Starters in the Processing of Meat Products

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ABSTRACT

tarter cultures applied in meat technology may contain molds, yeasts and bacteria. Molds have been selected with the imary intension to exclude any potential for mycotoxin production. Their sensorial effects and the requirements of chnology are also criteria for suitable starters. Their genetic potential can now be modified by genetic engineering. easts are applied for sensorial reasons mainly. The species in use are only weakly fermentative and do not enhance rate reduction. Bacterial components of starters consist of micrococci, staphylococci, lactic acid bacteria (LAB) and, th minor importance, *Streptomyces griseus* or *Aeromonas* spec.. For selection of the appropriate organisms and for suring optimum performance in the fermentation process, their technological, ecological, physiological and genetical operties should be well known. Most knowledge is present for LAB which represent the most important group of arter organisms since they are involved in the fermentation of all types of products and contribute to each single aim the fermentation process. The study of the properties of the organisms may also contribute to reduction of potential gienic risks not only in the classical fields of starter application but also in new fields where they may be employed as otective cultures that inhibit the growth of food pathogens or even spoilage organisms. Strains with an improved tential to reduce these risks and to exhibit further useful properties can be obtained by means of genetic engineering. amples for successfully modified meat specific bacteria are *Staphylococcus carnosus* and lactobacilli (*L. curvatus* and *sake*).

e interest in starter cultures in meat fermentation has strongly increased and numerous excellent reviews deal with e history and the physiological and technical aspects of starter cultures (LÜCKE, 1985; HAMMES et al, 1990; INCZE, 992). This increased interest may result from both the obvious advantage provided by the existing starter preparations d the change of food manufacturing from small factories into industrial dimension. Especially the modern concepts of uality assurance require the application of every means to obtain products of a high standard in quality, and starter ltures contribute to an improvement in (i) hygienic safety, (ii) sensorial attractiveness, (iii) high and even level of uality and (iv) shelf life.

ese effects can be obtained when suitable raw materials and technologies are available together with appropriate arter cultures. With these preparations it is furthermore possible to improve the economy of the production process d to obtain new products which cannot be produced without starters. In this overview the microbial aspects of starter ltures will be treated together with the ecological, metabolic and genetical background that affect meat fermentations may be of use in some future.

HE MICROBIAL COMPONENTS OF STARTER CULTURES

Table 1 are compiled the microbial components of meat starter cultures that are commercially available in Europe AMMES et al, 1985). The history of the investigation of the microbiology and the application of starters in fermented eat products commenced with yeasts (CESARI, 1919, CESARI and GUILLIERMOND, 1920).

able 1: Starter cultures in meat fermentation

EASTS

ebaryomyces hansenii, Candida famata

UNGI

enicillium chrysogenum, P. nalgiovense

ACTERIA

Lactic acid bacteria

Lactobacillus plantarum, L. sake, L. curvatus, Pediococcus acidilactici, P. pentosaceus, Lactococcus lactis

Micrococci

Micrococcus varians

Staphylococci

Staphylococcus carnosus, S. xylosus

Actinomycetes

Streptomyces griseus

Enterobacteria

Aeromonas spec.

YEASTS

More recent investigations of yeasts involved in meat fermentation were performed by LEISTNER and BEM (197 authors described *Debaryomyces hansenii* as the most common species in fermented meat products and ROSS and LEISTNER (1972) used this species as starter. They observed positive effects with regard to the developm characteristic yeast flavour and stabilization of the reddening reaction. *D. hansenii* and the imperfect form *famata* are used in starter preparations and should be added to the sausage mixture at 10^6 cfu/g. characterized by high salt tolerance (growth above a_w = 0.87) and by aerobic or weak fermentative met Therefore, these yeasts grow mainly on the surface and in the outer part of the sausages. Nitrate reduction property of the yeast starter. It was observed that these yeasts may slightly inhibit nitrate reduction in fer sausages which is performed by micrococci (MEISEL et al, 1989).

D. hansenii is haploid and improved strains can be obtained by crossing experiments. For example ERGINKAYA has breeded strains with broader spectra in sugar fermentation in combination with nitrite utilization from paren that exhibited only restricted metabolic activity.

