains[a)]	PB	MnO_2
Lb. sakei (*Lb. bavaricus*)	DSM 20494	-	nd
Lb. sakei ssp. carnosus *(Lb. curvatus ssp. melibiosus)*	DSM 15740	+	++
Lb. sakei ssp. carnosus	Lb1047	-	+
Lb. sakei ssp. carnosus	DSM 15831^T	++	++
Lb. sakei ssp. carnosus (ssp. sakei)	23K	+++	++
Lb. sakei ssp. carnosus	Lb790	+++	++
Lb. sakei ssp. sakei	DSM 20017^T	+++	+
Lb. brevis	DSM 20054^T	++	-
Lb. farciminis	DSM 20180^T	-	nd
Lb. hilgardii	DSM 20176^T	-	-
Weissella paramesenteroides	DSM 20288^T	+++	nd
Weissella minor	DSM 20014^T	+++	nd
Leuconostoc carnosum	Lb1259	+	+
Leuconostoc carnosum	Lb1054	++	+
Leuconostoc carnosum	Lb1045	++	+

[a)] strains have been obtained from the German Collection of Microorganisms (DSM) and the strain collections of MRI Location Kulmbach (Lb) and INRA at Jouy-en-Josas (23K)

Table 2. Reaction of different LAB species on PB agar with BHI or MRS base, and on MnO_2 agar. -, no production of H_2O_2; +/++/+++, moderate to strong production of H_2O_2; nd, not determined [69].

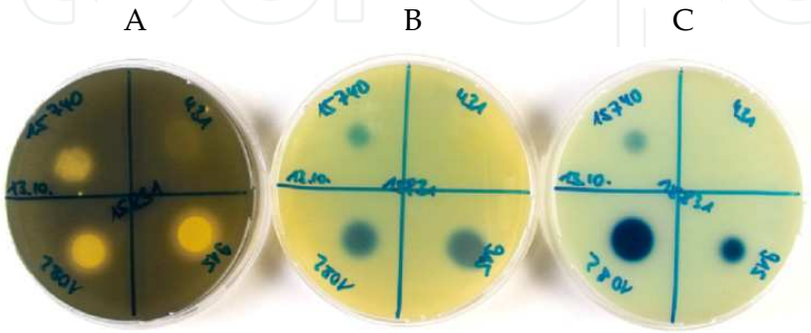

Figure 5. Reaction of different LAB species on MnO_2 agar (A) and on PB agar with MRS (B) or BHI (C) base. Production of H_2O_2 is indicated by bright and blue halos, resp. [69].

9. Formation of biogenic amines

Several LAB may produce biogenic amines by decarboxylation of amino acids, e.g. *Lb. buchneri, Lb. brevis, Lb. curvatus, Lb. hilgardii, Cb. maltaromaticum, Cb. divergens* [70]. Examples are such as tyramine and histamine during sausage fermentation. Strains of *Lb. plantarum, Lb. brevis* and *Lb. casei/paracasei*, and *Ec. faecium* and *Ec. faecalis* were identified as tyramine/histamine producers in the sausages [71]. Suitable starter cultures may contribute to reduction of biogenic amines in fermented sausages [72].

10. Identification of LAB

Identification of meat associated LAB is still wideley performed with phenotypic methods only, e.g API 50 CH [73]. These are, however, not always satisfying and may lead to misidentifications [74]. Nowadays, the application of PCR-DGGE and 16S rRNA gene sequencing allow the identification of a large number of strains in a quick and fast way [21,75]. Also various genomic fingerprinting methods are available. Nevertheless, conventional approaches remain important, especially when dealing with previously unknown species. Modern identification procedures rely on polyphasic approaches, integrating several lines of evidence to obtain a comprehensive description of a new species or of a microbiota [76].

11. Important LAB in meats

11.1. The Lb. sakei/curvatus cluster

In his 1983 review on lactic acid bacteria of meat and meat products EGAN mentions that according to recent findings of KANDLER and co-workers *Lb. sakei* (then *Lb. sake*) and *Lb. curvatus* were very common on German meat products [1]. Presently, two subspecies of *Lb. sakei* are known of which ssp. *carnosus* is the one characteristic for meats. It is common in fermented meat products, and is regularly found in vacuum-packaged meat and fermented plant material (sauerkraut). The subspecies *sakei* has been isolated from the Japanese sake starter and is regularly found in fermented meat products, vacuum-packaged meat, fermented plant material (sauerkraut), and human feces. The two subspecies can not be separated based on their physiological and biochemical characteristics [12]. The genomes of *Lb. sakei* 23K from a French dry-fermented sausage and *Lb. curvatus* CRL705 from an Argentinean artisanal fermented sausage have been sequenced [77,78]. Both genomes are highly similar. *Lb. curvatus* CRL705 lacks several genes present in *Lb. sakei* such as those related to fatty acid biosynthesis FASII, sucrose utilization, the arginine deiminase pathway, and citrate metabolism. The ones unique in *Lb. curvatus* CRL705 include genes for proteins and enzymes involved in the metabolism of carbohydrates, DNA, and fatty acids, as well as in the oxidative stress response and in bacteriocin production.

11.2. Lb. plantarum

The LAB species *Lb. plantarum* displays a high flexibility and versatility, and is able to colonize several ecological niches such as vegetables, meats, fish, milk substrates, and the human GI tract.

