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Review

Bioprotective cultures as a clean label strategy for food preservation: From concept to market

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ABSTRACT

Bioprotective cultures and their derived antimicrobial agents represent a promising clean label strategy to enhance food safety and extend shelf-life while aligning with consumer demand for minimally processed products. This review critically analyses the scientific foundations and mechanisms of action of bioprotective agents, with a particular focus on lactic acid bacteria. It elucidates how they inhibit spoilage and pathogenic microorganisms through different strategies (competitive exclusion, acidification, and the production of bacteriocins and other compounds). This work also evaluates international regulatory frameworks and outlines the key technological criteria that must be met for both bioprotective agents (both protective cultures and their derived antimicrobial agents) to be considered suitable for bioprotective applications. Under this practical perspective, we discuss the main strategies for incorporating these protective agents (direct addition into formulations, surface application via immersion or spraying, and integration into edible coatings and active packaging) and analyse published studies across diverse food matrices, including fruits, vegetables, dairy, cereals, meat, and seafood, showing how bioprotective agents can replace or complement conventional preservatives. Finally, we discuss emerging trends and real-world examples of successful industrial application across multiple food matrices. Overall, this review provides practical guidance to support the effective and strategic use of bioprotective cultures and their derived antimicrobial agents as a clean-label preservation strategy, connecting scientific and regulatory insights with actual implementation in industrial settings.

1. Introduction

According to the 2022 Data & Trends of the EU Food and Drink Industry report, the food industry is the principal sector within the manufacturing industry, accounting for 14.3% of the total and being the foremost employer with 15.3%. This sector comprises 290,000 companies and employs 4.6 million people.

The main objective of this dynamic industry is to market safe and quality products. In this sense, each food product has its own microbiota, which may contain pathogenic and spoilage microorganisms. These microorganisms can be introduced at multiple stages of food production, whether through contaminated ingredients, inadequate hygiene practices, or cross-contamination during processing and storage (Snyder et al., 2024). Foodborne pathogens (such as *Listeria monocytogenes, Escherichia coli, Staphylococcus aureus* and *Salmonella* spp.) are of particular concern due to their ability to cause severe foodborne illnesses, leading to significant public health risks and economic consequences (Ben et al., 2019). Spoilage microorganisms, on the other hand, cause negative organoleptic changes in food, resulting in financial impacts on producers, distributors, and consumers (Snyder et al., 2024). The presence of these spoilage microorganisms accounts for 30–40% of food waste, a challenge that both authorities and the industry aim to mitigate.

To mitigate the risks posed by the presence of pathogenic and spoilage microorganisms, the implementation of effective food preservation methods is essential. Many preservation strategies act primarily by inhibiting microbial growth through the control of environmental factors such as temperature, water activity, pH, or oxygen availability.

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These include refrigeration, freezing, drying, curing, vacuum packing, modified atmosphere packaging, acidification, fermentation, and the addition of preservatives (Gould, 2000). However, depending on the specific processing conditions and/or the food matrices, these methods may also result in microbial inactivation (El-Kest and Marth, 1992; Wang et al., 2025).

In contrast, thermal processes such as pasteurization and sterilization act by directly inactivating microorganisms. Additionally, aseptic processing and packaging further limit microbial access to food products (Gould, 2000). Due to consumer preferences shift towards minimally processed foods, emerging or modern technologies are being developed. These include preservation by high hydrostatic pressure (Aganovic et al., 2021), pulsed electric fields (García et al., 2003), ozone (Pandiselvam et al., 2019), plasma (López et al., 2019) and dense CO_2 (Spilimbergo & Bertucco, 2003). Essential oils and their individual constituents, known for their antimicrobial properties, are also being explored as natural preservatives in food systems due to their ability to inhibit bacterial and fungal growth (Corrêa & Ferreira, 2023). Other nature-extracted preservatives, such as propolis (Luis-Villaroya et al., 2015) or chitosan (Raafat & Sahl, 2009), are also gaining interest due to their broad-spectrum antimicrobial activity and potential for application in different food matrices. These preservatives can be incorporated directly into food, encapsulated in nanoemulsions for controlled release (Maurya et al., 2021; Pagán et al., 2018), or integrated into edible coatings to enhance preservation (Zhang et al., 2022). Combined preservation techniques (also referred to as hurdle technology) are widely recognized for their effectiveness in inhibiting microbial growth and survival without significantly compromising the organoleptic and nutritional qualities of food (Leistner, 2000). However, the combination of preservation techniques does not always result in increased efficacy compared to the application of individual methods. Indeed, the effectiveness of certain technologies, such as high-pressure processing, may be reduced when foods are formulated with high sodium chloride concentrations, low water activity, or the presence of compounds such as lactate, which can interfere with microbial inactivation mechanisms (Mataragas et al., 2003; Melhem et al., 2022). Despite these limitations, this strategic simultaneous or sequential application of multiple preservation methods, like essential oils and heat or other physical hurdles, has been successfully applied in various food products such as fruits, vegetables and juices, fish and fish products, and meat products (Aaliya et al., 2021).

Either used alone or as part of combined preservation strategies, chemical additives are often perceived by consumers as incompatible with the demand for "natural" and "healthy" food (Negowetti et al., 2022). Current consumer preferences reflect a growing emphasis on health, sustainability, and transparency, driving demand for *clean-label* foods, which are mainly understood as minimally processed and without additives, preservatives, artificial colors, or ingredients (Aschemann-Witzel et al., 2019; Negowetti et al., 2022). Simultaneously, the popularity of convenient, ready-to-eat (RTE) foods continues to increase (Aschemann-Witzel et al., 2019; Asioli et al., 2017). In this context, biopreservation offers a promising alternative to conventional preservatives that aligns with these expectations by enabling the development of *clean-label* foods that are both convenient and have a long shelf life (Anumudu et al., 2022).

Over the last few years, numerous review articles have emerged addressing food biopreservation from different perspectives, including general biopreservation strategies (Gomes Da Silva et al., 2024; Fischer & Titgemeyer, 2023; Silva et al., 2018; Singh, 2018; Vaishali et al., 2019), bacteriocins (Gálvez et al., 2007; Lahiri et al., 2022; Parada Fabián et al., 2025; Simons et al., 2020; Soltani et al., 2021; Sugrue et al., 2024), and lactic acid bacteria as bioprotective agents (Ayivi et al., 2020; Barcenilla et al., 2022; Simons et al., 2020). However, comparatively limited attention has been given to the diversity of available formats and application strategies, as well as to practical commercial examples, despite their central role in enabling the transfer of research outcomes to industrial implementation.

This manuscript provides an overview of the use and application methods of protective cultures and culture-derived antimicrobial agents as an alternative to conventional preservatives proposed by the food industry in order to satisfy the needs of both consumers and the industry, addressing the aforementioned reasons, and ensuring the safety, quality, and extended shelf-life of products within the context of the clean label trend.

2. Methodology

In this study, a narrative bibliographic review was conducted using the scientific databases Scopus, Science Direct, and Web of Science. Google Scholar was occasionally consulted to identify potentially relevant studies not previously retrieved. Only articles in English published in journals indexed in Scopus were included.

A first search was conducted to cover the reviews, including the terms "bioprotection", "bioprotective cultures", and "bacteriocins". These review articles were further examined to delineate the main thematic sections addressed in the literature.

For Sections 3–5 (covering definitions, regulatory frameworks, and key bioprotective microorganisms), an additional comprehensive literature search was conducted using terms related to regulation and microbial groups, including "regulation", "lactic acid bacteria", "LAB", "Bacillus", "bioprotective yeasts", and "bioprotective molds". Boolean operators (AND, OR) were applied to refine these search queries. The reference lists of the retrieved review articles were also consulted.

In a third phase, a targeted search of primary research articles was performed to provide an applied perspective on the use of bioprotective cultures and culture-derived antimicrobial agents, particularly with respect to the mode of incorporation and the food matrices involved. This search included combinations of the terms "bacterial culture", "cell-free supernatant", "CFS", "bacteria", "lyophilized", "packaging", "supernatant", "edible coatings", "purified bacteriocins", together with food-related terms such as "fruits", "vegetables", "fresh-cut", "kiwi", "tomatoes", "strawberries", "apples", "pears", "slices", "dairy products", "cheese", "bread", "cereal-based", "ready-to-eat", "salads", "seafood products", "cold-smoked", and "meat". Boolean operators (AND, OR) were applied to refine these queries. In addition, the reference lists of the selected articles were manually screened to broaden the collection of relevant reviews.

For this section and to facilitate the creation of Tables 3–7, a dedicated database was compiled to organize the selected studies. Only articles convering the period from 2014 to 2025 were included, although literature from previous years was retained to facilitate the general discussion. Records referring to duplicate experimental work were excluded, along with articles not aligned with the objective of evaluating bioprotective cultures against spoilage or pathogenic microorganisms naturally present in food matrices. The final dataset comprised over 100 studies, which were categorized according to the type of food matrix to which the bioprotective culture or postbiotic preparation was applied. Studies were further subdivided based on the strategy and format of incorporation of the microbial preparation into the food matrix.

Finally, the Mintel Global New Products Database (Mintel GNPD) was consulted using the terms "bioprotective cultures", "protective cultures", and "postbiotics" to identify commercial applications of bioprotective strategies in food products. In addition, market studies conducted between 2022 and 2029 by Mordor Intelligence (2023–2028), Maximize Market Research (2022–2029), Market Data Forecast (2023–2028), and Data Bridge Market Research (2022–2029) were consulted to identify the most frequently cited producers and major industrial stakeholders in this sector.

3. Concept of protective cultures and bioprotective agents and their growing interest

Biological preservation refers to the extension of the shelf-life of food products and improvement of their microbial safety using natural or controlled microbiota and/or their antimicrobial compounds (Ross et al., 2002). When specific strains of microorganisms (typically bacteria or yeasts) are intentionally added to a food product to inhibit the growth of spoilage and pathogenic microbes, they are referred to as protective cultures (Leroy & De Vuyst, 2004). The preservative effect works by inhibiting the growth of undesirable microorganisms (such as foodborne pathogens and spoilage bacteria) through natural mechanisms like competitive exclusion, acidification, and antimicrobial compound production (e.g., bacteriocins, organic acids, or hydrogen peroxide) (Ayala et al., 2019; Gálvez et al., 2007; Leroy & De Vuyst, 2004).

Although both protective and starter cultures involve the intentional addition of selected microorganisms, their purposes differ. Starter cultures are used to drive fermentation processes that actively transform the food's sensory characteristics (such as texture, flavor, and pH) through microbial metabolism. In contrast, protective cultures are primarily aimed at preventing microbial spoilage and contamination without significantly altering the product's sensory properties. Their function is centered on preservation and food safety rather than fermentation-driven changes.

In addition to the direct action of live microorganisms, food bioprotection may also be achieved through culture-derived antimicrobial agents. These include (i) non-viable microbial cultures that have been intentionally inactivated while retaining antimicrobial functionality; (ii) cell-free supernatants (CFSs), defined as the extracellular fraction of a microbial culture obtained after removal of viable cells; (iii) and bioactive molecules derived from microbial metabolism, such as bacteriocins, which can be isolated and applied as purified or semi-purified substances (Adesina & Oluwafemi, 2022; Moradi et al., 2019; Yap et al., 2022). In this review, the term “bioprotective agent” is used as an umbrella concept encompassing both protective cultures and culture-derived antimicrobial agents.

The application of bioprotective agents as ingredients to extend the shelf-life of various products has garnered interest for approximately thirty years. However, it is only within the past decade that the food industry has focused on incorporating these bioprotective agents for preservation and the development of new products, due to new technologies or new approaches in the preservation of food. This trend is evident in the literature, as indicated by the Scopus database, which shows that the earliest studies on bioprotection in food date to 1995, with the most recent ones published in 2024 (Fig. 1).

This graph reveals a gradual overall increase (despite year-to-year fluctuations) in the number of publications from 1995 to 2013. This period of gradual increase suggests an initial phase of exploration and foundational research in the field of protective cultures and their related compounds, as well as their application in the food industry. However, from 2013 onwards, there is a marked acceleration in the growth rate of publications. This indicates a growing interest and development in the field of bioprotection and their application in the food industry. Additionally, this increase may be attributed to regulatory changes, shifts in consumer and industry demands, and the adoption of innovation as an alternative to conventional methods.

On the other hand, it is noteworthy that European countries, including France, Italy, and Spain, have contributed the highest number of publications. This trend underscores the significant interest of the European agrifood industry in these types of ingredients.

4. Regulatory frameworks for the production of protective cultures

The commercialization of bioprotective agents in food production is tightly regulated to ensure microbial safety, functional efficacy, and compliance with regional food laws. Regulatory frameworks differ significantly across different countries, reflecting distinct approaches to risk assessment, approval processes, and labeling requirements. Table 1 summarizes the key requirements for protective cultures in the European Union and in the United States of America, and are further explained below. Information on the legal regulation in other major jurisdictions can be found in a review by Fischer and Titgemeyer (2023).

Given the regulatory complexity associated with different forms of bioprotective agents, this section first addresses regulatory frameworks applicable to protective cultures (i.e., bioprotective agents based on viable microorganisms). Regulatory considerations relevant to culture-derived, non-viable bioprotective agents are discussed separately for the European Union (Section 4.1.7), whereas in the United States these products are generally addressed within the same GRAS/food additive framework and are therefore discussed jointly in Section 4.2.

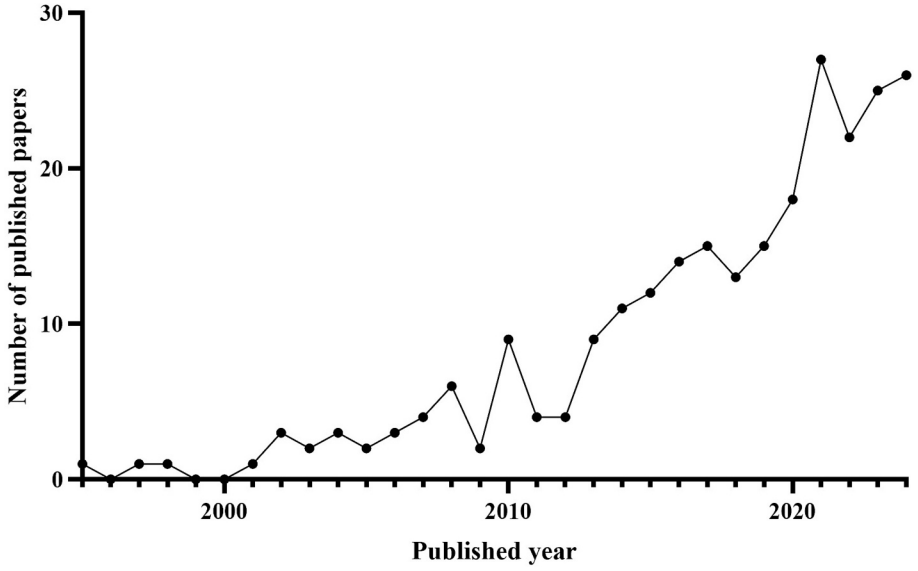

Fig. 1. Publications in bioprotective cultures (1995–2024). Source: Scopus (Elsevier). database.

