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New Developments in Meat Starter Cultures

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ABSTRACT

Meat starter cultures containing one or more strains of lactic acid bacteria, Actinobacteria, *staphylococci,* Halomonas elongata, Aeromonas *spec., and moulds or yeasts are widely in practical use. The progress in microbial systematic has led to changes in the taxonomy of familiar bacterial species which are described. Studies of flavour genesis led to the identification of the contribution of the enzyme activities endogenously present in the meat matrix as well as of those exerted by the starter cultures. Characteristic compounds of the aroma of fermented meat products originating from the starter organisms were also described. New knowledge was accumulated on the physiology and genetics of starter bacteria and some insight has been gained in the regulation of the expression of genes encoding important properties such as bacteriocin production or catalase activity. The applicability of gene technology to starter strains has been shown and strains have been constructed that have the potential to further improve the technological and hygienic suitability of starter cultures. New applications of the micro-organisms as protective or probiotic cultures have been developed for application in meat science.* © *1998 Elsevier Science Ltd. All rights reserved*

INTRODUCTION

'It is unbelievable that such a tiny streamlet, as it was our initial test trial with the first bacterial starters exactly 40 years ago, grew to a big river for an important industry. Who might have believed at those times that the use of starter cultures are a matter of course today'.

We should like to introduce our communication with these sentences of the pioneer of meat starter cultures Niinivaara (1994) concluding a paper on the history of starter utilisation in meat processing. More recent reviews on these starter cultures have been published by Hammes and Knauf (1994) and Jessen (1995). It is the purpose of this paper to focus on new developments starting from about the time covered by the above reviews.

MICRO-ORGANISMS EMPLOYED IN STARTER CULTURES

In Table 1 an overview is presented of the known species employed in starter preparations. For simplicity species used in so called protective and probiotic cultures are also

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TABLE 1
Current Status of Species Employed in Meat Starter Cultures

Bacteria

Lactic acid bacteria

Lactobacillus acidophilus[a], *L. alimentarius*[b], *L. casei*[c], *L. curvatus*, *L. plantarum*, *L. pentosus*, *L. sakei*, *Lactococcus lactis*, *Pediococcus acidilactici*, *P. pentosaceus*

Actinobacteria

Kocuria varians[c]
Streptomyces griseus
Bifidobacterium spec.[a]

Staphylococci

Staphylococcus xylosus, *S. carnosus* subsp. *carnosus*, *S. carnosus* subsp. *utilis*, *S. equorum*[b]

Halomonadaceae

Halomonas elongata[b]

Enterobacteria

Aeromonas spec.

Fungi

Penicillium nalgiovense, *P. chrysogenum*, *P. camemberti*

Yeasts

Debaryomyces hansenii, *Candida famata*

[a]Used in probiotic cultures.
[b]Used in pre-market studies at industrial scale (Laboratorium Wiesby, Niebüll and Rudolf Müller and Co., Gießen, pers. comm.).
[c]Formerly *Micrococcus varians*.

included in this compilation. These new preparations serve purposes which differ from those of classical starter cultures and shall be considered further below. The borderline between the classical starters and the new culture types cannot always clearly be drawn, as certain properties of a microbial strain may contribute to purposes that can be ascribed to various culture types. When referring to meat starter cultures, we shall rely on the following definition (Hammes, 1995):

> Starter cultures are preparations which contain living or resting forms of micro-organisms that develop in the fermentation substrate the desired metabolic activity. In the rule, but not necessarily, the organisms grow (multiply) in this substrate.

Their quality should be considered under microbiological as well as technological and sensory aspects of their performance in the meat matrix. Arguments were provided by Hammes (1995) that the following aspects should be included in the definition of the microbiological quality of these preparations:

- known identity of the micro-organisms on the taxonomic level
- stability of the desired physiological properties
- known cell counts
- biological purity (no microbial contamination interfering with the desired properties of the preparation)
- hygienic safety (free from any contamination interfering with consumer health).

The desired properties of meat starter cultures with regard to ensuring food quality and technological suitability were addressed in recent reviews (Cook, 1995; Jessen, 1995; Kröckel, 1995; Hammes and Hertel, 1996; Lücke, 1998).

