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SKLM



Microbial food cultures

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SKLM Commission Secretariat

Food Chemistry and Toxicology, University of Kaiserslautern

Erwin-Schroedinger-Str. 52, 67663 Kaiserslautern, Germany

E-Mail: sklm@rhrk.uni-kl.de • Tel.: +49 631 2054200 • Fax: +49 631 2054005

In the EU, there are no specific legal regulations regarding microbial food cultures. However, at European and national level there are regulations that require microbial cultures to be checked in terms of their compliance with legal requirements. Due to the lack of definitions for microbial food cultures with various applications, there are uncertainties regarding how they are to be assessed. The increased elaboration of microbial ecology and modern taxonomy has allowed the description of numerous new species that are attractive for use in food cultures or are already in use, on which however only limited experience is available. In view of these developments, the SKLM has prepared this statement, focusing on definitions, gaps in knowledge and further research needs. It aims to support producers and users of microbial cultures as well as authorities responsible for consumer health protection with respect to safety assessment and to contribute to consumer information. The scientific status concerning these cultures in food technology, the traditional roots of their application and their potential for sustaining and/or furthering food variety and quality have not been adequately described up to now. This is the subject of the present SKLM statement. In addition, definitions are proposed for cultures used in food technology that may also be useful for the assessment in a legal context.

Microbial food cultures

1. Introduction

Humans have been storing food at least since the beginning of the Neolithic period, around 10,000 years ago. The availability of storable and hygienically safe food was a decisive prerequisite for the development of mankind and society. Traditional methods for preserving food, such as drying, smoking, salting and fermentation are still in use today. The active principles of fermentation were unknown until modern times; Louis Pasteur was the first to recognise its nature, describing it as a (microbial) “life without air”. The causers of the process were called ferments and classified into ‘formed’ and ‘unformed’ variants. Hans Buchner showed that they correspond to microorganisms and enzymes, respectively. Subsequently, food fermentation processes underwent a process of continuous improvement, and microbial cultures became essential components of food production. They form a continuum, encompassing the historically ancient use of fermenting food substrates up to pure cultures that are characterised taxonomically, physiologically, biochemically and genetically. From cultures traditionally used in food fermentations, new fields of application have arisen on the basis of extensive scientific studies, which allowed utilizing specific features of the culture organisms for specific applications. An understanding of the historical relationships as well as the scientific and practical background for the use of cultures is not common knowledge.

The Senate Commission on Food Safety (SKLM) last addressed the issue of microbial cultures in its communication entitled *Starter cultures and enzymes for food processing technology* (SKLM, 1987). This work described safety aspects and recommendations for the safety assessment of non-traditional culture strains before their introduction into practical use. Prior to utilizing 'novel' organisms, studies on pathogenicity and infectivity, the formation of antibiotics and toxins, and other toxicologically potentially relevant properties were required. The communication opened with the following statement: *"The starter cultures currently used by the food industry in the Federal Republic of Germany have not given rise to health problems in consumers, according to current scientific knowledge. They have added to the range of available products and have significantly improved production compared with the earlier practice of fermentation with uncontrolled organism populations."*(SKLM, 1987). In the opinion of the SKLM, this is still true today. However, new findings in the areas of taxonomy, physiology, ecology and genetics of microorganisms and nutritional physiology, biotechnology and food technology have lead to new applications that (now) make an update necessary.

2. Definitions

While microbial cultures and their application in foods have been the subject of official statements and legal provisions, a scientific definition that includes all microbial cultures used in foods - taking history as well as existing practice into account - is not currently available.

2. 1. Fermented foods

Modern industrial microbiology defines fermentation as a process of biotransformation carried out by microorganisms or their enzymes, irrespective of whether it is based on fermentation in the classic sense (anaerobic catabolism of organic substrates without the involvement of exogenous electron acceptors) or oxidative metabolism (respiration).

Definition 1: Fermented foods are consumable products that are generated from thermally treated or untreated food raw materials of plant or animal origin. They have characteristic sensory and nutritional value as well as properties determining shelf life,

hygiene or practical value that are decisively affected by microorganisms and/or enzymes (from the raw material).

Fermentation processes exclusively achieved by enzymes naturally occurring in the substrate (e.g. in tea and tobacco fermentation) and through the addition of enzymes from other sources (enzyme treatment) are included in principle, but are not taken into account here.

