Starter Cultures in Foods

Subjects: Veterinary Sciences
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Definition

Starter cultures can be defined as preparations with a large number of cells that include a single type or a mixture of two or more microorganisms that are added to foods in order to take advantage of the compounds or products derived from their metabolism or enzymatic activity.

1. Introduction
Starter cultures can be defined as preparations with a large number of cells, either of a single type or a mixture of two or more microorganisms that are added to foods in order to take advantage of the compounds or products derived from their metabolism or enzymatic activity [1].

Since starter cultures are used to perform fermentation processes in food production, its use is a common practice in the food industry worldwide. This has resulted in the commercialisation of several products such as bioprotective cultures, starters or probiotics aimed to provide foods with specific sensory and nutritional characteristics, potential health benefits and guarantee food safety [2].

Starter cultures are used in a wide range of food industries such as the dairy industry for cheese, yogurt and other fermented dairy products’ manufacture [3], the meat industry, mainly for sausage manufacture [4], alcohol production for the beer and wine industry [5][6], vinegar production [7], preparation of oriental products based on rice and soy [8], baking, fermented cereals [9] and production of fermented fruits and vegetables [10][11][12]. Since starter cultures are adapted to the substrates, they allow us control of the fermentation process to obtain predictable results [13].

The most promising microorganisms selected as starter culture are those that are isolated from the native microbiota of traditional products [14] since they are well adapted to the environmental conditions of food and are capable of controlling spoilage and pathogenic microbiota of food [15].

To select a microorganism(s) as a starter or starter culture, it is necessary to carry out a proper study regarding its metabolism and activities, since in some cases, its effects and/or properties may vary between laboratory conditions and food products [16]. Also, starter culture must be recognised as safe, capable of being produced on a large scale and remain viable and stable during storage [17].

Microorganisms used as starter cultures are bacteria, moulds and yeast. Within the group of bacteria, lactic acid bacteria (LAB) are the most representative group, being used in fermentation processes of meat and dairy products [18]. In addition, other bacterial groups such as Gram-positive, catalase-positive cocci, mainly coagulase-negative staphylococci (CNS), and Micrococcaceae are also used [19][20]. Yeasts are mainly used for the fermentation of alcoholic beverages [21], with wine and beer production being the most representative. Regarding starter moulds, they are used to obtain fermented vegetable products, cheeses and meat products [22].

2. Use of Starter Cultures to Improve the Food Safety in Fermented Meat Products
Fermented meat products represent the oldest known way of preserving meat to achieve a microbiologically stable product with particular sensory characteristics that can be kept for several months [23]. Fermented meat sausages are products that are made with minced meat and fat mixed with salt, spices and authorised additives which are mixed and stuffed into natural or artificial casings and subjected to a drying process in which a microbial fermentation takes place, resulting in a drop of pH and
water activity (aW) levels [24]. Traditionally, the fermentation process of these meat products is developed by the natural microbiota existing in meat. However, the use of commercial starter cultures is currently widespread in the meat industry. Starter cultures can be defined as microorganisms selected according to their specific properties that are added to meat batter to improve some characteristics such as appearance, texture, aroma and flavour. Use of starter cultures enables homogenisation of production and avoids possible defects. In addition, they improve the safety of fermented meat products by production of several compounds such as lactic acid, acetic acid, propionic acid, benzoic acid, hydrogen peroxide or bactericidal proteins (i.e., bacteriocins), among others [25]. Thus, starter cultures become the predominant microbiota, directing the fermentation and excluding the undesirable flora, decreasing hygienic and manufacturing risks due to deficiencies of microbial origin.

Regarding organic acids, they inhibit spoilage and foodborne pathogens mainly by reduction of pH. The acid environment interferes with the maintenance of the cell membrane that alters both the structure and functionality, leading to cell death. The antimicrobial effect of organic acids in food has been investigated [26][27][28]. Thus, acetic acid is used to inhibit the growth of both Gram-positive and Gram-negative bacteria, yeasts and fungi. Its inhibitory effect is more pronounced at low pH and presented special importance in fermented vegetables and vinegar industry but is less interesting in foods of animal origin [29]. Benzoic acid occurs naturally in fermented milk products (e.g., kefir, yogurt), produced by microorganisms such as Lactobacillus acidophilus, Lacticaseibacillus casei or Lactobacillus helveticus [30].

Its antimicrobial effect against Staphylococcus aureus and Pseudomonas has been recently evaluated [31]. Antimicrobial effect of diacetyl, acetic acid and propionic acid against Salmonella typhimurium, Escherichia coli, S. aureus and Listeria monocytogenes has also been evaluated [32][33]. Regarding phenylactic acid, produced by several LAB genera [34], it displayed both bactericidal (against L. monocytogenes and S. aureus) [35] and antifungal effects [36]. As described, the organic acids produced by LAB contribute to the safety of foods by creating an adverse environment (by low pH) that interferes in the cell membrane permeability. However, it is important to highlight that research about the antimicrobial effect of these organic acids has been carried out by addition as a “natural additive” and not during fermentation processes in foods. Indeed, based on the low quantity of organic acid produced [30], the inhibitory effect of these organic acids may result from the synergistic action together with other metabolites produced by starters and not by the individual action of each one [37].

There are many microbial genera used as starter cultures for fermented meat products. Although the most used belong to the group of lactic acid bacteria and Gram-positive catalase-positive cocci (GCC+), mainly represented by Staphylococcus spp. and Kocuria spp. [4], other starter cultures belong Lactococcus spp., Leuconostoc spp., Enterococcus spp. and Pediococcus spp. are also used [13]. Moreover, yeast and moulds, that confer specific sensory characteristics, are also added as starter cultures. Starter yeast and moulds are mainly represented by Debaryomyces spp. and Aspergillus spp., respectively. Moulds, since they are aerobic, are used as surface microbiota aimed to improve particular sensory and external characteristics.

