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Biopreservatives

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Abstract Food producers of today are met with inherently contradictory demands as seen from a microbiological point of view: producing foods that are less stable (due to nutritional and taste requirements) by processes that confer less control of the detrimental microflora (due to trends of convenience, minimal processing, and reducing or removing additives including preservatives). How should food producers manage to develop such products with a sufficiently long shelf-life and at a competitive price? Some of the most promising tools to this end are the so-called biopreservatives, which are various types of products derived from lactic acid bacteria and other suitable microorganisms, namely bacteriocins and other antimicrobials, fermentates, bioprotective cultures, and bacteriophages. This chapter provides an overview of the scientific background and functionality, as well as food applications and further commercial aspects of each of these categories of biopreservatives.

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1 Introduction

The exploitation of biopreservation is by no means a new concept. Biotechnological processes for preserving food have already been used for thousands of years, even though the underlying mechanisms were not understood. Today, biopreservation of foods is as relevant as ever before because it is one of the few possible answers to what at first glance appears to be totally contradictory trends and demands:

- Health trends: The levels of salt, sugar and fat in foods are under pressure to be reduced. These changes are beneficial for human health, but they also all confer an increase in water activity, which provides a friendlier environment for microorganisms.
- Taste preferences: In many products, trends are towards a milder (i.e. less acidic) taste, which results in a higher pH that again is less adverse for microorganisms.
- Perception of "natural": This results in milder or minimal processing, which results in a fresher appearance of the food but also less inactivation of unwanted microorganisms. Furthermore, it increases the demand for "preservative-free" products.
- Convenience trends ("practically homemade"): There are two main risks associated with this trend—namely, more extensive processing, which results in more steps in which contamination with detrimental microorganisms can occur, and the need for proper handling by the consumer (e.g. sufficient heating), which may be neglected.
- Durability and open shelf-life: Market access and economically viable logistics require a long shelf-life. Furthermore, a sufficient open shelf-life is required to ensure customer loyalty.
- Ethical issues: Concerns such as corporate social responsibility, carbon dioxide (CO_2) footprint, and fair-trade and organic products put restrictions on which solutions a food producer can employ.

All in all, these trends lead to food formulations that provide better growth conditions for microorganisms, milder processing that results in less initial reduction, more processing steps that increase the risk of contamination, a need for longer shelf-life, and pressure to reduce food waste. In addition, many of the conventional preservatives are deemed to be unacceptable by trendsetters and consumers. Everyone wants preservative-free food, but most will agree that we cannot maintain our present society and standard of living—and certainly cannot reduce the global food waste problems—with food that is not preserved.

There is thus a strong market need for natural food protection solutions that can ensure both food safety (i.e. reduce the number and/or outgrowth of pathogenic microorganisms) and food shelf-life (i.e. delayed development of the spoilage microflora). One of the few possible solutions is biopreservation based on the concept of using food-grade microorganisms as so-called cell factories (Fig. 1).

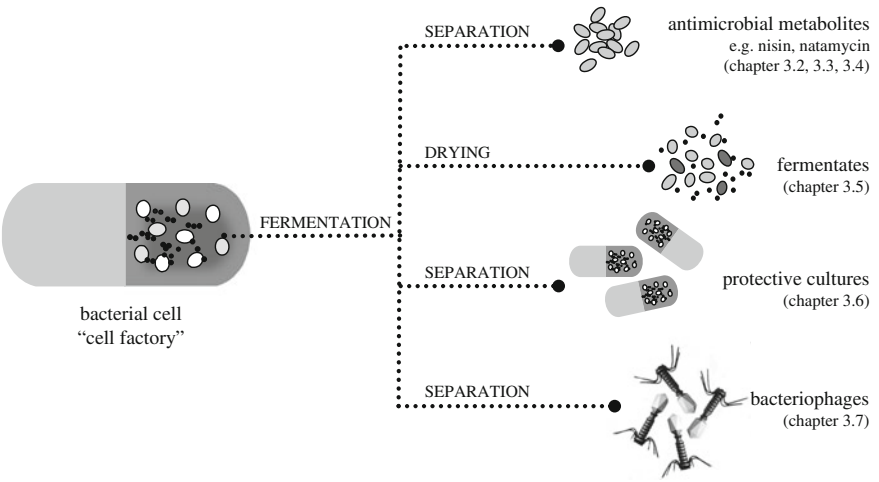

Fig. 1 Overview of the main categories of biopreservatives that can be produced by using lactic acid bacteria and other suitable microorganisms as "cell factories"

Food-grade microorganisms can form a multitude of different substances that are inhibitory to other microorganisms. These mechanisms are part of the natural balance in complex microbial ecosystems. By exploiting the fittest of the naturally occurring microorganisms in organoleptically appealing food products, it is conceivable to design preservation systems that ensure an adequate safety and shelf-life while maintaining the desired quality of the food product.

