Journal of Asian Scientific Research
ISSN(e): 2223-1331
ISSN(p): 2226-5724
DOI: 10.55493/5003.v15i1.5333
Vol. 15, No. 1, 111-121.

URL: www.aessweb.com

Novel applications of Lactobacillus in the food industry

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Article History
Received: 5 February 2025
Revised: 11 March 2025
Accepted: 26 March 2025
Published: 4 April 2025

Keywords
Biocontrol agents
Fermented products
Lactobacillus
Natural preservatives
Secondary metabolites.

ABSTRACT

The continuous growing interest of consumers in sustainable, natural, and safe products, together with the problems associated with the use of chemical pesticides and food preservatives, makes it necessary to search for new approaches. Food industries have widely used lactic acid bacteria (LAB) as a natural source due to their ability to produce antibacterial/antifungal substances capable of effective biocontrol. Lactobacilli have the futuristic potential for utilization in a diversity of industrial applications and hence can be considered a significant candidate. This paper highlights and emphasizes the commercial applications of Lactobacilli in food, feed, wine, and agriculture, providing a snapshot of the contribution of biocontrol agents as a suitable alternative in the food industry. Furthermore, several raw materials such as cereals, fruits, and vegetables have recently been investigated in this paper to determine their suitability for designing valuable products. The novelty brought by this review is the presentation not only of the summarized results and diverse applications of the Lactobacillus genus, new bioprocess technology, but also an expanded view of the new patents, creating a valid database in the future for scientific researchers and the food industry, evidencing the interest in this area.

Contribution/ Originality: This review presents its originality in the new strains of Lactobacillus, in the methodology used, and in specifying the concentrations necessary for inhibition and improvement of technological processes. The summary of results in tabular form provides a more accurate overview and contributes to scientific research. The presentation of new patents, which has not been done before, could help design novel probiotic products or starter cultures in the food industry.

1. INTRODUCTION

In recent years, growing interest has been shown in the use of biological control agents, including living organisms or natural substances, to improve the taste or texture of foods, reduce the use of chemicals, increase food shelf life, and prevent fungal growth, especially related to Aspergillus, Penicillium, Fusarium, and Claviceps spp. contamination [1]. Biological control is defined as the process by which natural enemies reduce pest populations, usually with human intervention. Lactic acid bacteria (LABs) have been particularly studied as biological control agents for the secondary metabolites produced by the aforementioned fungi [2]. Moreover, LABs have been shown to be capable of degrading or binding mycotoxins, e.g., in cereals, serving as a tool to reduce their contamination [3].

Since some of the metabolites produced by these microorganisms require high concentrations to be effective, there is a need to standardize the spectrum of antimicrobial activity and the proper form of application, e.g., granules, powders, solutions, sprays (aerosols), emulsions, or suspension concentrates, where the effect would be most lethal, maximizing the efficacy of these compounds [4].

1.1. Lactobacillus Strains: An Overview

Lactic acid bacteria (LAB) belong to the Lactobacillus order, which includes 6 families, 36 genera, and more than 262 species [5]. They are generally regarded as safe (GRAS) and play an important role in the preservation of foods and fermented food products, where they produce organic acids, enzymes, and metabolites that contribute to the organoleptic properties, flavor, and long shelf life of the final products [6]. The secondary metabolites produced by these strains exhibit a natural preservative effect and can inhibit the growth of harmful microbes in anaerobic environments, including food-borne diseases, molds, yeasts, and spoilage bacteria. Pore creation, cellular Deoxyribonucleic Acid (DNA) degradation, disruption by selective cleavage of 16S rDNA, and suppression of peptidoglycan production are some examples of their bactericidal mechanisms [7]. The ability of lactic acid bacteria to preserve food is primarily attributed to their production of organic acids like lactic acid. Consequently, the pH is lowered, which prevents many germs from growing. In addition to lactic acid, several other metabolites are crucial for the preservation of food and animal feed, including acetic acid, hydrogen peroxide, diacetyl, reuterin, and so-called bacteriocins [8]. Different methods have been proposed for the applications of these secondary metabolites: i) direct inoculation of LAB into the food; ii) addition of bacteriocins to food in a purified or semi-purified form; iii) use of products fermented with a bacteriocin-producing strain [9]. The chemical structures of selected secondary metabolites produced by LABs and their applications are reported in Figure 1 and Table 1, respectively.