With regard to starter species listed in Table 1 few changes have taken place during the past 4 years (Hammes and Knauf, 1994). On the other hand, research in systematic bacteriology led to changes in their taxonomic position as well as nomenclature. For example, the epithet of *Lactobacillus sake* had to be replaced by *sakei* (Trüper and De Clari, 1997). The species *L. curvatus* and *L. sakei* have been divided each into two subspecies: *L. curvatus* subsp. *curvatus* and subsp. *melibiosus*, *L. sakei* subsp. *sakei* and subsp. *carnosus* (Torriani *et al.*, 1996). The separation into the subspecies rested on biochemical and physiological differences which are not clearly supported by data on the genomic level (Klein *et al.*, 1996). With regard to properties with importance in characterising the ecological and food technological traits of the various new subspecies no specific criteria are available. Furthermore, it has not been studied to which of the respective subspecies the commercially available starter strains have to be allotted. Therefore, in Table 1 the subspecies of *L. curvatus* and *L. sakei* are not considered.

Micrococcus varians was placed into the genus *Kocuria* which belongs to the class *Actinobacteria*, subclass *Actinobacteridae*, order *Actinomycetales*, suborder *Micrococcineae*, family *Micrococcaceae* (Stackebrandt *et al.*, 1995, 1997).

The staphylococci are located together with the lactic acid bacteria in the so-called *Clostridium* branch consisting of Gram-positive bacteria with a low DNA G+C content. Within the genus *Staphylococcus* the widely applied species *S. carnosus* was divided into the two subspecies (Probst *et al.*, 1998) and both subspecies have been identified as components of starter preparations. The strains of subspecies *utilis* can be differentiated from those of subspecies *carnosus* on the basis of physiological as well as genotypical criteria and, remarkably, by their inability to form biogenic amines and to exhibit hemolysis. The use of *S. equorum* in starter preparations with application in curing of raw ham may be favourable as strains of this species grow well at temperatures $< 10°C$. This unusual property of a *Staphylococcus* is consistent with a temperature optimum for growth of 30°C (Schleifer *et al.*, 1984) as compared to 37°C commonly found in that genus. In addition, an involvement of *Staphylococcus equorum* in maturation of Iberian dry cured ham was demonstrated by Rodríguez *et al.* (1994). In this process numerous Gram-positive, catalase positive cocci are involved and grow on the surface to numbers of $> 10^8$ cfu g^{-1}. *S. xylosus* represented 70% of that flora and *S. equorum* 8%. It was shown (Rodríguez *et al.*, 1996) that among *S. xylosus* isolates, strains exist that exhibit the potential for staphylococcal enterotoxin formation. The prevention of their presence by application of starter cultures containing competitive strains of *S. equorum* might contribute to achieving highest hygienic standards for the cured ham. According to Montel *et al.* (1993) strains of *S. saprophyticus* and *S. warneri* are contained in commercial French starter cultures. The former species is however deemed as potentially pathogenic and needs careful consideration.

Halomonas elongata may be a good candidate for use in starter cultures in brines used for cured raw ham (German Patent, DE 4035836 C2). Strains of this genus are commonly detected in brines as shown by Meisel (1988). The strains may belong to numerous species as indicated by great differences in the SDS-PAGE patterns of their proteins. They have in common to be psychrophilic and halophilic, and strongly reduce nitrate and nitrite. Strains of the genus *Halomonas* may be favourable in ensuring low nitrate concentration in and improving the sensory quality of ham.

SENSORY QUALITY

The development of the flavour of fermented sausages was subject of extensive investigations. Contributions to the flavour derive from the meat raw material, added compounds

(e.g. carbohydrates, curing aids, spices, smoke), and from the microbial metabolism. As discussed by Dainty and Blom (1995) glycolysis, proteolysis, lipolysis, and lipid oxidation are key activities for flavour generation. These originate from endogenous enzymes of the meat and from the fermenting micro-organisms. Depending on the technological factors, such as temperature, relative humidity, nature and diameter of casings, size of meat particles, etc., a multifactoral effect will result. Therefore, the role of the starter culture may vary in the various types of sausages. The effect of basic activities of lactic acid bacteria (lactic fermentation) and of micrococci and staphylococci (nitrate/nitrite reduction, proteolytic and/or lipolytic properties, catalase) have been reviewed extensively (e.g. Dainty and Blom, 1995; Hammes and Hertel, 1996; Lücke, 1998). The link between these biochemical activities and their effect on the aroma of sausages was recently studied by Montel *et al.* (1996). It was shown that the aroma can be modulated by use of starters containing different species of staphylococci. Remarkably, the strongest dry-sausage aroma was obtained with strains exhibiting the lowest level of lipolytic, esterase or proteolytic activity. The authors concluded that the tissue enzymes are sufficiently active to provide fatty acids and amino acids necessary for the development of aroma. The use of strains of *S. equorum* resulted in an objectionable dairy-product odour. This species is commonly found at low numbers in fermented sausages and was found to belong to the predominant species of staphylococci in traditional French cheese (Irlinger *et al.*, 1997).