The biopreservation principles from food-grade microorganisms can be categorized according to the antimicrobial compound (e.g. bacteriocin, other metabolites, bacteriophages, enzymes) as well as product format (purified antimicrobial, fermentate, protective culture), as illustrated in Fig. 1. Not all microbial inhibitory mechanisms are fully understood, and not all antimicrobial metabolites from food-grade microorganisms have yet been discovered. It is highly likely that the near future will bring new understanding and discoveries, which will further expand the options for natural food biopreservation systems.

When investigating biopreservation systems, one should not search for "the silver bullet". As described by Roller [76]: "Antimicrobial compounds in nature rarely function in isolation; combination systems such as those found in the hen's egg are far more common." This principle is central for developing sound biopreservation solutions. Targeted intelligent strategies based on multifactorial systems are the most likely to succeed for protecting food against detrimental microorganisms.

This chapter gives an overview of the current knowledge on biopreservative compounds and concepts, covering solutions that are actually being used industrially today, and points out perceivable directions for future solution development.

2 Nisin

Nisin is a cationic, amphiphilic peptide produced by various strains of *Lactococcus lactis*, which has a relatively broad target spectrum that inhibits a wide range of gram-positive bacteria. The antimicrobial property of nisin was first observed in 1928, when it was reported that inhibition of a dairy starter culture was caused not by phages but by a strain of *L. lactis* (formerly called lactic streptococci and group N streptococci) [75]. The inhibitory compound was further studied the following years and given the name nisin, alluding to its origin as a "group **N** streptococci **I**nhibitory **S**ubstance" [65]. The application of nisin for preservation of dairy products was suggested already in 1951 for inhibiting blowing of Swiss-type cheese [49]. Soon after, the first commercial preparation was made by Aplin and Barret in 1953. The use of nisin as a food preservative was approved by the Food and Agriculture Organization of the United Nations and World Health Organization (WHO) in 1969, by the European Union (EU) in 1983 (E 234), and granted Generally Recognized As Safe (GRAS) status by the U.S. Food and Drug Administration (FDA) in 1988.

Thus, nisin has a long history of safe use in food. It is the only purified bacteriocin that is widely approved as food additive—a fact which presumably also reflects its early discovery. Through the years, a substantial number of scientific papers have described the biosynthesis, chemical and physical properties, mode of action, and practical applications of nisin, and furthermore the accrued knowledge has been extensively reviewed. (For comprehensive reviews, see [13, 25, 87, 89]. A short summary is given below, with focus on aspects that affect industrial applications.

Nisin belongs to the lanthionine-containing bacteriocins, which are designated as class I bacteriocins (Table 1). Production of bacteriocins containing the unusual lanthionine residues, which are formed by posttranslational modifications, is not uncommon amongst lactic acid bacteria; linear, globular, and two-peptide variants have been characterized. Many of these peptides are effective at low concentrations against a wide range of gram-positive bacteria, which has been attributed to a common mode of action: nisin and other lantibiotics bind with high affinity to a docking molecule in the cell envelope of target bacteria, lipid II, an intermediary molecule for building bacterial cell walls [52]. Nisin is a linear lantibiotic that exerts its antibacterial action through inhibiting cell wall formation as well as forming membrane pores; it is furthermore active against spores. Several variants of nisin occur naturally; the two that are currently available as commercial products, nisin A and nisin Z, differ in one amino acid, which confers a difference in charge and solubility.

Lipid II is an essential and highly conserved molecule, providing the broad target spectrum of lantibiotics against gram-positive bacteria. However, in gram-negative bacteria, lipid II is protected under the outer membrane. These organisms are therefore only sensitive to lantibiotics in cases where their outer membrane has been disrupted. The producer organisms, being gram-positive bacteria themselves,

Table 1 Classification of bacteriocins produced by lactic acid bacteria as suggested by Cotter et al. [13]

Classification	Remarks/suggestions	Examples
Class I		
Lanthionine-containing bacteriocins/ lantibiotics	Includes both single- and two-peptide lantibiotics; up to 11 subclasses have been proposed	Single-peptide: nisin, mersacidin, lacticin 481 Two-peptide: lacticin 3147, cytolysin
Class II		
Non-lanthionine-containing bacteriocins	Heterogeneous class of small peptides; includes pediocin-like (subclass a bacteriocins), two-peptide (subclass b bacteriocins), cyclic (subclass c; formerly class V), non-pediocin single linear peptides (subclass d)	Class IIa: pediocin PA1, leucocin A Class IIb: lactacin F Class IIc: enterocin AS48, reuterin 6 Class IId: lactococcin A, divergicin A
Bacteriolysins (Suggested that these are no longer considered bacteriocins)		
Non-bacteriocin lytic proteins	Large, heat-labile proteins, often murein hydrolases	Lysostaphin, enterolysin A

are protected by a dedicated immunity system that is encoded in conjunction with the biosynthesis genes [2].