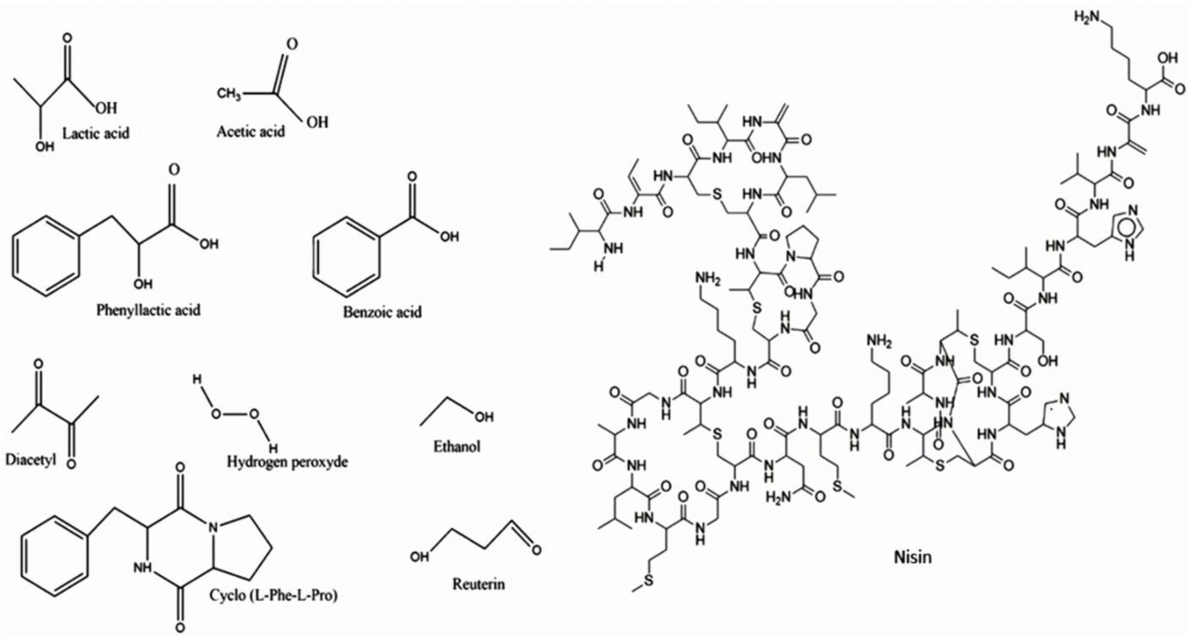

Figure 1. Chemical structures of selected secondary metabolites produced by *Lactobacillus* strains.

Table 1. Application of secondary metabolites produced by *Lactobacillus* strains.

Strain	Antimicrobial metabolite	Application	Results	References
L. lactis	Nisin, Lacticin 3147, Lactisin 481	Milk, meat, egg, cheese.	Nisin at 1-25 ppm prevent the growth of: *L. monocytogenes, S. aureus.* *B. cereus* were reduced by 80% in soup, in the presence of 1% (w/w) lacticin 3147	Branen and Davidson [8] and Morgan, et al. [10]
L. plantarum acr2	Pediocin PA-1	Meat, dairy products, wine	*Oenococcus oeni* in wine resulted sensitive to pediocin PA-1 (IC_{50} = 19 ng/ml)	Díez, et al. [11]
L.plantarum 220 *L.plantarum 221* *L.plantarum 748*	Hydroxibenzoic acid, acetic acid phenyllactic acid lactic acid, ethanol, hydrogen peroxide	Bread, grain, rice.	50 mg /mL of organic acids inhibitory effect range from 75.5% to 99.9%	Broberg, et al. [12] and Luz, et al. [13]
L. reuteri	Reuterin	Yogurt, cottage cheese, skim milk	Fungicidal activity 99.9% at concentrations 15.6 mM.	Vimont, et al. [14]
L. casei	Diacetyl	Bakery products, fermented dairy products	100 ppm of diacetyl bactericidal effect against *E. coli* and *S. aureus.*	Jyoti, et al. [15] and Lanciotti, et al. [16]
L. amylovorus	Nucleosides;cytidine and 2-deoxycytidine, Ciclo (L-Phe-L Pro)	Bakery products	82mM can inhibit *P. roqueforti* and *A. fumigates*	Ström, et al. [17]