This is the basis of many applications in the food and health areas. As a starter culture for salamis *Lb. plantarum* is used since decades. More recently also probiotic strains have been described. With a size of 3.3 Mb its genome is one of the largest of LAB. A recent study on the phenetic and genetic diversity of the species revealed a high phenetic diversity which generally correlated with the origin of the isolates, e.g. from meat fermentations, kimchi, sourdough, egg plants and cheese. Four main clusters were determined: (i) meat, (ii) vegetable, (iii) sourdough, (iv) mixed sources with high meat content. On the genome level there were seven main clusters. The core genome contains more than 2000 genes, 121 genes being specific for *L. plantarum*. None of the strains could grow in milk, or at 4°C, or in the presence of 10% NaCl. A limited number grew at 17°C, or at 6% NaCl [79]. One of the earliest and most successful starter cultures for raw fermented sausages on the German market, "DuploFerment 66", contains a strain of *Lb. plantarum*. This is also the case for the "Saga II" starter from the US. In contrast to the first one, the latter strain does not grow at 10°C. Both strains are homofermentative for lactate and grow at 42°C but not at 8°C [25]. They provide rapid acidification of the raw sausage batter. On the other hand, *Lb. plantarum* is not very well adapted to meat and fails to maintain sufficiently high cell numbers to outcompete indigenous LAB. Sometimes it even does not grow in the meat batter [80,81]. In Italian natural fermented sausage the initial dominant populations of *Lb. plantarum* were accompanied by *Lb. sakei* and *Lb. curvatus* from the 10th day of fermentation and were finally competed out by the latter [21]. But, in certain traditional Greek fermented sausages *Lb. plantarum* and *Lb. plantarum/pentosus* may predominate [82,83].

11.3. Lb. brevis

In combination with *Pc. pentosaceus*, *Lb. brevis* has been used as an indigenous starter culture for a Vietnamese fermented meat product [84]. While *Lb. brevis* strongly acidifies the product, *Pc. pentosaceus* acts as a mild acidifier. The combination of both species resulted in a product with an intermediate taste (not too mild and not too sour) preferred by the sensory panel. Meat isolates of *Lb. brevis* may produce bacteriocins with antagonistic activity against *Li. monocytogenes* [85].

11.4. Lb. versmoldensis

This species was first reported in 2003 as the dominant LAB in some German raw fermented poultry salamis. The species was present in high numbers and frequently dominated the lactic acid bacteria (LAB) populations of the products [86]. Later, the species has been isolated also from Scandinavian fermented meats, Egyptian Domiati cheese and Japanese traditional fermented fish products [87-89]. There are no studies to date on the general behaviour of this species in meat ecosystems. The genome of strain KCTC 3814, an isolate from poultry salami, has been recently sequenced by the Korea Research Institute of Bioscience & Biotechnology [90].

11.5. Carnobacteria

Carnobacteria are non-aciduric and, therefore, are preferentially isolated from meats with elevated pH. *Cb. divergens* and *Cb. maltaromaticum* frequently constitute a major component

of the microflora of packaged raw meats as well as of refrigerated, prepackaged, sliced cooked deli meats. Meat spoilage by *Cb. maltaromaticum* has been associated with "dairy", "spoiled-meat", and "mozarella cheese" perception [31,91,92]. The major volatiles on meat, acetoin, 1-octen-3-ol and butanoic acid, are volatile organic compounds with low sensory impacts. Butanoic acid in stored beef was also associated with *Cb. divergens*. It has a rancid cheese-like odor and can derive from leucine metabolism, microbial consumption of free amino acids via the Stickland reaction or from tributyrin hydrolysis.

The metabolites from leucine degradation are involved in dry fermented sausage aroma. The catabolism of leucine by a strain of *Cb. maltaromaticum* was studied directly in the growth medium with H-3-labelled leucine to investigate the effect of five parameters: phase of growth, pH, oxygen, glucose and alpha-ketoisocaproic acid. Leucine catabolism was most important during the exponential phase of growth. The addition of alpha-ketoisocaproic acid at 1%, glucose at levels of 0.5% to 2% and shaking of the growth medium increased leucine catabolism. At pH 5.4 and 7.2, the main metabolites detected were 3-methyl butanal, 3-methyl butanol and alpha-ketoisocaproic acid. At pH 6.5, the leucine catabolism was maximum and was characterised by a high production of 3-methyl butanoic acid [93].

Positive and negative effects of carnobacteria in the environment and in foods have recently been reviewed [94]. Because *Cb. divergens* and *Cb. maltaromaticum* show good growth in refrigerated meats and some of the strains produce potent anti-listerial bacteriocins, they may have some role as bioprotectants in meat environments. However, carnobacteria are associated with unpleasant spoilage metabolites in meats, such as acetic and butanoic acid as well as gas production in vacuum packed beef. An undesirable trait is also their ability to produce the biogenic amine tyramine from tyrosine. Carnobacteria are not regarded as human pathogens, but *Cb. maltaromaticum* is a well known fish pathogen and catagorised as a safety-level-2 microorganism. The genome of *Cb. maltaromaticum* ATCC 35586 carries putative virulence genes which probably play a role in fish pathogenesis [95]. Since carnobacteria are inhibited by acetate they do not grow well on routine LAB media such as MRS. A selective enumeration medium using a combination of three antibiotics (gentamicin, nalidixic acid, vancomycin) and an alkaline pH value (8.8) has recently been proposed for *Cb. maltaromaticum* from cheese [96].

11.6. Leuconostoc

Leuc. gelidum is a major spoilage organism in Finnish fresh meats [97]. Certain strains of *Leuc. gelidum* may produce yellow discolourations on prepackaged refrigerated German 'Weisswurst' and cold cuts (Figure 6, 7) [98]. Recently, the genome of a plant isolate of *Leuc. gelidum* has been sequenced [99].

The responsible pigment for the intensive 'neon-like' yellow discolouration is a bacterial carotenoid, the non-polar C30-carotenoid 4,4'-di-apo-7,8,11,12-tetra-hydro-lycopene. On fat-containing substrates this compound does not only stain the bacterial cells but also the substrate and, in the case of 'Weisswurst' does stain the natural casing (porc intestine) of the

sausage as well as the sausage surface beneath. This triterpenoid is an intermediate in the microbial synthesis of 4,4'-diaponeurosporene which represents the main carotenoid in pigmented enterococci, Leuc. citreum and Lb. plantarum. Identification of the pigment was achieved by using UV-VIS spectroscopy in combination with available data from literature [100].