A general concern with the prolonged use of an antimicrobial is the potential development of resistance in the target microorganisms. A moderate reduction in nisin sensitivity due to various modifications in the cell envelope has been described for laboratory-acquired mutants [55]; a prevalent mechanism involved enhanced expression of a penicillin-binding protein [41], which could reduce the accessibility or the affinity of nisin to lipid II. However, high level or complete resistance to nisin has not been observed, presumably due to the essential nature of the docking molecule. Transfer of the immunity system from producer to target organisms has similarly not been reported.

The tested and actual applications of nisin are numerous. Initially, nisin was used in conjunction with heat treatment to prevent spoilage of processed cheese by heat-resistant spores. Since then, effective use of nisin has been demonstrated both for shelf-life and safety purposes in various types of food, including dairy products and processed meats and vegetables [34, 81]. Nisin is particularly effective in heat-treated low pH products.

Technical limitations to be aware of relate to the characteristics of the nisin molecule as well as the mode of action. Nisin is sensitive to degradation by peptidolytic enzymes (e.g. in raw meat) and can be sequestered in food matrices (e.g. in the fat phase). In addition, nisin is relatively heat-stable at low pH but not at neutral or higher pH. Furthermore, if used in fermented products, nisin will inhibit gram-positive starter cultures.

The efficacy and application range of nisin, like any other antimicrobial, can be expanded by use in a multifactorial system. Nisin can be protected from peptidolytic enzymes or sequestering by incorporation in liposomes [63] or

incorporation in edible coatings or films [11]. Efficacy and target range can be enhanced by combination with plant extracts or essential oils or with physical treatments, such as high hydrostatic pressure [48, 91].

As mentioned previously, nisin was first approved for food applications in 1969. The initial approvals were based on toxicity testing results from 1962 [33, 46]. Recently, two independent studies have shown that even a very high daily intake was not toxic [44, 45]. In the EU, nisin is currently approved as additive in ripened and processed cheese, clotted cream, puddings such as semolina or tapioca, mascarpone, and pasteurized liquid egg. In the United States and Australia/New Zealand, further approvals have been granted, such as for use in sauces, soups, salads, dressings, and ready-to-eat and processed meat products.

Nisin-containing products on the market are manufactured by a batch fermentation process followed by concentration, drying, milling, and standardization [21]. For many years, Nisaplin, which contains 2.5 % nisin A and is standardized with salt, was the main product on the market (developed by Aplin and Barrett, now DuPont). In recent years, other producers have emerged, and both nisin A and nisin Z are now commercially available. However, toxicology studies were performed with nisin A.

In addition to the products consisting of standardized nisin A or Z, some combination products are also available, such as those combined with rosemary extract. Furthermore, nisin-producing cultures are available (see Sect. 5).

3 Natamycin

Natamycin was discovered in the 1950s. As described by Struyk et al. [84], "A new crystalline antibiotic, pimaricin, has been isolated from fermentation broth of a culture of a *Streptomyces* species, isolated from a soil sample obtained near Pietermaritzburg, State of Natal, Union of South Africa. This organism has been named *Strepyomyces natalensis*". The original name "pimaracin" can be found in earlier publications but it is no longer accepted by the WHO [24]. Natamycin is classified as a macrolide polyene antifungal and is characterized by a macrocyclic lactone-ring with a number of conjugated carbon–carbon double bonds (Fig. 2). The full chemical name is 22-(3-amino-3,6-dideoxy-β-D-manno pyranosol) oxy-1,3,26 trihydroxy-12-methyl-10-oxo-6,11,28-trioxiatri [22.3.1.05.7] o catosa-8,14,16,18,20-pentanene-25-carboxylic acid.

Natamycin has a low solubility in water (approximately 40 ppm), but the activity of neutral aqueous suspensions is very stable. Natamycin is stable to heat and it is reported that heating processes for several hours at 100 °C lead to only slight activity losses. Natamycin is active against almost all foodborne yeasts and molds but has no effect on bacteria or viruses. The sensitivity to natamycin in vitro (minimal inhibitory concentration) is in most cases below 20 ppm (see Table 2).

Natamycin acts by binding irreversibly with ergosterol and other sterols, which are present in the cell membranes of yeasts and vegetative mycelium of molds. It

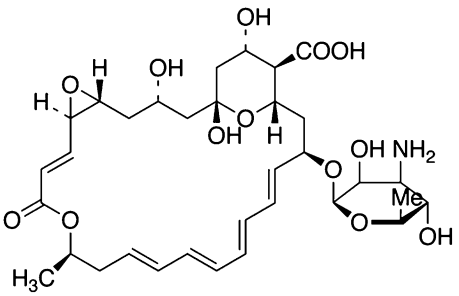

Fig. 2 The chemical structure of natamycin

Table 2 Sensitivity to natamycin of fungi occurring on sausages (Adapted from [83]