Regarding food safety, fermented meat products are considered as safe products due to the development of unfavourable or inhibitory conditions to the growth of spoilage and/or pathogenic microorganisms. Low values of pH and aW, presence of salt, nitrites, spices and other ingredients, called hurdle technology, are responsible for the pathogenic and spoilage microorganism inhibition [38]. But these hurdles, in some cases, are not enough, and foodborne pathogens can survive, causing outbreaks [39].

Thus, some industrial practices such as the reduction of fermentation times to increase the production yield, slicing, decreased salt content or decrease/absence of nitrites allow conditions for the survival of foodborne pathogens [40][41][42][43]. In addition, low initial natural microbial load of meat batter for fermented sausage manufacture may pose a risk for pathogen multiplication due to the reduced competition [44]. In this context, starter cultures present a key role in the guarantee of the safety of these products. Starter cultures are also used in combination with other techniques (e.g., essential oils, packaging) to improve its efficiency, guaranteeing the food safety [45].
2.1. Antimicrobial Effect of Selected Starter Cultures Against Foodborne Pathogens

LAB represent the main starter cultures used in the meat industry. Its antimicrobial effect has already been described decades ago, not only based on the reduction of the pH derived from the transformation of sugar into lactic acid but also by the competitive effect against natural microbiota, production of other organic acids (e.g., lactic acid, acetic acid, propionic acid, benzoic acid), hydrogen peroxide, enzymes or bactericidal peptides called bacteriocins, for which the action mechanism has been described elsewhere.

The antimicrobial effect of organic acids lies in the reduction of pH and in the action of undissociated acid molecules. Also, low pH facilitates the diffusion of organic acids across the cell membrane, collapsing the electrochemical proton gradient, affecting the cell membrane permeability and leading to the cell death. Bacteriocins, most of them produced by LAB, are peptides or proteins of low molecular weight, synthesised in the ribosomes of the producer bacteria. Most bacteriocins act on the cellular membrane, destabilising and permeabiling through the formation of ionic channels or pores, which will release compounds such as phosphate, potassium, amino acids and adenosine triphosphate (ATP), decreasing the synthesis of macromolecules and consequently, cell death.

As previously discussed, starter cultures improve the safety of fermented meat products but evaluation of its antimicrobial effect, both in vitro and in food matrix, should be previously investigated. This study should be carried out both for commercial starters as well as in-house starters isolated from meat products or its environment of a specific meat industry. This fact is important since less antimicrobial effect is usually described in real meat sausages than in in vitro assays related to the interaction with food compounds. Thus, Reference verified that 1 out of 13 strains of Latilactobacillus sakei isolated from traditional meat sausages displayed an in vitro antimicrobial effect against L. monocytogenes, Salmonella spp. and S. aureus. Other research observed that only 14 out of 39 commercial starter cultures for meat sausage manufacture displayed antimicrobial effect. In contrast, other authors observed, both in broth and in fermented Greek sausage, that autochthonous strains of Lb. sakei displayed antimicrobial effect against E. coli and L. monocytogenes. Similar results were described in meat model media and fermented sausage against L. monocytogenes using Enterococcus mundtii as a starter culture. However, differences observed in the antimicrobial effect of starter cultures can be related to the microorganism, strain, the target microorganism and/or characteristics of sausage manufacture. Thus, it was observed that addition of Lacticasebacillus rhamnosus as a starter culture, isolated from human intestinal tract, did not suppress the growth of enterotoxin-producing S. aureus.

Antimicrobial effect of meat-borne LAB has been described in the literature against main foodborne pathogens and main spoilage bacteria. The antimicrobial effect is characterised by reducing or eliminating pathogenic and/or spoilage microorganisms in a shorter time during the manufacturing process. Thus, it allows meat producers to obtain safer products more quickly, being able to optimise the production processes. It is important to remark that the ability of starter cultures to compete with the natural microbiota of the raw material and to undertake the metabolic activities expected is conditioned by its growth rate and survival in the conditions prevailing in the fermented sausage (i.e., anaerobic atmosphere, NaCl concentration, ingredients, temperature of fermentation and ripening and low pH).