A report from Canada also described the yellow discolouration phenomenon on cooked sliced meats which had been stored for an extended time period under refrigeration [101]. These authors, employees of a big Canadian food company (then Canada Packers Inc.), tentatively identified an *Enterococcus* sp. as the causative agent.

Figure 6. Yellow discolourations on prepackaged refrigerated German 'Weisswurst' after targeted inoculation with *Leuc. gelidum* and incubation at 5°C for 14 days [98].

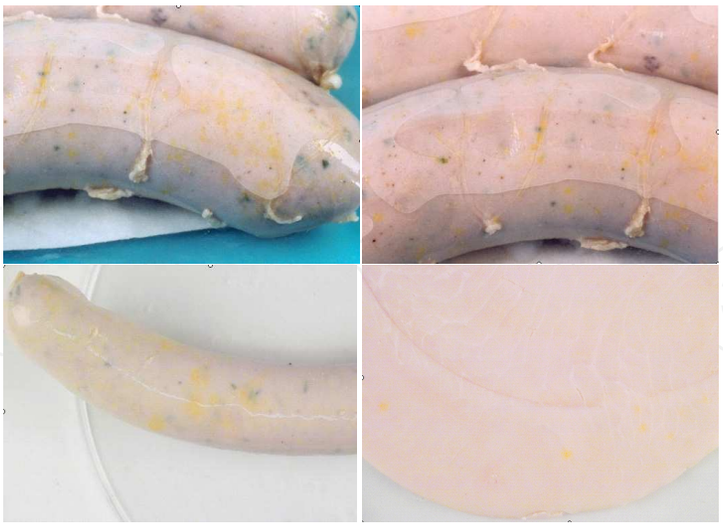

Figure 7. Yellow discolourations on pre-packaged meat products produced by *Leuc. gelidum*. A and B, 'Weisswurst' from organic production; C, grill sausage from conventional production; D, sliced cooked turkey breast from conventional production [98].

Leuc. gasicomitatum has been recognized as a specific spoilage organism in cold-stored Finnish MAP meats. It emerged as a spoilage problem of tomato-marinated, raw broiler

meat strips. Due to CO_2 production the packages already showed clear bulging more than a week before the expected shelf life [102]. It is a psychrotrophic species and, because of its dominance in marinated meats and fish as well as in vegetable sausages, probably of plant origin. But, it was also detected in minced meat and high-oxygen modified-atmosphere packaged raw, beef steaks injected with sugar-salt solutions, so-called moisture-enhanced or value-added meats [97,103]. Recently, the genome of the type strain *Leuc. gasicomitatum* LMG 18811^T has been sequenced [55].

11.7. Weissella

Weissella spp. are heterofermenters producing CO_2, ethanol and/or acetate from glucose. The species *Ws. viridescens*, *Ws. halotolerans* and *Ws. hellenica* have been associated with meat and meat products. *Ws. viridescens* is considered as heat resistant and may cause green discolouration in cured meats [104]. This species is frequently isolated from refrigerated sliced cooked meats [43] and was reported to produce cavities in the muscles of hams after cooking [105].

11.8. Pediococcus

The homofermentative pediococci are mostly applied for rapid and strong acidification at elevated temperature, especially in US summer sausage fermentation. Usually *Pc. acidilactici* and *Pc. pentosaceus* are the species involved. *Pediococcus* sp. are among the most common starter cultures in the US [11,21]. A pediocin producing *Pc. acidilactici* is also commonly used by the Spanish meat industry as a starter culture [66].

11.9. Enterococcus

In mediterranian traditional dry-fermented sausages enterococci are found in relevant numbers and are believed to contribute to the characterisic product flavor. *Ec. faecalis*, e.g., is common in Portuguese 'alheira' [19].

On the other hand, the presence of enterococci in foods is debatable, since some strains carry antibiotic resistances and virulence determinants relevant in human medicine [22,106]. Also, *Ec. faecium* and *Ec. faecalis* were identified as tyramine/histamine producers in the sausages [71]. The use of *Ec. faecium* strains has been suggested to control the growth of undesirable microorganisms such as listeria on material and environmental surfaces in meat plants [107].

12. Outlook

Meat and meat products provide a concentrated source of protein of high biological value and can make a valuable contribution to human diets. However, they are also highly perishable commodities which rapidly spoil and may even allow the growth of food-borne pathogenic microorganisms if no suitable preservative actions are taken. Meat fermentation

involving beneficial LAB has become an important and sustainable preservation technology, and today a number of suitable species and strains are successfully applied as starter and protective cultures in various fermented meats all over the world. These cultures not only prevent the growth of common food pathogens but also of undesirable food spoilage bacteria, including heterofermentative LAB. The answer to the question which strains we should use for which products largely depends on consumer expectations and technological needs. Much has been learned over the years, however, we are still far from understanding the complex metabolic interactions of LAB in meats.

Systems biology has become an important approach in LAB microbiology and will become even stronger in the future [108]. It links quantitative microbial physiology with population dynamic modelling and ecological theories. In comparative systems biology of LAB, the so-called ”omics”-techniques (”genomics”, ”proteomics”, ”transcriptomics”, ”metabolomics”) and mathematical and statistical methods are of crucial importance [109, 110]. Comparative analyses between various species is expected to deliver understandable models of the metabolism of these species. Whole genome sequencing has made a quantum leap in the past few years and it is likely that very soon all genomes of meat associated LAB species and even of different strains will be available for comparative studies. Diversity and differences within each of the species at the strain level will have to be considered. The ripening, packaging and storage of meats could benefit from improved systems knowledge of the diverse meat microcosms with respect to microbial survival and growth, as well as desired and unwanted microbial transformations of meat components to ensure high-quality, healthy, safe and tasty products. The beneficial aspects of LAB in meat preservation could be explored using systems techniques and will decrease our dependence on chemical preservatives. Likewise, the impact of microbes on meat spoilage could be better managed with a systems understanding of the interplay of microbes, raw materials, additives and processing technologies.