The importance of meat endogenous lipases was also demonstrated in studies of sausages produced under aseptic conditions (García *et al.*, 1992; Hierro *et al.*, 1997). It was observed that lipolysis by tissue enzymes of dry fermented sausages accounts for more than 60% of total free fatty acids release (Hierro *et al.*, 1997). A similar situation appears to apply for proteolysis, as it was found that myosin and actin are degraded by the tissue proteinase cathepsin D at levels of up to 80% and 50%, respectively (Demeyer, 1995). Although results are published indicating the importance of bacterial proteinases, it can be concluded that the endogenous enzymes in meat play the crucial role in the development of flavour in fermented sausages.

Lipolysis *per se* and the accumulation of free fatty acids do not significantly affect the desired flavour of fermented sausages. However, the fatty acids are aroma precursors for autoxidation processes and modifications yielding compounds such as short-chain fatty acids and carbonyls (Dainty and Blom, 1995). The studies of the aroma generation focus on the monitoring of the different profiles of volatile compounds found in the various products and on the effect of microbial metabolism and process parameters on their generation (e.g. Mateo and Zumalacárregui, 1996; Montel *et al.*, 1996; Stahnke, 1995*a*,*b*). For example, Montel *et al.* (1996) showed that each species of the staphylococci led to a characteristic profile of volatile aroma compounds in the sausages. The investigations permitted conclusions to be drawn on the relationship between staphylococci and specific aroma notes. It was concluded that strong dry-salami odour obtained with strains of *S. carnosus* or *S. xylosus* correlated with high desorption of 3-methylbutanal, methylketones and ethyl esters. This is consistent with the results of Stahnke (1995*b*) who observed that the typical salami odour of fermented sausages produced with *S. xylosus* correlated with the presence of ethyl esters, 2-alkanones, and likely 2- and 3-methylbutanal. Similar results with special regard to 3-methylbutanal were obtained in studies of ham flavour by Andersen and Hinrichsen (1995).

Yeasts and moulds play traditionally an important role in sausage fermentation as well as maturation of hams (Cook, 1995) and bring about characteristic flavours and surface appearance. The contribution of these organisms to the typical aroma of the products is based on their primary and secondary metabolites and, again, lipases and proteinases are key activities. Recent *in-vitro* studies have focused on generation of volatile compounds by moulds. For example, Jacobsen and Hinrichsen (1997) investigated the formation of

volatile compounds on agar media by *Geotrichum candidum* and the commercial meat starter *Penicillium camemberti* (*candidum*) and *P. nalgiovense*. Volatiles were formed from free fatty acids by the penicillia whereas those from *G. candidum* originated from the amino acids metabolism. The *Penicillium* strains produced the volatiles 1-octen-3-ol and octan-3-one which had been identified as typical volatiles from penicillia and being responsible for the mushroom-like flavour (Sprecher and Hanssen, 1985). The formation of these volatiles was independent of components added to the medium such as glucose, peptone, maize oil, or meat extract.

TECHNOLOGICAL IMPROVEMENTS

The key role of enzymes in flavour generation suggests that their deliberate application might contribute to an economical and sensorial improvement of the process of fermented sausage production. This application is a technological measure which is basically independent of the application of starter cultures. However, the results obtained in practical investigations of the enzymes in sausage fermentation provide knowledge of the potential of these activities which may be employed in developing specifically designed starter cultures. These cultures might, for example, produce the desired enzymes during the fermentation process. Enzymes were used in sausage production to achieve an improved flavour and accelerated ripening of fermented sausages. The effect of proteinases from *Lactobacillus paracasei* subsp. *paracasei* (Hagen *et al.*, 1996), pronase E from *Streptomyces griseus* (Diaz *et al.*, 1993), or papain (Diaz *et al.*, 1996) was investigated in dry fermented sausages. The proteolytic activities were confirmed by chemical analysis of the sausages. No effect of pronase E and papain on the microbial flora was detected during ripening. It was observed that proteolysis can be increased in the products which however did neither improve the flavour or accelerate ripening. On the other hand, it was observed by Hagen *et al.* (1996) that the *Lactobacillus* proteinase activities increased not only the viable counts of the starter *L. sakei* but also the drop in pH. The sensory analysis also confirmed that the ripening of sausages was accelerated by the bacterial proteinases and resulted in a maturation time of 14 days in contrast to 28 days for the control.