<table>
<thead>
<tr>
<th>Starter(s) Culture(s) Used</th>
<th>Origin of the Starter Culture</th>
<th>Characterisation of the Inhibition Mechanism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Starter cultures selected against L. innocua</td>
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<tr>
<td>Starter(s) Culture(s) Used</td>
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<tr>
<td>Pediococcus acidilactici</td>
<td>Isolated from alheira (Portuguese fermented pork sausage)</td>
<td>Not determined</td>
<td>[56]</td>
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<tr>
<td>(b) Starter cultures selected against L. monocytogenes</td>
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<tr>
<td>Lactiplantibacillus plantarum (strain 178) (formerly Lactobacillus plantarum)</td>
<td>Isolated from pork meat</td>
<td>Not determined</td>
<td>[57]</td>
</tr>
<tr>
<td>Lactiplantibacillus plantarum</td>
<td>Isolated from poto-poto, an ethnic maize fermented food</td>
<td>Production of plantaricin</td>
<td>[58]</td>
</tr>
<tr>
<td>Latilactobacillus sakei (formerly Lactobacillus sakei)</td>
<td>Isolated from chouriço (fermented cured pork sausage) made from wine-marinated meat</td>
<td>Not determined</td>
<td>[16]</td>
</tr>
<tr>
<td>Latilactobacillus curvatus 54M16 (formerly Lactobacillus curvatus)</td>
<td>isolated from traditional fermented sausages of Campania region (Italy)</td>
<td>Bacteriocin genes detection by PCR</td>
<td>[59]</td>
</tr>
<tr>
<td>Pediococcus pentosaceus</td>
<td>IOTEC culture collection (Thailan)</td>
<td>Not determined</td>
<td>[60]</td>
</tr>
<tr>
<td>Mix of Staphylococcus xylosus DD-34, Pediococcus acidilactici PA-2, Lactobacillus bavaricus MI-401</td>
<td>Commercial starter cultures (FloraCarn LC, Møller RM 52)</td>
<td>Production of pediocin (indicated by manufacturer)</td>
<td>[61]</td>
</tr>
<tr>
<td>Pediococcus acidilactici</td>
<td>Commercial stater cultures from Chr. HansenLaboratories (Denmark)</td>
<td>Bacteriocin purification and amino acid sequencing</td>
<td>[50]</td>
</tr>
<tr>
<td>Latilactobacillus sakei 8416 Latilactobacillus sakei 4413</td>
<td>Natural Greek dry-fermented sausage</td>
<td>Not determined</td>
<td>[61]</td>
</tr>
<tr>
<td>Lacticaseibacillus rhamnosus E-97800 (formely Lactobacillus rhamnosus)</td>
<td>Lacticaseibacillus rhamnosus E-97800: isolated from human faeces; Lacticaseibacillus rhamnosus LC-705: isolated from dairy; Lactiplantibacillus plantarum ALC01: commercial starter Pediococcus pentosaceus RM2000: commercial starter</td>
<td>Not determined</td>
<td>[62]</td>
</tr>
<tr>
<td>(c) Starter cultures selected against Clostridium perfringens</td>
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<tr>
<td>Lactiplantibacillus plantarum PCS20</td>
<td>Microbial Strain Collection of Latvia,</td>
<td>Not determined</td>
<td>[63]</td>
</tr>
<tr>
<td>Pediococcus acidilactici</td>
<td>Commercial stater cultures from Chr. HansenLaboratories (Denmark)</td>
<td>Bacteriocin purification and amino acid sequencing</td>
<td>[50]</td>
</tr>
<tr>
<td>(d) Starter cultures selected against Salmonella spp.</td>
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<tr>
<td>Enterococcus faecalis (strains A-48-32 and S-32-81)</td>
<td>Isolated from cheese</td>
<td>Production of enterocin</td>
<td>[64]</td>
</tr>
<tr>
<td>Latilactobacillus sakei</td>
<td>Isolated from chouriço (fermented cured pork sausage) made from wine-marinated meat</td>
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<tr>
<td>Latilactobacillus sakei 23K</td>
<td>Latilactobacillus sakei 23K: isolated from a French sausage</td>
<td>Not determined</td>
<td>[65]</td>
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<tr>
<td>Latilactobacillus sakei BMG 95</td>
<td>Latilactobacillus sakei BMG 95: isolated from anchovies</td>
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<tr>
<td>Latilactobacillus sakei BMG 37</td>
<td>‘Latilactobacillus sakei BMG 37: isolated from sheep meat</td>
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<tr>
<td>Staphylococcus xylosus</td>
<td>Staphylococcus xylosus: isolated from artisanal Tunisian fermented sausages</td>
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<tr>
<td>(e) Starter cultures selected against Escherichia coli</td>
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</tr>
<tr>
<td>Lacticaseibacillus rhamnosus (strains GG, E-97800 and LC-705) and Pediococcus pentosaceus</td>
<td>Lacticaseibacillus rhamnosus (strains GG, LC-705): commercial starter (Valio Ltd., Helsinki, Finland)</td>
<td>Not determined</td>
<td>[66]</td>
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<td></td>
<td>Lacticaseibacillus rhamnosus E-97800: commercial starter (VTT Biotechnology, Finland)</td>
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<tr>
<td></td>
<td>Pediococcus pentosaceus: commercial (Gewurzmuller, Germany)</td>
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<tr>
<td>Latilactobaciullus sakei 8416</td>
<td>Fermented game meat sausages</td>
<td>Not determined</td>
<td>[67]</td>
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<tr>
<td>Latilactobaciullus sakei 4413</td>
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<tr>
<td>(f) Starter cultures selected against Staphylococcus aureus</td>
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<td>Lacticaseibacillus rhamnosus</td>
<td>Not determined</td>
<td>[54]</td>
</tr>
<tr>
<td>FERM P-15120</td>
<td>Isolated from intestinal tracts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lacticaseibacillus paracasei subsp. paracasei FERM P-15121</td>
<td>(formerly Lactobacillus paracasei)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(g) Starter cultures selected against Enterobacteriaceae</td>
<td>(g) Starter cultures selected against Enterobacteriaceae</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Mix of *Pediococcus acidilactici* (MC184, MS198 and MS200) plus *Staphylococcus vitulus* RS34 Isolated from traditional Iberian dry-fermented salchichón Not determined [68]

(h) Starter cultures selected against *Yersinia enterocolitica*

*Latilactobacillus sakei* ATCC 15521: obtained from the American Type Culture Collection *Pediococcus acidilactici*: obtained from the Food Microbiology Culture Collection (Kansas State University, Manhattan, Kan., USA) Not determined [69]

PCR: polymerase chain reaction. The nomenclature of the genus *Lactobacillus* was presented according to the new taxonomic classification [83].

Thus, technological agents such as salt and curing agent may interfere in the bacteriocin production of *Lb. sakei* [56]. Also, spices seem to influence the growth of starter cultures. Thus, it was observed [57] that garlic enhanced bacteriocin production, lactic acid production was stimulated by pepper, while nutmeg decreased the bacteriocin production. In contrast, addition of garlic in Turkish soudjouk manufacture did not present any significant effect on the survival of *S. typhimurium* [58].

In addition, in case of high microbial contamination, the antimicrobial effect of starter cultures can be compromised. For example, if the initial contamination level is high, the use of a starter culture cannot improve the quality of the food product [44]. Thus, it has been reported [70] that the antimicrobial effect of natural microbiota cannot be enough in high microbial contamination of meat batter of Italian salami with 7 log cfu/g of *Salmonella* spp. and *L. monocytogenes*. Although *Salmonella* spp. decreased about 4 log cfu/g after fermentation, *L. monocytogenes* reduced less than 1 log cfu/g.