In a global perspective, the role of starter and protective cultures for the safety and quality of meats is expected to increase. Although the chemical preservatives currently applied to prevent the growth of pathogens and spoilage bacteria in deli meats perfectly serve this purpose, there is an increasing consumer demand for more natural products. This is in part reflected by the so-called clean label strategies of the big manufacturers. Many chemical additives not only contribute to the sodium burden of the meats, but also leave an undesirable numb mouthfeel which negatively effects the sensory perception of the meat aroma. Innovations in fermented meat production will benefit from an improved knowledge of systems microbiology of LAB in the various meat environments on the one hand, and the gastrointestinal environment on the other. A future challenge will be to link intraspecies diversity to a specific sensory profile [21]. The application of probiotic starter microorganisms in dry-fermented sausages remains appealling for the wellness-oriented consumers even if immediate health claims should be difficult to establish. In this sense beneficial LAB will vitally contribute to a sustainable and diversified food production.

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13. References

[1] Egan AF (1983) Lactic Acid Bacteria of Meat and Meat Products. Antonie van Leeuwenhoek 49: 327-336.

[2] Björkroth J, Ridell J, Korkeala H (1996) Characterization of *Lactobacillus sake* Strains Associated with Production of Ropy Slime by Randomly Amplified Polymorphic DNA (RAPD) and Pulsed-Field Gel Electrophoresis (PFGE) patterns. Int. j. food microbiol. 31: 59–68.

[3] Marshall VM (1987) Lactic Acid Bacteria: Starters for Flavour. FEMS microbiol. lett. 46: 327-336.

[4] Caplice E, Fitzgerald GF (1999) Food Fermentations: Role of Microorganisms in Food Production and Preservation. Int. j. food microbiol. 50: 131-149.

[5] Lücke FK (2000) Utilization of Microbes to Process and Preserve Meat. Meat sci. 56: 105-115.

[6] Schlafmann K, Meusburger AP, Hammes WP, Braun C, Fischer A, Hertel C (2002) Starter Cultures to Improve the Quality of Raw Ham. Fleischwirtschaft 82 (11): 108-114.

[7] Kröckel L, Dederer I, Troeger K (2011) Starter and Protective Cultures for Meat Products. Fleischwirtschaft 91 (3): 93-98.

[8] McIntyre L, Hudson JA, Billington C, Withers H (2012) Biocontrol of Foodborne Bacteria. In: McElhatton A, Sobral PJA, editors. Novel Technologies in Food Science – Integrating Food Science and Engineering Knowledge into the Food Chain 7. Springer Science+Business Media. pp. 183-204.

[9] Katla T, Moretro T, Sveen I, Aasen M, Axelsson L, Rorvik LM, Naterstad K (2002) Inhibition of *Listeria monocytogenes* in Chicken Cold Cuts by Addition of Sakacin P and Sakacin P-Producing *Lactobacillus sakei*. J. appl. microbiol. 93: 191-196.

[10] Vermeiren L, Devlieghere F, Vandekinderen I, Rajtak U, Debevere J (2006) The Sensory Acceptability of Cooked Meat Products Treated with a Protective Culture Depends on Glucose Content and Buffering Capacity: A Case Study with *Lactobacillus sakei* 10A. Meat sci. 74: 532-545.

[11] Hammes WP, Knauf HJ (1994) Starters in the Processing of Meat Products. Meat sci. 36: 155-168.

[12] Hammes WP, Hertel C (2009) Genus I. *Lactobacillus* Beijerinck 1901, 212AL. In: De Vos P, Garrity GM, Jones D, Krieg NR, Ludwig W, Rainey FA, Schleifer KH, Whitman WB, editors. The Firmicutes. Bergey's Manual of Systematic Bacteriology, 2nd Ed., Vol. 3. Springer, Dordrecht, Heidelberg London, New York; pp. 465- 511.

[13] Urso R, Comi G, Cocolin L (2006) Ecology of Lactic Acid Bacteria in Italian Fermented Sausages: Isolation, Identification and Molecular Characterization. Syst. appl. microbiol. 29: 671-680.