Table 1
Regulatory frameworks for the safety assessment of protective cultures in the European Union and the United States according to the European Commission (EC), the European Food Safety Authority (EFSA), and the U.S. Food and Drug Administration (FDA).

Criterion	European Union (EC/EFSA)	United States (FDA)
General safety framework	• QPS: A pre-approved list of TUs. Strains of a QPS TU are presumed safe, subject to mandatory strain-level verification of qualifications. • Non-QPS strains require a full safety assessment.	GRAS or Food Additive Petition: safety is established through scientific consensus based on a history of common use in food or through a formal pre-market approval process.
Taxonomic identification	Mandatory precise identification, typically requiring WGS to confirm species and strain.	Requires published taxonomic evidence and a well-documented history of identification. WGS is recommended but not explicitly mandated.
Antibiotic resistance assessment	• The QPS list carries specific AMR qualifications for many TUs. • For a new strain of a QPS TU: Mandatory WGS screening for acquired AMR genes and phenotypic MIC testing against relevant antibiotics is required to verify compliance with QPS qualifications. • For non-QPS strains, a full, two-fold assessment is always required.	Primarily requires genomic evidence (e.g., from WGS) to demonstrate the absence of transferable antibiotic resistance genes. Phenotypic susceptibility testing is not explicitly required.
Absence of virulence & toxins	• **For QPS listing: Rigorous assessment for the entire TU.** • **For a new QPS strain: Mandatory verification of taxon-specific qualifications (e.g., absence of toxigenic potential in *Bacillus* spp.). The absence of other virulence factors is presumed unless specific risks are indicated.**	Relies on a combination of genomic data (to exclude known virulence factors) and toxicological studies to confirm the absence of toxin production.
Evidence of safe use	• For QPS, relies on the established **"body of knowledge"** for the species. • For non-QPS, requires **published scientific literature** for the strain or a closely related strain.	Requires published scientific evidence and a documented history of common use in food or through scientific procedures.
Technological & functional properties	Must be technologically viable and compatible with the food matrix and other starter cultures without adverse effects.	Must be functional for its intended purpose and must not negatively alter the product's safety or quality.
Stability in Food	Must remain viable and metabolically stable at effective levels throughout the product's declared shelf life.	Must demonstrate functional stability, meaning it remains effective for its intended purpose throughout the product's shelf life.

AMR: Antimicrobial Resistance; GRAS: Generally Recognized as Safe; MIC: Minimal Inhibitory Concentration; QPS: Qualified Presumption of Safety; TU: Taxonomic Unit, WGS: Whole Genome Sequencing.

4.1. Regulatory frameworks in Europe

4.1.1. Regulatory context in the European Union

In the European Union, the European Food Safety Authority (EFSA) conducts independent scientific evaluations to assess the safety of microorganisms used in food, including protective cultures. Based on EFSA's risk assessments, the European Commission establishes legally binding regulations governing their use in the food industry. The Commission, in collaboration with EU member states, is responsible for final regulatory approvals and enforcement under specific regulations such as the Novel Food Regulation (EU) 2015/2283 and the food additive legislation (EC) N° 1333/2008, **and the food information and labelling framework established by Regulation (EU) No 1169/2011**, which regulates the labelling of foodstuffs.

4.1.2. The qualified presumption of safety (QPS) system

To harmonize risk assessment across microbial applications, EFSA introduced the Qualified Presumption of Safety (QPS) framework in 2007 (EFSA, 2007). QPS is applied at the taxonomic unit (TU), typically the species level for bacteria and yeasts. To propose a TU for QPS consideration, a dossier must be submitted to EFSA with supporting evidence addressing the four pillars of the framework: (i) unambiguous taxonomic identity, (ii) the body of knowledge concerning history of use and biological properties, (iii) possible safety concerns such as pathogenicity, virulence factors, or toxin production, and (iv) the intended end use (EFSA, 2007; Herman et al., 2019). The EFSA BIOHAZ Panel evaluates the evidence and, if the TU is considered safe, it may be included in the QPS list, sometimes with certain exclusions (such as pathogenic subspecies) or qualifications (e.g., "for production purposes only").

After this taxonomic identification, the body of knowledge regarding the history of use, industrial applications, interaction with other microorganisms, etc. (Herman et al., 2019) is investigated based on scientific literature. The assessment also evaluates the possible presence of virulence factors that could cause pathogenicity in humans or animals, as well as the production of biologically active substances like antimicrobials and toxins.

The QPS list is a dynamic tool, subject to continuous monitoring and six-monthly updates, in which the BIOHAZ Panel assesses new TUs notified to EFSA and reviews existing listings. The most recent BIOHAZ Panel Statement published in the EFSA Journal (current version at the time of writing: EFSA BIOHAZ Panel, 2025a) represents the authoritative source for QPS status. Associated datasets are available on Zenodo (EFSA BIOHAZ Panel, 2025b), together with publicly accessible protocol (EFSA BIOHAZ Panel, 2025c) and the extensive literature search strategies (BIOHAZ Panel, 2025d).

4.1.3. Strain-level safety assessment

The QPS status, granted at the TU level, provides a foundational presumption of safety that must be validated through mandatory strain-level verification. This critical step confirms that the specific isolate in question does not possess unique risk factors absent from the broader TU, such as acquired antimicrobial resistance (AMR) or toxigenic potential.

The initial and non-negotiable prerequisite is unambiguous strain identification. This is achieved through Whole-Genome Sequencing (WGS) and confirmed by precise genomic taxonomic methods, such as Average Nucleotide Identity (ANI) and digital DNA-DNA Hybridization (dDDH), which provide definitive classification against type strains.

For most bacterial TUs, the primary safety requirement is demonstrating the absence of acquired AMR genes (EFSA BIOHAZ et al., 2023). This necessitates a dual analytical approach: (i) genotypic analysis, in which WGS data is screened against curated AMR databases to identify acquired resistance determinants, carefully distinguishing them from intrinsic, non-transferable resistance; and (ii) phenotypic confirmation through determinations of the Minimum Inhibitory Concentration (MIC) for relevant antimicrobials and comparing results to established clinical breakpoints.

Furthermore, the strain must comply with all taxon-specific qualifications attached to its QPS listing. For *Bacillus* species, this involves verifying the absence of toxigenic activity through genetic and biochemical assays (EFSA FEEDAP, 2014). Finally, it should be noted that the QPS assessment primarily focuses on the deliberate introduction of viable microorganisms. If a TU is used only for the production of

specific compounds and no viable cells are present in the final product, it may receive a "for production purpose only" qualification.

This two-tiered framework builds on existing safety data from well-characterized taxa and adds focused evaluations to address strain-specific risks, ensuring microbial strains are used safely.

4.1.4. Impact of QPS status on the regulatory pathway

QPS status significantly influences the regulatory pathways for microorganisms used in food and feed. QPS-listed strains benefit from an expedited approval process, as their QPS status eliminates the need for a full safety assessment at the TU level (Herman et al., 2019). This considerably shortens the approval timeline compared to non-QPS strains or novel foods. In contrast, non-QPS strains fall under the Novel Food Regulation (EU 2015/2283), necessitating a comprehensive dossier with detailed toxicology, exposure, and antimicrobial resistance data.

4.1.5. Criteria to be considered a protective culture in Europe

Beyond meeting safety criteria, microorganisms intended to function as protective cultures must demonstrate technological efficacy and functional relevance within a specific food matrix (Ben Said et al., 2019). In the EU regulatory context, this means that the strain must (i) exert a measurable antimicrobial effect against relevant foodborne pathogens and/or spoilage organisms under realistic processing and storage conditions for the targeted food category; (ii) remain viable and metabolically active throughout the intended shelf life to ensure a sustained protective effect without undesired overgrowth or metabolic drift; (iii) be technologically compatible with starter or adjunct cultures so as not to interfere with fermentation dynamics or desirable sensory characteristics; (iv) demonstrate matrix specificity; and (v) be applied in a way that is consistent with its regulatory status, which determines whether the culture is classified as a processing aid or as a food additive depending on the presence and technological action of viable cells in the final product. These requirements are summarised in Table 1.

4.1.6. The Nagoya protocol

The Nagoya Protocol (European & Council of the European, 2014) establishes a framework for Access and Benefit-Sharing of genetic resources, which is of relevance when considering the origin of the cultured microbial species. It mandates that genetic resources, including those utilized in protective cultures for the food industry, are accessed legally from the providing country with "Prior Informed Consent", and that "Mutually Agreed Terms" for benefit-sharing are in place. This aims to prevent "biopiracy" and ensure that benefits arising from the utilization of these genetic resources are shared fairly with the countries and often local communities that provided them, fostering biodiversity conservation and sustainable use.

For the EU food industry, this translates into rigorous documentation, traceability, and contractual obligations when sourcing or developing protective cultures derived from genetic resources. The United States has not signed or ratified this protocol, so there is no federal legislation equivalent to the Nagoya Protocol, and the obligations are not uniformly enforced across sectors.

4.1.7. Regulatory considerations for culture-derived antimicrobial agents

Bioprotective agents which do not contain viable microorganisms fall outside the scope of the QPS framework, which is exclusively applicable to live microbial taxa deliberately introduced into the food chain.

Within the European Union, the regulatory classification of these agents depends primarily on their technological function in the final product, rather than on the identity of the producing microorganism. When the principal function of a culture-derived preparation is the inhibition of spoilage or pathogenic microorganisms throughout the shelf life of the product, it may be classified as a food additive (preservative) under Regulation (EC) No 1333/2008. In such cases, the active compound must be authorised and listed in Annex II of the regulation, with an assigned *E*-number, as exemplified by nisin (E234), **which is currently the only bacteriocin authorised for use in foods in the European Union**. Preparations not covered by the positive list must undergo a full safety evaluation prior to authorisation.

Alternatively, if a culture-derived preparation exerts a primary sensory effect, such as flavor modification, it may be classified as a flavouring under Regulation (EC) No 1334/2008. However, this classification only applies when the sensory effect clearly outweighs any antimicrobial function, and may be challenged by regulatory authorities if the primary purpose is food preservation.

A further regulatory pathway arises when culture-derived antimicrobial agents are used as complex preparations rather than as isolated compounds. CFSs may in some cases be considered functional ingredients of microbial origin. However, in the absence of a documented history of significant consumption in the EU prior to May 1997, such preparations may be classified as novel foods under Regulation (EU) 2015/2283, requiring EFSA evaluation and authorisation by the European Commission.

In light of the considerations outlined above, the labelling of bioprotective agents must reflect their regulatory classification and technological function in the final food, rather than their microbial origin or the terminology commonly used in scientific and technical contexts.

4.2. Regulatory frameworks in the United States of America

In the United States, the safety of substances intentionally added to food is regulated under the Federal Food, Drug, and Cosmetic Act (1938). The U.S. Food and Drug Administration (FDA) is the federal agency responsible for implementing this legislation and ensuring the safety of the national food supply.

As a general principle under the FD&C Act, substances intentionally added to food are considered **food additives** and require premarket approval unless they qualify for an exemption. The most common exemption relevant to microbial ingredients is the Generally Recognized as Safe (GRAS) designation (U.S. Food and Drug Administration, 2024). GRAS status can be established through two main pathways: 1) scientific procedures, including publicly available data and consensus among qualified experts, or 2) for substances used in food prior to 1958, based on a history of common use.

Microorganisms and microbial-derived ingredients intentionally added to food are subject to this regulatory framework. GRAS status is contingent upon the intended use, conditions of use, and demonstrated safety profile of the substance**. For example, a microorganism may be considered GRAS for use as a live starter or protective culture in fermented foods, whereas its metabolites, cell-free preparations, or purified antimicrobial compounds may constitute distinct substances requiring independent safety evaluation. Furthermore,** purified bacteriocins typically require independent GRAS determinations or food additive approvals (Soltani et al., 2021).

Finally, the regulatory approach to genetic resources differs significantly between the U.S. and the EU. The United States has not ratified the Nagoya Protocol on Access and Benefit-Sharing (ABS). Consequently, its provisions are not enforced at the federal level. However, U.S. researchers and companies utilizing genetic resources from other countries may still be subject to the ABS laws and obligations of those provider countries. In response, some U.S. institutions have adopted internal policies to ensure compliance with international norms.

5. Key protective cultures in the Food industry

Among the diverse array of microorganisms employed for biopreservation, **lactic acid bacteria (LAB)** stand out as the most known and widely utilized (Ayivi et al., 2020; de Souza et al., 2023).

5.1. Lactic acid Bacteria (LAB)

These are Gram-positive, non-spore-forming, facultative anaerobic or aerobic bacteria capable of fermenting the free sugars of the medium or food in which they are found, producing lactic acid as the major product.

5.1.1. Classification and taxonomy of LAB

Depending on their carbohydrate fermentation pathways, LAB can be classified into homofermentative and heterofermentative (Mokoena, 2017). Homofermentative LAB, such as *Lactococcus*, *Streptococcus* and *Pediococcus*, primarily yield two molecules of lactate from one glucose molecule. In contrast, heterofermentative LAB, including *Leuconostoc*, *Weissella*, and some *Lactobacillus* species, produce lactate, ethanol, and carbon dioxide from a single glucose molecule (Ayivi et al., 2020; Mokoena, 2017).

It should be noted that the genus *Lactobacillus*, which is particularly relevant in biopreservation due to its diverse antimicrobial capabilities and its wide distribution across various fermented foods (Ayivi et al., 2020), has undergone a significant taxonomic reclassification. See Zheng et al. (2020) for the table summarizing the newly defined genera and their phylogenetic relationships.

5.1.2. Mechanism of action of antimicrobial compounds of LAB

LAB, through different metabolic processes, can produce various compounds with broad-spectrum antimicrobial activity such as organic acids, hydrogen peroxide, bacteriocins, diacetyl, and ethanol (Ayivi et al., 2020;Ben Said et al., 2019; de Souza et al., 2023). Given that the first three are considered the primary and most extensively studied antimicrobial compounds contributing to LAB's biopreservative effects, a detailed explanation of their mechanisms of action is offered below.