Table 1 (Appendix) lists examples of fermented foods well-known in Europe, as well as the groups of microorganisms involved in the process. About one third of all food currently being consumed is fermented. These fermented foods feature a number of advantages:

- They offer a high degree of hygienic safety.¹
- They have an increased shelf life compared to the raw product.
- Raw materials are refined by improving quality-determining properties.
- Toxic or harmful substances derived from the raw material, such as cyanides, hemagglutinins, goitrogens, proteinase inhibitors, phytic acid, oxalic acid, glucosinolates and indigestible carbohydrates, are partly degraded.
- Manufacture requires only basic technology and low energy consumption.
- They meet a demand for natural and organic food.

The organisms involved in fermentation often occur in typical associations, i.e. communities of different microorganisms developed under different environmental influences displaying different metabolic properties. In the multitude of fermented foods these associations have different compositions and accordingly have different metabolic pathways (see Appendix in **Table 2**). The group of lactic acid bacteria is involved in achieving all of the desired effects and is therefore the most important element of the fermentation associations. Species of practically all genera of the group are involved in indigenous fermentations. The genera can include a huge variety of species, such as the *Lactobacillus* genus with over 150 species, most of which have a food association (Hammes and Hertel, 2009). The potential of their intended use as selected organisms for fermentation processes or as so called ‘starter cultures’ is based on this.

¹ Beer, the production of which has been known for more than 7000 years, was, like wine, a source of safe beverages free from life threatening microbial contamination.

2. 2. Starter cultures

Definition 2: Starter cultures are preparations of live microorganisms or their resting forms, whose metabolic activity has desired effects in the fermentation substrate, the food.

The preparations may contain unavoidable residues from the culture substrate and additives that support the vitality and technological functionality of the microorganisms (such as antifreeze or antioxidant compounds).

This definition includes a multitude of preparations, which is based on the history of starter cultures. The development of fermented foods was determined by the fact that originally a special microbial association developed under the influence of ecological factors prevailing in the respective substrate. Such spontaneous processes in the fermentation of sauerkraut (pickled cabbage), olives or pickles are still state-of-the-art. In other areas, fermenting substrate was used to inoculate new fermentation methods. Still standard practice today is for instance ‘old-new inoculation’ with cheeses, ‘back-slopping’ or ‘back shuffling’ with sourdough. Vinegar is also produced in this way. The inoculum obtained in this way (having been propagated many times) undergoes a high level of organism selection and is practically synonymous with starter cultures. Such ‘**undefined cultures**’ are still in use today, for example ‘Flora Danica’, used as a milk starter culture from more than 100 *Leuconostoc* and *Lactococcus* strains or ‘Reinzuchtsauer’ used as a sourdough starter. These cultures are subject to a continuous change in composition, as strains may disappear or mutate, or may change their properties after phage attacks.

The use of ‘**defined cultures**’ allows for a greater degree of control over the fermentation process. Mäyrä-Mäkinen and Bigret, (1998) made a distinction between:

- **Single-strain cultures:** contain one strain of a species;
- **Multi-strain cultures:** contain more than one strain of a single species;
- **Multi-strain mixed cultures:** contain different strains from different species.

These different cultures are used in the fermentation of milk, meat, wine, fruit, vegetables and cereals. Review articles provide information about characterising starter cultures for various food groups (dairy products: Teuber, 2000; meat products: Hammes, W. P. and Hertel, C. 1998; sourdough: Hammes, W. P. and Vogel, R. F. 1997; wine: Lonvaud-Funel, 1997; Krieger-Weber, 2009; beer: Bohak et al., 1998; fruit- and vegetable juice as well as fermented

vegetables: Buckenhüskes, 2001). To maintain their stability, effectiveness and applicability, they are prepared, packaged, chilled, frozen or freeze-dried. Using such starter cultures offers a number of benefits, such as:

- Food production at a uniform level of high quality;
- Control of fermentation time;
- Economic process management through reductions in process time and/or improvement of the substrate turnover;
- Reduction of hygienic risks;
- Access to new products that cannot be produced by spontaneous fermentation.