FUNGI

Mold fermented foods are found in many human cultures. In Asian countries these products are based on raw m of plant origin, whereas in Europe substrates of animal origin are fermented such as cheese, fermented saus ham. In Northern Europe smoking of sausages is the common practice, but mold ripened sausages have tradition and are preferred in mediterranean and south-east European countries (LEISTNER, 1986). Molds characteristic surface appearance and flavour. The latter originates mainly from the proteolytic and lipolytic ac the molds. Further effects of mold growth consist in prevention of adverse effects of oxygen and thus, of ranci discolouration (GEISEN et al, 1990). In addition, the drying of the sausages proceeds more evenly. Tradition inocula for the sausages originate from the environment and, therefore, a great number of different species are fo the sausages. An extensive survey on mold species isolated from fermented sausages is given by LEISTNER and (1967). Apparently, the most common species belong to the genera *Penicillium* and *Scopulariopsis*. Objections the practice of inoculation by chance arise from the fact that many species of *Penicillium* are mycotoxin produc strains of *Scopulariopsis brevicaulis* are involved in skin and nail infections. LEISTNER and ECKARDT (1981) that about 80 % of the *Penicillium* isolates from sausages were able to produce mycotoxins on artificial media out of 17 investigated mycotoxins could also be found in meat products (LEISTNER, 1984). Thus, mold starter developed without any potential for toxin production (proven by chemical and biological testing). MINTZLA LEISTNER (1972) selected a non-toxic strain of *P. nalgiovense* which possessed good technological propert which was later on used in a starter preparation. The different toxicological tests necessary to select non-toxic were described by FINK-GREMMELS et al (1988).

Based on the experiments with mold starter preparations essential properties of optimally performing strains described which are compiled in Table 2.

able 2: Important properties of molds applied in starter preparations for meat fermentation

no toxigenic and no pathogenic potential
competitive against microorganisms growing on the surface
firm and lasting surface mycelia of a white, yellowish or ebony colour
well balanced proteolytic and lipolytic activity
characteristic moldy aroma

France non-toxic strains of *P. chrysogenum* are applied which have been selected for their ability to produce the eferred ebony colour instead of the green colour which is characteristic for the wild type strain. HWANG (1991) lated strains of *P. nalgiovense* and *P. chrysogenum* that proved to be non-toxic, competitive against contaminants, ting on the surface and gave rise to sausages of good quality. He also demonstrated that the combination in starter eparations of a strain of *Penicillium* and one strain of *Scopulariopsis candida* resulted in greatly improved visual pearance and firmness of the mycelium on the surface, but for reasons mentioned above, strains of *Scopulariopsis* ould not be used in starters.

s remarkable that *P. nalgiovense* has been subjected to genetic modification. GEISEN and LEISTNER (1989) developed method by which heterologous genes can be introduced into *P. nalgiovense*. With this method the lysostaphin gene m *Staphylococcus staphylolyticus* was transferred into *P. nalgiovense* (GEISEN et al, 1990b). The transformed strain s able to lyze cells of the food pathogen *Staphylococcus aureus*.

CTERIA

e use of bacterial starter cultures commenced with the application of *Micrococcus* M53 by NINIVAARA (1991) who scribed the effect of the starter organism as follows:

increased speed of
- colour formation
- drop in pH
- attaining the desired texture
- total processing

improvement in
- economy of the process
- controlling the growth of food pathogens and spoilage organisms

ter a phage attack *Micrococcus* M53 was substituted (POHJA, 1960) by other organisms among which a fermentative ain of *Micrococcus* was initially identified as *Staphylococcus simulans* and later on was allocated to *Staphylococcus rnosus* (SCHLEIFER and FISCHER, 1982). In addition, other micrococci and staphylococci were incorporated into rious starter preparations.

th genera share the property that they possess nitrate reductase activity but do not reduce nitrite. This nitrite may be her the product of a microbial nitrate reductase activity or is added directly as the curing aid. During the fermentation riod, nitrite is subjected to chemical reactions among which acid catalyzed disproportioning is most important. trogen monoxid is a product of this reaction. Nitrogen monoxid reacts with myoglobin to form nitrosomyoglobin which presents the stable and characteristic red cured meat colour. Additional properties attributed to micrococci and aphylococci consist in adding to the fermenting mixture the activities of catalase, lipase and protease, ensuring the velopment of the desired flavour. Catalase activity is considered to be important for the removal of hydrogenperoxide hich may be formed as a metabolite by LAB and represents a strong oxidizing agens exerting adverse effects on lour, aroma and shelf life of the sausages.

Micrococci and staphylococci are only distantly phylogenetically related but they share certain habitats, e.g. skin and ucosa of men and animals and, in addition, they exert similar technologically desired effects on sausage ripening.