5.1.2.1. Organic acids. LAB can produce a wide range of organic acids, such as lactic acid, acetic acid, and propionic acid, among others. These acids are obtained after fermentation of the carbohydrates present in the matrix (Ben Said et al., 2019; de Souza et al., 2023). The concentration of these acids depends on the bacterial strain, as well as the pH and temperature of the medium, and their interactions (Bangar et al., 2022).

The antimicrobial activity of organic acids is attributed to several mechanisms, including the disruption of nutrient absorption and the reduction of adenosine triphosphate (ATP) production (Kovanda et al., 2019). However, the most widely accepted explanation is the weak organic acid theory, illustrated in Fig. 2. According to this theory, at low pH, the undissociated (lipophilic) forms of organic acids can readily penetrate the cell membrane of pathogenic and spoilage bacteria. Once inside the cell (where the pH is typically higher), these acids dissociate, releasing H+ ions, which lowers the intracellular pH, leading to cellular damage and forcing the cell to expend significant ATP to pump out the excess protons and maintain pH homeostasis (Bangar et al., 2022; Ben Braïek & Smaoui, 2021; Kovanda et al., 2019). The accumulated anions within the cell can also be toxic, inhibiting essential metabolic reactions, increasing osmotic pressure, and damaging cytosolic enzymes (Ben Braïek & Smaoui, 2021; Gómez-García et al., 2019; Yoon et al., 2024).

Organic acids exhibit antimicrobial properties that are contingent upon their chemical characteristics, including their pKa values, as well as the concentration of their undissociated forms. At low pH, undissociated acid molecules, which are lipophilic, can cross microbial cell membranes and once inside the cell, these acids dissociate, releasing H+ ions that lower the intracellular pH, leading to cellular damage. The resultant anions are toxic, inhibiting metabolic reactions, increasing osmotic pressure, and damaging cytosolic enzymes.The pH of the medium influences in the determination of the ratio between the dissociated and undissociated forms of an acid near its dissociation constant (pKa), thereby influencing the inhibitory efficacy of organic acids. The toxicity of these acids is strongly correlated with an increase in the concentration of protonated free acids and the pKa of the acids utilized (Ben Braïek & Smaoui, 2021; Gómez-García et al., 2019; Yoon et al., 2024).

5.1.2.2. Hydrogen peroxide. In the presence of oxygen, LAB produce hydrogen peroxide due to the action of flavoprotein oxidase and/or nicotinamide adenine dinucleotide hydrogen peroxide (NADH) peroxidases. This can lead to the formation of free radicals and hydroxyl radicals, which are precursors to oxidative damage. Such damage permeabilizes the membrane and damages the DNA of target bacteria (Ben Said et al., 2019). Optimal production of hydrogen peroxide by LAB

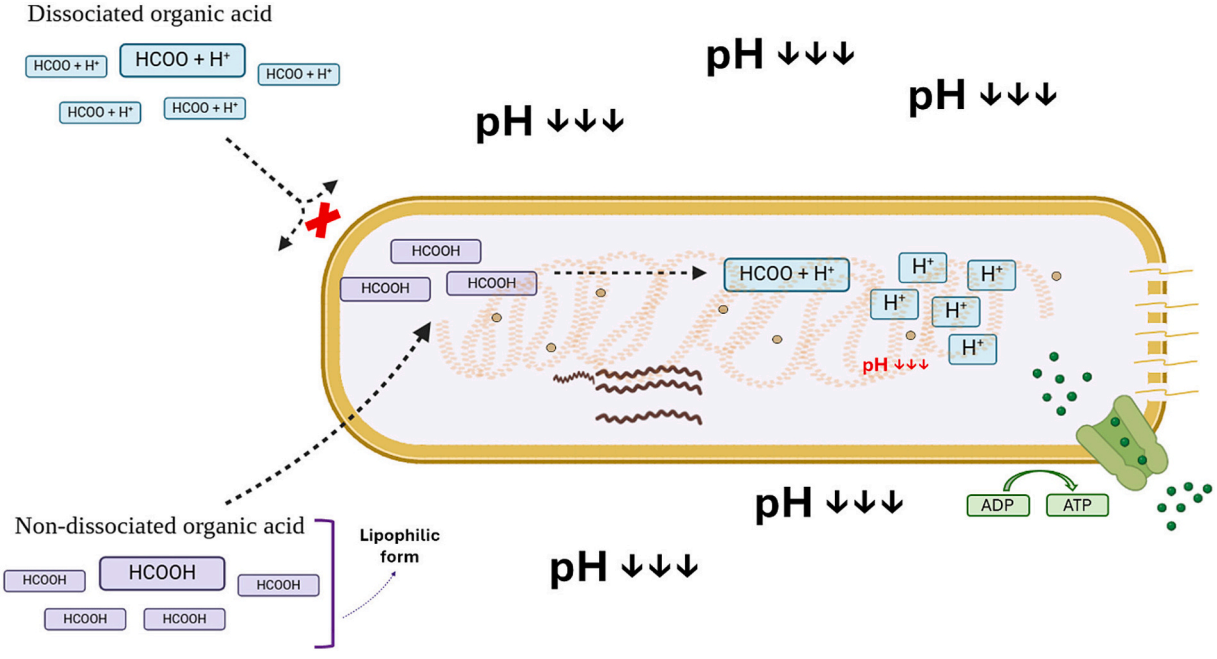

Fig. 2. Intracellular mechanism of action of the organic acid through membrane diffusion and proton release. Figure created by the authors based on conceptual models from previously published literature.

occurs at 37 °C and pH 5.5 (Ben Said et al., 2019). However, the antimicrobial efficacy and stability of hydrogen peroxide are influenced by the presence of catalase, either produced by LAB themselves or present in the food matrix, which can rapidly degrade hydrogen peroxide into water and oxygen, thereby limiting its activity (Bryukhanova et al., 2022).

5.1.2.3. Bacteriocins. While bacteriocins are produced by a wide array of bacteria, including notable examples from Gram-negative species, this section will primarily focus on the bacteriocins synthesized by LAB. Bacteriocins are a diverse group of antimicrobial peptides and proteins ribosomally synthesized by various bacterial species, many of them belonging to the LAB group. These non-toxic compounds are primarily known for their inhibitory activity against other often closely related bacterial strains, playing a crucial role in microbial competition within diverse environments (Cintas et al., 2001; da Costa et al., 2019).

While many bacteriocins exhibit a narrow spectrum, targeting specific species or genera, others, like nisin, demonstrate broader efficacy against a range of Gram-positive pathogens and spoilage microorganisms (Cotter et al., 2013; da Costa et al., 2019). A key characteristic of bacteriocins is their sophisticated self-protection mechanisms, ensuring that the producing bacteria remain unaffected by their own antimicrobial agents (da Costa et al., 2019; Lahiri et al., 2022). Furthermore, many bacteriocins, particularly those from food-grade bacteria, are generally considered safe for human consumption. They are often susceptible to inactivation by digestive enzymes, minimizing potential adverse effects on the human gut microbiota (Nes et al., 2007). Consequently, bacteriocins are garnering significant interest as promising antimicrobial alternatives in various fields, offering solutions to challenges such as antibiotic resistance and food spoilage (Cotter et al., 2013; Simons et al., 2020; Sugrue et al., 2024).

The production of bacteriocins is a complex biological process. While some are thermoresistant, their synthesis is often induced by environmental stress conditions, such as high cell density (population increase) or nutrient limitation (Cintas et al., 2001). Their production is also significantly influenced by the availability of carbon and nitrogen sources in the growth medium and is intricately regulated by quorum sensing, a cell-to-cell communication system (da Costa et al., 2019; Todorov et al., 2022). Besides, they are generally produced during or at the end of the exponential growth phase, with their synthesis often linked to biomass production and regulated by inducing peptides or pheromones (da Costa et al., 2019; Todorov et al., 2022).

The genetic information encoding bacteriocins, along with their associated processing and immunity proteins, is typically organized into operon clusters. These gene clusters can be located on the bacterial chromosome, plasmids, or mobile genetic elements, highlighting their adaptability and horizontal transfer potential. Their expression is inducible, often requiring specific regulatory signals for both secretion and extracellular accumulation of the active peptides (Chanos & Mygind, 2016). While the primary antimicrobial mechanisms often involve pore formation in bacterial membranes or degradation of the cell wall, the exact modes of action can vary widely depending on the bacteriocin class. For instance, the mechanisms by which certain bacteriocins exert their effects against Gram-negative bacteria, which possess an outer membrane as an additional barrier, are more complex and require further comprehensive investigation (Pérez-Ramos et al., 2021).

Bacteriocins are currently grouped into several major classes according to their molecular size, structural features, stability, and mechanisms of action. This classification provides a useful framework for understanding their functional diversity and their potential roles in food preservation and safety. In Gram-positive bacteria (and particularly LAB), bacteriocins are typically categorized into lantibiotics (Class I), non-lantibiotic peptides (Class II), and larger heat-labile bacteriolysins (formerly Class III). Although a fourth class comprising lipid- or carbohydrate-associated peptides has been proposed in earlier classifications, this group remains poorly defined and is not included here. Table 2, adapted and expanded from Cotter et al. (2005) and Singh (2018), illustrates this information and provides representative examples relevant to food biopreservation applications.

Although information about bacteriocins is increasingly available, the most challenging aspect lies not in the technical aspect but in the legal one (Barcenilla et al., 2022). This challenge stems from the complexity of approving new bacteriocins as food additives and the necessity to determine the precise concentration of each bacteriocin to demonstrate antimicrobial activity in various foods without causing undesirable changes in the final product, such as alterations in color or flavor (Gálvez et al., 2007; Lahiri et al., 2022; Sugrue et al., 2024). For their use to be approved, extensive research on bacteriocin immunogenicity and toxicity is essential to confirm their safety. Additionally, clinical studies are required for the approval of bacteriocins by the World Health Organization and regulatory agencies (Parada Fabián et al., 2025).

The EFSA- and FDA-approved food-grade bacteriocin nisin exhibits antimicrobial activity against bacterial spores and Gram-positive bacteria, including *Clostridium botulinum, L. monocytogenes*, and *S. aureus*. It is marketed as a partially purified product for use in both dairy and non-dairy fermentations (Soltani et al., 2021).

In addition to LAB, there are now other microorganisms described as protective cultures. Among them are the following species.

5.2. *Bacillus subtilis*

Bacillus subtilis, a Gram-positive, endospore-forming bacteria, has emerged as a protective culture due to its well-recognized potential as a

Table 2
Updated classification of bacteriocins based on structural features, mode of action, and representative examples of bacteriocins with the producing strains.

Class	Description	Structural Features	Mode of Action	Bacteriocin examples
Class I	Lantibiotics	Small, heat-stable, post-translationally modified peptides	Pore formation and inhibition of cell wall synthesis	Nisin A (*Lactococcus lactis*)
Class IIa	Non-lantibiotic peptides. Antilisterial, Pediocin-like	Small, heat-stable, linear with bisulfide bridges	Pore formation	Pediocin PA-1 (*Pediococcus acidilactici*) Sakacin A (*Lactobacillus sakei*) Leucocin A (*Leuconostoc gelidum*)
Class IIb	Non-lantibiotic peptides. Two-peptide	Two peptides	Pore formation	Plantaricin EF (*Lactiplantibacillus plantarum*) Lactococcin G (*Lactococcus lactis*)
Class IIc	Non-lantibiotic peptides. Circular	Circular peptides, with one or two cysteine residues	Pore formation	Enterocin AS-48 (*Enterococcus* spp.) Gassericin A (*Lactobacillus gasseri*)
Class IId	Non-lantibiotic peptides. Linear non-pediocin	Linear peptides	Pore formation	Lactococcin A (*Lactococcus lactis*) Garvicin A (*Lactococcus garvieae*)
Class III	Large, heat-labile	Large proteins	Enzymatic activity	Helveticin J (*Lactobacillus helveticus*) Enterolysin A (*Enterococcus faecalis*)

biocontrol agent, contributing to shelf-life extension by exerting antimicrobial activity against pathogenic and/or spoilage microorganisms (Hinarejos et al., 2016; Rocha et al., 2023). Its application is primarily focused on crop and plant biocontrol (Ben Khedher et al., 2020), although its use has also expanded to minimally processed products, fruits and vegetables.

The primary mechanism of action of this bacterium is based on the production of lipopeptides secondary metabolites with antimicrobial activity, such as surfactin, iturin, fengycin, bacillomycin, and bacilysin, among others (Rocha et al., 2023; Jiawen Xiao et al., 2021). These compounds exhibit bactericidal and fungicidal activity by disrupting the cellular membrane integrity of bacterial pathogens such as *L. monocytogenes, Salmonella enterica* subsp. *enterica* serovar Enteritidis or *S. aureus* (T. Chen et al., 2023; Fernandes et al., 2007). They have also been described as active against molds like *Fusarium oxysporum* (Assena et al., 2024) or *Botrytis cinerea* (Jiling Xiao et al., 2023). Additionally, *B. subtilis* is capable of forming biofilms on food-contact surfaces, enabling it to compete for nutrients with other microorganisms and thereby inhibit their growth (Bridier et al., 2011; Carrascosa et al., 2021).

5.3. Yeasts and molds

Not only bacteria are used as protective cultures, but certain yeasts and molds also have shown a significant role as natural preservatives in clean label products. Although they do no produce classical bacteriocins like LAB or *B. subtilis,* they do synthesize other antimicrobial compounds that contribute to the inhibition of pathogenic and spoilage microorganisms in food.

One of the most recognized yeasts is *Debaryomyces hansenii*, a halotolerant species naturally present in cured meats and cheeses. Its protective role is based on competition for nutrients and space, as well as the production of antimicrobial compounds such as volatile fatty acids, ethanol, and phenolic compounds, which modify the surface microenvironment (Ramos-Moreno et al., 2021; Zhao et al., 2022). Another well-known yeast is *Saccharomyces cerevisiae*, widely used in the fermentation of products such as beer and bread (El Dana et al., 2025). It can also exert a protective effect through the production of compounds like ethanol, carbon dioxide, and proteinaceous toxins that inhibit the growth of other microorganisms (El Dana et al., 2025; Fakruddin et al., 2017).