The effect of various lipases from moulds on the improvement of flavour and ripening of dry fermented sausages was subject of several studies. It was observed that the addition of a lipase preparation from *Aspergillus* sp. resulted in an increase of free fatty acids (Zalacain *et al.*, 1997*a*) without affecting growth of the starter organisms. The sensor analysis revealed only a slight increase in odour intensity in sausages with added lipase. Similar results were obtained in studies of application of a lipase from *Rhizomucor miehei* in a pilot plant and at industrial level (Zalacain *et al.*, 1997*b*). It was shown that levels of free fatty acids and short-chain fatty acids were increased and did not result in rancidity. Although the ripening was accelerated with regard to lipolysis, the sensory analysis revealed rather similar sensory profiles for the sausages with and without lipase.

Malfermentations may occur in sausages when ecological factors and technological conditions are unfavourable during the fermentation process. For example, in presence of oxygen hydrogen peroxide may be formed by lactic acid bacteria. The accumulation of this strong oxidising compound leads to undesired effects in foods such as rancidity and discoloration (Rozier, 1971). Studies of the distribution of catalases in lactic acid bacteria showed that numerous species possess this desired property (Engesser and Hammes, 1994). With regard to meat lactobacilli it was shown that strains of *L. sakei* and *L. plantarum* exhibit true catalase and strains of *L. plantarum* can also possess the so-called non-heme, pseudo-, or manganese catalase. The corresponding genes have been already cloned and characterised (Knauf *et al.*, 1992; Igarashi *et al.*, 1996). To prevent the deleterious

effects caused by hydrogen peroxide starter cultures can be used containing catalase positive strains. A further strategy is to endow catalase negative starter organisms such as *L. curvatus* with the property to produce catalase. This genetic manipulation permits to produce starter preparations without the need to combine organisms such as *L. curvatus* with catalase positive species such as *Staphylococcus carnosus* and *Kocuria varians*.

The feasibility of the latter approach was demonstrated by Hertel *et al.* (1998). The authors used the following elements: the catalase gene *katA* of *L. sakei* LTH677 (Knauf *et al.*, 1992); the *Lactobacillus* cloning vector pJK356 (Klein *et al.*, 1993) based on the cryptic plasmid pLC2 of the meat starter *L. curvatus* LTH683; and *L. curvatus* LTH1432 as host, a plasmid-cured derivative of strain LTH683. As a result of the gene dose effect, the catalase activity of aerobic cultures of the recombinant strain *L. curvatus* LTH4002 was four-fold higher than that of *L. sakei* LTH677. For application of catalase containing organisms, the regulation of this enzyme should be well known. Investigations at physiological and genetic level revealed that the catalase activity is strongly increased upon addition of hydrogen peroxide to anaerobic cultures as well as switching to aerobic conditions (Hertel *et al.*, 1998). The analysis of RNA revealed that the regulation takes place on the transcriptional level. A regulatory sequence of at least 25 bp within the promoter region of *katA* was identified as a putative binding site for a transcriptional activator. Under inducing conditions the recombinant strain LTH4002 did no longer accumulate hydrogen peroxide and remained viable in the stationary phase in contrast to the host strain LTH1432.

The increasing interest in genetics of *L. sakei* and *L. curvatus* as important meat adapted lactobacilli is reflected by the concomitant increase of the numbers of genes and genetic elements characterised for these species (Table 2). This progress in the genetics was facilitated by application of basic knowledge acquired in the corresponding studies of *Lactococcus lactis*. This organism is of paramount importance in dairy fermentations. On the basis of nearly extensive experience with this species numerous new applications have been made possible (Venema *et al.*, 1996). It may be foreseen that a similar status can be achieved for the meat lactobacilli such as *L. sakei* and *L. curvatus*.

The competitiveness of starter cultures strongly affects the outcome of the fermentation process. It is remarkable that little is known about the factors contributing to this important property of meat lactobacilli. The effect of ecological factors on the competitiveness of *Lactobacillus pentosus* LTH985 and *L. sakei* LTH681 was investigated in a liquid model system under simulated practical conditions (Doßmann *et al.*, 1998). These lactobacilli are characterised by poor and high competitiveness, respectively, in fermenting sausages. It was shown that under the influence of the ecological factors characterising fermenting meat *L. sakei* achieved a higher cellmass production rate at lower growth rates than the competitor. In addition, the maintenance coefficient of *L. pentosus* was 73% higher than of *L. sakei*. In mixed culture *L. pentosus* showed hardly reproduction. Remarkably, adaptation of pre-cultures of *L. sakei* to low temperature (22·5°C) and high salt concentration (5%) led to a significant reduction of the lag-phase as compared to non-adapted cells and this property was maintained in revitalised cultures that had been subjected to freeze-drying.