1.2. Recent Evidence of the Research in the Last Decade

Nowadays, there has been a growing interest in bio preservation, which is a biological technique for preserving food using microbes and their byproducts. This interest has been driven by consumers' increased knowledge of the harmful effects of chemical preservatives on health.

The presence of probiotic microbes and increased nutrients in the product contribute to its positive impact on the health-promoting value of food, as they have the potential to enhance microbiological safety [18].

Food industry concerns are centered around foodborne pathogens, food spoilage bacteria, and biocontrol agents. Food can be preserved using a variety of approaches, such as high-pressure or thermal processing, food radiation, and the addition of additives, all of which have the potential to significantly modify the chemical and organoleptic properties of foodstuffs [19]. Recent approaches to ensure preservation products have increasingly directed towards using effective biocontrol microorganisms, e.g., LABs. These refer to the use of epiphytic or regulated microbiota, or their metabolites, to prevent the growth of microorganisms, prolong food shelf life, and ultimately improve food safety.

Lactic fermentation is employed for milk acidification, which is essential for the manufacturing of several fermented dairy products, including yogurts, cheese, butter, and sour cream [18]. There is thus a growing interest in antifungal compounds from LABs, as few strains exhibit antifungal activity [20]. In this regard, food industries face a new challenge, and research is being triggered by this challenge to study possible solutions. Several studies have been conducted to explore the antimicrobial potential and possible biotechnological applications of natural fermented products. Table 2 shows the relevant published studies in the last ten years regarding Lactobacillus strains as biocontrol agents.

Table 2. Examples of LABs application as biocontrol agents in foodstuff.

Biocontrol agent	Pathogen target	Food application	Results	Reference
Lactobacillus plantarum LR/14	*Aspergillus niger, Rhizopus stolonifer, Mucor racemosus, Penicillium chrysogenum*	Cereal grains	The peptide AMPs from LR/14 suitable for food-related applications.	Gupta and Srivastava [20]
Lactobacillus plantarum AF1	*Aspergillus flavus*	Soybean; (*Glycine max*)	*A. flavus* (10^7spores/mL) did not germinate in soybeans treated with LAB	Yang and Chang [21]
Lactobacillus plantarum	*Aspergillus carbonarius*	Grape berries	10^5 CFU/mL of LAB reduced 92% Ochratoxin A.	Lappa, et al. [22]
Lactobacillus sakei GM3	*Pseudomonas aeruginosa; Klebsiella pneumonia, Salmonella; Candita albicans*	Goat milk	Bacteriocin by *L. sakei GM3* showed inhibitory activity (45.60 ± 0.5%)	Devi Avaiyarasi, et al. [23]
Lactobacillus plantarum 220, 221, 748	*Fusarium graminearum, Penicillum expansum, Aspergillus flavus,*	Freeze dried whey	*L. plantarum 220* increased the shelf-life of food.	Essia Ngang, et al. [24]
Lactobacillus sakei ST22Ch, ST153Ch, ST154Ch	*Enterococcus faecium; Listeria ivanovi*	Smoked pork meat	Bacteriocins showed 99% antibacterial spectrum	Todorov, et al. [25]
Lactobacillus amylovorus DSM19280	*Aspergillus niger; Fusarium culmorum; Penicullum roqueforti*	Bakery products	Seventeen novel antifungal compounds were isolated	Ryan, et al. [26]
Lactobacillus curvatus MBSa2	*Listeria monocytogenes*	Salami (10% bovine meat/ 75% pork / 15% lard)	Bacteriocin decrease *L. monocytogenes* up to 2 log	Barbosa, et al. [27]
Lactobacillus brevis	*Fusarium culmorum*	Barley malt	Synergism between bacteroicins were detected	Peyer, et al. [28]
Lactobacillus amylovorus	*Penicillum expansum*	Cheddar cheese	Shelf-life of cheeses for 12 days were detected	Lynch, et al. [29]
L. plantarum B4496; *L. brevis* 207; L. *sanfranciscensis* BB12	*Aspergillus carbonarius, Aspergillus niger, Aspergillus ochraceus*	Cacao beans	In vitro activities towards the three ochratoxin-producing fungi	Chheda and Vernekar [30]