Certain molds, such as *Penicillium nalgiovense* and *Penicillium chrysogenum*, are used in meat and dairy products. These molds exhibit both a competitive exclusion effect (their rapid growth on the food surface quickly depletes available nutrients and oxygen) and synthesis of specific antifungal and antibacterial metabolites (Guzmán-Chávez et al., 2018; Papagianni & Papamichael, 2007).

6. Methods of application of bioprotective cultures in food

The increased use of protective cultures and culture-derived antimicrobial agents as food preservation ingredients is evidenced by the growing innovation within the industry to incorporate them in different ways, tailored to the characteristics of the product and its presentation to consumers in the market.

Considering the physicochemical and organoleptic properties of the product, as well as its specific processing conditions and microbiological characteristics, the bioprotective agent can be added in the formulation, on the surface, or in the packaging.

When added to formulations, bioprotective agents are included during product development, typically alongside starter cultures. Bioprotective agents may be applied in different formats depending on their biological nature, reflecting the categories defined in Section 3. Accordingly, they can be added: as live bacteria that produce and release antimicrobial compounds during manufacturing; as CFSs, acting as an antimicrobial ingredient; in powder or freeze-dried forms; as microencapsulated or spray-dried CFSs; or as purified or semi-purified bacteriocins (Adesina & Oluwafemi, 2022; Moradi et al., 2019; Yap et al., 2022).

For some food products sourced directly from nature, bioprotective agents cannot be added during formulation. Instead, they are applied directly to the food's surface. This can involve spraying, washing, or soaking the product in solutions containing the bioprotective agents. Similar to their use in formulations, these bioprotective agents come in various forms for surface application, including **live bacterial cultures, CFSs, powdered/lyophilized preparations, or purified bacteriocins (Aymerich et al., 2019; Mostafa, 2020; Vijayakumar & Muriana, 2017)**.

Traditional methods of incorporating antimicrobial compounds into food have certain drawbacks, such as low diffusion, uneven distribution, and incompatibility with the food matrix or a spectrum of activity that is not suitable for certain foods (da Costa et al., 2019). To overcome these limitations, and in response to the increasing trend of RTE foods and consumer preference for convenience and familiar ingredients (Aschemann-Witzel et al., 2019; Asioli et al., 2017), an innovative approach is emerging using packaging as a carrier for antimicrobial compounds. This strategy involves the incorporation of protective cultures or their components in packaging surfaces or coatings, often including often include thickeners and gelling agents like carboxymethyl cellulose, glycerol, or konjac (Mohammadi et al., 2022; C. C. Silva et al., 2018). After obtaining the film, protective cultures are added in the following formats: as a lyophile, as pellets or immobilized cells, as encapsulated bacteriocins, or as purified and lyophilized bacteriocins (Ceylan & Atasoy, 2023; Khodaei & Hamidi-Esfahani, 2019; Mohammadi et al., 2022; Silva et al., 2018; Tumbarski Y., 2019).

It should be noted that the addition of bioprotective agents is only a part of the barrier technology applied to food, since they are usually accompanied by other techniques such as pasteurization (Vijayakumar & Muriana, 2017), high hydrostatic pressure (Stratakos et al., 2016), light pulses (Pirozzi et al., 2021), active packaging (Ceylan & Atasoy, 2023), or modified or controlled atmospheres (Guimaraes et al., 2018), among others.

6.1. Scientific interest in different application methods over the last decade

In the last decade, scientific publications have systematically included various strategies for incorporating antimicrobial compounds into foods, such as the addition of bacterial cultures, CFS,and freeze-dried cultures or supernatants. Each of these methods has been the subject of an average of 3–4 scientific publications annually. Fig. 3 shows the evolution of the number of studies publishing the application in food of bioprotective strategies beyond protective cultures, highlighting the importance of each method in the scientific literature.

As shown in Fig. 3, the method of addition most frequently mentioned in scientific literature, and therefore arguably the most widely used in the food industry, is the CFSs of bacteria, followed by purified bacteriocins. In particular, the use of CFSs has seen a notable increase, with the number of related publications rising from an annual average of 60 publications (2014–2018) to 180 (2019–2024). Notably, in the periods 2020–2021 and 2023–2024, the total number of publications increased by 150% compared to the previous year. This trend seems to continue in 2025, with 22 publications related to this bioprotective agent up to the date of writing this review. This trend suggests that the food industry is increasingly focusing on the application and legal acceptance of these ingredients. On the other hand, the incorporation of purified bacteriocins has historically shown an upward trend, although this seems to have stagnated over the past five years. However, the limited regulatory authorisation of purified bacteriocins in the European Union may contribute to the recent slowdown in publications describing this approach.

A third and increasingly relevant strategy is the integration of

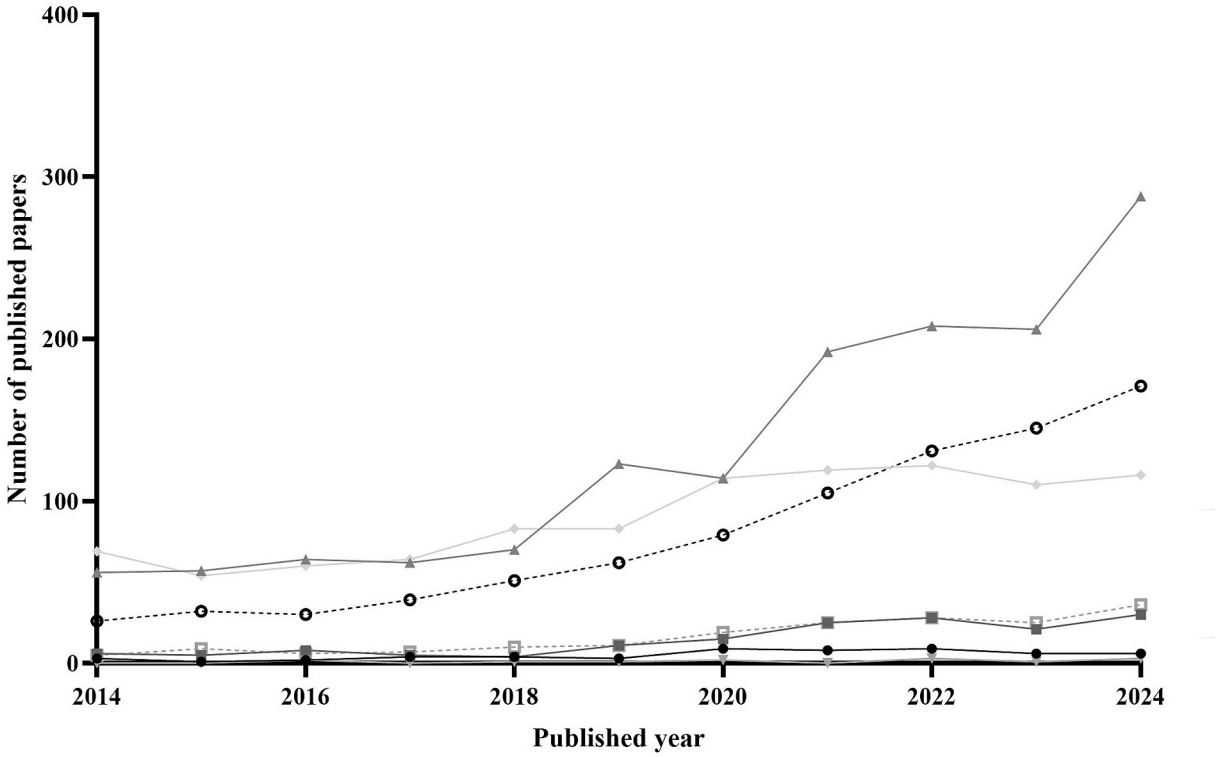

Fig. 3. Publications about the addition of protective cultures to foods over the last ten years, depending on the strategy of incorporation: bacterial culture (—●—), supernatant (—■—), cell free supernatant (—▲—),lyophilized (—▼—), purified bacteriocins (—◆—), edible coatings (—-○—), packaging material (—-□—). Source: Scopus (Elsevier) database.

bioprotective agents into edible coatings, which has grown considerably since 2017, likely reflecting the interest in multifunctional preservation systems (Fig. 3). Edible coatings are thin layers of edible materials (like alignate or chitosan) containing the culture or its metabolites (Gregirchak et al., 2020). In contrast, the use of bioprotective agents incorporated directly into packaging materials or applied in the form of bacterial supernatants shows only a modest but steady increase. Meanwhile, strategies based on lyophilised cells or the direct addition of live cultured bacteria remain comparatively marginal and have stayed at consistently low publication levels throughout the period analysed.

7. Current uses of bioprotective agents in specific foods

Tables 3–7 list some of the trends, according to the matrix, in the use of microorganisms as bioprotective agents, highlighting the specific products in which these microorganisms are used and the methods of their addition to the food or if they are used with another preservation treatment. In this section, studies are presented according to **food category** and further grouped by **strategy of incorporation**.

7.1. Fruits and vegetables (table 3)

Fruits and vegetables are available in various formats in the market, catering to evolving lifestyle and consumer consumption trends. These formats include whole fruits and fresh-cut or RTE options, with the latter gaining prominence due to their convenience.

Cut and RTE fruits are highly perishable; thus, a key objective in this sector of the food industry is to develop preservation techniques that extend the shelf life by inhibiting the growth of pathogens or spoilage microorganisms of these products while maintaining their organoleptic properties. Commonly employed methods include modified atmosphere packaging (MAP) and the addition of additives such as ascorbic acid.

To meet consumer demand for clean label products, an emerging alternative is the incorporation of live bacteria that act as probiotics. These bacteria serve dual roles: acting as antimicrobials and, when labelled as probiotics, providing additional health benefits.

The literature identifies three primary methods for incorporating microorganisms in fruits and vegetables: edible coatings, immersion, and matrix/formulation. Within these methods, microorganisms can be added as cultures or CFSs.

Edible coatings are commonly applied to perishable fruits that are cut fresh in various formats or are prone to rapid browning. These coatings also serve as carriers for live bacteria, which can act as probiotics. The literature extensively documents the use of edible coatings on fresh strawberries, with alginate and carboxymethyl cellulose (CMC) being two primary ingredients. LAB, including *Lactiplantibacillus plantarum* as pellet (Khodaei & Hamidi-Esfahani, 2019) and *Lacticaseibacillus rhamnosus* (Li et al., 2024; Temiz & Özdemir, 2021), as well as *Bacillus* spp. (Menéndez-Cañamares et al., 2024; Tumbarski Y., 2019), are incorporated into these formulations effectively inhibiting the growth of molds and yeasts responsible for spoilage and the reduced shelf life of strawberries. Other products that utilize these techniques include fresh-cut apple (Alvarez et al., 2021; Ceylan & Atasoy, 2023; Hashemi & Jafarpour, 2021; Wong et al., 2021), fresh-cut kiwifruit (Hashemi & Jafarpour, 2021), and fresh-cut pineapple (Tenea et al., 2020). The primary microorganisms added to edible coatings in these products are *Lp. plantarum* and *Lc. rhamnosus*, in their pellet, culture or CFS format, which serve as protection against pathogens such as *E. coli*, *Listeria* spp., and *Salmonella* spp., as well as spoilage agents like yeasts and molds.

Another method for incorporating protective cultures or CFSs into fruits is immersion, as described for pears (Iglesias et al., 2018), pineapples (Russo et al., 2014), apples (Siroli et al., 2015), peppers (Saravanakumar et al., 2020), or lettuce (Siroli et al., 2015). The CFS of *Lp. plantarum* is the most used ingredient for immersion, effectively acting against pathogenic microorganisms such as *L. monocytogenes*, *Salmonella* spp., and *E. coli*.

The method of adding protective cultures or CFSs to feed varies depending on the product type. Various studies have reported the

Table 3
List of publications on bioprotective agents applied in fruits and vegetables, according to different strategies of incorporation.

Strategy: Edible coatings

Food	Microbial source	Pathogen/ spoilage microorganism	Other technologies	Storage	Author
Fresh-cut apple slices	*Lacticaseibacillus rhamnosus* *Bifidobacterium animalis* subsp. *lactis*	*Listeria innocua* CIP 80.11 and *Escherichia coli* O157:H7 FP605/03	Alginate-based edible coating + polypropylene containers with a polyolefine film	5 ± 1°C for 8 days (in darkness)	Alvarez et al., 2020
Cherry tomatoes	*Lactiplantibacillus plantarum* A6	*Fusarium* spp. and *Rhizopus stolonifer*	Exopolysaccharide (*Weisella confusa* JCA4)-based coating + PET packages	4 °C for 8 days/ 30 °C for 8 days	Alvarez et al., 2021
Fresh-cut apple slices	*Lacticaseibacillus rhamnosus* B-445	Aerobic mesophiles, and yeasts and molds	Alginate-based edible coating + packaging	5 ± 1°C for 13 days (70–75% relative humidity)	Elabd, 2019
Table grapes	*Lactiplantibacillus plantarum* 11-A	*Aspergillus niger* CECT 2805	Alginate-based edible coating + plastic containers under passive atmosphere	4°C for 14 days	De Simone et al., 2024
Fresh-cut kiwis	*Lactiplantibacillus plantarum* LP3 *Lactiplantibacillus plantarum LU5* *Lactiplantibacillus plantarum* AF1	Yeast and molds	Konjac-based edible coating + polyethylene terephthalate	4°C for 5 days	Hashemi & Jafarpour, 2021
Avocado	*Meyerozyma caribbica*	*Colletotrichum gloeosporioides* Pa14	Sodium alginate-based edible coating	25 °C for 15 days (75% RH)/ 6°C (95% RH) for 10 days and ripened at 25 °C (75% RH) for 5 days	Iñiguez-Moreno et al., 2020
Fresh strawberries	*Lactiplantibacillus plantarum*	Yeast and molds	Carboxymethyl Cellulose (CMC) edible coating	4°C for 16 days	Khodaei and Hamidi-Esfahani, 2019
Strawberries	*Lacticaseibacillus rhamnosus* GG (LGG) CICC 6224	*Molds* *Escherichia coli O157:H7 9637* *Staphylococcus aureus*	Alginate-based edible coating + polypropylene containers	28 °C for 8 days (80 ± 2% relative humidity)	Li et al., 2024
Strawberries	*Bacillus subtilis* SB8	*Botrytis cinerea*	Sodium-alginate and glycerol based edible coating + sterilized food containers with damp paper	Room temperature for 5 days (in darkness)	Menéndez-Cañamares et al., 2024
Mushrooms cream	*Lactococcus lactis* subsp. *lactis CECT 539/ ATCC 11454*	*Listeria monocytogenes* CECT 934/ ATCC 19114	(PVOH + Hgel) films +thermo-sealed bags	4°C for 15 days	Settier-Ramírez et al., 2021
Carrots sticks	*Lactobacillus acidophilus* La-14	Aerobic mesophiles, and yeasts and molds	Sodium-alginate based edible coating + polystyrene trays sealed with vinyl polychloride film	8 ± 2°C for 19 days	Shigematsu et al., 2018
Fresh strawberries	*Lacticaseibacillus rhamnosus*	Aerobic mesophiles, and yeasts and molds	Gelatin-based edible coating Gelatin-inulin based edible coating	4°C for 16 days	Temiz & Özdemir, 2021
Pineapple	*Lactiplantibacillus plantarum* UTNCys5–4 and *Lactococcus lactis* subsp. *lactis* Gt28	*Escherichia coli* ATCC25922, *Escherichia coli* UTNEc1, *Salmonella enterica* subsp. *enterica* ATCC51741, *Salmonella* UTNSm2 and *Shigella sonnei* ATCC25931	CFS peptide-based formulation	Refrigerated temperature for 5 days	Tenea et al., 2020
Fresh strawberries	*Bacillus velezensis* BM47	Yeast and molds	Carboxymethyl Cellulose (CMC) edible coating + plastic bags	4°C for 16 days (75% relative humidity)	Tumbarski et al., 2019
Fresh-cut apple slices	*Lactiplantibacillus plantarum* 299v	*Listeria monocytogenes* BAA-839	Carboxymethyl Cellulose (CMC) edible coating (1st coating) + zein coating (2nd coating) + polypopylene fresh-keeping boxes	4°C for 7 days	Wong et al., 2021
Fresh pistachio	*Lacticaseibacillus casei* XN18	Yeast and molds	*Lallemantia royleana* mucilage based edible coating + polyethylene plastic bags	4°C for 35 days (85% relative humidity)	Zibaei-Rad et al., 2024