The taxonomic status is a basic requirement for characterising starter cultures but often has errors or gaps. Apart from these starter cultures, there are diverse cultures poorly documented in taxonomical terms that are propagated 'in-house' or are produced by biotech firms for practical application. Therefore, knowledge about culture organisms is primarily limited to those cultures that are commercially available. Basically it is known that these may contain bacteria, yeasts and moulds. Bacteria are almost exclusively Gram-positive organisms, as these can be prepared much more easily than their Gram-negative counterparts.

The most advanced state of application of starter cultures has been achieved in the dairy industry. The manufacture of fermented dairy products made from pasteurised milk – therefore containing very low levels of bacteria - would be practically impossible without cultures. However, high-quality cheese is also produced using raw milk or 'in-house' cultures.

Scientific analysis of the microbiology of fermented foods shows that new organisms are continuously being added to the cultures and new cultures are finding their way into practice. Special physiological properties and their genetic background are increasingly being characterised in order to use the specific benefits of defined strains and to minimise adverse effects. It has been shown for example that the process of 'old-new' inoculation entails the risk of selecting listeria in red smear cheeses such as Tilsit, Harzer, Romadour and Münster. Microbiological and ecological investigations of red smear cheese associations create new opportunities for reducing the incidence of listeria through the use of a variety of newly described organisms that can be used as starters (Bockelmann et al., 2005). The addition of mesophilic lactobacilli such as *L. paracasei*, *L. plantarum*, *L. rhamnosus* and *L. curvatus* is an example of the use of so-called secondary cultures or 'culture adjuncts' in dairy cultures

(Chamba and Irlinger, 2004). Such types of species can basically be allotted to the starter organisms. When added, they do not exert their influence on the primary fermentation process and sensory properties like the classic culture in cheese milk, but rather in the second fermentation stage of cheese fermentation and ripening. Another example is the use of cultures in the production of malt as a way to reduce embryonic rooting, thereby reducing malting losses (Schehl et al., 2007). Simultaneously, the risk of mycotoxin formation in the malting process may be reduced (Laitila et al., 2002).

Based on in-depth scientific research with such starter cultures, so-called probiotics and protective cultures have developed from them.

2. 3. Probiotics

The FAO/WHO issued a definition of probiotics² from as far back as 2001.

Definition 3: Probiotics are live microorganisms which when consumed in adequate amounts confer a health effect on the host” (FAO/WHO, 2001)

Probiotic microorganisms are normally not involved in the microbial fermentation process in food. It cannot be excluded that organisms used in classical food fermentation may also be metabolically active and probiotic in the gastrointestinal tract. In addition, it should also be considered that probiotic effects ascribed to living microorganisms can often be triggered by cell (wall) fractions of these organisms. Although the borderlines to the therapeutic effects are sometimes unclear, probiotics are regarded as functional foods. The assessment of functional foods was the subject of a comprehensive statement by the SKLM (2004). A guidance for demonstrating the beneficial effects of probiotics has been worked out in a very recent workshop (ILSI 2010).

Only lactobacilli and bifidobacteria are present in probiotic foods in the European market. In a review (Mercenier et al., 2003), 30 strains of the genera *Bifidobacterium* (7), *Enterococcus* (1), *Lactobacillus* (19), *Lactococcus* (1) and *Streptococcus* (2) were listed. Fermented yoghurt-like dairy products that in particular contain *Lactobacillus casei*, *L. johnsonii*, *L.*

² The use of probiotics in feed, as pharmaceuticals, cosmetics or as food additives is not covered by the statement above.

plantarum, *L. rhamnosus* and *Bifidobacterium animalis* are of considerable importance to the market. Breakfast cereals, muesli, ice cream, cheese, various beverages and uncooked sausages are also available as probiotic foods. Unlike the above, probiotic dietary supplements contain a wide variety of organism groups, e.g. *Bacillaceae* (e.g. *B. coagulans*, *B. subtilis*), enterococci (*E. faecium*), *Propionibacterium freudenreichii* and yeast (*Saccharomyces cerevisiae*, synonym *S. boulardii*).