Molds and yeasts	Minimal inhibitory concentration (ppm)	Source of microorganisms
Aspergillus flavus	10–20	Air
Aspergillus niger	<5	Fruit
Cladosporium cladosporioides	<5	Meat stamp
Eurotium appendiculatum	<5	Smoked sausage
Mucor racemosus	<5	Sausage
Penicillium chrysogenum	<5	Meat
Penicillium nalgiovense	<5	Sausage
Rhizopus stolonifer	5–10	Bread
Candida zeylandoides	<5	Sausage
Debaryomyces hansenii	<5	Sausage
Rhodutorula mucilaginosa	<5	Air
Trichosporon pullulans	<5	Frozen beef

disrupts the cell membrane and increases the cell permeability, which finally leads to cell death. The fungicidal of natamycin is an "all-or-none" effect, which destroys the cell membrane of the target cells [57].

Due its interaction with ergosterol, which is a major constituent of fungal cells, it is unlikely that fungi will develop resistance. So far, after many decades of use, no development of resistance has been reported. Natamycin is mostly used for surface applications, particularly for treating surfaces of hard cheese and salami-type sausages. One of the advantages over sorbate is that even the dissolved fraction of natamycin hardly migrates into the food matrix. As shown in Table 3, natamycin can be applied by spraying the surface (e.g. of cheese), by dipping, by applying natamycin via coating emulsions or by direct addition.

The antifungal efficacy of natamycin has been extensively studied and a substantial amount of scientific papers have been published. Comprehensive overview articles are available [20, 24, 83]. However, due to its long history of use, no data on application studies have been published recently.

Natamycin does not have acute toxicity. In animal studies, the lowest median lethal dose found was 450 mg/kg. The long history of safe use in food products

Table 3 Applications of natamycin, with suggested dosage levels and methods of application

Food application	Suggested natamycin dosage levels (ppm)	Method
Hard/semihard cheese	1,250–2,000	Surface treatment by spray or immersion
	500	Direct addition to coating emulsion
Meat products: dry sausage	1,250–2,000	Surface treatment by spray or immersion
Yogurt	5–10	Direct addition to yogurt mix
Bakery products	1,250–2,000	Surface treatment by spray
Tomato purée/paste	7.5	Direct addition during mixing
Fruit juice	2.5–10	Direct addition
Wine	30–40	Direct addition to stop fermentation
	3–10	Added after bottling to prevent yeast/mold growth

Source [88]

confirms that natamycin is a safe antifungal preservative. As acceptable daily intake, 0.3 mg/kg body weight/day was suggested Smith and Moss [82]. In the scientific opinion on the use of natamycin (E 235) as a food additive from the European Food Safety Authority [26], "The Panel considered that the proposed use of levels of natamycin are not of safety concern if it is only used for the surface treatment of the rind of semi-hard and semi-soft cheese and on casings of certain sausages. The Panel concluded that there was no concern for the induction of antimicrobial resistance."

Natamycin is allowed as antifungal preservative in many countries, but details on authorization vary from country to country. In the European Union, natamycin is permitted for the surface treatment of hard, semihard, and semisoft cheese and dried cured sausages. According the EU Directive 1333/2008, the maximum permitted level is 1 mg/dm^2 surface.

Commercial preparations are produced by fermentation of sugar-based substrates by *Streptomyces natalensis*. Natamycin is then recovered by extraction, filtration, and spray drying. The dried powder can be stored for years without any activity loss.

4 Other Bacteriocins

Microorganisms produce a diverse range of microbial defense molecules, including the classical antibiotics, numerous types of protein exotoxins, lytic agents, metabolic byproducts, and bacteriocins [74]. The latter group has received particular attention due to a perceived high potential for exploitation for food preservation [13].

Bacteriocins are ribosomally synthesized, extracellularly released antimicrobial peptides that have a bacteriocidal or bacteriostatic effect on other microorganisms.

Bacteriocin production is a common feature of food-grade lactic acid bacteria (LAB). The first discovered bacteriocin was nisin, as described previously, and it was subsequently estimated that up to 99 % of all bacteria produce at least one bacteriocin [56]. Since then, the variety of both bacteriocin-producing LAB and bacteriocin molecules has proven to be very diverse. It has thus become evident that the natural presence of bacteriocins in microbial ecosystems (e.g. fermented foods) is quite common.

Parallel to the discovery of new bacteriocins, schemes for classifying the molecules have been proposed and modified. Table 1 shows the modification of Klaenhammer's original classification [56], which was suggested by Cotter et al. [14]. However, it is likely that the classification will still be modified as more knowledge on the LAB bacteriocins emerges (e.g. [47]. Class I, the lantibiotics (including nisin), and class II, the unmodified bacteriocins (including the class IIa pediocin-like antilisterial bacteriocins), constitute the most abundant, the best characterized, and presumably also the most useful of the food-grade bacteriocins.