Micrococcus varians is the only species in use within the genus *Micrococcus*. It is only weakly fermentative and grows only poorly in the anaerobic environment of the interior parts of the sausages. The organism possesses a reductase which is active even at temperatures below 15 °C (down to 5 °C) (MEISEL, 1988), whereas *Staphylococcus carnosus* this activity is no longer detectable below this temperature (HAMMES, 1986). A advantage of *Micrococcus varians* arises from a rather psychological background. Based on a safe history, this is safe with regard to any toxigenic and pathogenic potential. On the other hand, the genus *Staphylococcus* several species that have been isolated from diseased persons (e.g. *Staphylococcus xylosus*) (GEMMELL, GEMMELL and THELESTAM, 1981). No such reports exist, however, for *Staphylococcus carnosus*.

Staphylococcus carnosus is the most important component of starters among the non lactic acid bacteria. It is remarkable for a Gram positive organism that the genetics of *Staphylococcus carnosus* is exceptionally well investigated genetic engineering was applied on it successfully. Genetical modifications that have been performed with *Staphylococcus carnosus* are compiled in Table 3.

Table 3: Gene cloning in *Staphylococcus carnosus*

Phenotype	Analyzed gene(s) or gene products	Origin
Ribose degradation	ribokinase ribose uptake protein	*Staphylococcus hyicus*
Arabinose degradation	L-arabinose-5-P, 4-epimerase	*S. xylosus*
Xylose degradation	xylose isomerase xylulosekinase[2] repressor[2]	*S. xylosus*
Urea hydrolysis	urease	*S. xylosus*/*S. aureus*
Triglyceride hydrolysis	lipase	*S. aureus*/*S. hyicus*/ *S. xylosus*
Endopeptidase	lysostaphin	*S. staphylolyticus*
DNA hydrolysis	DNase	*S. carnosus*
Sucrose degradation	sucrose transport[3]	*S. xylosus*
Sugar transport	enzyme I[1]	*S. carnosus*
Starch hydrolysis	α-Amylase	*Bacillus stearothermophilus*
Cellulose hydrolysis	cellulase	*Clostridium thermocellum*

The data were derived from GÖTZ (1990) and are supplemented with reports of KOHLBRECHER et al (1992)[1], SIZEMORE et al (1992)[2] and WAGNER et al (1992)[3].

In our studies (HAMMES, unpublished results) strain TM300 used by GÖTZ proved to be not suitable as starter because of its poor competitiveness. Nevertheless, based on the available knowledge, it appears possible to rapidly modify other, more competitive strains of *Staphylococcus carnosus*.

LACTIC ACID BACTERIA

The application of lactic acid bacteria (LAB) in meat fermentation is of paramount importance for the success fermentation process. These organisms are applicable to all types of fermented sausages and contribute to the process (HAMMES et al, 1990). Historically, the introduction of LAB into the market proceeded on different the U.S.A. and in Europe. *Pediococcus cerevisiae* (later identified as *Pediococcus acidilactici*) was introduced by et al (1955) in the U.S.. With this organism a successful starter culture was created for production of summer The high fermentation temperature (37° C), the use of a nitrite cure and short ripening times are specific for this sausage and *P. acidilactici* was well acting in controlling the process because the optimum growth temp 42 °C. In addition, *Pediococcus pentosaceus* was introduced for ripening processes at lower temperature optimum of this species is 35° C.

Europe at first *L. plantarum* was introduced in combination with micrococci (NURMI, 1966). *L. plantarum* and the ococci are still essential components of starter cultures on the market (HAMMES et al, 1985). As further specie *urvatus* and *L. sake* were incorporated into starter preparations. These two species have in common that they stitute the characteristic microflora governing the microbial process in uncontrolled meat fermentations. They are y competitive and well adapted to this environment. When incorporated in starter preparations these organisms inate the fermentation process from the point of inoculation up to the time of consumption (REUTER, 1967; MMES et al, 1990). In our group these organisms and especially *L. sake* were studied with regard to technological, ological, physiological and genetical properties.

LOGY

interest of food technology in the ecology of the starter organisms arises from the necessity to control their tabolic activity by factors that can be influenced by technological means. For fermentation of meat it is, therefore, portant to know how the organisms respond to parameters as temperature, water activity, formula etc.. This type of eraction in sausages was studied by LANDVOGT and FISCHER (1990, 1991) who provided data that allow both to ose starters that are most suitable for a given process and to automate the fermentation process. In this estigation, strains of *L. curvatus* were characterized as being able to lower the pH linearly over a broad range of mperatures.

th, *L. curvatus* and *L. sake* can be isolated from various habitats. However, those strains isolated from fermented eat are especially well adapted to this habitat and outnumber isolates from other habitats (HAMMES, unpublished sults). They are generally psychrotrophic and differ from the more known lactobacilli that are also associated with rmenting meat by their low pH-tolerance (pH limit 3.9-4.1) (HAMMES et al, 1990).