The different strains were usually isolated from human faeces and, as far as the lactobacilli are concerned, belong to such species that are also involved in traditional processes of food fermentation. They are not ‘novel organisms’ in the sense of Regulation (EC) No. 258/97 (EU, 1997). The isolation of lactobacilli from faeces does not permit to conclude that these are intestinal bacteria, as this bacteria group varies greatly from individual to individual, and the organisms may also originate from the oral cavity or from consumed foods and could have survived their passage through the gastrointestinal tract. As part of a safety assessment of these species the history of safe use according to the QPS concept (see below), the exposure of consumers and the special status of target groups must be considered

2. 4. Protective cultures

The discovery that certain strains amongst the fermentation organisms are noticeably competitive, and in particular that they can also inhibit pathogenic and toxigenic microorganisms in foods, opens up the possibility that these properties can be used for extended application in foods in general. The term ‘**bioprotection**’ was coined for such applications (Bech Hansen, 2002); the cultures used are called ‘protective cultures’.

Definition 4: Protective cultures are preparations consisting of live microorganisms (pure cultures or culture concentrates) that are added to foods with the aim of reducing risks by pathogenic or toxigenic microorganisms.

They develop their protective effect via metabolic pathways in food, although they do not usually determine the typical nature of a fermented food characterised by the starter culture. The protective cultures available in practice in fact contain the same microorganisms as found in the starter cultures. Their active metabolism is a prerequisite for their effectiveness, which also allows to define them as ‘fermenting’ according to definition 1. The distinction between protective- and starter cultures lies in their intended use.

The effectiveness of protective cultures is based on the following principles:

- 1. Competitive exclusion**, for example through competition for nutrients and/or binding sites on the substrate, or through better adaptation to the oxygen content.
- 2. Formation of antagonistically active substances**, e.g. with lactobacilli organic acids (e. g. lactic, acetic, propionic, formic, benzoic acid), ethanol, H₂O₂, CO₂, bacteriocins (ribosomal synthesized peptides, proteins and polypeptide compounds such as Nisin), as well as antibiotics or other antagonistically active principles with antimycotic or antibacterial activity.

Such principles may be equally allocated to the spectrum of methods that are in general use in the food industry (e.g. drying, salting, cooling, freezing, oxygen removal, acidification, chemical preservation) (Hammes, 2010).

Protective cultures may have particular importance when used in non-fermented foods with a neutral pH value and high water activity ($a_w > 0.96$) that are subject to an increased hygiene risk.

3. Legal aspects of the application of microbial cultures in foods

There is no clear allocation of microbial food cultures to one of the categories ‘ingredient’, ‘additive’ or ‘processing aid’. Instead, categorisation is handled separately by Member States. For instance, although yeast used in baking or brewing is functionally a starter culture, according to the EU it is an ‘ingredient’.

A review by Wessels et al. (2004) shows that within the EU, only Denmark has a legal regulation for the use of cultures (Danish statutory order on food additives of 11 January 2005, Statutory order No 22 of 11 January 2005, Annex 5, information to be provided in connection with the evaluation of a bacterial culture and mould or yeast fungus). Cultures are considered to be additives and require notification and approval, including the documentation on safety and efficacy. Of the mixed cultures for the dairy industry, only those that were already present in the Danish market prior to 1973 (when the law came into effect) are permitted. Fermented foods from other EU countries have free access to the Danish market, even if they were not produced with the aid of such mixed cultures.

There is no specific legal regulation regarding microbial cultures for foods in Europe. However, they must satisfy the legal requirements of Regulation (EC) No. 178/2002 or, in Germany, the Food and Feed Code (Lebensmittel- und Futtermittelgesetzbuch, LFGB), i.e. they must be safe for their intended use. The sole responsibility for this lies with the distributor. More specific legal regulations can be found in the appendix.

The EU Standing Committee on the Food Chain and Animal Health (SANCO, 2006) has made a proposal for the categorisation of different cultural applications. According to this proposal, classic cultures for achieving the distinctive nature of a (fermented) food that are used before or during the process, should not be considered as ‘additives’. The same also applies to cultures that are added without a technological purpose, such as probiotics. However, cultures intended to achieve a technological effect (e.g. preservation), should be regarded as additives (with all associated consequences with respect to registration, labelling, etc.).

Thus, protective cultures would also fall under this category. However, according to definition 4 of the present opinion, these are more likely to be attributed rather to the starter cultures (from which they have developed). They have the supplemental use of reducing the potential risk of pathogenic or toxigenic microorganisms.