Food-relevant class I bacteriocins include the lactococcal lantibiotics, lacticin 3147 and lacticin 481, which have shown good efficacy for both shelf-life and food safety purposes, particularly in dairy products [43]. The lantibiotics generally have a wide target range conferred by their interaction with lipid II, similarly to nisin, as described in detail in Sect. 2.

The class IIa bacteriocins are small, heat-stable unmodified peptides with a conserved YGNGV motif in their N-terminal domain. They are often referred to as pediocin-like, reflecting that some of the first characterized IIa bacteriocins were produced by *Pediococcus* [70]. It is now apparent that this is a really widespread type of family of peptides [14]. As listed in Table 4, IIA bacteriocins are produced by a variety of LAB in addition to *Pediococcus* including *Lactobacillus*, *Enterococcus*, *Carnobacterium*, *Leuconostoc*, *Streptococcus* and *Weissella*. The IIa-producing LAB have been isolated from various dairy, fermented sausage, and vegetable products and also from the mammalian gastrointestinal tract. Furthermore, the non-LAB species *Bifidobacterium bifidum*, *Bifidobacterium infantis*, *Bacillus coagulans* and *Listeria innocua* have also been reported to produce IIa bacteriocins.

In contrast to nisin, the class IIa bacteriocins have a relatively narrow target spectrum. They are generally active against *Listeria* species, and against some species of *Clostridium, Enterococcus, Carnobacterium*, *Lactobacillus, Pediococcus* and occasionally *Streptococcus* and *Leuconostoc*. These bacteriocins have thus primarily been tested in foods for their antilisterial properties. The class IIa specificity reflects their mode-of-action: the IIa bacteriocins bind to a target molecule, a sugar uptake system called the mannose phosphotransferase system (man-PTS), and subsequently form membrane pores and kill the sensitive cells. The man-PTS sequence is not completely conserved, and the variation in man-PTS sequences are reflected in varying sensitivities to IIa bacteriocins, from highly sensitive to completely insensitive [14]. Furthermore, elimination of the man-PTS expression can confer high-level resistance to class IIa bacteriocins in otherwise sensitive organisms [55], and this appears as a general mechanism for spontaneous IIa resistance

Table 4 Examples of class IIa bacteriocins

Bacteriocin[a]	Producing species and strains	Source
Pediocin AcH	*Lactobacillus plantarum* WHE92	Muenster cheese
	Pediococcus acidilactici H	Fermented meat
Pediocin PA-1+	*Pediococcus acidilactici* Pac 1.0	Culture collection
	Pediococcus parvulus AOT 77	Vegetables
Enterocin A	*Enterococcus faecium* CTC 492	Fermented sausage
Divercin 41	*Carnobacterium divergens* V41	Fish viscera
Leucocin A	*Leuconostoc gelidum* UAL 187	Processed meat
Sakacin A	*Lactobacillus sakei* Lb 706	Raw meat
Piscicocin V16	*Carnobacterium piscicola* V1	Fish
Mundticin ATO6	*Enterococcus mundtii* ATO6	Vegetables
Bavaricin MN	*Lactobacillus sakei* MN	Meat

[a] Several of the published class IIa bacteriocins have the same amino acid sequence, but were originally named differently
Source [73]

development in *Listeria monocytogenes* [40]. The risk arising from possible resistance development can best be circumvented by including class IIa bacteriocins, and in fact bacteriocins in general, by multifactorial approaches in which the bacteriocins are combined with plant extracts or suitable processes [48, 91].

In addition to the well-characterized bacteriocins of class I and IIa, two more recently discovered groups of peptides could potentially provide new opportunities for food applications. One group, which appears to be a subclass of class II peptides, was reported to have the unusual property for LAB bacteriocins of high efficacy against gram-negative bacteria, including *Campylobacter* [85]. However, the potential for commercial exploitation of these compounds for food preservation remains to be realized. The second group constitutes bacteriocins produced by propionic acid bacteria (PAB). The PAB peptides characterized so far display some unusual properties. However, they have a relatively narrow target spectrum with activity against other PAB and, in some cases, certain lactobacilli [30], which may limit their usefulness for biopreservation. The PAB bacteriocins are not yet as well characterized as their LAB counterparts, and it is conceivable that future investigations on PAB bacteriocins may disclose new opportunities for food applications.

In summary, the number of known LAB and PAB bacteriocins and the number of publications reporting their potential use for food preservation is steadily increasing [6, 22, 36, 43]. However, so far the industrial options for use in food production have not developed accordingly. As described in Fig. 1, there are in principle three different possible formats for applying bacteriocins in food: as a concentrated antimicrobial, as a fermentate, or as a live culture that produces the bacteriocins in the food product. With the fermentation technologies of today, supplying such products at an economically feasible cost does not represent a hurdle. However, the regulatory situation and the general perception have a strong impact on which solution may be viable in different regions. Promotion of other

bacteriocins as antimicrobial additives similarly to nisin is unlikely, partly because of the high investment needed today for approval and partly because approval as an additive would in itself defeat the purpose: the main driver for the use of bacteriocins is the demand for "preservative-free" foods (i.e. to provide a natural alternative to chemical preservatives). If a bacteriocin was approved, it would no longer be perceived as natural as it would have become an "E-number". Therefore, the potential industrial food applications of bacteriocins constitute the fermentates and the cultures; these are described in detail in Sects. 5 and 6, respectively.