nsitivity to bacteriophages and the presence of prophages have been described for *L. sake* (LEUSCHNER et al, 1992). ajor practical problems caused by phages in sausage fermentation were however not yet described. Apparently, the mi-solid matrix of the fermenting meat mixture is not suited for phage diffusion.

markably, bacterocins were described for *L. sake* and *L. curvatus* (SCHILLINGER and LÜCKE, 1989; TICHACZEK et al, 92). However the lactic acid bacteria present in the available starter cultures do not exhibit this property. Bacteriocins e proteinaceous compounds and may contribute to an improved competitiveness of the starter strains in the rmenting substrate. There is hope that the application of bacteriocin producing strains helps to reduce hygienic risks, hich is supported by the fact that these compounds are especially active against *Listeria monocytogenes*. In fact, EGEDING et al (1992) could show that under practical conditions the application of a pediocin producing strain of *acidilactici* suppressed the growth of that food pathogen.

sed on results obtained from studies of bacteriocins, applications of bacteriocin producing cultures are possible on itherto unknown fields and products, e.g. for the treatment of non fermented sausages and carcasses. In this cases the eparations are called protective cultures, thus, expressing their protective potential against spoilage organisms or the owth of food pathogens.

HYSIOLOGY

AB used in meat fermentation are facultatively homofermentative, i. e. lactic acid is virtually the only product of ucose fermentation. The potential is however present for production of other metabolites as for example CO_2, acetate, rmate, succinate and acetoin. It is known that aberrant flavour notes may occur in fermented sausages and, therefore, is necessary to study the physiological potential of starter organisms with the aim to exclude, that undesired ompounds may be produced during fermentations. In our group we have observed that the empirically elaborated rmentation technology leads to a clean lactic acid fermentation. For example, nitrate and nitrite both inhibit yruvate:formate lyase of lactobacilli, whereby formation of formate is prevented in fermented sausage (CSELOVSZKY al, 1992). In addition, the anaerobic conditions together with the initially high concentration of sugar counteract the rmation of acetate and CO_2.

he formation of H_2O_2 by LAB is of practical importance (*vide supra*) and the same is true for the presence in these rganisms of catalase, which enzyme activity removes this noxious compound. The presence of catalase in meat LAB

and their potential to form H_2O_2 are compiled in Table 4.

Table 4: Potential of meat LAB to produce H_2O_2 or to exhibit catalase activity

Species	*formation of H_2O_2	presence of the activities of pseudocatalase	true catalase
L. plantarum	±	±	±
L. sake	+	-	+
L. curvatus	+	-	-
P. pentosaceus	-	+	-
P. acidilactici	+	-	+

*, determined as described by LÜCKE et al (1986); +, property is present;
-, property is absent; ±, strain dependent property

From this table it can be concluded that accumulation of H_2O_2 can be expected only from *L. curvatus* whereas *L. plantarum* and the pediococci exhibit catalase activity. Remarkably, these bacteria contain two different enzymes, namely pseudocatalase (or manganese catalase) and a true catalase. The latter requires for activity that is present in the environment which is abundantly the case in meat. Thus, both types of enzymes are effective fermenting sausages. A corresponding heme dependent activity was also described for the nitrite reductase activity strains of *L. plantarum.* On the other hand, a heme independent nitrite reductase is present in *L. sake* (HAMMES 1990)

For development of flavour the activities of proteases and lipases are essential prerequisites. Results reported DEMEYER and SAMEJIMA (1991) suggest that the major protease activity is, however, derived from the endogenous enzymes of the meat. On the other hand, lipases appear to be sufficiently present in lactobacilli and contribute significantly to the formation of flavour compounds derived from lipid metabolism (BERGER et al, 1990; NIELSEN KEMNER, 1989).

When selecting starter organisms their potential to form biogenic amines should be known. Lactobacilli decarboxylate certain amino acids and form tyramine, phenylethylamine or histamine. These compounds exert toxic effects on human health. Putrescine and cadaverine may also be formed and strengthen the effect of the former. The low pH and water activity prevailing in the fermenting substrates enhance amine formation and so does the presence of precursor amino acids or peptides (STRAUB et al, 1991). By selecting competitive starter organisms without the potential to decarboxylate amino acids this hygienic risk can be reduced. In our investigations (HAMMES unpublished results) we have observed that decarboxylating activity is not present in strains of *L. sake*, whereas species *L. curvatus* contains strains without any potential and others that form up to four different amines.