However, if during culture production, inhibitors are formed in the fermenter in active concentrations and added to the food with the culture organisms, the fermentate is to be considered like an additive. By determining the microbial counts and the inhibitory effect of the culture preparation *per se* (i.e. without the fermentation taking place in the food), a reliable distinction can be made between a ‘protective culture’ and an ‘inhibitory fermentate’. For the use of ‘novel microorganisms’ as protective cultures, such as certain *Pseudomonas* strains that are active against food pathogenic bacteria in sprouts, as well as packaged ready-to-eat fresh salads (Wei et al., 2006, Weiss et al., 2007), a comprehensive safety assessment is necessary.

4. Safety aspects

Food must not endanger the health of the consumer. Responsibility for consumer safety lies with the distributor. One of the factors in determining microbiological safety is long-term experience ('history of safe use³'). Experience going back a long way can be called upon for a variety of spontaneously fermented foods. In such foods, a microbial association will always develop, the composition of which depends on the ecological factors acting on the microorganisms⁴ that are characteristic to the specific food. With the aid of the refined testing methods of today, it is possible to isolate and identify from such microbial associations previously unknown microorganisms that can in part be described as a new species. **Table 3** (Appendix) gives an example from sourdough fermentation. These strains identified as 'new' can actually be viewed as being traditional and not 'novel' in the legal sense.

As long as the history of use shows that foods contain no microorganisms that are hazardous to health, the content of microorganisms in food will generally be tolerated up to the spoilage limit. The same also applies to food produced by spontaneous fermentation in the traditional way. For foods produced with defined cultures however, sufficient long-term experience is not always available. For their assessment it should also be taken into account that consumer exposure to a particular microorganism may be higher compared with spontaneously fermented foods. Nevertheless, individual strains could also easily reach relatively high numbers in spontaneous fermentation. In fact, these are the preferred starting associations for starter culture development.

For the introduction of novel microorganisms (including GMOs), a safety assessment is mandatory. A safety assessment should also be carried out for undefined cultures. It should be noted, however, that the strain composition therein cannot be kept constant and therefore is also not suited for the adequate characterisation of such cultures.

³ SKLM will comment on the concept of the 'history of safe use' in a separate statement.

⁴ Microbial ecology describes the interaction of microorganisms with their environment.

4. 1. Qualified Presumption of Safety (QPS)

At European level, the EFSA has proposed a ‘generic’⁵ approach to the safety assessment of microorganisms (EFSA, 2005a). It relates to microorganisms that are affected by current legislation and therefore require a safety assessment. These include microorganisms that are used in food production and animal feed, those used as pesticides, genetically modified microorganisms as defined in gene technology legislation, as well as microorganisms subject to Regulation (EC) No 258/97 (EU, 1997). It also applies to microorganisms in traditional foods with a history of safe use that allows for a qualified presumption of safety (QPS).

QPS is based on the four pillars of pathogenicity, taxonomy, familiarity and use and includes basic knowledge of the organism (EFSA, 2005b). The confirmation of a QPS status is a case-by-case decision made on the basis of a decision tree (EFSA, 2005b). In the Scientific Committee's view (EFSA, 2007), thorough knowledge of the microorganism is sufficient for awarding the QPS status for numerous species from the groups of lactic acid bacteria and yeast. The QPS status is not a regulatory requirement for microbial cultures used in foods. However, organisms that are not listed in the ‘EFSA's list’ of QPS organisms (EFSA, 2008a), will only be considered safe after a comprehensive assessment. In this respect, the QPS status and practice of safety assessment of cultures used in food technology corresponds in principle to the contents of an earlier SKLM statement (SKLM, 1987).