Strategy: Immersion

Food	Microbial source	Pathogen/ spoilage microorganism	Other technologies	Storage	Author
Hazelnuts	*Metschnikowia* aff. *Pulcherrima* DN-HS	*Aspergillus flavus*		25 °C for 7 days	Dikmetas et al., 2023
Cut iceberg lettuce	*Lactococcus lactis* 537	*Listeria monocytogenes* ATCC 7644	Commercial plastic zip lock bags	4°C for 10 days	Dong et al., 2021
Fresh-cut pear	*Lacticaseibacillus rhamnosus* GG (LGG)	*Listeria monocytogenes* CECT 4031 (serovar 1a), CECT 933 (serovar 3a), CECT 940 (serovar 4d), CECT 4032 (serovar 4b) and serovar 1/2a	Polypropylene trays sealed with non-peelable polypropylene plastic film	5 °C for 9 days	Iglesias et al., 2018
Fresh-cut pear	*Pseudomonas graminis* CPA-7	*Salmonella enterica* subsp. *enterica* ATCC BAA-707, ATCC BAA-709 and CECT 4300 *Listeria monocytogenes* CECT 4031 (serovar 1a), CECT 933 (serovar 3a), CECT 940 (serovar 4d), CECT 4032 (serovar 4b) and 1/2a	Antioxidant solution (2% ascorbic acid +2% sodium citrate +1% CaCl2) and MAP	5 °C for 10 days/ 10 °C for 10 days	Iglesias et al., 2018
Pineapple	*Lactiplantibacillus plantarum* B2 (CECT 8328)	*Listeria monocytogenes* CECT 4031 and *Escherichia coli* O157:H7 CECT 4267	Polypropylene plastic film bags with thermally sealed in MAP	5 °C for 8 days	Russo et al., 2014

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Table 3 (*continued*)

Strategy: Immersion					
Food	Microbial source	Pathogen/ spoilage microorganism	Other technologies	Storage	Author
Red and yellow fresh-cut bell pepper	*Lactobacillus fermentum* PBCC11.5 (CECT 8448)	*Listeria monocytogenes* ATCC 19118	Biochemical additive solution (sodium benzoate + ascorbic acid + DL-α-tocopherol acetate) + polypropylene container	4°C for 15 days/ 15 °C for 15 days	Saravanakumar et al., 2020
	Lacticaseibacillus rhamnosus GG (LGG) (ATCC 53103)	*Salmonella Typhimurium* ATCC 14028			
Apple slices	*Lactiplantibacillus plantarum* CIT3	*Escherichia coli, Salmonella* spp. and *Listeria monocytogenes*	2-(*E*)-hexenal/ hexanal and citral/2-(E)-hexenal + active modified atmosphere (7% O2/ 0% CO2)	6°C for 16 days	Siroli et al., 2015
Lamb's lettuce	*Lactiplantibacillus plantarum* V7B3		Thyme essential oil + artificial ordinary atmosphere		

Strategy: Matrix/formulation					
Food	Microbial source	Pathogen/ spoilage microorganism	Other technologies	Storage	Author
Fresh-cut melon	*Pseudomonas graminis* CPA-7	*Salmonella enterica* subsp. *enterica*ATCC BAA-709, ATCC BAA-710 and ATCC BAA-711 *Listeria monocytogenes CECT 4031 (serovar 1a), CECT 933 (serovar 3a), CECT 940 (serovar 4d), CECT 4032 (serovar 4b) and serovar 1/2a*	Passive MAP with polypropylene trays sealed with a non peelable polypropylene plastic film/ air packaging with non peelable polypropylene plastic film with ten holes of 400 μm	5 °C for 8 days/ 10 °C for 8 days	Abadias et al., 2014
Water-melon juices Orange juices	*Pediococcus pentosaceus* IO1	*Staphylococcus aureus*	Sterilized glass bottles	Room temperature for 72 h	Adesina & Oluwafemi, 2022
Cherries	*Bacillus subtilis* Y17B	*Alternaria alternata* ACT-1	Sterilized six-well culture cluster flat-bottom plates and covered with a zipper bag	28 °C for 7 days	Ahmad et al., 2023
Peach juice	*Levilactobacillus brevis* HL6	*Staphylococcus aureus* *Listeria monocytogenes* *Salmonella enterica* serovar Typhimurium *Shigella dysenteriae* *Bacillus cereus* *Streptococcus pyogenes*		4°C for 21 days/ 25 °C for 21 days	Behbahani et al., 2024
Orange fruit	*Lactiplantibacillus plantarum* CKXP13 and CWXP24	*Penicillium digitatum*	Plastic bags	25 °C for X days (high relative humidity)	Chen et al., 2021
Apple juice Fresh-cut apple Grapes Banana Sugarcane juice	*Ralstonia* sp. SL312 *Lactiplantibacillus plantarum* DMR14	Patulin(*Penicillium* spp., *Aspergillus* spp. *y Byssochlamys* spp.) *Shigella boydii*		37 °C for 32 h 37 °C for 48 h	He et al., 2022 Islam et al., 2023
Tomatoes	*Wickerhamomyces anomalus*	*Botrytis cinerea*	Plastic baskets and wrapped with cling film	20 °C for 4 days (95% relative humidity)	Lanhuang et al., 2022
Cream of potato / meat soups	*Lacticaseibacillus rhamnosus* GG (LGG)	*Listeria monocytogenes* ATCC 19112 *Salmonella Typhimurium* ATCC 13311	Hot-filled into multi-layered plastic pouches sealed with the time knob at 6	5 °C for 21 days/ 10 °C for 21 days/ 15 °C for 21 days	Muñoz et al., 2019
Ready-to-eat salads	*Weissella viridescens*	*Listeria monocytogenes* LR102 (serotype 1/2a), VI 51028 (serotype 4),0227e359 (serotype 1),0113e131 (serotype 1) and VI 51010 (serotype 4b)	High Hydrostatic Pressure (HHP) (400 MPa/1 min) + packaged using polyethylene/polyamide vacuum pouches	4°C for 21 days/ 12 °C for 21 days	Stratakos et al., 2016
Cherry tomatoes Fresh-cut apple Fresh-cut kiwi Fresh-cut strawberries	*Bacillus velezensis* A4	*Botrytis cinerea*	Air dried	25 °C (95% relative humidity)	Zhao et al., 2022

Table 4
List of publications on bioprotective agents applied in dairy products, according to different strategies of incorporation.

Strategy: Active film

Food	Microbial source	Pathogen/ spoilage microorganism	Other technologies	Storage	Author
White semi-firm cheese slices	*Enterococcus faecium* ES216	*Listeria innocua* ATCC 33090	Triticale flour film + bacteriocin like substance (BLIS)	7°C for 15 days	Salvucci et al., 2019

Strategy: Biodegradable films

Food	Microbial source	Pathogen/ spoilage microorganism	Other technologies	Storage	Author
Sliced Prato cheese	*Latilactobacillus curvatus* P99	*Listeria monocytogenes* Scott A	Filmogenic solution with starch, distilled water, glycerol, Tween 20	4°C for 10 days	de Lima Marques et al., 2017

Strategy: Edible coating

Food	Microbial source	Pathogen/ spoilage microorganism	Other technologies	Storage	Author
Sliced cheese	*Lacticaseibacillus rhamnosus*		Sodium caseinate, glycerol-based edible coatings	4°C for 30 days	Ceylan & Atasoy, 2023
Mongolian cheese	*Lacticaseibacillus paracasei* ALAC-4	*Candida albicans* CICC 32819 *Zygosaccharomyces rouxii* LJL117 *Rhodotorula glutinis* CICC 33037 *Penicillium* spp. *Mucor niemalis* CICC 40868 *Aspergillus* spp. *Staphylococcus aureus* 8325 *Salmonella Typhimurium* 14,028 s *Listeria monocytogenes* 598 *Escherichia coli* O157:H7 9637 *Bacillus cereus* B-3711 *Aspergillus* spp.	Alginate-based edible coating	4°C for 15 days	Dong et al., 2023
Soft cheese	*Bifidobacterium animalis* subsp. *lactis*, *Lactobacillus acidophilus* and *Lacticaseibacillus casei*		Chitosan, sodium alginate and carboxymethyl cellulose (CMC)-based edible coating	7°C for 45 days	El-Sayed et al., 2021
Tybo cheese Handcraft goat cheese	*Enterococcus avium* DSMZ 17511	*Listeria monocytogenes* 01/155	Agar-based (0.8% *w/v*) edible coating	4°C for 14 days/ 8°C for 14 days	Guitián et al., 2019
Fresh cheese	*Pediococcus pentosaceus* 147	*Listeria monocytogenes* ATCC 7644	Glycerol and chitosan-based edible coatings + individual aseptic bags	4°C for 21 days	Jutinico-Shubach et al., 2020
Fresh-cheese	*Lactococcus lactis* L3A21M1 *Lactococcus garvieae* SJC17	*Listeria monocytogenes* ATCC 7466 Yeasts Molds *Pseudomonas* spp. *Clostridium* spp.	Alginate, maltodextrin and glycerol-based edible coating	4°C for 10 days/ 10 °C for 10 days	Silva et al., 2022
Artisanal acid-curd cheese	*Lactobacillus helveticus* MI-LH13		Glycerol-based edible coating	4 ± 1°C for 14 days/ 23 ± 1°C for 14 days	Vasiliauskaite et al., 2022

Strategy: Matrix/formulation

Food	Microbial source	Pathogen/ spoilage microorganism	Other technologies	Storage	Author
Fresh cheese	*Lacticaseibacillus rhamnosus* LRH05 *Latilactobacillus sakei* LSK04 *Carnobacterium maltaromaticum* CNB06	*Pseudomonas* spp. and *Enterobacteriaceae*	Modified atmosphere (MAP) (70% N2/ 30% CO2)	8 for 5 weeks/ 14°C for 5 weeks	Bassi et al., 2020
Kefir	*Lacticaseibacillus rhamnosus* *Propionibacterium freudenreichii* subsp. *shermanii*	*Escherichia coli* ATCC 25922 And*enterococcus faecalis* 29,212	200 mL packages	4°Cfor 21 days	Balta & Yerlikaya, 2024
Fresh-cheese	*Enterococcus faecalis*	*Listeria monocytogenes* ATCC 7644	Sterile stainless steel circular cheese containers and mesh covered	4°C for 15 days	Coelho et al., 2014
Sheep cheese whey Goat cheese whey	*Lacticaseibacillus casei*	Total psychrotrophic bacteria count and total bacteria count		7°C for 28 days	dos Santos et al., 2024
High-moisture model cheese	*Lactiplantibacillus plantarum*	*Listeria monocytogenes*lm 108 (serovar 1/2b), LM 301 (serovar 1/2a), LM 310 (serovar 4b), R2–500 (serovar 4b) and R2–501 (serovar 4b)	EVOH plastic + vacuum	4 ± 0.5 °C for 8 to 10 weeks	Engstrom et al., 2021

(*continued on next page*)

Table 4 (*continued*)