Also, the way in which starter cultures are added to the meat batter may influence its antimicrobial effect. Thus, microencapsulation of *Limosillactobacillus reuteri* decreased its antimicrobial effect against *E. coli* O157:H7 in dry fermented sausages [63].

Use of starter cultures combined with other compounds (Table 2), such as essential oils, organic acids, wine or spices, have been added to meat batter to improve the safety of these products [68,71,72,73,74,75]. However, previous assessment on potential interaction with starter cultures must be addressed since an inhibitory effect may be present, as discussed above.

**Table 2.** Combination of starter cultures and other hurdle technology to improve antimicrobial effect against foodborne pathogens in meat products’ manufacture.

<table>
<thead>
<tr>
<th>Starter Culture</th>
<th>Origin of Starter Cultures</th>
<th>Combined by</th>
<th>Antimicrobial Effect against</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Latilactobacillus sakei</em></td>
<td>Isolated from meat sausages</td>
<td>Garlic powder and wine</td>
<td><em>L. monocytogenes</em></td>
<td>[71]</td>
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<td></td>
<td></td>
<td></td>
<td><em>L. monocytogenes</em></td>
<td>[71]</td>
</tr>
<tr>
<td>Mix of starters</td>
<td>Commercial starter cultures</td>
<td>Mustard</td>
<td><em>E. coli</em></td>
<td>[72]</td>
</tr>
<tr>
<td><em>Latilactobacillus sakei</em></td>
<td>Isolated from meat sausages</td>
<td>Garlic powder and wine</td>
<td><em>Salmonella spp.</em></td>
<td>[71]</td>
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<td>[71]</td>
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<td></td>
<td></td>
<td></td>
<td><em>E. coli</em></td>
<td>[72]</td>
</tr>
</tbody>
</table>
### 2.2. Control of Biogenic Amine Formation in Meat Products by Addition of Selected Starter Cultures

Biogenic amines (BA) are nitrogenous compounds that are found in fermented foods and beverages formed by the microbial decarboxylation of amino acids \[^76\]. The main BAs in foods are histamine, tyramine, putrescine, cadaverine, tryptamine, spermine and spermidine. In some cases, they have been considered hazardous substances due to their ability to react with nitrites and form potentially carcinogenic nitrosamines \[^77\].

Regarding consumers' health, ingestion of BA may display some adverse dose-dependent effect, from allergy symptoms (e.g., skin rash, hives, itching) to systemic clinical signs (e.g., difficulty breathing, diarrhoea, vomiting, abdominal pain, joint pain, fatigue, seasickness, among others) \[^78\]. In addition, due to the fact that BA are thermostable, further processing of foods will not eliminate them once formed \[^79\].

Since concentration of BA in foods can display negative effects on the health of consumers, research about application of some manufacturing techniques and/or procedures, such as use of high hydrostatic pressure, control of NaCl concentration, freezing of raw materials, use of starter cultures, seasoning mixtures, product diameter, reduction in the amount of sugar added, use of additives, variation of the time/temperature parameters during fermentation and ripening, among others, have been investigated to decrease the BA contents in final product \[^80\]–\[^85\]. The microbiological quality of meat sausage ingredients is related to the aminogenesis process. Although hygienic quality is essential, other technological measures are needed. Thus, use of starter cultures represents one of the main measures to control BA formation \[^83\]. The action mechanism of starter cultures is based on its competitive effect against the natural microbiota. Several studies have demonstrated the role of starter cultures in reducing the accumulation of BA in meat products. For example, combination of S. xylosus and Lactiplantibacillus plantarum decreases the content of cadaverine, putrescine, tryptamine, 2-phenylethylamine, histamine and tyramine by about 50% in Chinese Harbin dry sausage \[^88\]. Addition of Lb. plantarum decreases the BA content by about 20%, but addition of both of them displayed a synergistic effect in which starter mix reduced tryptamine, phenylethylamine, putrescine, cadaverine, histamine and tyramine contents by nearly 100%, 100%, 86%, 63%, 82% and 43%, respectively \[^89\].

Other research indicated that the combination of Enterococcus thailandicus and Enterococcus faecalis displayed better antiobigenic formation than the combination of Staphylococcus carnosus and Lb. sakei \[^90\]. Since pH affects the BA formation, the lower level of pH achieved by the combination of E. thailandicus/E. faecalis than those achieved by the combination of S. carnosus/Lb. sakei may explain this difference on the anti-biogenic properties.

In contrast, addition of starter Lb. sakei and S. xylosus in the manufacture of Italian sausages displayed a higher level of BA compared to those made without starters \[^91\]. This result can be explained by the aminobiogenic capacity of both starter cultures in which histidine \[^92\] and tyramine \[^93\] decarboxilase activity was reported in artisanal fermented sausages. This fact was also reported in foal dry sausage, in which the use of a mix of Pediococcus pentosaceus and S. xylosus displayed higher accumulation of BA than those made without starter \[^94\]. Combination of Staphylococcus equorum S2M7/Lb. sakei CV3C2 displayed better anti-biogenic performance than S. xylosus CECT7057/Lb. sakei CECT7056 in finished dry-
cured sausage Paio Alentejano. However, addition of yeast 2RB4 together S. equorum S2M7/Lb. sakei CV3C2 reduced the BA content in the finished product by about 10%. The yeast effect may probably be associated to an improved competitive effect against other naturally bacterial strains presented in dry-cured sausage able to produce biogenic amines [95].

As indicated above, starter cultures may prevent the BA formation by its competitive effect against spoilage bacteria, however, recent research reported that LAB have been considered as main BA producers [77]. It indicates that selected starter cultures used in fermented sausage manufacture must be previously assessed regarding their decarboxilase activity. Since starters used in fermented meat products are usually isolated from natural microbiota, aminobiogenic capacity vary among LAB [96]. Thus, it has been reported that 80% of indigenous E. faecium and E. faecalis presented tyramine-producing capacity [97][98]. However, the combination of E. thailandicus and E. faecalis produced the lowest BA concentration [99]. With regards to S. xylous, only 7 out of 50 strains isolated from artisanal Italian sausages presented potential capacity to produce spermine, spermidine, tryptamine or tyramine [93]. Regarding Lb. sakei, this LAB has been reported as non-aminobiogenic [96] and may explain why manufacture of Italian sausage with a mix of Lb. sakei and S. xylous displayed lower BA content than addition of a starter mix composed of P. pentosaceus and S. xylous [99].