1.3. Literature Quantitative Analysis

To further evidence the interest in the challenges mentioned in the proposed review paper and the innovative aspects of the current research in the area of interest, a survey of the Scopus database (www.scopus.com) was performed. It was recorded on February 3rd, 2025, using the following keywords and operators: “Lactobacillus” AND “Biocontrol AND Agents” OR “Bacteriocins” OR “Antifungal AND Activity,” which resulted in a total of 3,283 published papers indexed in the Scopus core collection from 2000 to 2025. VOSviewer software version 1.6.16 (https://www.vosviewer.com) was used for data analysis, generating the bibliometric maps shown in Figure 2.

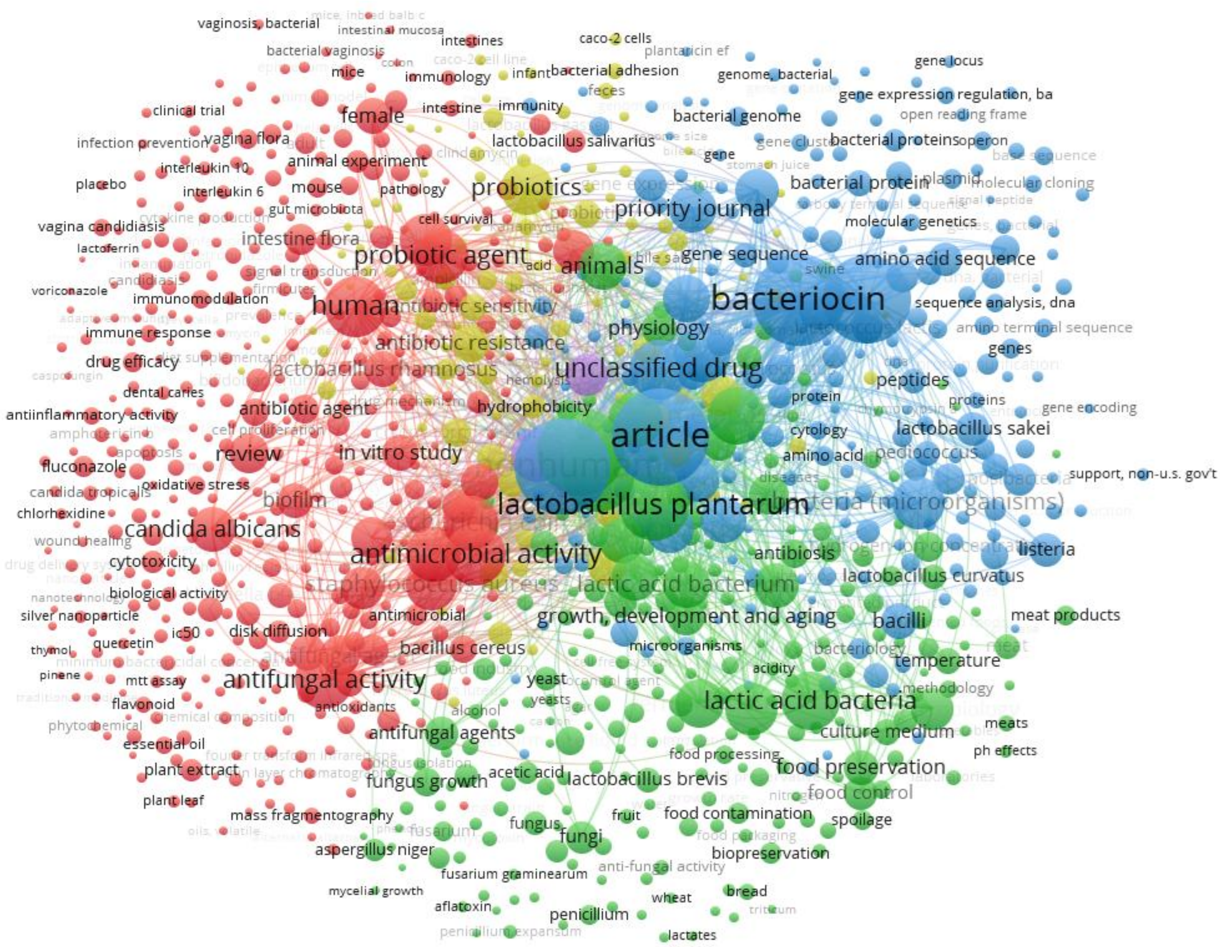

Figure 2. Bibliometric maps obtained by VOS viewer software version 1.6.16 (https://www.vosviewer.com), recorded from Scopus database (accessed on February 3rd, 2024).

The outputs were based on 3,283 papers (the VOS setting was set to a minimum of five occurrences of a keyword). Under these condition settings of the evaluated keywords, 320 reached the threshold, divided by the software into 13 clusters, which can be distinguished in the term maps (bubble maps) evidenced by the different colors in Figure 2. One of the clusters is ethanol, which should not be considered since it is not pertinent to the search. Among the shown clusters, the most relevant cover the topics of bacteriocins, lactic acid bacteria, bio-preservation/bio-preservatives, antifungal, and antifungal activity. As shown in Figure 3, the papers (represented as percentages) were linked to Agricultural and Biological Sciences, Immunology and Microbiology, Multidisciplinary, Environmental Science, Chemical Engineering, Pharmacology, Toxicology, and Pharmaceutics, which, together with the food sector, represent the areas of interest for the use of Lactobacillus.