GENETICS

The starter cultures presently in use have been selected based on the natural endowment of microorganisms with properties and on the requirements of technology. Nevertheless, the more we know about microbiology the more we realize that even better performing cultures may be created.

The optimization of starters by means of mutation and selection is time consuming and does not add new properties because these are not within the genetic potential of the specific species or strains.

On the other hand, some useful properties can be found in other organisms, whose genes can be used to transform them into competitive starter strains by means of genetic engineering (HAMMES and VOGEL, 1990). The application of these techniques affects various aspects of the useful organisms which can be summarized as follows:

improved knowledge of the genetic potential of the starter organisms
understanding (and modifying) the regulation of the expression of properties naturally present and
introducing new properties not naturally present and, thus, combining useful properties of different organisms in one strain.

Although the main emphasis in genetic engineering is put on species employed in the dairy industry, species used in meat processing are also increasingly investigated. Here lactobacilli are of more importance. Their genetics and state of genetic engineering has been reviewed by KNAUF et al (1992b).

In the following, examples are described which show the strategies followed to improve the properties of meat associated lactobacilli. As a prerequisite for genetic modification a method for introducing DNA had to be established. The most suited method for lactobacilli proved to be electroporation which had been adapted to strains of *L. curvatus* and *L. sake* by GAIER et al (1990). Suitable vectors (carriers for the DNA of interest) were constructed by POSNO et al (1991). However, these vectors still have to be improved with regard to their stable preservation in the cells. Useful genes may be obtained from lactobacilli, other LAB or even non-lactics. *Lactobacillus* genes have been cloned which may serve as marker genes in vectors and/or help to improve the metabolic potential of strains. Examples for this type of genes from lactobacilli are the β-galactosidase gene (OBST et al, 1992) and the catalase gene of *L. sake* (KNAUF et al, 1992a). Improvement of the expression of the latter and introducing it into competitive starter strains can help to prevent the adverse effects of hydrogen peroxide. Micrococci normally providing catalase activity may be omitted from starter preparations thus rendering the fermentation process more convenient and safe.

Research has also concentrated on antimicrobial substances produced by lactobacilli with the intention to improve the hygienic status and the shelf life of foods (*vide supra*). Genetic engineering may help in understanding the expression of these substances and provide starter strains with the ability of bacteriocin production, i.e. strains of superior technological properties have not to be abandoned but can be further used together with their improved properties.

An example for the cloning of a gene from a non lactic acid bacterium is the transfer of the lysostaphin gene of *Staphylolyticus* into meat starter lactobacilli. By this modification the growth of *S. aureus* can be prevented and, thus, hygienic status of meat products increased. The feasibility of this approach was shown by GAIER et al (1992).

STREPTOMYCETES

Streptomyces griseus is a component in the preparation of one starter producer, and it is claimed that it improves the flavour of the fermented sausages. In sausages produced under uncontrolled conditions, streptomycetes may be found in low numbers and they do not grow in the fermenting meat mixture.

ENTEROBACTERIACEAE

Enterobacteriaceae are common contaminants of meat and, therefore, can be found in all fermenting substrates at numbers depending on the initial load of the raw material, the type of sausage and the stage of the ripening process. Under the conditions of a nitrite curing and the use LAB-containing starters, their numbers decline constantly, which process is considered to be essential in order to inhibit food pathogens as for example *Salmonella*, *Shigella* or strains of *E. coli*. Sausages prepared by a nitrate curing and without LAB may give rise to higher numbers of *Enterobacteriaceae*. TAJÄ (1977) isolated strains of *Aeromonas* without any detectable pathogenic or toxigenic potential. They affect the flavour positively when added to sausages and are applied by one factory in Spain (NINIVAARA, 1991).

FIELDS OF APPLICATION OF STARTER CULTURES IN MEAT TECHNOLOGY

An overview of the fields of application of LAB containing starters in meat technology was presented by HAMMES (1992). It was shown that practical application is restricted to fermented sausages and to cooked meat products (bacon) in Spain (LÜCKE et al, 1990). For the latter products cultures containing *Lactococcus lactis* are employed. There are several further fields of application which have shown their useful potential in pilot studies (for use as protective cultures and the perspectives of gene technology: *vide supra*). A remarkable new field was introduced by URLINGS and VAN KNAPEN (1992), who fermented meat byproducts for use as feed for carnivores. They observed an impressive improvement of the hygienic status of these products which efficiently interrupted the chain of infection with food pathogens and, thereby, improved environmental hygiene. Thus, it can be expected that the increased world wide interest in starter organisms will result in starter applications in several fields which were hitherto largely unknown.

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