Overall, the aminobiogenic capacity of LAB together with the BA capacity of spoilage microbiota (naturally presented in raw meat) represent a chemical hazard concern in fermented meat products. It highlights the importance of selecting strains with oxidase activity instead of decarboxilase activity as starter cultures [100]. Although production of BA during sausage manufacture is inevitable, rapid overgrowth of selected (non-aminobiogenic starter) LAB at the beginning of fermentation may improve the chemical safety of these products.

Nitrates and nitrites in cured meat products are responsible for the characteristic red colour, inhibit the growth of pathogenic bacteria such as Clostridium botulinum, contribute to the development of the typical aroma of cured meats and act as antioxidants by delaying the development of rancidity and avoiding the appearance of alterations [101].

Overall, nitrates are not toxic, except in case of ingestion of large amounts. However, nitrites may pose a risk derived from their consumption since they can lead to allergic reactions and even cause methemoglobinemia situations. The main concern of nitrites is related to the possibility to act as precursors in the formation of carcinogenic nitrosamines, both in foods and at the organic level (for example, under acidic pH of mouth or stomach, nitrites or nitrates added to food or naturally occurring may combine with amines to form nitrosamines) [102]. Nitrosamine is a general term used to designate a vast group of N-nitroso compounds (NOCs). Its importance relies on the evidence of their carcinogenic properties [103]. Specifically, nitrosamines are formed by the reaction of compounds derived from nitrates, such as nitrous acid, with secondary amines throughout a nitrosation reaction. The presence of amines and the addition of nitrates and nitrites during the preparation of cured meat products can favour the development of this type of reaction in them. In meat products, the most commonly detected volatile nitrosamines are N-nitrosodimethylamine (NDMA), N-nitrosopyrrolidine (NPyR), N-nitrosopiperidine (NPIP), N-nitrosodiethylamine (NDEA), N- nitrosodi-n-butylamine (NDBA) and N-nitrosomor-folin (NMOR) [40]. Regarding these health issues for consumers, several food processing techniques have been investigated to reduce or replace the use of nitrates and nitrites in meat products (i.e., irradiation and n-nitrosamine blockers such as ascorbic acid) [103].

In this context, starter cultures appear to have a role in the reduction of nitrite levels in cured meat products. Thus, some authors [104][105][106] reported that addition of L. plantarum, L. pentosus, Lb. sakei or Lb. curvatus as starter culture decreased the nitrite content, suggesting the existence of nitrite reductase and heme-independent nitrite reductase that converts nitrite to NO, NO₂ or N₂O under anaerobic conditions [107]. Also, it has been referred that the rate of nitrite dissipation increases with pH reduction [108]. In contrast, the use of starter culture increased the N-nitrosopiperidine levels in heat-treated Turkish...
2.3. Control of Polycyclic Aromatic Hydrocarbons in Meat Products by Addition of Selected Starter Cultures

Polycyclic aromatic hydrocarbons (PAH) constitute a large group of organic compounds widely distributed in the environment with carcinogenic effects. Food contamination can occur by atmospheric deposition processes as well as during processing mainly related to heat treatments such as smoking, either by traditional methods or by the addition of smoke extracts directly into foods by spraying or dipping [110].

Since PAHs represent a health hazard and meat and meat products are one of the food categories contributing most to the dietary PAHs intake per day of the European Union, maximum levels in foods have been set by specific policy [111] to reduce its exposition. Research about the influence of starter cultures on PAH reduction is scarce.

Recently, it has been reported that immersion of cold smoked pork sausages in a LAB suspension of Lb. sakei, P. acidilactici and P. pentosaceus before ripening or in finished products decreased the benzo[a]pyrene contents [112]. Although the action mechanism of PAHs’ reduction is still unknown, it has been suggested that toxins are removed by specific enzymes produced by cells [113]. However, other studies suggested that biodegradation may be related to PAH binding to wall components of LAB cells [114]. Also, binding mechanisms of ion-exchange and hydrophobic bonds between exopolysaccharides and PAH have been suggested as a biodegradation route [115].

However, the effect of commercial starter (Lactobacillus spp., Micrococcaceae and yeasts) vs experimental starter (Lb. sakei and S. xylosus) on the PAH content in finished Portuguese Paio Alentejano (dry-cured pork sausage) did not evidence significant differences among starters [116]. It may suggest that the presence of specific enzymes or the presence of specific membrane compounds, as previously indicated, can be associated to specific microorganisms and/or strains.

3. Use of Starter Cultures to Improve the Safety in Dairy Products

3.1. Improving the Food Safety of Cheese by Use of Starter Cultures

LAB are the main starter cultures used in the dairy industry for cheese and yogurt production. Most of them are grouped into the genera Lactococcus, Lactobacillus, Leuconostoc and Pediococcus. Along with LAB, species of other genera such as Propionibacterium and Bifidobacterium are also occasionally used.

As previously discussed, use of starter cultures allows manufacturers to control and optimise the fermentation processes aimed to confer specific characteristics to the final product. Thus, starter cultures are related to the flavour and aroma characteristics, proteolytic and lipolytic activities, as well as inhibition of pathogenic microorganisms. In this section, we discuss the use of starter cultures to improve the safety of cheese and yogurt.

In cheese manufacturing, lactic acid bacteria play different roles in the cheese making process. Some species participate more in fermentation while others are mainly involved in ripening. Regarding food safety, the importance of LAB is related to the antimicrobial effect against foodborne and spoilage bacteria throughout production of organic acids, competitive effect and production of antimicrobial substances [25].