BACTERIOCINS AND LYTIC ACTIVITIES

The potential of lactic acid bacteria to produce bacteriocins (BCs) has attracted much attention as they can be used to prevent food spoilage and to inhibit growth of food pathogens (Abee *et al.*, 1995; Montville *et al.*, 1995). Up to now nisin is the only legally permitted BC employed as food preservative. Cultures producing antagonistic compounds

TABLE 2
Characterised Genes and Genetic Elements of *Lactobacillus curvatus* and *L. sakei*

Genes or genetic element	*Function*	*Reference*
L. curvatus		
IS*S1*	Insertion element	Scholl (1996)
curA	Structural gene of curvacin A	Tichaczek *et al.* (1993)
pLC2	Cryptic plasmid	Klein *et al.* (1993)
manA, B, C, D	PTS glucose transport	Veyrat *et al.* (1996)
L. sakei		
IS*1163*	Insertion element	Skaugen and Nes (1994)
lacL/lacM	β-galactosidase	Obst *et al.* (1995)
ptsHI operon	General enzymes of the PTS	Stentz *et al.* (1997)
ldhL	L-lactate dehydrogenase	Van den Berg (1996); Zagorec *et al.* pers. comm.)
rbsKR	Ribose utilisation	Stentz and Zagorec (pers. comm.)
Tagatose pathway genes	Enzymes for tagatose pathway	Alpert and Zagorec (pers. comm.)
Leloir pathway genes	Enzymes for Leloir pathway	Alpert and Zagorec (pers. comm.)
Arginine catabolism gene cluster	Enzymes of arginine deiminase pathway	Zuñiga and Pérez-Martinez (pers. comm.)
katA	Catalase	Knauf *et al.* (1992)
dnaK operon	Proteins for heat shock response	Schmidt *et al.* (pers. comm.)
sakP	Structural gene of sakacin P	Tichaczek *et al.* (1994)
Sakacin P gene cluster	Sakacin P production	Hühne *et al.* (1996)
Sakacin A gene cluster	Sakacin A production	Axelsson and Holck (1995)
Lactocin S gene cluster	Lactocin S production	Skaugen *et al.* (1997)

in the food matrix, such as pediococci producing pediocin, have been employed in meat practice. Bacteriocinogenic starter cultures may be used with three different intentions: (i), to improve the competitiveness of the starter strain (Vogel *et al.*, 1993), (ii), to prevent growth of food pathogens, and (iii), as protective cultures (Stiles, 1996). The study of the effect of bacteriocinogenic cultures on food pathogens under the practical conditions of sausage fermentation has shown that effects against *Listeria monocytogenes* can be achieved (Berry *et al.*, 1990; Foegeding *et al.*, 1992; Hugas *et al.*, 1995). It was, however, observed (Hugas *et al.*, 1997) that the effect is not always reproducible when different types of sausages are produced. The authors investigated in challenge experiments with *L. monocytogenes* the killing and fermentative effect of five bacteriocinogenic starter strains (four *L. sakei* and one *L. curvatus*). The sausages were prepared under the practical conditions common in Spain (series A) and in Germany (series B), respectively. In all batches of series A, including the controls produced with non-bacteriocinogenic starters, the listeria counts dropped during ripening by 1 to 4 log cycles. Two strains (*L. curvatus* LTH1174 and *L. sakei* CTC494) reduced the listeria count 1·5 to 2 log cycles below the values determined for the control. In series B the listeria counts remained virtually unchanged in four batches (including the control) and in two batches, containing the sakacin A producing strain *L. sakei* Lb706 and again the *L. curvatus* LTH1174, respectively, the counts dropped by 1 log cycle. Thus, the efficiency of the bacteriocinogenic strains with regard to reducing listeria counts needs confirmation in a specific type of fermented sausages, as not all strains exhibit their desired effect consistently. For example, ecological factors prevailing in the specific food matrix may affect the BC production and the antimicrobial activity, as dicussed below.