Lactic acid bacteria in food and human mucous membranes as well as intestinal microorganisms are considered to be safe organisms, i.e. neither pathogenic nor toxigenic. Certain strains of lactobacilli, however, have the potential for forming biogenic amines and are therefore not suitable for use as culture organisms. In a few cases, their contribution to human infections has been documented. In such cases it was shown that the persons affected were usually already suffering from an underlying illness or had an impaired immune system (Klein et al., 1992; Aguirre and Collins, 1993; Gasser, 1994). In such a group of people, even baker's yeast (*Saccharomyces cerevisiae*), or *Lactococcus lactis* occurring in sour milk, as well as in particular the bacteria in the intestinal flora could have caused infections. Streptococci and enterococci (Hancock and Gilmore, 2000), which both also belong to the group of lactic acid bacteria, may contain both obligatory and potentially pathogenic species

⁵ Referring to similar members of the same group.

or strains. The presence of a gene coding for a pathogenicity characteristic is, however, not sufficient to decide whether or not the corresponding organism is considered pathogenic. Such a characteristic (e.g. adherence to the intestinal epithelium, stress tolerance) may even, in the case of probiotics, contribute to the improvement of the hosts health by the microorganism.

Moulds should have no potential for forming mycotoxins or antibiotics. The same applies also to staphylococci in cultures used in the meat fermentation, or for smear cheese (Hammes, 2009). In this genus there is a variety of pathogenicity and toxigenicity properties present, e.g. in *Staphylococcus aureus*, that must be excluded for the organisms in starter cultures.

4. 2. Probiotics

Compared with fermented foods, probiotics are relatively new products used for health maintenance which may not have a therapeutic claim. In people with a critical immune status, probiotics should only be used after careful consideration (see below and WHO recommendations). For instance, strains present in probiotics were also isolated from infected persons. Reviews have been published by Boriello et al. (2003) and Vankerckhoven et al. (2008). The latter publication references the findings from an EU-funded research project entitled *Biosafety Evaluation of Probiotic Lactic Acid Bacteria Used for Human Consumption* (acronym: PROSAFE). These publications can be used to derive recommendations that basically are in agreement with FAO/WHO recommendations (2001):

1. The taxonomic position of the strain must be ascertained with state-of-the-art methods in molecular biology, biochemistry and physiology.
2. Consumers must be monitored closely for unusual effects on health and well-being in connection with their consumption. This especially applies for potentially vulnerable sub-populations (e.g. the elderly, infants, pregnant women and immunocompromised persons).
3. Lactic acid bacteria containing known and confirmed virulence genes, in particular enterococci, should not be used as probiotics.
4. The strains should not contain any transferable gene encoding resistance to antibiotics used therapeutically.

4. 3. Antibiotic resistances

The possibility of the transfer of a resistance-conferring gene from the organisms in cultures used in foods is being given particular attention in light of the increase in pathogens that are resistant to antibiotics (EFSA, 2008b).

Microbiological resistance occurs when a bacterium tolerates higher concentrations of antibiotics than phenotypically related bacteria or wild-type strains, resulting from the acquisition of resistance mechanisms via gene transfer or mutation. Classifications are made by determining the minimum inhibitory concentration (MIC) of a large number of strains of one species and defining so-called ‘epidemiological breakpoints’ (ECOFF values, Vankerckhoven et al. 2008). They are critical for assessing acquired resistance or intrinsic resistance. **Intrinsic resistance** is a constitutive property of a **bacterial species**. Examples of this include the lack of transport through the cytoplasmic membrane, the absence of a target, or accelerated degradation or transport of the antibiotic out of the cell. Such bacteria are clinically resistant to antibiotics (or more precisely: insensitive). Meanwhile, there are ECOFF values for 13 antibiotics in the case of 12 species of lactic acid bacteria encompassing the genera *Lactobacillus*, *Pediococcus* and *Lactococcus* (Klare et al., 2007).

The EFSA’s ‘Panel on Biological Hazards (BIOHAZ)’ has concluded that the fermenting bacteria associated with food, whether resistant to antibiotics or not with the possible exception of enterococci do not pose a clinical problem (EFSA, 2008b). However, they can act as a reservoir for transferable resistance genes. Strains with genes transferable in such a way could disseminate into the food chain and increase the probability of a transfer to food-associated intestinal pathogenic organisms. However, it is difficult to assess whether such an event would have clinical consequences. In a qualitative risk assessment, the extent to which food could serve as a source for bacteria with antimicrobial resistance or resistance genes originating from microorganisms was estimated for human beings. A ranking of identified risks was drawn up and options for reducing exposure named. From the SKLM's point of view, strains with transferable genes encoding resistance to antibiotics with therapeutic use or those inducing cross-resistances should not be used in food, feed or as probiotics. This conclusion appears plausible in view of the lack of verified data, following the precautionary principle for consumer protection.