Strategy: Matrix/formulation

Food	Microbial source	Pathogen/ spoilage microorganism	Other technologies	Storage	Author
Cottage cheese	*Lacticaseibacillus rhamnosus* (+ *Bifidobacterium animalis* subsp. *lactis* A026)	*Penicillium chrysogenum* LMA-212		6°C for 21 days (in darkness)	Fernandez et al., 2017
Sheep cheese whey Goat cheese whey	*Lacticaseibacillus casei* BGP1–304799 A	Psychrotrophic bacteria		7°C for 28 days	Junior & Tribst, 2022
Domiati-like cheese	*Lactiplantibacillus plantarum* FFNL1810 *Lactiplantibacillus plantarum* FFNL739	*Staphylococcus aureus* ATCC 6538		6 ± 1°C for 56 days	Khalil et al., 2022
Torta del Casar cheese	*Lacticaseibacillus casei* 116 *Lactococcus garvieae* 151	*Listeria monocytogenes*		35 days at 6 °C and 90% relative humidity (RH), 10 days at 8°C and 80% RH, 10 days at 9°C and 80% RH, and 35 days at 10 °C and 80% RH	Martín et al., 2022
Fermented milk	*Schleiferilactobacillus harbinensis* K.V9.3.1 Np	*Yarrowia lipolytica* UBOCC-A-211004		10 °C for 2 weeks	Mieszkin et al., 2017
Yogurt	*Lacticaseibacillus rhamnosus*	*Debaryomyces hansenii* CHCC 16374 *Yarrowia lipolytica* CHCC 16375 *Saccharomyces cerevisiae* CHCC 16590 *Kluyveromyces marxianus* CHCC 16601	Microtiter with a breathable lid	7°C for 31 days/ 12 °C for 31 days and 16 °C for 31 days	Nielsen et al., 2021
Sour cream	*Lactiplantibacillus plantarum* CH1	*Mucor racemosus* *Penicillium commune*	Sterile containers	Incubation at 25 °C for 5–10 days	Ouiddir et al., 2019
Laboratory Cheese Model	*Lactococcus lactis* 16FS16–9/ 20234 - 11FS16 *Lactiplantibacillus plantarum* 1/ 14537 - 4 A/20045	*Listeria monocytogenes* ATCC 7644	Plastic container	10 °C for 10 days (85% humidity)	Pisano et al., 2022
Brazilian cheese	*Lactiplantibacillus paraplantarum* FT-259 *Lactococcus lactis* QMF 11	*Listeria monocytogenes* ATCC 7644 *Staphylococcus aureus* ATCC 25923	Polyethylene packaging	8°C for 21 days	Ribeiro et al., 2021
Tomato cheese spreads	*Latilactobacillus sakei* subsp. *sakei* 2a	*Listeria monocytogenes* serovar 4b	Plastic vials for food products	4°C for 28 days/ 15 °C for 28 days	Martinez et al., 2015
Sour cream	*Lactiplantibacillus plantarum* L244 + *Schleiferilactobacillus harbinensis*L172 *Lactiplantibacillus plantarum* L244 + *Lacticaseibacillus rhamnosus* CIRM-BIA1113	*Penicillium commune* UBOCC-A-116003/ *Mucor racemosus* UBOCC-A-116002/ *Rhodotorula mucilaginosa* UBOCC-A-216004		Refrigeration for 24 days	Leyva Salas et al., 2018
Semi-hard cheese	*Lactiplantibacillus plantarum* L244 + *Schleiferilactobacillus harbinensis*L172 *Lactiplantibacillus plantarum* L244 + *Lacticaseibacillus rhamnosus* CIRM-BIA1113	*Penicillium commune* UBOCC-A-116003/ *Mucor racemosus* UBOCC-A-116002		12 °C for 24 weeks (96% relative humidity)	
Crescenza cheese	*Penicillium caseifuvulm* FUA 5008 and *Penicillium roqueforti* 3969	*Lacticaseibacillus rhamnosus* FUA3185 *Lacticaseibacillus paracasei* FUA3413		9°C for 1 week, 13 °C for 3 weeks, 9°C for 4 weeks and 5 °C until 90 days	Zhao et al., 2024
Gouda cheese	*Debaryomyces hansenii* FUA4064	*Lactiplantibacillus plantarum* FUA 3183 *Lactiplantibacillus plantarum* FUA 3247			
Whey beverages	*Lactiplantibacillus plantarum* N7	*Pichia pastoris, Aspergillus niger, Geotrichum candidum, Kluyveromyces marxianus* and *Penicillium chrysogenum*		4 ± 2°C for 10 days/ room temperature for 10 days	Xu et al., 2021

Strategy: Sprayed

Food	Microbial source	Pathogen/ spoilage microorganism	Other technologies	Storage	Author
Ricotta fresca cheese	*Carnobacterium* spp.	*Pseudomonas* spp.	Modified atmosphere (MAP) (70% N2/ 30% CO2)	4°C for 21 days/ 10 °C for 10 days	Spanu et al., 2018

Strategy: Surface inoculation

Food	Microbial source	Pathogen/ spoilage microorganism	Other technologies	Storage	Author
Cottage cheese	*Lactiplantibacillus plantarum*	*Penicillium commune*		Room temperature for 29 days	Cheong et al., 2014

incorporation of CFSs in fruit juices; for instance, Adesina and Oluwafemi (2022) documented the use of CFS from *Pediococcus pentosaceus* in orange and watermelon juices to combat the pathogen *S. aureus*. Similarly, Behbahani et al. (2024) added *Levilactobacillus brevis* CFS to peach juice to inhibit the growth of *L. monocytogenes*, *Salmonella* spp., and other spoilage microorganisms. Conversely, for cut fruits such as strawberries (Zhao et al., 2022), kiwis (Zhao et al., 2022), melon (Abadias et al., 2014), oranges (Chen et al., 2021), and apples (Islam et al., 2023; Zhao et al., 2022), the predominant method involves creating a cavity and inoculating both the indicator microorganism and the protective culture or CFS. In these cases, *Lp. plantarum* and various strains of *Bacillus* spp. are commonly used to inhibit the growth of molds such as *B. cinerea* or *Penicillium* spp., as well as *L. monocytogenes* and *Salmonella* spp.

Table 5
List of publications on bioprotective agents applied in cereals, according to different strategies of incorporation.

Strategy: Edible coating					
Food matrix	Microbial source	Pathogen/ spoilage microorganism	Other technologies	Storage	Author
Wheat bread	*Streptococcus salivarius* subsp. *thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Lactobacillus acidophilus*	*Aspergillus* spp. and *Penicillium* spp.	Sodium alginate, whey and glycerol-based edible coating	23 ± 2°C for 120 h (relative humidity of 75 ± 5%)	Gregirchak et al., 2020

Strategy: Immersion					
Food matrix	Microbial source	Pathogen/ spoilage microorganism	Other technologies	Storage	Author
Maize seeds	*Levilactobacillus brevis* MYSN105	*Fusarium verticillioides* *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538, and *Salmonella Paratyphi* ATCC 9150	Sterile petri dishes	28 °C for 7 days	Somashekaraiah et al., 2021

Strategy: Matrix/ formulation					
Food matrix	Microbial source	Pathogen/ spoilage microorganism	Other technologies	Storage	Author
Fresh based-pizza	*Lactobacillus acidophilus*, *Lacticaseibacillus casei*, *Bifidobacterium* spp. and *Bacillus coagulans*	*Alternaria infectoria*	Modified Atmosphere Packaging (MAP) (50% CO2/ 50% N2)	4°C for 70 days	Calasso et al., 2023
Focaccia		*Alternaria alternata*			
Bread	*Pediococcus pentosaceus*	*Aspergillus niger* ATCC 9029		Room temperature (25 °C) for 7 days	Ebrahimi et al., 2020
Fresh pasta	*Lactobacillus acidophilus*, *Bifidobacterium animalis*, *Lacticaseibacillus paracasei*, *Lacticaseibacillus casei* and *Bacillus coagulans*	Aerobic mesophilic m.o. at 30 °C/ Mesophilic LAB, yeasts and molds	Modified Atmosphere Packaging (MAP) (40% CO2/ 60% N2)	4 ± 2°C for 120 days	Marzano et al., 2022
Bread	*Lactiplantibacillus plantarum* CH1	*Aspergillus tubingensis* *Aspergillus flavus*		Incubation at 25 °C for 5–10 days	Ouiddir et al., 2019
Wheat sourdough	*Limosilactobacillus reuteri*	*Aspergillus niger* ATCC 9029 (aflatoxins)		Room temperature (25 °C) for 7 days	Sadeghi et al., 2019
Wheat grains	*Lactobacillus* sp. RM1	*Aspergillus parasiticus* ITEM 11	Sterilized grinder	28 °C for 15 day	Shehata et al., 2019
Cereal-based, potentially probioticand/or synbiotic fermented vegan product (vegan dessert)	*Lactiplantibacillus plantarum* O21	*Enterobacteriaceae, Escherichia coli,* coliforms, yeasts and molds		4°C for 21 days	Szydłowska et al., 2021
Fava bean and wheat bread	*Levilactobacillus brevis* AM7	*Penicillium paneum, Penicillium roqueforti DPPMAF1, and PI, Penicillium crustosum, and Peniciilium albocoremium, Eurotium herbariorum Aspergillus niger*	Polyethylene bags	Room temperature for 15 days	Verni et al., 2023

Strategy: Sprayed					
Food matrix	Microbial source	Pathogen/ spoilage microorganism	Other technologies	Storage	Author
Cake and milk bread rolls	*Leuconostoc citreum* L123 and L32, *Latilactobacillus sakei* O25, *Limosilactobacillus reuteri* 5529 *and Levilactobacillus brevis* Lu 35	*Cladosporium sphaerospermum* UBOCC-A-112116 + *Wallemia sebi* UBOCC-A-11209 *Eurotium repens* UBOCC-A-112075 + *Aspergillus niger* UBOCC-A-112064 + *Penicillium corylophilum* UBOCC-A-112081		25 °C for 1 week (humid environment)	Le Lay et al., 2016

7.2. Dairy products (table 4)

Historically, fermented dairy products products have been recognized as fermented due to the addition of starters for fermentation to achieve the final product. Beyond this primary function, the incorporation of other bacteria, acting as probiotics and protective cultures, due to their production of antimicrobial compounds, is being investigated to enhance their organoleptic properties and extend shelf-life.

The most significant proliferation issues in these foods are caused by various species of *Listeria* spp. and LAB, leading to their alteration and deterioration, and posing public health risks.

The incorporation of protective cultures or their CFSs into the formulation or matrix is frequently highlighted in scientific literature due to its simplicity during the dairy manufacturing process. Nielsen et al. (2021) demonstrated the use of *Lc. rhamnosus* in yogurt production to inhibit the growth of *D. hansenii* yeast. Similarly, Leyva Salas et al. (2018) and Ouiddir et al. (2019) employed various strains of *Lp. plantarum* to combat *Mucor racemosus* in sour cream. Balta and Yerlikaya (2024) successfully inoculated *Lc. rhamnosus* into kefir formulations, effectively inhibiting the growth of *E. coli* and *Enterococcus faecalis*, which are known to cause product spoilage.

Lp. plantarum is frequently cited in the literature as a key microorganism incorporated into cheese formulations to inhibit the growth of various undesirable microorganisms. For instance, Leyva Salas et al. (2018) utilized *Lp. plantarum* in semi-hard cheese to inhibit the molds *Penicillium commune* and *M. racemosus*. Additionally, other researchers have employed *Lp. plantarum* to inhibit *L. monocytogenes* in high-moisture model cheese (Engstrom et al., 2021), laboratory cheese

Table 6
List of publications on bioprotective agents applied in meat products, according to different strategies of incorporation.

Strategy: Active films

Food matrix	Microbial source	Pathogen/ spoilage microorganism	Other technologies	Storage	Author
Fresh ground beef	*Lactiplantibacillus plantarum* subsp. *Plantarum* ATCC 14917	*Listeria monocytogenes* ATCC 19115	Polyethylene containers and BNC film	4 ± 1°C for 9 days	Shafipour-Yordshashi et al., 2020
Sliced cooked ham	*Lactococcus lactis* subsp. *Lactis* CECT 539/ ATCC 11454	*Listeria monocytogenes* CECT 934/ ATCC 19114	PLA/(PVOH + Hgel) films	4°C for 15 days	Settier-Ramírez et al., 2021

Strategy: Edible coatings

Food matrix	Microbial source	Pathogen/ spoilage microorganism	Other technologies	Storage	Author
Lamb meat slices	*Lactococcus lactis* NJ414	Total viable count and psychrotrophic bacteria	*Plantago major* mucilage (PMM)	4°C for 9 days	Behbhani et al., 2024
Sliced ham	*Bifidobacterium animalis* Bb-12 *Lacticaseibacillus casei*-01	*Staphylococcus* spp., *Pseudomonas* spp., *Enterobacteriaceae and yeasts/molds*	Whey protein and glycerol-based edible coating	4°C for 45 days	Pereira et al., 2018

Strategy: Immersion

Food matrix	Microbial source	Pathogen/ spoilage microorganism	Other technologies	Storage	Author
Sliced cooked ham	*Carnobacterium maltaromaticum* CM_B824, CM_B827 and CM_B289	*Pseudomonas fluorescens* ATCC 1355 and *Brochothrix thermosphacta* ATCC 11509 + indigenous	PP/EVOH/PPR trays with modified atmosphere (70% N2/ 30% CO2) sealed with PET/PP film	4°C for 10 days and 8°C for 18 days	Cavalari et al., 2024
Fresh chicken breast	*Lacticaseibacillus paracasei*FX-6	*Pseudomonas* spp. *Hafnia-Obesumbacterium Serratia* spp.	Sterile petri dishes covered with parafilm	4°C for 14 days	Duan et al., 2020
Chilled beef	*Latilactobacillus sakei* (Bactoferm B-2) *Latilactobacillus curvatus* (SafePro B-LC-48)	*Enterbacteraceae*, *Pseudomonas* spp. and *Brochothrix thermosphacta*	Vacuum bags were composed of polyamide/ polyethylene film	4°C for 38 days	Zhang et al., 2018
Frankfurters	*Pediococcus acidilactici* B-LC-20	Total viable count	Vacuum-packaged at−1 bar vacuum level a	4 ± 1°C for 28 days	Incili et al., 2023
Fresh raw top round beef	*Lactiplantibacillus plantarum* NRRL B-4496	*Escherichia coli* *Salmonella Typhimurium* *Listeria monocytogenes* *Staphylococcus aureus* ATCC 29213 and ATCC 25923	Marinade	4°C for 14 h	Arrioja-Betrón et al., 2020
Ground beef	*Carnobacterium maltaromaticum* CM_B824, CM_B827 and CM_B289	*Pseudomonas fluorescens* ATCC 1355 and *Brochothrix thermosphacta* ATCC 11509 + indigenous	PP/EVOH/PPR trays with modified atmosphere (66%O2/ 4% N2, 30% CO2) sealed with PET/PP film	4°C for 3 days and 8°C for 4 days (0% relative humidity)	Cavalari et al., 2024

Strategy: Matrix/ formulation

Food matrix	Microbial source	Pathogen/ spoilage microorganism	Other technologies	Storage	Author
Beef hamburgers	*Latilactobacillus sakei* subsp. *carnosus*/ *Latilactobacillus sakei* + *Staphylococcus xylosus*	*Brochothrix thermosphacta*	Modified atmosphere packaging (70% N2/ 30% CO2) + PET/PE/EVOH/PE/ANTIFOG – EVOH trays laminated with a film consisting of PET/PE/EVOH/PE/ ANTIFOG - EVOH	4 ± 2°C for 12 days	Comi et al., 2015
Sardinian fermented sausage	*Latilactobacillus sakei, Pediococcus acidilactici, Staphylococcus carnosus* and *Staphylococcus carnosus* subsp. *utilis* *Pediococcus acidilactici*	*Listeria monocytogenes*	Nitrate and nitrite, ascorbic acid	12–15 °C for 20 days (70–75% humidity)	Sidi et al., 2022