Regarding foodborne pathogens, the most commonly involved in cheese outbreaks are enteropathogenic E. coli, particularly 0157:H7, Salmonella spp., S. aureus and L. monocytogenes [1]. This last microorganism represents the most concerning pathogen since they can survive in a wide range of conditions during manufacture, ripening and storage (even in chilled storage).

Use of starter cultures to control L. monocytogenes in cheese has been largely described in the literature
(Table 3), mainly based on the bacteriocinogenic properties of starter cultures. Thus, use of bacteriocinogenic starter cultures of sakacin, nisin, pediocin or enterocin represents the most important tool to control L. monocytogenes in cheese [117][118]. However, control of surface contamination by L. monocytogenes by LAB, during ripening or storage, should be carefully assessed since susceptibility of Listeria spp. to the antimicrobial activity of LAB is strain-dependent [119]. This strain susceptibility has been reported by other authors [118], in which addition of starter Lactococcus lactis in fresh cheese displayed a modest decrease of L. monocytogenes counts. The authors of Reference [120] reported that spraying surfaces with E. faecium in Munster cheese did not decrease L. monocytogenes levels but acts as a bacteriostatic. It can be concluded that starter cultures play an important role in the control of L. monocytogenes, but antimicrobial properties should be previously assessed in vitro (as described above for meat products) since L. monocytogenes susceptibility is strain-dependent. This fact was reported in Reference [122], in which nearly one third out of eight hundred LAB strains displayed anti-listerial activity. Also, hygienic practices must be guaranteed since this pathogen and outbreaks are still detected [122][123]. To improve the safety of cheese, combination of starter cultures and other antimicrobial treatments have been studied. Thus, enhancing the anti-listerial effect of starter Lc. lactis with lactic acid and sodium lactate [124] or plus sodium acetate or sodium lactate [118] have been reported. Addition of tartaric, fumaric, lactic or malic acid improves the inhibition of L. monocytogenes, but differences among organic acids may be explained by differences in the synergistic effect with lactic or acetic acid naturally produced by LAB during cheese ripening.

Table 3. Antimicrobial effect of selected starter cultures (added as ingredients during cheese manufacture) against main foodborne pathogens.

<table>
<thead>
<tr>
<th>Cheese</th>
<th>Starter(s) Culture(s) Used</th>
<th>Origin of Starter Cultures</th>
<th>Characterisation of the Inhibition Mechanism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Starter cultures selected against L. monocytogenes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anari cheese</td>
<td>Enterococcus faecium</td>
<td>Donkey milk</td>
<td>Not determined</td>
<td>[126]</td>
</tr>
<tr>
<td>Cottage cheese</td>
<td>Lactococcus lactis</td>
<td>Italian fermented food</td>
<td>Nisin producer. PCR detection of bacteriocin genes</td>
<td>[127]</td>
</tr>
<tr>
<td>Portuguese Pico cheese</td>
<td>Lactococcus lactis Enterococcus faecium</td>
<td>Isolated from cheese</td>
<td>PCR detection of bacteriocin genes</td>
<td>[128]</td>
</tr>
<tr>
<td>Fresh Minas cheese</td>
<td>Lactiplantibacillus plantarum 59</td>
<td>Isolated from fruits</td>
<td>Not determined</td>
<td>[129]</td>
</tr>
<tr>
<td>Munster cheese</td>
<td>Enterococcus faecium WHE 81</td>
<td>Isolated from cheese</td>
<td>Enterocin producer. Determination by sensitivity to proteolytic and other enzymes</td>
<td>[120]</td>
</tr>
<tr>
<td>Fresh cheese</td>
<td>Lactococcus lactis</td>
<td></td>
<td>Nisin producer. Bacteriocin gene determination</td>
<td>[130]</td>
</tr>
<tr>
<td>Cheese model</td>
<td>Lactiplantibacillus plantarum</td>
<td>Isolated from cheese</td>
<td>Plantaricin producer. Purification by HPLC</td>
<td>[131]</td>
</tr>
<tr>
<td>Gongonzola Cheese (Italy)</td>
<td>Lactiplantibacillus plantarum Latilactobacillus sakei Lactococcus lactis</td>
<td>Microbial collection (Institute of 108 Sciences of Food Production of the National Research Council of Italy</td>
<td>Nisin and enterocin P producers. Characterisation by bacteriocin gene identification</td>
<td>[119]</td>
</tr>
<tr>
<td>Fresh minas cheese</td>
<td>Enterococcus mundtii Enterococcus faecium CRL 35</td>
<td>Isolated from cheese</td>
<td>Enterocin identification by HPLC and sensitivity to proteolytic and other enzymes</td>
<td>[132]</td>
</tr>
<tr>
<td>Cheese</td>
<td>Starter(s) Culture(s) Used</td>
<td>Origin of Starter Cultures</td>
<td>Characterisation of the Inhibition Mechanism</td>
<td>Reference</td>
</tr>
<tr>
<td>-------------------------</td>
<td>------------------------------------------------------------------------------------------</td>
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</tr>
<tr>
<td>Ripened cheese</td>
<td>Pediococcus acidilactici 347, Lactococcus lactis ESI 515, Lactococcus lactis CL1, Lactococcus lactis CL2</td>
<td>Isolated from dairy products</td>
<td>Nisin and pediocin producers</td>
<td>[117]</td>
</tr>
<tr>
<td>Sicilian cheese</td>
<td>Lactococcus lactis, Lacticaseibacillus rhamnosus 971, Enterococcus faecium</td>
<td>Isolated from dairy environment</td>
<td>Not determined</td>
<td>[133]</td>
</tr>
<tr>
<td>Golka cheese</td>
<td>Lactococcus garvieae Lab428, Lactococcus mesenteroides Lab25, Lactiplantibacillus plantarum Lab572</td>
<td>Isolated from Golka cheese</td>
<td>Characterisation by bacteriocin gene identification</td>
<td>[121]</td>
</tr>
<tr>
<td>(b) Starter cultures selected against Staphylococcus aureus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Probiotic white cheese</td>
<td>Commercial lyophilised starter culture, Lacticaseibacillus rhamnosus, Lacticaseibacillus casei Shirota</td>
<td>Commercial starter cultures</td>
<td>Not determined</td>
<td>[122]</td>
</tr>
<tr>
<td>Commercial cheese</td>
<td>Lactococcus lactis L005, Lacticaseibacillus rhamnosus BGP2, Brevibacterium linens 004-0001, Microbacterium lacticum</td>
<td>Isolated from raw milk</td>
<td>Not determined</td>
<td>[134]</td>
</tr>
<tr>
<td>Ripened cheese</td>
<td>Pediococcus acidilactici 347, Lactococcus lactis ESI 515, Lactococcus lactis CL1, Lactococcus lactis CL2</td>
<td>Isolated from dairy products</td>
<td>Nisin producer Pediocin producer</td>
<td>[117]</td>
</tr>
<tr>
<td>Raw milk Montasio cheese</td>
<td>Lactiplantibacillus plantarum</td>
<td>Commercial starter mix</td>
<td>Not determined</td>
<td>[135]</td>
</tr>
<tr>
<td>Algerian’s goat cheese</td>
<td>Lactococcus lactis ssp. lactis KJ660075 strain</td>
<td>Isolated from raw goat milk</td>
<td>Detection of bacteriocin by sensitivity to proteolytic and other enzymes</td>
<td>[136]</td>
</tr>
<tr>
<td>(c) Starter cultures selected against Escherichia coli</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jben (Moroccan fresh cheese)</td>
<td>Lactococcus lactis subsp. lactis UL790</td>
<td>Not available</td>
<td>Nisin producer</td>
<td>[137]</td>
</tr>
<tr>
<td>Cheese</td>
<td>Starter(s) Culture(s) Used</td>
<td>Origin of Starter Cultures</td>
<td>Characterisation of the Inhibition Mechanism</td>
<td>Reference</td>
</tr>
<tr>
<td>--------------------------------</td>
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<td>-----------</td>
</tr>
<tr>
<td>Commercial cheese</td>
<td>Lactococcus lactis L005 Lacticaseibacillus rhamnosus BGP2 Brevibacterium linens 004-0001 Microbacterium lacticum</td>
<td>Isolated from raw milk</td>
<td>Not determined</td>
<td>[134]</td>
</tr>
<tr>
<td>Ripened cheese</td>
<td>Pediococcus acidilactici 347 Lactococcus lactis ESI 515 Lactococcus lactis CL1 Lactococcus lactis CL2</td>
<td>Isolated from dairy products</td>
<td>Nisin producer Pediocin producer</td>
<td>[127]</td>
</tr>
<tr>
<td>Goat cheese</td>
<td>Authochthonous Lactobacillus spp.</td>
<td>Raw goat milk</td>
<td>Not determined</td>
<td>[138]</td>
</tr>
<tr>
<td>Raw milk Montasio cheese</td>
<td>Lactiplantibacillus plantarum</td>
<td>Commercial starter cultures</td>
<td>Not determined</td>
<td>[135]</td>
</tr>
<tr>
<td>White Brined Cheese</td>
<td>Streptococcus thermophilus Lactobacillus delbrueckii subsp. bulgaricus Lactcaseibacillus paracasei K5</td>
<td>Isolated from Greek Feta cheese</td>
<td>Not determined</td>
<td>[139]</td>
</tr>
</tbody>
</table>