In addition to the restraints originating from BC expression by the micro-organisms in the food matrix, the properties of the BC *per se* need to be well known before a culture can be safely applied. The effect of ecological factors prevailing in sausage fermentation was studied by Gänzle *et al.* (1997). The authors investigated the antilisterial efficiency of sakacin P produced by *L. sakei* LTH673 depending on pH value and concentration of sodium chloride, nitrite, and nitrate. It was observed that the BC activity increased synergistically at low pH and high concentrations of sodium chloride, whereas nitrite had only minor effect synergistic to that of low pH. The effect of food components and ecological factors on nisin, sakacin P, and curvacin A activity against *L. curvatus*, *Listeria innocua*, *Salmonella* Heidelberg and strains of *E. coli*, including *E. coli* O157:H7 was studied by Gänzle *et al.* (1998) It was observed that Mn^{2+} and Ca^{2+} were antagonistic to the BC activities at concentrations of $> 10\,mmol\,l^{-1}$ and so were lecithin at concentrations $> 0{\cdot}1\%$ and, to a minor extent, casein at $> 1\,g\,l^{-1}$. On the other hand, synergistic effects were observed with EDTA and ethylparabene, respectively. The Gram-negative organisms were sensitive to the latter compounds although they are commonly highly resistant to the BCs from lactic acid bacteria. Special practical importance was seen in the observation that growth inhibition of *E. coli* O157:H7 and *Salmonella* was achieved at $pH < 5{\cdot}5$ or $> 5\%$ sodium chloride concentrations, as these conditions can occur in fermented meat products.

The scientific evidence for the efficiency of bacteriocin producing culture in protecting meat and meat products from spoilage or presence of certain food pathogens has been exclusively reviewed by Stiles (1996). Remarkably, the protective effect does not necessarily rely on bacteriocin formation by the culture. This was shown with two preparations that were actually marketed as protective cultures (Andersen, 1995). These preparations contain *L. alimentarius* BJ-33 either alone or in combination with *S. carnosus*. The *Lactobacillus* strain is psychrophilic and grows at 2°C, and the protective effect was attributed to its competitiveness to the psychrophilic flora that normally dominates in chilled-stored meat products. It is claimed that the sensory properties of the products are not affected as

the strain acidifies poorly and exhibits only limited proteolytic or lipolytic activity. The cultures can be applied to cooked as well as uncooked meat products and have shown to prevent the growth of *L. monocytogenes*.

Meat lactobacilli form various types of BCs. The cationic peptide BCs (class II BCs) are most often found and it was observed that at the genetic level they share common principles (Nes *et al.*, 1996). As an example the *sppA* operons involved in regulation and synthesis of sakacin P are shown in Fig. 1 (Hühne *et al.*, 1996).

Most class II BCs are synthesised as a preform consisting of a N-terminal extension, the so-called double-glycine leader and the active bacteriocin. This pre-BC is processed and externalised by a transmembrane translocator belonging to the ATP-binding cassette (ABC) transporter superfamily (Håvarstein *et al.*, 1995). Some class II BCs, e.g. sakacin P, are transcriptionally regulated in a quorum-sensing mode (Kleerebezem *et al.*, 1997) by a BC-like induction factor (IF), also termed pheromone. During growth of potentially bacteriocinogenic cultures IF accumulates in the medium ensuring that in the late logarithmic growth phase sufficient IF is present to induce transcription of genes involved in BC synthesis. The extracellular IF signal is transduced by a two component regulatory system, a sensor histidine kinase and a response regulator. The IF is required not only for the transcriptionally induction of BC expression but also to maintain BC synthesis. This regulation mechanism might complicate the applicability of producer cultures in practice. For example, when IF is degraded or bound to components of the meat matrix. To circumvent this limitation it can be reasoned that producer strains are employed that express BCs constitutively, as for example the sakacin A producing *L. sakei* Lb706 (Axelsson and Holck, 1995). A different approach was chosen by McCormick *et al.* (1996). The authors redesigned the genetic information for BC synthesis by fusing the BC structural gene devoid of its natural leader sequence with genetic elements coding for Sec-dependent leader sequences of the general secretory pathway. The strategy was as follows (Fig. 2).

Few BCs are externalised via the general secretory pathway, e.g. divergicin A (*Carnobacterium divergens*) or acidocin B (*Lactobacillus acidophilus*). These BCs possess the characteristic N-terminal leader sequence of the Sec-type. The leader peptide of divergicin A was fused with the leaderless carnobacteriocin B2 structural gene (*cbnB2*) of *Carnobacterium piscicola* LV17 together with the gene (*cbiB2*) coding for the immunity to this bacteriocin (McCormick *et al.*, 1996). This experiment shows that the genetic information for BC formation can be integrated into more generally transport mechanism and opens ways to endow bacteria with one or more structural genes coding for useful antagonistic compounds.