5. Conclusions and recommendations

According to the SKLM, the use of microbial cultures not only allows the production of quality foods, but also, in particular, an increase in the reproducibility of their manufacturing process and, thereby, also of food safety. These cultures include both defined single-strain and multi-strain cultures and multi-strain mixed cultures, as well as undefined multiple-strain mixed cultures. The risk assessment should be performed by comparing the risks of microbial cultures for food with those risks arising from the use of spontaneous fermentation or ‘in-house’ cultures or in the ‘back shuffling’ process. These traditional methods are considered safe based on a long history of use. Undefined multi-strain mixed cultures that are characterised down to the species level, for which a QPS status was confirmed, can be regarded as safe. Strains of species for which a QPS status cannot be confirmed, e.g. enterococci, lactobacilli of risk group 2 (without a proven history of safe use), staphylococci or moulds, require a comprehensive safety assessment. Protective cultures should be assessed on a case-by-case basis. If the organisms contained therein are also used as starter cultures, based on identical properties they should also be considered starter cultures. Organisms contained within protective cultures that are not used as starter cultures need a comprehensive safety assessment.

The largest possible taxonomic unit should be used for the assessment in each case (e.g. genus > species > strain).

It is the opinion of the SKLM that the microbial ecology, i.e. the association of different microorganisms resulting from environmental factors and metabolic properties, should be used as the basis for assessing the novelty of newly-described organisms. The microbial ecology is determined by the fermentation technology and the specific raw materials. The use of this approach may reveal that strains identified as ‘novel’ may as well be considered ‘traditional’.

In addition to the WHO/EFSA recommendations discussed, the SKLM recommends that the moulds used in food fermentation should not have the potential to form antibiotics. Also recommended is the use of the QPS status proposed by the EFSA as a tool in the safety assessment of microbial cultures whose approval is not subject to any formal legal regulation. For the most important group - the lactic acid bacteria with QPS status - the assessment may

then be restricted to a small number of properties, such as ensuring the absence of the potential to form physiologically active biogenic amines, or of transferable antibiotic resistance.

6. Research needs

There is a need for further research in terms of an adequate safety assessment of currently undefined cultures, with the aim of clarifying whether or not such cultures can be adequately characterised through a description at the level of species, or even the genus alone. Although they may be regarded as safe based on their history of safe use, only a limited assessment of e.g. the potential to form biogenic amines or the presence of transferable antibiotic resistance is possible in such a complex mixture of 'QPS-organisms'.

In the context of research into resistance, more attention should be given to the aim of collecting data allowing a scientifically sound quantitative risk assessment. A major challenge here is determining the presence of transferable resistance, since the transferability is a quantitative event and non-transferable genes are difficult to define in bacteria. Moreover, the significance of such an event for humans after consumption of fermented food products has practically not been investigated up to now. This is all the more valid if **one** resistant strain is involved among many others.

Further research beyond the QPS concept is needed with respect to defining pathogenicity characteristics in microbial cultures. The regulation and interaction of such characteristics in culture preparation and food processes - but also with regard to relations between microorganisms and humans - should therefore be thoroughly investigated and understood.

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APPENDIX

Table 1 Examples of fermented foods in the European market

<i>Raw material</i>	<i>Product</i>	<i>Microorganism</i>
Olives, cabbage, cucumbers, tomatoes	Fermented olives, sauerkraut, pickles	Lactactic acid bacteria (LAB)
Dough and pastes from cereals	Sourdough, yeast dough, Kisra	LAB , yeasts
Malt, Koji, made from cereals	Beer, sake, spirits	LAB , yeasts, moulds
Beer, wines and spirits	Vinegar	Acetic acid bacteria
Grapes and other fruits	Wine	Yeasts, LAB
Soya, Carob	Soy sauce, tempeh, natto, Dawadawa	LAB, <i>Bacillus spp.</i> , moulds, yeasts
Milk	Sour milk products: Sour milk, sour cream, yogurt, kefir, kumis	LAB, yeasts Acetic acid bacteria
	Sour cream butter	LAB
	Cheese	LAB, yeasts, moulds, propionic acid bacteria
Meat	Fermented sausages	LAB, yeasts, moulds, staphylococci Micrococci, <i>Streptomyces</i>
	Ham	LAB, yeasts, moulds, staphylococci
Fish	Fish sauce, fermented fish	Staphylococci, <i>Vibrio costicola</i> , LAB