The nomenclature of the genus Lactobacillus was presented according to the new taxonomic classification [83].

Combination of essential oils and starter cultures during cheese manufacture should be assessed since survival of starter can be compromised with further impacts on sensory and safety characteristics [130, 131].

*S. aureus* is a concerning pathogen in cheese making. The importance of its control is related to the capacity of toxin production that, once formed in food, are extremely difficult to eliminate. These toxins are responsible for most staphylococcal food poisoning associated with the consumption of contaminated food. Thus, control of *S. aureus* contamination is of great importance, with special relevance in those cheeses made from raw milk since prevalence of *S. aureus* in milk is high [140]. Indeed, microbiological criteria for *S. aureus* in cheese has been set by law [141].

Research about control of *S. aureus* by addition of starter cultures is less than observed for *L. monocytogenes* (Table 3). Reduction of *S. aureus* was only achieved in bacteriocin producer *Lc. lactis* strains [127]. However, other reports suggest that the presence of *S. aureus* in raw milk is inhibited at different stages of ripening [134]. It has been observed that *S. aureus* survives in 60-day ripened white cheese made with commercial starter, although combination with probiotic [142] *L. rhamnosus* and Lactobacillus casei Shirota displayed an inhibitory effect up to 5 Log cfu/g, probably associated with the increased effect of bacteriocins arising during the ripening period. In contrast, use of starter *L. rhamnosus* did not display an inhibitory effect against *S. aureus* in Brazilian minas frescal cheese [143]. Survival of *S. aureus* in Jben, a Moroccan fresh cheese, was also reported [137], but addition of nisin-producer starter Lactococcus lactis subsp. lactis UL730 increased the safety of fresh cheese by elimination of *S. aureus*.
after 4 days. Combination of Lc. lactis subsp. cremoris and oregano essential oil (EO) to inhibit L. monocytogenes and S. aureus has been studied [144], however its efficacy may be compromised due to the inhibitory effect of oregano EO against added starter culture. A similar inhibition effect of EO was observed in a combination of thymus EO and starter Lc. lactis subsp. lactis and Lc. lactis subsp. cremoris against S. aureus [145]. Combination of Mentha longifolia L. EO in combination with starter Lb. casei in concentrations over 50 ppm displayed a synergistic effect against growth of S. aureus [146]. In addition to the negative effect of EO on starter LABs, as described above, sensory cheese analysis is necessary since inhibitory concentrations of EO may be incompatible with consumer acceptance.