These compounds need not necessarily be BCs. As it was shown by Cavadini *et al.* (1996), a specific bacteriolytic property can be cloned and expressed in meat lactobacilli.

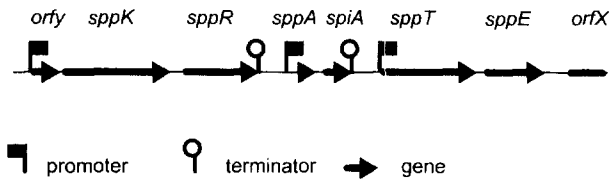

Fig. 1. Genetic organization of the gene cluster involved in sakacin P production (according to Hühne *et al.*, 1996) *sppA*, the structural gene encoding the prebacteriocin; *spiA*, the immunity gene conferring specific immunity against sakacin P; *sppT*, encoding a dedicated ABC-transporter externalizing the prebacteriocin concomitantly with splitting of the leader peptide; *sppE*, encoding an accessory protein for the export of sakacin P; *sppK* and *sppR*, encoding a histidine kinase and the corresponding response regulator, respectively; *orfY*, encoding the sakacin P induction factor (IF).

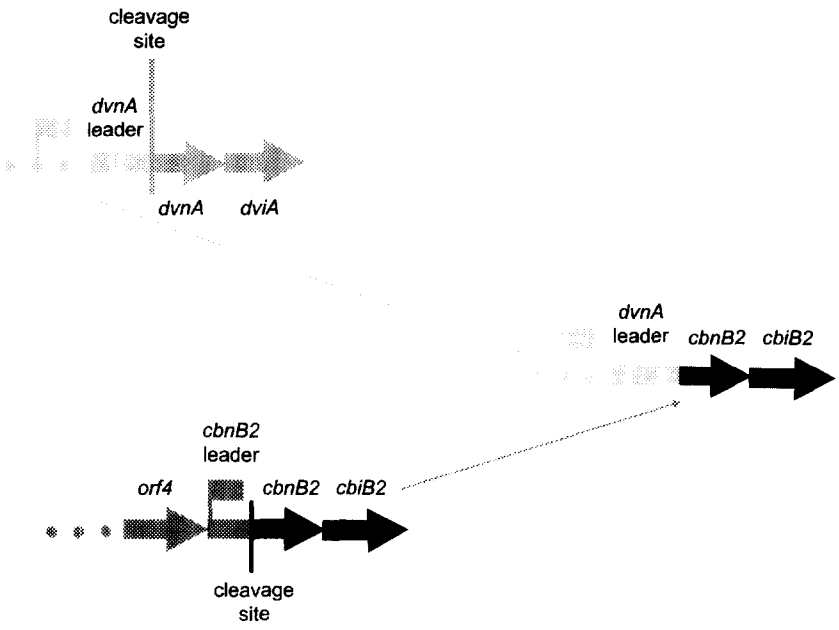

Fig. 2. Strategy of fusion of the divergicin A leader sequence of Carnobacterium divergens with the structural gene coding for the mature carnobacteriocin B2 of Carnobacterium piscicola (according to McCormick *et al.*, 1996). *dvnA*, the structural gene encoding divergicin A; *dviA*, the immunity gene conferring specific immunity against divergicin A; *cbnB2*, the structural gene encoding carnobacteriocin B2; *cbiB2*, the immunity gene conferring specific immunity against carnobacteriocin B2; *orf4*, putative bacteriocin.

Pursuing the aim to reduce the numbers of *Staphylococcus aureus* in meat products, the truncated lysostaphin gene *lys* of *Staphylococcus simulans* was cloned and expressed in strains of *L. curvatus* and *L. sakei*. In one example the natural sakacin P producer *L. sakei* LTH673 was used as host of *lys* and it was achieved to broaden the antimicrobial spectrum to include activity against the food pathogens: *L. monocytogenes* and *S. aureus*. The applicability of the lysostaphin producing strains as starter and protective cultures was demonstrated in fermenting sausages and mayonnaise based meat salads (Cavadini *et al.*, 1998). It was observed in the practical experiments that the recombinant lysostaphin was sufficiently expressed and was responsible for the rapid decrease of the staphylococcal population under the limit of detection. The application of a protective culture of *L. sakei* as a producer of the two antimicrobial principles for hamburgers patties resulted in a killing effect against both target organisms (Hertel *et al.*, 1997). The listerial and staphylococcal counts remained in the order of the respective inoculum, whereas in the control the food pathogenic models grew to a density of up to 10^9 cfu g^{-1}. The experiments have shown that the risk of food poisoning by *S. aureus* in various foods can be reduced by employing genetically modified starter or protective organisms. It has to be considered that the use of genetically modified organisms in foods has to pass serious hurdles such as consumer acceptance and regulatory requirements. The question of the safety of genetically modified micro-organisms in foods has been addressed in more detail by Heller *et al.* (1995), Klijn *et al.* (1995), and, with special reference to meat, by Hertel *et al.* (1995).