5 Fermented Food Ingredients: The Fermentates

As the name indicates, fermentates are fermented food ingredients. These products may be produced from a variety of raw materials (typically milk, sugar, or plant-derived material), and the fermentation is done using food-grade microorganisms such as lactic acid bacteria (LAB) or propionic acid bacteria (PAB). The fermentation is designed to provide a high yield of antimicrobial metabolites, which may comprise organic acids (lactic, acetic or propionic acid), diacetyl, bacteriocins, and other secondary metabolites, depending on the properties of the strain(s) used for the fermentation. Fermentates are thus complex products that inherently do not have a well-defined composition. Fermentates are usually supplied as a dry, cell-free powder.

The currently commercially available fermentates for use in foods are the MicroGARD range (DuPont), the DuraFresh range (Kerry), which includes the former Alta and Perlac products from Quest, and various other products that are promoted as shelf-life extenders—namely spray-dried vinegar or fermented wheat flour products. There are only limited scientific reports available on the functionality of fermentates in foods. The original Alta and Perlac were whey-based products for use as shelf-life extenders. The initial MicroGARD products, which were produced by fermenting skimmed milk or dextrose with *Propionibacterium shermanii* or specific lactococci, were demonstrated to inhibit the psychotropic spoilage flora and thereby enhance the shelf-life of cottage cheese [1]. Inhibition of *Pseudomonas*, *Salmonella*, *Yersinia,* and certain fungi was shown.

Conversely, the MicroGARD and Alta products had no significant effect on aerobic mesophilic counts, *Escherichia coli* or *Brochothrix thermosphacta* when tested in an acidified chicken meat model stored at 22 °C [58]. In hamburgers, addition of 1 % MicroGARD provided some initial reduction of *E.* coli O157:H7 and a bacteriostatic effect against *L. monocytogenes* during refrigerated storage [19]. In fresh salmon stored at 6 °C, a combination of nisin and MicroGARD reduced the total aerobic count by 2 log, which provided 3–4 days prolongation of shelf-life and furthermore reduced the outgrowth of *L. monocytogenes* [94]. Similarly, certain combinations of MicroGARD and nisin provided an adequate control of *Listeria innocua* in liquid cheese whey [92]. The anti-listerial effect of

nisin in seasoned salmon roe was furthermore enhanced by combining with a *Pediococcus pentosaceus* fermentate and pectin [5].

Even though the scientific documentation for the fermentates is much sparser than for the bacteriocins, the industrial applications are much wider. The main markets for fermentates are in the United States, where such products are labeled as "cultured milk" or "cultured sugar", for example, according to the substrate used for producing the fermentate. Toxicity tests have been performed for a cultured dextrose version of MicroGARD and no detrimental effects were observed [9]. The MicroGARD products are used for a wide range of food applications including cottage cheese, yogurt, sour cream, dairy desserts, sauces, dressings, pasta, baked goods, and prepared meals. An estimated 30 % of the US cheese production is made with MicroGARD [78]. Durafresh was approved by the FDA in 2011 for use in cottage cheese to control *Listeria*, being labeled as "cultured grade A skim milk and skim milk powder".

Labeling as an undefined cultured raw material is not an option in the EU. In the EU, it would be required to label all active components in the fermentate, which presents two main problems: not all active components are known, and most of the known ones have E-numbers. Therefore, the use of fermentates as natural preservatives is so far quite limited in the EU.

6 Bioprotective Cultures

Food fermentation using microbial starter cultures is one of the oldest known uses of biotechnology. Fermentation of perishable food raw materials to provide more stable products has been used by man since approximately 10,000 BC [72]. Fermented food and beverages are still today an important part of the human diet and constitute an estimated 20–40 % of the global food supply [67].

The raw materials used for producing fermented foods are very diverse, covering the range from milk, meat, fish, vegetables, fruits, cereals, and honey. The main desired functionalities provided by the fermentation processes comprise the following: (1) enhanced durability through formation of antimicrobial metabolites (e.g. organic acids, bacteriocins, ethanol), often in conjunction with decreased water activity (drying and/or salting); (2) enhanced safety by reducing the level of either pathogenic microorganisms or their toxins at the time of consumption; (3) enhanced nutritional value; and (4) enhanced organoleptic quality [7].

In addition, there are various detrimental properties that are evidently unwanted and unacceptable in food cultures, including virulence, toxicity, and antibiotic resistance. In the US, acceptable food microorganisms are granted the GRAS status, and in EU they are included in the Qualified Presumption of Safety list. An inventory list of currently used microbial food cultures, comprising 195 bacterial species and 69 fungal species, has recently been compiled [7].