Application of high-pressure treatment (HPT) at lower pressure in combination with bacteriocin-producing LAB [147] improves the safety of raw cheese against S. aureus. Since HPT disrupts the structure of S. aureus, including its cell membrane, it may explain the enhanced effect of bacteriocins produced by starter LAB.

In cheese processing, Salmonella spp. decreases along the ripening and storage periods [148][149]. Factors such as salt concentration, storage temperature and pH are the main barriers that disrupt its growth. However, Salmonella spp. may survive until the finished product [150][151]. Thus, it has been suggested that reduction of S. typhymurium along ripening in Montasio cheese is associated with the drop of pH after the negative antagonistic effect of starter Lb. plantarum by the spot method [152]. Survival of Salmonella spp. in low-salt cheddar cheese made with commercial starter Lactococcus lactis, Lc. lactis subsp. cremoris and Lb. helveticus was detected for up to 90 days when stored at 4 or 10 °C and for up to 30 days at 21 °C. Addition of starter cultures in cheese making improved the decrease of Salmonella spp. [138][139], probably associated to the enhanced effect of the pH by lactic acid production [152]. However, the survival of this pathogen indicates that the antimicrobial effect of starter cultures used in cheese making must be previously verified together with high hygienic quality of ingredients and storage temperature conditions.

Presence of E. coli in cheese has been reported in the literature. During cheese making, E. coli increased in the first hours of ripening [153][154]. Thus, the use of starter cultures to inhibit E. coli growth has been investigated as a biopreservative tool [155]. Addition of nisin- and pediocin-producing Lc. lactis CL2 inhibited E. coli after 15 and 30 days of ripening. However, addition of non-bacteriocinogenic Lc. lactis ESI 153 [117] displayed an unexpected better inhibitory effect than pediocin-producer P. acidilactici.

It was also reported that the inhibitory effect of starter cultures (Hafnia alvei, Lb. plantarum and Lc. Lactis) against E. coli may be influenced by the initial LAB load of raw milk [155]. It suggests that the acidification rate carried out by natural LAB microbiota together with starter cultures is related to the inhibitory effect of E. coli. However, it has been suggested that survival of E. coli during ripening may be associated to the initial microbial load of raw milk [156]. The synergistic effect of essential oil and starter cultures to control E. coli have been also studied [157], in which the combination of Zataria multiflora EO and Lb. acidophilus decreased the growth rate of E. coli. In contrast, total growth inhibition of E. coli was achieved by combination of Lb. acidophilus LA-5 with oregano and rosemary EO [158].

Combination of bacteriocinogenic starter cultures and high hydrostatic pressure can reduce E. coli counts with lower pressure intensity in ripened cheese [117]. Other authors showed that addition of Lb. reuteri or glycerol in semi-hard cheese manufacture does not inhibit the growth of E. coli O157:H7 up to 30 days of ripening. However, combination of Lb. reuteri and glycerol eliminates E. coli completely after 24 h [159].

### 3.2. Improving the Food Safety of Yogurt by Use of Starter Cultures

Yogurt is a food product obtained by lactic fermentation of milk previously subjected to a heat treatment, at least, to pasteurisation, through the action of some microorganisms such as Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus. Also, other lactobacilli and bifidobacteria are sometimes added during or after culturing yogurt as probiotics [160]. Yogurt is considered a safe food since its manufacture includes two hurdle steps that make the survival of foodborne pathogens difficult,
such as heated milk and low pH resulting from fermentation. To the best knowledge of the authors, no information regarding recent bacterial outbreaks in yogurt is available, although older scientific studies have reported the presence of foodborne pathogens such as L. monocytogenes, E. coli or Yersinia enterocolitica related to cross-contamination issues.

Because yogurt manufacture is carried out by addition of Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus starters, scarce research is available regarding the role of other starter cultures added to improve its safety.

Addition of bacteriocinogenic Streptococcus thermophilus as a starter displayed an inhibitory effect against L. monocytogenes during fermentation. However, a scarce inhibitory effect was observed against S. aureus by the same starter. Counts of L. monocytogenes and E. coli increased during fermentation but decreased during storage, influenced by storage temperature, with a higher decrease at 10 than at 4 °C. In addition, fermentation at two consecutive periods (43 °C for 3 h and 30 °C for 21 h) revealed better inhibition effect against E. coli O157:H7, L. monocytogenes 4b and Y. enterocolitica O3. Greater inhibition results against E. coli were also observed at 17 and 22 °C than at 4 and 8 °C during yogurt storage, suggesting that E. coli presented more adaptation capacity to pH variation than refrigeration temperatures.

Regarding Salmonella spp., it was observed that S. enteritidis and S. typhimurium survived throughout the fermentation process. Also, S. enteritidis can survive up to 12 days at 4 °C and up to 60 h at 25 °C. Overall, Gram-negative bacteria discussed above presented variable capabilities of survival throughout fermentation and storage of yogurt. So, contamination after fermentation may represent a risk for foodborne poisoning. This survival on acid conditions can be related to development of acid, gene-encoded survival mechanisms. It indicates that safety of yogurt cannot be based on the antimicrobial effect of starter cultures added. Since one of the antimicrobial effects of starters is based on the competitive effect, the survival mechanisms of enterobacteriaceae at acid environment may overlap the growth capacity of the starter. Also, the fact that not all starter cultures presented bacteriocinogenic capacity may imply a previous testing, as already discussed in the text. In consequence, good microbial quality of milk, proper thermal treatment of milk, together with good hygienic manufacturing practices and proper starter cultures selection must be implemented.

References


142. Kalkan, S. Predicting the antimicrobial effect of probiotic lactic acid bacteria against Staphylococcus aureus in white cheeses, using Fourier series modeling method. J. Food Saf. 2020, 40, e12724.


Keywords

start cultures; foodborne pathogens; fermented meats

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