[14] Cocolin L, Dolci P, Rantsiou K, Urso R, Cantoni C, Comi G (2009) Lactic Acid Bacteria Ecology of Three Traditional Fermented Sausages Produced in the North of Italy as Determined by Molecular Methods. Meat sci. 82: 125-132.
[15] Cocolin L, Dolci P, Rantsiou K (2011) Biodiversity and Dynamics of Meat Fermentations: The Contribution of Molecular Methods for a Better Comprehension of a Complex Ecosystem. Meat sci. 89: 296-302.
[16] Hugas M, Garriga M, Aymerich T, Monfort JM (1993) Biochemical Characterization of Lactobacilli from Dry Fermented Sausages. Int. j. food microbiol. 18: 107-113.
[17] Santos EM, González-Fernández C, Jaime I, Rovira J (1998) Comparative Study of Lactic Acid Bacteria House Flora Isolated in Different Varieties of `Chorizo'. Int. j. food microbiol. 39: 123-128.
[18] Samelis J, Maurogenakis F, Metaxopoulos J (1994) Characterisation of Lactic Acid Bacteria Isolated from Naturally Fermented Greek Dry Salami. Int. j. food microbiol. 23: 179-196.
[19] Albano H, van Reenen CA, Todorov SD, Cruz D, Fraga L, Hogg T, Dicks LMT, Teixeira P (2009) Phenotypic and Genetic Heterogeneity of Lactic Acid Bacteria Isolated from "Alheira", a Traditional Fermented Sausage Produced in Portugal. Meat sci. 82: 389-398.
[20] Adams MR, Nicolaides L (1997) Review of the Sensitivity of Different Foodborne Pathogens to Fermentation. Food control 8: 227-239.
[21] Cocolin L, Rantsiou K (2012) Meat Fermentation. In: Hui YH, editor. Handbook of Meat and Meat Processing, Second Edition. CRC Press, pp. 557-572.
[22] Fontana C, Fadda S, Cocconcelli PS, Vignolo G (2012) Lactic Acid Bacteria in Meat Fermentations. In: Lahtinen S, Ouwehand AC, Salminen S, von Wright A, editors. Lactic Acid Bacteria – Microbiological and Functional Aspects, 4th Ed. CRC Press, Taylor & Francis, Boca Raton, London, New York. pp. 247-264.
[23] Buckenhüskes HJ (1993) Selection Criteria for Lactic Acid Bacteria to be Used as Starter Cultures for Various Food Commodities. FEMS microbiol. rev. 12: 253-271.
[24] Dodds KL, Collins-Thompson DL (1984) Nitrite Tolerance and Nitrite Reduction in Lactic Acid Bacteria Associated with Cured Meat Products. Int. j. food microbiol. 1: 163-170.
[25] Nordal J, Slinde E (1980) Characteristics of Some Lactic Acid Bacteria Used as Starter Cultures in Dry Sausage Production. Appl. environ. microbiol. 40: 472-475.
[26] Nissen H, Holck A (1998) Survival of *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella kentucky* in Norwegian Fermented, Dry Sausage. Food microbiol. 15: 273–279.
[27] Leistner L (2000) Basic Aspects of Food Preservation by Hurdle Technology. Int. j. food microbiol. 55:181–186.
[28] Lindqvist R, Lindblad M (2009) Inactivation of *Escherichia coli*, *Listeria monocytogenes* and *Yersinia enterocolitica* in Fermented Sausages During Maturation/Storage. Int. j. food microbiol. 129: 59-67.
[29] Heir E, Holck AL, Omer MK, Alvseike O, Hoy M, Mage I, Axelsson L (2010) Reduction of Verotoxigenic *Escherichia coli* by Process and Recipe Optimisation in Dry-Fermented Sausages. Int. j. food microbiol. 141: 195–202.
[30] Erkes M (2011) Einsatz von Kulturen bei Rohpökelwaren. Fleischwirtschaft 91 (11): 39-43.
[31] Jones RJ (2004) Observations on the Succession dynamics of Lactic Acid Bacteria Populations in Chill-Stored Vacuum-Packaged Beef. Int. J. food microbiol. 90: 273– 282.

[32] Schillinger U, Lücke FK (1987) Lactic Acid Bacteria on Vacuum-Packaged Meat and their Influence on Shelf Life. Fleischwirtschaft 67: 1244-1248.
[33] Castellano P. Gonzalez C, Carduza F, Vignolo G (2010) Protective Action of *Lactobacillus curvatus* CRL705 on Vacuum-Packaged Raw Beef. Effect on Sensory and Structural Characteristics. Meat sci. 85: 394-401.
[34] Castellano P, Belfiore C, Vignolo G (2011) Combination of Bioprotective Cultures with EDTA to Reduce *Escherichia coli* O157:H7 in Frozen Ground-Beef Patties. Food control 22: 1461-1465.
[35] Fadda S, Lopez C, Vignolo G (2010) Role of Lactic Acid Bacteria During Meat Conditioning and Fermentation: Peptides Generated as Sensorial and Hygienic Biomarkers. Meat sci. 86: 66-79.
[36] Borch E, Kant-Muermans ML, Blixt Y (1996) Bacterial Spoilage of Meat and Cured Meat Products. Int. j. food microbiol. 33: 103–120.
[37] Korkeala HJ, Bjørkroth KJ (1997) Microbiological Spoilage and Contamination of Vacuum-Packaged Cooked Sausages. J. food prot. 60: 724–731.
[38] Nattress FM, Jeremiah LE (2000) Bacterial Mediated Off-Flavours in Retail-Ready Beef after Storage in Controlled Atmospheres. Food res. int. 33: 743-748
[39] Björkroth KJ, Vandamme P, Korkeala HJ (1998) Identification and Characterization of *Leuconostoc carnosum*, Associated with Production and Spoilage of Vacuum-Packaged, Sliced, Cooked Ham. Appl. environ. microbiol. 64: 3313-3319.
[40] Kröckel L (1998) Lactic Acid Bacteria as Protective Cultures in the Preservation of Meat. In: Adria-Normandie, editor. Les Bactérie Lactic – Quelles Souches? Pour quels Produits? Lactic Acid Bacteria – Which Strains for which Products? – Actes du colloque LACTIC 97, Caen, 10-12 Sept 1997 – Adria Normandie, Villers-Bocage, pp. 229-242.
[41] Laursen BG, Bay L, Cleenwerck I, Vancanneyt M, Swings J, Dalgaard P, Leisner JJ (2005) *Carnobacterium divergens* and *Carnobacterium maltaromaticum* as Spoilers or Protective Cultures in Meat and Seafood: Phenotypic and Genotypic Characterization. Syst. appl. microbiol. 28: 151-64.
[42] Lücke FK, Raabe C, Hampshire J (2007) Changes in Sensory Profile and Microbiological Quality During Chill Storage of Cured and Uncured Cooked Sliced Emulsion-Type Sausages. Arch. Lebensmittelhyg. 58: 57-63.
[43] Kröckel L (2008) Mikrobiologische Qualität Vorverpackter Aufschnittware - Aktuelle Untersuchungen von Erhitztem, Vorverpacktem Brühwurst- und Kochschinkenaufschnitt auf *Listeria monocytogenes*. Fleischwirtschaft 88 (11): 112-116.
[44] Leistner L (1995) Principles and Applications of Hurdle Technology. In: Gould GW, editor. New Methods of Food Preservation. Springer; pp. 1–21.
[45] Leistner L, Gorris LG (1995) Food Preservation by Hurdle Technology. Trends food sci technol 6: 41-46.
[46] Hui YH (2012) Hazard Analysis and Critical Control Point System. In: Hui YH, editor. Handbook of Meat and Meat Processing, 2nd Ed. CRC Press, pp. 741-767.
[47] Jacobsen T, Budde BB, Koch, AG (2003) Application of *Leuconostoc carnosum* for Biopreservation of Cooked Meat Products. J. appl. microbiol. 95: 242–249.