Microbial cultures used in food production are often referred to as either starter cultures (providing nutritional and organoleptic characteristics) or protective

cultures (providing durability and safety). However, these properties are inherently linked, such that durability is enhanced by formation of organic acids, which also contribute to the characteristic taste and texture of many fermented foods. All starter cultures are per se also protective cultures, but not all protective cultures are also starter cultures. A clear distinction between starter cultures and bioprotective cultures is therefore neither possible nor meaningful.

In the last two or three decades, substantial research activities have aimed to develop cultures that (1) enhance food safety by directly killing or inhibiting the outgrowth of pathogenic bacteria or by suppressing toxin formation or (2) improve durability by reducing or inhibiting growth of spoilage microorganisms. The resulting scientific papers have been summarized in comprehensive reviews, including solutions for fish and seafood [10, 59], dairy products [6, 39], and antifungal cultures in general (Dalié et al. [17, 18]. Overall, most of the reports can be allocated to one of the following main categories: (1) use of bacteriocin-producing LAB cultures to control *L. monocytogenes* in various ready-to-eat foods, (2) use of antifungal LAB and/or PAB to delay spoilage of various types of food, or (3) use of nonbacteriocinogenic LAB with other competitive properties.

The mode of action of the bacteriocinogenic antilisterial cultures relies on the production of class I or IIa bacteriocins, as described in Sect. 4, and is relatively well characterized. The antifungal cultures, on the other hand, have been discovered more recently, and scientific evidence has been gathered during the last 10–15 years (Dalié et al. [17, 18, 27, 50, 77]. The antifungal cultures have been reported to produce a variety of different metabolites, and the current understanding indicates that they work by a complex antifungal mechanism obtained by the combined effects of the described and also as yet not elucidated metabolites [60, 79]. Finally, nonbacteriogenic cultures with antibacterial properties have been reported; these seemingly rely on a variety of competitive advantages over the unwanted microbiota.

In the following lists, recent examples of application studies within each category for various food segments are provided.

Anti-listerial bacteriocinogenic LAB cultures:

- In meat products: *Lactobacillus sakei* together with 50 % CO_2 prevented outgrowth of *L. monocytogenes* in bologna-type sausage without an unacceptable pH drop [54]. *Pediococcus acidilactici* was efficient in reducing *L. monocytogenes* in dry-fermented Spanish sausages [68]. *L. sakeii* prevented listerial growth in a pork meat system while enhancing protein hydrolysis [12].
- In fish and seafood: Listerial control was achieved in cold-smoked salmon using *Carnobacterium divergens* [86] or *Lb. sakei* [93].
- In dairy products: *Lactococcus lactis* used as starter culture in cottage cheese [15] or *Enterococcus faecium* in smear of soft cheese [53] controlled outgrowth of *L. monocytogenes.*
- In vegetable products: *Leuconostoc mesenteroides* was used for reduction of *L. monocytogenes* in apples and iceberg lettuce [90].

Antifungal LAB and/or PAB cultures:

- In bakery products: *Lactobacillus plantarum* was used for delaying *Penicillium* spoilage of bread [16, 37].
- In dairy products: *Lactobacillus rhamnosus* and *Propionibacterium freudenreichii* ssp. *shermanii* were used for inhibiting yeast in yogurt [61]. *Lactobacillus harbinensis* was used as an antifungal cultures in yogurt [23].

Non-bacteriocinogenic LAB with other competitive properties:

- In cooked peeled shrimp, *Lactococcus piscium* inhibited outgrowth of *L. monocytogenes* and delayed sensory spoilage [28, 29] and inhibited outgrowth of *Staphylococcus aureus* [64].
- *Leuconostoc gelidum* delayed spoilage of shrimp and cold-smoked salmon [64].
- *Staphylococcus xylosus* was used for inhibiting biogenic amine formation in anchovies [62].
- Plant-associated *Pseudomonas* was used for inhibiting *Salmonella enterica* on alfalfa sprouts [31].
- Commercial culture had a protective effect by depletion of oxygen [80].

Commercial products of protective cultures are produced in the same way as starter cultures: by batch fermentation, subsequent concentration by centrifugation, and final formulation as frozen pellets or freeze-dried powders. Approaches for continuous fermentation have also been described, such as cultivation of *Lactococcus lactis* in a fixed bed reactor [71]. Even though protective cultures were first introduced about 10–15 years ago, they are now well established in the food industry and recognized as an efficient tool to ensure the safety and durability of food products. Table 5 summarizes the main functionalities, species, and producers of protective cultures.

7 Bacteriophages

Phages are the most abundant living creatures on the planet: the estimated total number of phages is 10^{31}. As example, one milliliter of sea water contains about 1,000,000 bacteria but 10,000,000 phages. Phages are also widely spread in foods of various origins [8]. Today, it is recognized that the interaction between phages and bacteria plays an important part in maintaining the natural balance in our ecosystems.