Table 2: : Effect of fermentation organisms on food

desired effect	Group of organisms	Food
Nutritional quality	Lactic acid bacteria (LAB) Yeast	Improving digestibility, breakdown of anti-nutritional agents in cereals, pulses, vegetables Vitamin enhancement
Taste	LAB Acetic acid bacteria	fermented milk products, bread, pickles, olives, fermented sausage, wine, beer vinegar
Aroma	LAB Propionic acid bacteria, staphylococci, yeast, Brevi bacteria, Arthrobacter spp. staphylococci, Kocuria ssp, Yeast moulds	As for taste Hard and semi-hard cheese Red smear cheese Fermented sausage, fish sauce, Bread, fermented milk products, fermented sausage, cheese, fermented sausage, soya sauce
Texture/consistency/gas formation	LAB Yeast moulds, staphylococci, Brevi bacteria, Arthrobacter spp. propionic acid bacteria	Fermented milk products, cheese, fermented sausages, pickles, bread, beer, sparkling wine, kefir, bakery products Cheese Hard/semi-hard cheese
Colour	LAB, Kocuria spp., staphylococci, Brevibacteria	fermented sausages Red smear cheese
Shelf life	LAB Yeasts, Zymomonas, propionic acid bacteria, acetic acid bacteria	All lactic fermented foods Alcoholic beverages Cheese Vinegar

Table 3: Microbial associations in continuously propagated sourdoughs

Lactic acid bacteria	Yeasts
<p><i>L. acidifarinae</i>, <i>L. alimentarius</i>, <i>L. amylophilus</i>, <i>L. brevis</i>, <i>L. crustorum</i>, <i>L. fructivorans</i>, <i>L. hammesii</i>, <i>L. mindensis</i>, <i>L. namurensis</i>, <i>L. nantensis</i>, <i>L. nodensis</i> <i>L. paralimentarius</i>, <i>L. pentosus</i>, <i>L. plantarum</i>, <i>L. pontis</i>, <i>L. reuteri</i>, <i>L. rossiae</i> <i>Lactobacillus sanfranciscensis</i>, <i>L. secaliphilus</i>, <i>L. siliginsis</i>, <i>L. spicheri</i>, , <i>L. zymae</i>, <i>Leuconostoc mesenteroides</i>, <i>Weissella confusa.</i>, <i>W. cibaria</i> <i>Pediococcus spp</i></p>	<p><i>Canida humilis</i> syn. <i>C. milleri</i>, <i>S. cerevisiae</i>, <i>Kasachstania exiguus</i> syn. <i>Saccharomyces exiguus</i> syn. <i>S. minor</i>, anamorph <i>Torulopsis holmii</i> syn. <i>C. holmii</i>, <i>Pichia kudriavzevii</i> syn. <i>Issatchenkia orientalis</i>, anamorph <i>C. krusei</i>, <i>C. boidii</i>, <i>Torulasporia delbruecki</i>,</p>

Bold: newly-described species after 2000

Appendix: Additional legal regulations

More specifically, EU Guideline 95/2/EC, Appendix IV states that non-pathogenic L(+)-lactic acid producing cultures may be used for the manufacture of acidified milks in infant formulae and follow-on formulae, in other word, only the lactic acid configuration is important. Further legal regulations are:

a) the use of lactic bacteria in wine: Regulation (EC) No. 1622/2000 (Appendix VIII) allows the use of species of genera *Leuconostoc*, *Lactobacillus* und *Pediococcus*, for the biological degradation of malic acid when these were isolated from grapes, grapes, must, wine or products made from grapes,

b) the use of microbial preparations in organic products (Regulation (EC) No. 834/2007); and

c) the use of microorganisms in use after 1997 (when the Novel Food Regulation (EC) 258/97 came into effect), which were not consumed in significant amounts in the EU prior to this date. For genetically-modified microorganisms (GMO), there has been a modification to the original authorisation; according to this regulation they are now subject to Regulations (EC) 1829/2003 and (EC) 1830/2003. Up to now, no application has been made concerning an authorisation of a GMO strain for food cultures.