[48] Vermeiren L, Devlieghere F, Debevere J (2004) Evaluation of Meat Born Lactic Acid Bacteria as Protective Cultures for the Biopreservation of Cooked Meat Products. Int. j. food microbiol. 96: 149–164.
[49] Jones RJ, Wiklund E, Zagorec M, Tagg JR (2010) Evaluation of Stored Lamb Biopreserved Using a Three-Strain Cocktail of *Lactobacillus sakei*. Meat sci. 86: 955-959
[50] Jones RJ, Zagorec M, Brightwell G, Tagg JR (2009) Inhibition by *Lactobacillus sakei* of Other Species in the Flora of Vacuum Packaged Raw Meats During Prolonged Storage. Food microbiol. 26: 876-881.
[51] Ananou S, Maqueda M, Martínez-Bueno M, Valdivia E (2007) Biopreservation, an Ecological Approach to Improve the Safety and Shelf-Life of Foods. In: Méndez-Vilas A, editor. Communicating Current Research and Educational Topics and Trends in Applied Microbiology, pp. 475-486. Available: http://www.formatex.org/microbio/pdf/Pages475-486.pdf. Accessed 2012 Apr 3.
[52] www (2012) Ingredients with Potential: Chr. Hansen A/S - Bactoferm Rubis. Available: http://www.safoodcentre.com.au/__data/assets/pdf_file/0015/131451/innova_ingredients_with_potential.pdf. Accessed: 2012 Apr 4
[53] Anonymous (2011) Veröffentlichte Mikrobiologische Richt- und Warnwerte zur Beurteilung von Lebensmitteln (Stand: November 2011) Eine Empfehlung der Fachgruppe Lebensmittelmikrobiologie und -hygiene der Deutschen Gesellschaft für Hygiene und Mikrobiologie (DGHM); http://www.dghm.org/m_275. Accessed 2012 Apr 3.
[54] Kröckel L (2010) Mikrobiologisch-Genetische Ressourcen bei Fleisch - Biodiversität und Nachhaltige Nutzung bei der Herstellung von Fleisch-Erzeugnissen. Forschungsreport Ernährung, Landwirtschaft, Verbraucherschutz 1/2010: 24-26.
[55] Johansson P, Paulin L, Säde E, Salovuori N, Alatalo ER, Bjørkroth KJ, Auvinen P (2011) Genome Sequence of a Food Spoilage Lactic Acid Bacterium, *Leuconostoc gasicomitatum* LMG 18811(T), in Association with Specific Spoilage Reactions. Appl. environ. microbiol. 77: 4344-4351.
[56] Hugas M, Monfort JM (1997) Bacterial Starter Cultures for Meat Fermentation. Food chemistry 59: 547-554.
[57] Kröckel L (2006a) Use of Probiotic Bacteria in Meat Products. Fleischwirtschaft 86:109–13.
[58] Ammor MS, Mayo B (2007) Selection Criteria for Lactic Acid Bacteria to be used as Functional Starter Cultures in Dry Sausage Production: an Update. Meat sci. 76: 138-146.
[59] De Vuyst L, Falony G, Leroy F (2008) Probiotics in Fermented Sausages. Meat sci. 80: 75-78.
[60] Huang CH, Lee FL (2009) Development of Novel Species-Specific Primers for Species Identification of the *Lactobacillus casei* Group Based on RAPD Fingerprints. J. sci. food agric. 89: 1831-1837.
[61] Khan MI, Arshad MS, Anjum FM, Sameen A, Aneeq-ur-Rehman, Gill WT (2011) Meat as a Functional Food with Special Reference to Probiotic Sausages. Food res. int. 44: 3125-3133.
[62] Leroy F, Verluyten J, De Vuyst L (2006) Functional Meat Starter Cultures for Improved Sausage Fermentation. Int. j. food microbiol. 106: 270-285.
[63] Abee T, Kröckel L, Hill C (1995) Bacteriocins: Modes of Action and Potentials in Food Preservation and Control of Food Poisoning. Int. j. food microbiol. 28:169-185.