The studies of the effect of antagonistic compounds formed by starter organisms are promising to further improve the safety of fermented meat products. A special challenge

in this regard is the elimination of enterohemorrhagic *E. coli* from meat products which are not subjected to thermal processing. Attempts to eliminate EHEC by use of starter cultures in minced meat have been found ineffective (Zeuthen *et al.*, 1997). For the production of fermented sausages in the US guidelines were imposed (Anon, 1995*a*) which require the proof that the process leads to a reduction of the EHEC counts by 5 log cycles. The achievement of such requirements is a challenge, especially in spreadable fermented sausages such as those consumed in Germany, e.g. 'Teewurst, Rohpolnische, Streichmettwurst', etc. The sausages are fermented but not subjected to a drying process. In challenge studies (Kofoth *et al.*, 1996) it was shown that in the presence of starter cultures containing *L. plantarum* and *S. carnosus* the EHEC did not grow but kept their numbers at the level of the inoculum. On the other hand, it was reported by Gareis (1997) that in salami type of sliceable fermented sausages challenge with up to 10^7 cfu g^{-1} EHEC a reduction of EHEC counts by 1 to 5 log cycles has been demonstrated. The nature of the starter cultures as well as the low pH ($< 4{\cdot}8$) did not clearly contribute to the reduced survival of EHEC (Glass *et al.*, 1992) but a remarkable effect of > 2 log cycles originated from the reduced water activity (from 0·96 to 0·98). According to Gareis (1997), under optimum conditions, i.e. low initial water activity of the meat mixture and controlled ripening and drying, it was possible to eliminate *E. coli* O157:H7 from sausages challenged with high inocula.

PROBIOTIC CULTURES IN FERMENTED SAUSAGES

In Table 1 probiotic species are included which have been marketed in culture preparations for production of fermented sausages. These species originate from isolates from the human intestinal tract. This ecological niche is the natural habitat for organisms such as bifidobacteria and *L. acidophilus*. On the other hand, *L. casei* may also be found as component of the flora of the oral cavity, in various fermented foods as well as in spoiled foods (Hammes, 1998). There is plenty evidence that the intestinal isolates are well adapted to their natural habitat and do not grow in and contribute to the fermentation of the food (meat) substrate.

Probiotics have been defined in an expert workshop organised by the Lactic Acid Bacteria Industrial Platform (LABIP) of the EU (Guarner and Schaatsma, 1997) as follows:

'Oral probiotics are living micro-organisms, which upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition'.

The success of probiotics in dairy foods is mainly based on the increasing scientific evidence for the beneficial effects of certain well defined strains. The question as to the usefulness of probiotics in meat products was discussed by Hammes and Haller (1998). The authors pointed out that a prove of a beneficial effect should be based on sound studies performed with the probiotics in the meat matrix as it is consumed. It appears not possible to conclude from studies with dairy products that a specific strain is as efficient when applied in a different food. This argument bases on the general knowledge that the expression of properties of a bacterium is controlled by environmental factors. There are no data available from the scientific literature which show that the respective effects are achieved under the specific conditions prevailing in fermented sausages. In addition, little is known about the numbers of the probiotic bacteria in the sausage that are required to obtain the claimed effect, and finally it should be clear that it is possible that a consumer is willing to eat fermented sausages in such amounts as they might be needed to establish an effective level of probiotic counts in the intestines. As probiotics in meat products might have a potential to contribute to the health of the consumer, their introduction into the market might become beneficial and useful after sound scientific studies have been performed.

It was argued by Hammes *et al.* (1997) that fermented foods including meat products have shown their beneficial effects in the long history of their consumption. Evidence was presented that *in vitro* certain properties of probiotics derived from intestine are also present in the food fermenting strains, e.g. in lactobacilli and staphylococci. It might be possible that true starter cultures can be developed that exhibit specific probiotic properties as well as achieving the required technological and sensory tasks in the meat matrix.

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