Strategy: Sprayed

Food matrix	Microbial source	Pathogen/ spoilage microorganism	Other technologies	Storage	Author
Chicken sausages	*Latilactobacillus sakei*B-2 *Latilactobacillus curvatus* B-LC-48	Psychrotrophic bacteria, and yeasts and molds	Modified Atmosphere (70% N2/ 30% CO2)	4°C for 60 days/ 10 °C for 60 days	Ataş et al, 2021

Strategy: Surface inoculation

Food matrix	Microbial source	Pathogen/ spoilage microorganism	Other technologies	Storage	Author
Fresh cooked ham slices	*Lactococcus lactis* ULE383, *Lactiplantibacillus plantarum* ULE639, ULE949, ULE1599 and ULE1841, and *Lacticaseibacillus paracasei* ULE721	*Listeria monocytogenes* CECT 911 (serovar 1/2c)	Vacuum packaging (25 mbar)	7°C for 19 days and 12 °C for 10 days	Barcenilla et al., 2023
Meatloaf			Modified atmosphere packaging (80% N2/ 20% CO2)		
Roasted pork shoulder			Vacuum packaging (25 mbar) + High Pressure Processing at 500 MPa (15 °C/ 3 min)		
Sliced emulsion type sausages	*Pediococcus* spp.	*Listeria monocytogenes*	MAP trays sealed with a surface film (PET, Top Tray M/O a 47) Vacuum packaging with boards inside in vacuum bags (PA/PE) and sealed with a vacuum sealer (Allpax V 50)	7°C for 21 days	Bungenstock et al., 2020

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Table 6 (*continued*)

Strategy: Surface inoculation					
Food matrix	Microbial source	Pathogen/ spoilage microorganism	Other technologies	Storage	Author
Cooked bacon	*Lactococcus lactis* subsp. *Lactis* (Rubis - CHR HANSEN) *Latilactobacillus sakei* (B-2 Safe Pro® - CHR HANSEN)	*Leuconostoc mesenteroides*	Packed under vacuum using the packaging film Combiflex PE/PA 2010 (60/150)	4 ± 2°C for 90 days	Comi et al., 2016

models (Pisano et al., 2022), and Brazilian cheese (Ribeiro et al., 2021). Furthermore, it has been used to inhibit *S. aureus* in domiati-like cheese (Khalil et al., 2022) and brazilian cheese (Ribeiro et al., 2021).

Another technique used to incorporate bacteria or their CFSs into cheeses is to wrap them with edible coatings. The formulation of these coatings varies depending on the authors and the type of cheese, achieving the goal of inhibiting pathogenic and/or spoilage microorganisms. An agar-based edible coating with the CFS of *Enterococcus avium* demonstrated to inhibit the growth of *L. monocytogenes* in tybo cheese and handcrafted goat cheese (Guitián et al., 2019). Other researchers also achieved antimicrobial activity against the same pathogen with different edible coatings. For example, Jutinico-Shubach et al. (2020) tested a glycerol and chitosan-based edible coating formulated with the CFS of *P. pentosaceus* in fresh cheese. Silva et al. (2022) tested in the same product an alginate, maltodextrin, and glycerol-based coating, supplemented with *Lactococcus lactis*, and another with *Lactococcus garviae*. In other cheeses such as mongolian cheese (Dong et al., 2023) and soft cheese (El-Sayed et al., 2021), two edible coatings were also tested. One was alginate-based with the CFS of *Lacticaseibacillus paracasei*, and the other was a combination of chitosan, sodium alginate, and CMC with *Bifidobacterium animalis* subsp. *lactis*, *Lactobacillus acidophilus*, or *Lacticaseibacillus casei*, respectively, against pathogenic microorganisms and *Aspergillus* spp. and *Mucor hiemalis*.

Finally, de Lima Marques et al. (2017) highlighted the use of biodegradable films formulated with the CFS of *Latilactobacillus curvatus* to be placed between slices of Prato cheese, aiming to inhibit the growth of *L. monocytogenes* on the surface and throughout the product. In addition to these types of films, Salvucci et al. (2019) proposed the use of active films incorporating bacteriocin-like substances from the bacterium *Enterococcus faecium* to inhibit the proliferation of *Listeria innocua* in semi-hard white cheese slices.

7.3. Cereals (*table 5*)

Cereals serve as the primary ingredient in fermented products such as bread, where starters are traditionally incorporated into the formulation or sourdough to facilitate fermentation and achieve the desired final product (Calasso et al., 2023).

The incorporation of these microorganisms primarily occurs as an ingredient in the formulation of sourdough or cereals. The most extensively described microorganisms are LAB, specifically *Lp. plantarum* and *Lc. casei*. These bacteria not only promote fermentation to produce the desired food but also exhibit antimicrobial activity against different spoilage microorganisms. The use of these bacteria against *Aspergillus* spp. has been described by multiple researchers (Ebrahimi et al., 2020; Ouiddir et al., 2019; Shehata et al., 2019) in products like bread or wheat sourdough. Another important mold to combat is *Alternaria* spp., for which Calasso et al. (2023) proposed the use of LAB in pizzas and focaccias.

Other methods of application have been described, such as the spraying of two combinations of LAB onto a cereal based dessert known as milk bread rolls, as detailed by Le Lay et al. (2016), which showed that way of addition as a viable alternative for inhibiting the growth of spoilage microorganisms such as *Eurotium repens, Aspergillus niger* and *Penicillium corylophilum.*

Additionally, Gregirchak et al. (2020) proposed the use of edible coatings formulated with various LAB, sodium alginate, yeast, and glycerol to combat molds such as *Aspergillus* spp. and *Penicillium* spp. in wheat bread.

In the case of seeds, not only are studies being developed in which the inoculation of the protective culture or CFS is carried out, but in 2021, Somashekaraiah et al. proposed, with positive results, the immersion of maize seeds in the CFS of *Lb. brevis* MYSN105 at 16% to inhibit the growth of pathogenic microorganisms such as *E. coli, S. aureus*, and *Salmonella* spp. that commonly appear in these ingredients.

7.4. Meat products (*table 6*)

Traditionally, LAB have been incorporated into various meat formulations to produce fermented products. However, their use has evolved, and they are now being utilized as alternatives to artificial preservatives with *E*-numbers.

The most described methods for incorporating these bacteria into meat include dipping, addition to the matrix or formulation, and surface inoculation. For dipping, strains of LAB species such as *Lc. paracasei* (Duan et al., 2020), *Latilactobacillus sakei* and *Lt. curvatus* (Zhang et al., 2017), or *Pediococcus acidilactici* (İncili et al., 2023) have been used on fresh chicken breast to combat *Pseudomonas* spp., *Hafnia alvei*, or *Serratia* spp.; on refrigerated beef to inhibit the growth of *Enterobacteriaceae*, *Pseudomonas* spp., and *Brochothrix thermosphacta*; and on frankfurters, respectively. Additionally, *Carnobacterium maltaromaticum* has been proposed by de Andrade Cavalari et al. (2024) as a potential protective culture for sliced cooked ham.

Surface inoculation involves the addition and spreading of bacteria using an L-shaped seeding loop. Barcenilla et al. (2023) highlighted the effectiveness of this method in fresh cooked ham slices, meatloaf, and roasted pork, using *Lco. lactis*, *Lp. plantarum*, and *Lc. paracasei* bacteria, which showed positive results against *L. monocytogenes*. Additionally, Bungenstock et al. (2021) demonstrated the antimicrobial activity of surface inoculation with *Pediococcus* spp. against *L. monocytogenes.*

The incorporation of these bacteria has also been achieved by inoculating them into the formulation or matrix of the final product. This method aims to inhibit the growth of pathogenic bacteria such as *L. monocytogenes* or *E. coli* in fresh raw top round beef by adding *Lp. plantarum* to the marinade (Arrioja-Bretón et al., 2020). Other bacteria, such as *P. acidilactici*, *Lt. sakei*, and *Staphylococcus* spp., have shown positive effects on shelf-life extension in beef hamburgers by inhibiting the growth of *B. thermosphacta* (Comi et al., 2015) and in Sardinian fermented sausages by inhibiting the growth of *L. monocytogenes* (Siddi et al., 2022). Additionally, *C. maltaromaticum* can be added in this format to inhibit the growth of *Pseudomonas fluorescens* and *B. thermosphacta* (De Andrade Cavalari et al., 2024).

In addition to these conventional techniques, more innovative methods such as the use of active films and edible coatings as carriers of probiotics have been explored. These carriers act as protective cultures due to their antimicrobial activity. This type of film or plastic has been validated in sliced meat products; for instance, Settier-Ramírez et al. (2021) and Pereira et al. (2018) added *Lco. lactis*, *Lc. casei*, and *B. animalis* cultures to sliced ham in active film and edible coating formats, respectively, to inhibit the growth of pathogenic microorganisms such as *L. monocytogenes* and *Pseudomonas* spp.. Additionally, the incorporation of *Lp. plantarum* into the film has been noted for its ability

Table 7
List of publications on bioprotective agents applied in seafood products, according to different strategies of incorporation.

Strategy: Immersion

Food matrix	Microbial source	Pathogen/ spoilage microorganism	Other technologies	Storage	Author
Cold-smoked salmon	*Latilactobacillus sakei*CTC 494 *Latilactobacillus curvatus* CTC 1742 *Carnobacterium maltaromaticum* CTC1741	*Listeria monocytogenes* CTC1500 *Listeria monocytogenes* CTC1500 (in vitro some samples)	Vacuum packing in individual bags	8°C for 21 days	Aymerich et al., 2019
Young hake	*Latilactobacillus curvatus*	Total viable counts			Gómez-Sala et al., 2016
Megrim	BCS35	*Listeria monocytogenes* CECT 4032	Plastic film	0–2°C for 14 days	
Cold-smoked sea bass	*Latilactobacillus sakei* LAK-23	*Listeria monocytogenes*	Vacuum packed in plastic bags (PA/PE)	4°C for 60 days	
Ribbonfish	*Lactiplantibacillus plantarum* SKD4 *Pediococcus stilessi* SKD11	Total viable count	Container	4°C for 5 days/ 25 °C for 5 days	Jo et al., 2021
Cold-smoked salmon (CSS), gravlax, and sushi	*Carnobacterium* spp. *Leuconostoc* spp. *Weissella* spp.	*Listeria monocytogenes* (cepas CCUG 15527 y F11) *Listeria innocua* (cepa CCUG 15531) *Escherichia coli* (cepa CCUG 38079)	Vacuum packing	6 ± 2°C for 60 days	Stupar et al., 2021

Strategy: Sprayed

Food matrix	Microbial source	Pathogen/ spoilage microorganism	Other technologies	Storage	Author
Fresh salmon filets	*Lactiplantibacillus pentosus* 39	*Listeria monocytogenes* ATCC 19117 *Aeromonas hydrophila*atcc 14,715	Sterile plastic bag containers with normal stmospheric air	4°C for 96 h/ 4°C for 144 h or 4 °C for 96 h/ 30 °C for 12 h	Anacarso et al., 2014
Sea bream	*Latilactobacillus sakei* CTC 494	*Listeria monocytogenes* CTC1034	Vacuum packed in plastic bags (PA/PE)	15 to 50 days at 2°C and from 6 to 10 days at 12 °C	Bolívar et al., 2021b
Young hake	*Latilactobacillus curvatus*	Total viable counts			Gómez-Sala et al., 2016
Megrim	BCS35	*Listeria monocytogenes* CECT 4032	Plastic film	0–2°C for 14 days	
Cold-smoked salmon	*Lactococcus piscium* EU2241	*Brochothrix thermosphacta* *Serratia proteamaculans*	Vacuum packed in plastic bags (PA/PE)	4°C for 1 week and 8°C for 3 weeks	Leroi et al., 2015
Cold-smoked rainbow trout	*Leuconostoc carnosum* and *Lactococcus lactis*	*Listeria monocytogenes* CECT 4032, CECT 5366, CECT 935, CECT 5725 and CECT 7467	Vacuum packaging and refrigeration storage		Sánchez-Martín et al., 2025
Fish filet (Grouper family)	*Lacticaseibacillus rhamnosus* GP1 and bacteriocin GP1	Coliforms *Aeromonas* spp. *Lactobacillus* spp. *Vibrio* spp.	Enfolded in sterile aluminum foil and placed in separate sterile boxes	0°C for 28 days/ 4°C for 28 days	Sarika et al., 2019
Cooked and peeled shrimps	*Lactococcus piscium* CNCM I-4031 *Carnobacterium divergens* V41	*Listeria monocytogenes* RF191	Modified atmosphere packaging (50% O2/ 50% N2	8°C for 28 days	Saraoui et al., 2017

Strategy: Sterile syringe

Food matrix	Microbial source	Pathogen/ spoilage microorganism	Other technologies	Storage	Author
Surimi-based product Tuna paté	*Latilactobacillus sakei* CTC 494	*Listeria monocytogenes* CTC1034	Aluminum containers with lids	15 to 50 days at 2°C and from 6 to 10 days at 12 °C	Bolívar, Tarlak, et al., 2021b
Sea bream	*Latilactobacillus sakei* CTC 494	*Listeria monocytogenes* CTC1034	Vacuum-packaged	3 to 12 °C	Bolívar, Costa, et al., 2021a

Strategy: Surface inoculation

Food matrix	Microbial source	Pathogen/ spoilage microorganism	Other technologies	Storage	Author
Surimi-based product	*Latilactobacillus sakei* CTC 494	*Listeria monocytogenes* CTC1034	Vacuum packed in plastic bags (PA/PE)	15 to 50 days at 2°C and from 6 to 10 days at 12 °C	Bolívar, Tarlak, et al., 2021b
Hake fillets	*Lacticaseibacillus paracasei* spp. *paracasei* LAFTI-L26 and *Bifidobacterium animalis* spp. *lactis* LAFTI-B94	*Pseudomonas phosphoreum* CECT 4192 *Shewanella putrefaciens* CECT 5349 T	Bioactive film (agar, glycerol, glucose) + vacuum packed in bags	4°C for 15 days	De Lacey et al., 2014

to inhibit *L. monocytogenes* in fresh ground beef (Yordshahi et al., 2020).

7.5. Seafood products (table 7)

Contemporary seafood products have evolved to meet consumer demands for clean labels, convenience, and rapid consumption. Consequently, ready-to-eat seafood items frequently encounter *L. monocytogenes* contamination, posing significant food safety concerns.

To inhibit the growth of both pathogenic and spoilage microorganisms while providing clean label products, the incorporation of LAB and/or their metabolites is a viable alternative supported by the literature.