[64] Kelly WJ, Asmundson RV, Huang CM (1996) Isolation and Characterization of Bacteriocin-Producing Lactic Acid Bacteria from Ready-to-Eat Food Products. Int. j. food microbiol. 33: 209-218.
[65] Hugas M (1998) Bacteriocinogenic Lactic Acid Bacteria for the Biopreservation of Meat and Meat Products. Meat sci. 49: S139-S150.
[66] Nieto-Lozano JC, Reguera-Useros JI, Peláez-Martínez MC, Hardisson de la Torre A (2002) Bacteriocinogenic Activity from Starter Cultures Used in Spanish meat Industry. Meat sci. 62: 237-243.
[67] Lücke FK, Popp J, Kreutzer R (1986) Bildung von Wasserstoffperoxid durch Laktobazillen aus Rohwurst und Brühwurstaufschnitt. Chem. mikrobiol. technol. lebensm. 10: 78-81.
[68] Condon S. (1987) Responses of lactic acid bacteria to oxygen. FEMS Microbiol. rev. 46, 269-280.
[69] Kröckel L (2011) Evaluation of a Novel Agar Medium for the Detection of Hydrogen Peroxide Producing Lactic Acid Bacteria. Fleischwirtschaft 91 (10): 97-101.
[70] Silla-Santos MH (1996) Biogenic Amines: their Importance in Foods. Int. j. food microbiol. 29: 213–231.
[71] Komprda T, Sládková P, Petirová E, Dohnal V, Burdychová R (2010) Tyrosine- and Histidine-Decarboxylase Positive Lactic Acid Bacteria and Enterococci in Dry Fermented Sausages. Meat sci. 86: 870-877.
[72] Lu S, Xu X, Zhou G, Zhu Z, Meng Y, Sun Y (2010) Effect of Starter Cultures on Microbial Ecosystem and Biogenic Amines in Fermented Sausage. Food control 21: 444-449.
[73] Samappito W, Leenanon B, Levin RE (2011) Microbiological Characteristics of ''Mhom'', a Thai Traditional Meat Sausage. The Open Food Science Journal 5: 31-36.
[74] Sohier D, Coulon J, Lonvaud-Funel A (1999) Molecular Identification of *Lactobacillus hilgardii* and Genetic Relatedness with *Lactobacillus brevis*. Int. j. syst. bacteriol. 49: 1075-1081.
[75] Rantsiou K, Cocolin L (2006) New Developments in the Study of the Microbiota of Naturally Fermented Sausages as Determined by Molecular Methods: A Review. Int. j. food microbiol. 108: 255-267.
[76] Vandamme P, Pot B, Gillis M, de Vos P, Kersters K, Swings J (1996) Polyphasic taxonomy, a consensus approach to bacterial systematics. Microbiol rev. 60: 407-38.
[77] Chaillou S, Champomier-Verges MC, Cornet M, Crutz-Le Coq AM, Dudez AM, Martin V, Beaufils S, Darbon-Rongere E, Bossy R, Loux V, Zagorec M (2005) The Complete Genome Sequence of the Meat-Borne Lactic Acid Bacterium *Lactobacillus sakei* 23K. Nat. biotechnol. 23: 1527-1533.
[78] Hebert EM, Saavedra L, Taranto MP, Mozzi F, Magni C, Nader MEF, Font de Valdez G, Sesma F, Vignolo G, Rayaa RR (2012) Genome Sequence of the Bacteriocin-Producing *Lactobacillus curvatus* Strain CRL705. J. bacteriol. 194: 538-539.
[79] Siezen RJ, Tzeneva VA, Castioni A, Wels M, Phan HTK, Rademaker JLW, Starrenburg MJC, Kleerebezem M, Molenaar D, van Hylckama Vlieg JET (2010) Phenotypic and Genomic Diversity of *Lactobacillus plantarum* Strains Isolated from Various Environmental Niches. Environ. microbiol. 12: 758-773.

[80] Marchesini B, Bruttin A, Romailler N, Moreton RS, Stucchi C, Sozzi T (1992) Microbiological Events During Commercial Meat Fermentations. J. appl. bacteriol. 73: 203-209.
[81] Kröckel L (unpublished observations)
[82] Drosinos EH, Mataragas M, Xiraphi N, Moschonas G, Gaitis F, Metaxopoulos J (2005) Characterization of the Microbial Flora from a Traditional Greek Fermented Sausage. Meat sci. 69: 307-317.
[83] Drosinos EH, Paramithiotis S, Kolovos G, Tsikouras I, Metaxopoulos I (2007) Phenotypic and Technological Diversity of Lactic Acid Bacteria and Staphylococci Isolated from Traditionally Fermented Sausages in Southern Greece. Food microbiol. 24: 260-270.
[84] Ho TNT, Nguyen NT, Deschamps A, Hadj Sassi A, Urdaci M, Caubet R (2009) The Impact of *Lactobacillus brevis* and *Pediococcus pentosaceus* on the Sensorial Quality of "Nem Chua" – a Vietnamese Fermented Meat Product. Int. food res. j. 16: 71-81.
[85] Coventry MJ, Wan J, Gordon JB, Mawson RF, Hickey MW (1996) Production of Brevicin 286 by *Lactobacillus brevis* VB286 and Partial Characterization. J. appl. bacteriol. 80: 91-8.
[86] Kröckel L, Schillinger U, Franz CMAP, Bantleon A, Ludwig W (2003) *Lactobacillus versmoldensis* sp. nov., isolated from raw fermented sausage. Int. j. syst. env. microbiol. 53: 513-517.
[87] Klingberg TD, Axelsson L, Naterstad K, Elsser D, Budde BB (2005) Identification of Potential Probiotic Starter Cultures for Scandinavian-Type Fermented Sausages. Int. j. food microbiol. 105: 419-431.
[88] El-Baradei G, Delacroix-Buchet A, Ogier JC (2007) Biodiversity of Bacterial Ecosystems in Traditional Egyptian Domiati Cheese. Appl. environ. microbiol. 73: 1248-1255.
[89] An C, Takahashi H, Kimura B, Kuda T (2010) Comparison of PCR-DGGE and PCR-SSCP Analysis for Bacterial Flora of Japanese Traditional Fermented Fish Products, Aji-Narezushi and Iwashi-Nukazuke. J. sci. food agric. 90: 1796-1801.
[90] Kim DS, Choi SH, Kim DW, Kim RN, Nam SH, Kang A, Kim A, Park HS (2011a) Genome Sequence of *Lactobacillus versmoldensis* KCTC 3814. J. bacteriol. 193: 5589-5590.
[91] Casaburi A, Nasi A, Ferrocino I, Di Monaco R, Mauriello G, Villani F, Ercolini D (2011) Spoilage-Related Activity of *Carnobacterium maltaromaticum* Strains in Air-Stored and Vacuum-Packed Meat. Appl. environ. microbiol. 77: 7382-7393.
[92] Ercolini D, Ferrocino I, Nasi A, Ndagijimana M, Vernocchi P, La Storia A, Laghi L, Mauriello G, Guerzoni ME, Villani F (2011) Monitoring of Microbial Metabolites and Bacterial Diversity in Beef Stored Under Different Packaging Conditions. Appl. environ. microbiol. 77: 7372-7381.
[93] Larrouture-Thiveyrat C, Montel MC (2003) Effects of environmental factors on leucine catabolism by *Carnobacterium piscicola*. Int. j. food microbial. 81: 177–184.
[94] Leisner JJ, Groth Laursen B, Prevost H, Drider D, Dalgaard P (2007) *Carnobacterium*: Positive and Negative Effects in the Environment and in Foods. FEMS microbiol. rev. 31: 592–613.
[95] Leisner JJ, Hansen MA, Larsen MH, Hansen L, Ingmera H, Sørensen SJ (2012) The Genome Sequence of the Lactic Acid Bacterium *Carnobacterium maltaromaticum* ATCC 35586 Encodes Potential Virulence Factors. Int J. food microbiol. 152: 107–115.