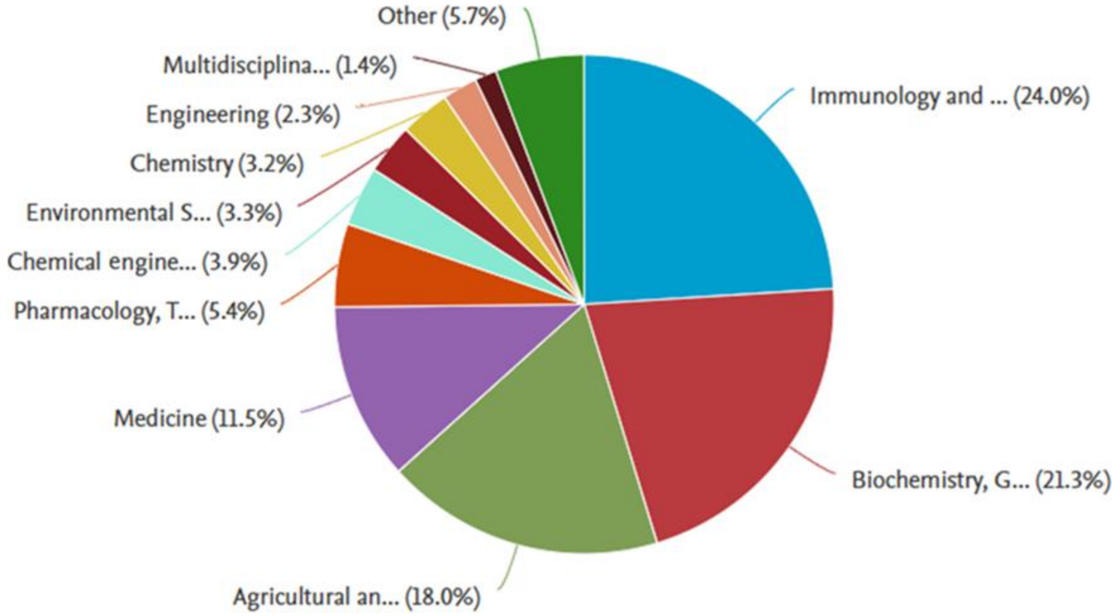

Figure 3. Documents by subject areas.

1.4. Novel Proposed Applications: Patents

The trends of modern consumers to choose natural preservatives over artificial additives motivate food scientists and the food sector to discover novel and potentially beneficial sources. The interest in natural bio-preservation is going beyond boundaries, so efforts have been made, and the industry has recognized the need to investigate to fulfill consumer demand and to offer more innovative products. While synthetic chemical fertilizers hold a significant portion of the pesticide business, biopesticides are becoming increasingly important. As a result, developing a patent that might open up new opportunities in this field has become a difficult task with fierce competition. The properties of Lactobacillus are used as a key factor that stimulates the industry to bring novel formulations and encourages further advancements in the future. The search for patents regarding these aspects has been performed on the official sites available online (public domain), e.g., the European Patent Office website (https://www.epo.org/index.html - last accessed February 5, 2025), the United States Patent and Trademark Office (USPTO) (https://www.uspto.gov/ - last accessed February 5, 2025), and on the datasets available online at the addresses: Google Patents, https://patents.google.com/, Global Dossier (United States Patent and Trademark Office), https://globaldossier.uspto.gov/#/home, and Justia Patents, https://patents.justia.com (last accessed February 5, 2025). The keywords used to search the patent datasets mentioned above were: Lactobacillus, novel inventions, new strains, and bio-preservative. This search provided the opportunity to select the patents and present a good overview of the available patents and their potential impact on the agro-food industry. Table 3 summarizes the recent patents that have been proposed for application to the market in the last decade.