Various methods for inoculating these microorganisms into fish or fish by-products have been described, with surface spraying being one of the most extensively studied techniques. For instance, Sánchez-Martín et al. (2025) highlighted the use of *Leuconostoc carnosus* and *Lco. lactis* as protective cultures against different strains of *L. monocytogenes* in cold-smoked rainbow trout. Similarly, Anacarso et al. (2014), Gómez-Sala

et al. (2016), and Bolívar et al. (2021a, b) reported positive results using different LAB strains, including *Lactiplantibacillus pentosus* in fresh salmon filets, *Lt. curvatus* in young hake and megrim, and *Lt. sakei* in surimi-based product and tuna paté, respectively, against *L. monocytogenes*. Other researchers have successfully inhibited the growth of various microorganisms. For example, Leroi et al. (2015) focused on *Serratia proteamaculans* and *Brochothrix thermosphacta* in cold-smoked salmon, using *Lactococcus piscium* bacteria to combat them. Similarly, Saraoui et al. (2017) inhibited the growth of *S. proteamaculans* in cooked and peeled shrimps by using *Lco. piscium* and *Carnobacterium divergens*. As shown in the table, other authors have also achieved positive results working with different products and microorganisms.

Another technique developed in these studies involves immersing the product in a broth containing protective cultures or their CFSs. Several authors have highlighted this methodology in cold-smoked fish to inhibit the growth of *L. monocytogenes* using *Lt. sakei*, *Lt. curvatus* and *C. maltaromaticum* (Aymerich et al., 2019), *Carnobacterium* spp. (Iacumin et al., 2021), *Leuconostoc* spp. and *Weisella* spp. The latter was also applied to two other products, gravlax and sushi (Stupar et al., 2021).

Finally, Bolívar, Tarlak, et al. (2021b) described a technique involving the use of a sterile syringe to inoculate *Lt. sakei* into tuna pate, effectively inhibiting the growth of *L. monocytogenes*.

8. The bioprotective agents market

Clean label is no longer a fad but a lifestyle or food philosophy (Cao & Miao, 2023). It is now a requirement increasingly demanded by consumers seeking alternatives to highly processed foods (Aschemann-Witzel et al., 2019; Asioli et al., 2017). In our view, strategies that enable the food industry to obtain clean label certification are consequently expected to grow in importance in the coming years. One such strategy is the use of bioprotective agents as an alternative to approved food additives.

According to these insights, the market of protective cultures and their culture-derived antimicrobial agents is expected to grow at an annual rate of 6.58% until 2029, increasing from 398.44 million USD in 2021 to 663.39 million USD in 2029. This increase is driven by various factors that have positively influenced the commercialization of these types of ingredients. One key factor is the growing public awareness of the effects of chemical additives, which has led to an increased demand for healthier and more natural products.

Another contributing factor has been the occurrence of the Covid-19 pandemic, which has not only had an economic impact on the food industry and associated industries but also heightened consumers and legislative awareness of the importance of clean label products. Due to this behaviour change and the varying consequences across countries, the bioprotective agents market is adopting different strategies for each of them. The increased consumption of dairy products and the use of protective cultures for the preservation of soured foods, including dairy products (yogurts, cheeses, sour cream) and meat products (sour sausages), also contributed to this trend.

Furthermore, improvements and advances in technology, as well as distribution channels, have facilitated the commercialization of these ingredients. Finally, the strict and variable standards for ensuring food security play a crucial role in driving the market for bioprotective agents.

8.1. Geographical trends in the bioprotective agents market

Europe leads the global market for protective cultures and bacteriocins, holding a 44% share according to recent market analyses. Furthermore, the top 10 list of the most important international commercial producers of protective cultures-related products consists of 70% of European companies. This prominent role could be attributed to the region's historical significance in the dairy sector, evolving consumer concerns, increased production and consumption of dairy products, and advancements in dairy food production. Additionally, the use of protective cultures and bacteriocins as additives in dairy, meat, and seafood products has risen, driven by the need for longer shelf-life products to address food corruption concerns.

In the case of Asia-Pacific region, market growth is anticipated due to a shift towards a more Westernized lifestyle. This includes increased consumption of organic and natural (clean label) products and dairy products, as well as heightened awareness and knowledge about bioprotective agents in countries such as India and China.

Finally, in North America, market growth is partly driven by increased consumption of dairy, meat, and seafood products, like trends observed in the Asia-Pacific region. Additionally, consumer concerns for safe and RTE foods, facilitated using natural food preservatives, are propelling the market for bioprotective agents in this region.

8.2. Examples of commercially available products

The bioprotective agents market is currently highly fragmented, with a mix of global suppliers and national specialists. Competition is intense within regions, and no single dominant operator can be pointed out.

Market analyses consistently identify a group of companies that are most frequently cited as key producers of protective culture–based products, consisting of Novonesis (formerly Chr. Hansen), THT S.A., BIOPROX, DSM-Firmenich and Kerry Group. Each of these companies offers a variety of starter and protective cultures tailored for different processes and food products.

Table 8 presents a selection of representative commercial solutions, including the microorganisms typically used, the target microorganisms, and the food matrices for which they are intended. Despite the commercial secrecy surrounding exact strain compositions and manufacturing processes, several trends can be inferred from the available information shown in this table. Across suppliers, LAB remain the primary protective cultures used today, manufactured primarily as multi-strain blends. *Lactococcus* appears most frequently, followed by *Streptococcus* and members of the former *Lactobacillus* group (e.g., *Lactiplantibacillus*, *Lacticaseibacillus*, and *Latilactobacillus*). The predominant foodborne and spoilage organisms of concern include *L. monocytogenes*, *Clostridium tyrobutyricum*, *Staphylococcus* spp., *Bacillus* spp., and spoilage yeasts and molds. These bacterial targets are predominantly Gram-positive, consistent with the natural activity spectrum of LAB bacteriocins (e.g., nisin, plantaricins, sakacins, pediocins), while yeasts and molds are additionally controlled through organic acid production, competitive exclusion, and other metabolic antifungal mechanisms. Furthermore, they are especially relevant in the selected food matrices which are typically high-moisture, minimally processed, and refrigerated foods.

Regarding formulation, a clear distinction exists between the major suppliers. The majority of products are marketed as live bacterial concentrates, typically supplied as freeze-dried powders or frozen pellets. One exception is the product listed from the Kerry Group, which is listed as a cell-free dried fermentate.

Overall, the composition and antimicrobial profiles of the presented commercial protective cultures are aligned with the published scientific literature, where LAB and bacteriocin-producing strains are the most widely and validated strategies for preserving refrigerated and fermented products.

9. Limitations and opportunities

Despite the growing interest in protective cultures and culture-derived antimicrobial agents as clean label strategy for food biopreservation, several limitations still constrain their broader implementation. One major challenge is the lack of clear and harmonized legislation, particularly the absence of detailed labelling requirements in commercial products. This regulatory ambiguity allows companies to

Table 8
Overview of selected commercial protective culture-based solutions, showing the main microorganisms included in each formulation, the target spoilage or pathogenic microorganisms, and the intended food applications.

Product (Company)	Microbial source	Target microorganism	Target food
FRESHQ® (Novonensis)	Not disclosed	Molds and yeasts	Yogurt, sour cream, quark, kefir, tvorog, fresh white cheese, cottage cheese, pasta filata
SAFEPRO® (Novonensis)	Not disclosed	*Listeria monocytogenes*	Meat and processed meat products
BIOSAFE®	Not disclosed	*Clostridium tyrobutyricum*	Cured cheeses
VEGA™ (Novonensis)	Not disclosed	Not disclosed	Fermented vegetables (kimchi, sauerkraut, pickles)
VEGA™ SAFEPRO® (Novonensis)	Not disclosed	Plant-based meat alternatives	Fermented vegetables
Not disclosed (THT S. A.)	*Lacticaseibacillus sakei, Lactiplantibacillus plantarum, Latilactobacillus curvatus*		
Not disclosed (THT S. A.)	*Pediococcus acidilactici, Pediococcus pentosaceus*	*Listeria monocytogenes*	Dry-cured sausages (salchichón, chorizo, fuet) and other cured sausages
Not disclosed (THT S. A.)	*Staphylococcus aureus, Staphylococcus xylosus*		
LACTO-PROX® (BIOPROX)	*Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus acidophilus*, *Bifidobacterium animalis* subsp. *lactis*, *Lactococcus lactis* and *Leuconostoc* spp.	*Listeria monocytogenes, Salmonella* spp. and *Escherichia coli*	Set-style yogurts, milk-based shakes, and drinkable yogurts
MEDIA-PROX® (BIOPROX)	*Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris, Leuconostoc mesenteroides, Streptococcus thermophilus, Lactobacillus* spp.	*Listeria monocytogenes* and *Clostridium* spp.	Mesophilic fermented dairy products, smooth yogurts and ambient yogurts, and fermented milks with specific textures
YO-PROX® (BIOPROX)	*Streptococcus thermophilus, Lactobacillus delbrueckii* subsp. *bulgaricus*	*Listeria monocytogenes, Salmonella* spp. and *Escherichia coli*	Yogurts (set-style, stirred, and drinkable yogurts; ambient yogurts; protein-enriched yogurts; and reduced-sugar)
DI-PROX® (BIOPROX)	*Lactococcus lactis* subsp. *lactis, Lactococcus lactis subsp. cremoris, Leuconostoc mesenteroides, Streptococcus thermophilus*	*Listeria monocytogenes, Clostridium* spp., *Staphylococcus aureus, Salmonella* spp.	Fermented and fresh cheeses
DI-PROX®K (BIOPROX)	*Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris, Leuconostoc mesenteroides, Streptococcus thermophilus*	*Listeria monocytogenes, Clostridium* spp., *Staphylococcus aureus, Salmonella* spp.	Kefir
DI-PROX MTTX® (BIOPROX)	*Lactobacillus delbrueckii* subsp. *bulgaricus, Streptococcus thermophilus*	*Listeria monocytogenes, Clostridium* spp., *Staphylococcus aureus, Salmonella* spp.	Dahi
AROMA-PROX® (BIOPROX)	*Lactococcus lactis* subsp. *lactis biovar diacetylactis*, *Leuconostoc mesenteroides, Lactococcus lactis* subsp. *cremoris*	Not disclosed	Fermented and fresh cheeses, and other dairy products
Dairy Safe™ (DSM-Firmenich)	*Lactococcus lactis*	*Clostridium tyrobutyricum, Bacillus* spp., *Staphylococcus* spp.	Semi-hard, hard, and continental cheeses (cow, goat, sheep)
Delvo®Guard (DSM-Firmenich)	*Lacticaseibacillus rhamnosus, Lacticaseibacillus sakei, Lactococcus* spp.	Molds and yeasts	Yogurts and other fermented dairy products
Delvo®One (DSM-Firmenich)	*Streptococcus thermophilus, Lactobacillus bulgaricus, Lacticaseibacillus rhamnosus, Lacticaseibacillus casei/paracasei* and *Bifidobacterium animalis* subps. *Lactis*	Molds (*Candida* spp., *Kluvyeronyces* spp.), *Clostridium tyrobutyricum, Listeria monocytogenes, Bacillus* spp., *Staphylococcus aureus, Lactobacillus* spp. and yeasts	Yogurts
DuraFresh™(Kerry Group plc)	Not disclosed	Yeasts and molds	Bakery products, dairy products, plant-based beverages, ready-to-eat meals

advance commercially, but often without transparent communication to consumers. However, many companies are actively collaborating with technological centres and universities to engage with regulatory bodies, aiming to accelerate approval processes and demonstrate the safety and efficacy of these bioprotective agents.

Additionally, the efficacy of the bioprotective agents is highly matrix dependent, which complicates generalization across products. Nevertheless, this limitation has encouraged early-stage, product-specific in situ testing, allowing researchers to tailor applications to specific food environments and improve reliability.

Importantly, **the absence of explicit regulatory guidance has not prevented the commercialization of bioprotective solutions**, and several products currently available on the market. However, there is a lack of standardization in labelling practices. Furthermore, consumer education remains a barrier, although protective cultures and their derived products align with clean label expectations, their microbial nature and function are often misunderstood. This highlights the need for **clear communication strategies based on scientific evidence**, emphasizing both safety and functional benefits. At the same time, this gap in understanding represents an opportunity to integrate targeted education, transparent labeling, and evidence-based marketing approaches.

From a research perspective, the detailed analysis of the selected studies (Tables 3–7) indicates that edible coatings are the most frequently explored application strategy, in line with the perishable nature of the foods considered and the suitability of surface-based interventions. Expanding research into this and other strategies of applications of known and novel bioprotective agents in different food matrices would be needed to advance in this research field. In particular, further work is needed to move beyond proof-of-concept studies towards transferable and scalable approaches, including validation across industrially relevant processing technologies, assessment of effects on product shelf life, and evaluation of impacts on sensory quality and product identity, including consumer perception.

10. Conclusions

Protective cultures and culture-derived antimicrobial agents are a

sustainable and effective clean label alternative for food preservation, aligning with consumer expectations for natural and minimally processed foods. Their ability to inhibit spoilage and pathogenic microorganisms without negatively affecting sensory properties makes them a valuable tool for the food industry. The scientific evidence and market trends confirm their increasing relevance across diverse food sectors, including fruits, vegetables, dairy, cereals, meat, and seafood. However, challenges remain regarding regulatory approvals, consumer acceptance, and ensuring consistent efficacy across food matrices. Continued research, including well-designed industrial trials, is essential to optimize the application of bioprotective agents, sustain the basis for the clarification of legal frameworks, and evaluate their effectiveness under real processing conditions. Overall, bioprotection represents a key strategy in advancing towards sustainable food preservation while meeting the demands of the clean label market.

CRediT authorship contribution statement

Nerea Garín-Murguialday: Writing – review & editing, Writing – original draft, Visualization, Investigation, Data curation. **Raquel Virto:** Supervision, Funding acquisition, Conceptualization. **Rafael Pagán:** Writing – review & editing, Funding acquisition, Conceptualization. **Laura Espina:** Writing – review & editing, Writing – original draft, Visualization, Funding acquisition.

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Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this manuscript, the authors used ChatGPT and/or Google's Gemini to assist with improving the clarity and readability of certain sentences in English. Following this assistance, the authors reviewed, edited, and approved all content, and take full responsibility for the final work.

Declaration of competing interest

The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of the data; in the writing of the manuscript; or in the decision to publish the results.

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Data availability

Data will be made available on request.

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