[96] Edima HC, Cailliez-Grimal C, Revol-Junelles AM, Tonti L, Linder M, Millière JB (2007) A Selective Enumeration Medium for *Carnobacterium maltaromaticum*. J. microbiol. meth. 68: 516-21.

[97] Vihavainen E, Björkroth J (2007) Spoilage of Value-Added, High-Oxygen Modified-Atmosphere Packaged Raw, Beef Steaks by *Leuconostoc gasicomitatum* and *Leuconostoc gelidum*. Int. j. food microbiol. 119: 340–345.

[98] Kröckel L (2006b) Yellow Discolourations of Prepackaged Refrigerated German Weisswurst are Due to *Leuconostoc gelidum*. Fleischwirtschaft 86 (9): 129-133.

[99] Kim DS, Choi SH, Kim DW, Kim RN, Nam SH, Kang A, Kim A, Park HS (2011b) Genome Sequence of *Leuconostoc gelidum* KCTC 3527, Isolated from Kimchi. J. bacteriol. 193: 799–800.

[100] Kröckel L (2007) Ein Bakterielles Carotinoid Färbt Vorverpackte, Kühl Gelagerte Weißwurst Gelb. Mitteilungsblatt der Fleischforschung Kulmbach 46, Nr. 178: 223-230.

[101] Whiteley AM, D'Souza MD 1989. A Yellow Discoloration of Cooked Cured Meat Products - isolation and characterization of the causative organism. J. food protect. 52: 392– 395.

[102] Björkroth KJ, Geisen R, Schillinger U, Weiss N, De Vos P, Holzapfel WH, Korkeala HJ, Vandamme P (2000). Characterization of *Leuconostoc gasicomitatum* sp. nov., Associated with Spoiled Raw Tomato-Marinated Broiler Meat Strips Packaged Under Modified-Atmosphere Conditions. Appl. environ. microbiol. 66: 3764–3772.

[103] Nieminen TT, Vihavainen E, Paloranta A, Lehto J, Paulin L, Auvinen P, Solismaa M, Björkroth KJ (2011) Characterization of Psychrotrophic Bacterial Communities in Modified Atmosphere-Packed Meat with Terminal Restriction Fragment Length Polymorphism. Int. J. food microbiol. 144: 360–366.

[104] Björkroth J, Dicks LMT, Holzapfel WH (2009) Genus III. *Weissella*. In: De Vos P, Garrity GM, Jones D, Krieg NR, Ludwig W, Rainey FA, Schleifer KH, Whitman WB, editors. The Firmicutes. Bergey's Manual of Systematic Bacteriology, 2nd Ed., Vol. 3. Springer, Dordrecht, Heidelberg London, New York; pp. 643- 654.

[105] Comi G, Iacumin L (2012) Identification and Process Origin of Bacteria Responsible for Cavities and Volatile Off-Flavour Compounds in Artisan Cooked Ham. Int. j. food sci. technol. 47, 114-121.

[106] Mathur S, Singh R (2005) Antibiotic Resistance in Food Lactic Acid Bacteria - A Review. Int. j. food microbiol. 105: 281-295.

[107] Ammor S, Tauveron G, Dufour E, Chevallier I (2006) Antibacterial Activity of Lactic Acid Bacteria Against Spoilage and Pathogenic Bacteria Isolated from the Same Meat Small-Scale Facility: 2 - Behaviour of Pathogenic and Spoilage Bacteria in Dual Species Biofilms Including a Bacteriocin-Like-Producing Lactic Acid Bacteria. Food control 17: 462-468.

[108] Teusink B, Bachmann H, Molenaar D (2011) Systems Biology of Lactic Acid Bacteria: a Critical Review. Microb. Cell. Fact. 10 (suppl 1): S11.

[109] McLeod A, Zagorec M, Champomier-Vergès MC, Naterstad K, Axelsson L (2010) Primary Metabolism in *Lactobacillus sakei* Food isolates by Proteomic Analysis. BMC microbiol. 10: 120.

[110] Nyquist OL, McLeod A, Brede DA, Snipen L, Aakra Å., Nes IF (2011) Comparative Genomics of *Lactobacillus sakei* with Emphasis on Strains from Meat. Molec. genetics and genomics 285: 297-311.