The short name *phage* comes from ancient Greek, meaning "eat"; bacteriophage thus means "bacteria-eater". Bacteriophages are host-specific. The specificity is due to the fact that a phage can only propagate on a certain bacterial species; the phage recognizes its specific host cell. Thus, they are harmless to humans, animals, and plants.

Phages are renowned in the dairy industry for attacking starter cultures during fermentation and thereby spoiling the production of yogurt. On the contrary,

Table 5 Examples of commercially available protective cultures

Protective function	Microorganisms	Fields of application	Producer
Growth inhibition of *Listeria monocytogenes*	Lactic acid bacteria (e.g. *Lb. sakei, Lb. curvatus, Lb. plantarum*)	Fermented meat products Dairy products	Chr. Hansen (Denmark) DuPont (USA)
	Carnobacterium sp.	Fish and seafood	Sacco (Italy)
Inhibition of mold and yeasts	*Lactobacillus sp.*	Fresh dairy products	Chr. Hansen (Denmark)
	Lb. rhamnosus, Lb. paracasei, Propionobacterium sps.	Fresh dairy products	DuPont (USA)
Inhibition of *Clostridia tyrobutyricum*; prevention of late blowing	*Lactococcus lactis*	Cheese	CSK (Netherlands)

phages which can inactivate pathogenic bacteria can be useful in food processing. So to speak, "the enemy of my enemy is my useful friend".

In the food chain, bacteriophages were initially reported to be useful as interventions in the primary production. Drug-resistant bacteria have become a global problem, urging for the prompt development of alternative control strategies in order to avoid growth promoters while maintaining or enhancing food quality and safety. Oral treatment of broilers with phages reduced the carriage of *Salmonella* [32]. Phage therapy has also been explored in aquacultures; however, a recent review emphasizes the need of further research in the field of the application [69].

In unprocessed foods, phages have been tested for reducing *Campylobacter* and *Salmonella* on chicken skin [4, 38]. A reduction of 1–2 log of the pathogens was achieved. In various ready-to-eat foods (hot dogs, sliced turkey, smoked salmon, seafood, sliced cabbage, and lettuce), application of bacteriophages against *L. monocytogenes* provided up to a 5-log reduction of the pathogen [42].

However, a recent review of the use of bacteriophages against pathogens in food products concluded that the technology has so far had a variable success [35]. This could perhaps partially be due to testing of unsuitable applications for this relatively new technology in food production; bacteriophage products are already in use in agricultural, food safety, and diagnostic applications, demonstrating the utility and viability of such approaches [66].

Bacteriophages differ from many bacteria in the respect that phages are not motile. Therefore, the application method must ensure that the phages are well distributed in the product, so the target cells are brought into contact with a suitable number of phages. Furthermore, phages will typically become inactivated or bound in the food matrix—that is, they will have an initial effect in reducing their target organism, but will often presumably not be able to prevent regrowth of

surviving cells. It is important to consider these factors for developing successful application of phages, such as by using them with other hurdles that provide growth inhibition or in multifactorial systems. As example, a combination of phages and a bacteriocinogenic culture of *Lb. sakei* was used to provide both initial reduction and suppression of outgrowth of *L. monocytogenes* in cooked ham [51]. Successful and plausible applications of bacteriophages in foods were recently reviewed by Garcia et al. [36].

Similarly to the scientific reports demonstrating successful use of phages in the food chain, the options for applying bacteriophage products in industrial food production is relatively new. Again, the regulatory situation varies in different regions. In the EU, the use of bacteriophages in the food chain is being reviewed by the European Food Safety Authority to assess its efficacy and safety for use with food producing animals and in food products [3].

In the USA, OmniLytics Inc. received FDA approval for an anti-*E. coli* and an anti-*Salmonella* phage-based product to treat live animals prior to slaughtering. Phage cocktails against *L. monocytogenes*—Listex (formerly EBI Food Safety, now Micreos Food Safety) and LMP 02 (Intralytics)—were approved by the FDA in ready-to-eat meat.

Other commercially available phage-based bioprotective products are Agri-Phage from Omnilytics (specific formulations for strains of *Xanthomonas campestris* or *Pseudomonas syringae*) and EcoShieldTM (targets *E. coli* O157), as well as ListShield (antilisterial phages) from Intralytix. Recently, the Korean CheilJedang Corporation has introduced BioTector, a bacteriophage product for reducing *Salmonella* in poultry [66].

A main limiting factor for the industrial application of bacteriophages in foods, in addition to developing functional applications and avoiding negative publicity (e.g. “they are putting viruses in our food”), has been the high production costs. This issue will presumably be solved as several companies are investing in development and production facilities, and it appears likely that we will see new bioprotective solutions based on bacteriophages as alternative measures for controlling detrimental bacteria in food production in the coming years.

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