Table 3. Recent reported patents regarding the Lactobacillus strains.

Patent number	Year	Patent description	Category of the invention	Country of origin
US20140341872A1	2014	Novel compositions and methods for reducing *L. monocytogenes* during food production by LAB	Bio-sanitizer; bactericide	United States
CN102559561A	2014	New food-sourced milk-acid bacteria strain *Lactobacillus radiumii* M2011381 against food pathogens	Food preservative	China
US20140023749A1	2014	New method utilized diacetyl produced by *Lactobacillus rhamnosus*	Starter culture in dairy industry	United States
US8980611	2015	New method by using different *Lactobacilus* spp strains to inhibit pathogen in meat	Bactericide	United States
US9468231B2	2016	Novel strain *Lactobacillus curvatus* DSM 18775 useful for preserving food products	Food bio preservative	France
US20120201795	2015	New compositions for inhibiting pathogenic growth achieved by LAB on plant materials	Bio pesticide	United States
EP2543246A1	2017	New formulation with emulsion polymer and a biopolymer dispersion by utilized LAB to protect cheese coatings	Food bio preservative	Netherland
AU2014230777B2	2017	Novel Lactobacillus strains and the uses thereof, in particular for preserving foods, animal feedstuff, pharmaceutical compositions and/Or cosmetic compositions.	Food bio preservative	Australia
US10174347B2	2019	New methods for producing lactic acid from organic waste during the fermentation	Food bio preservative	United States
US10588320B2	2020	Cell free supernatant composition of microbial culture from Lactobacillus spp for agricultural use	Bio pesticide	United States
CN116286520A	2023	New strain *Lactobacillus plantarum E2* application thereof in preventing and treating visceral ichthyophthiriasis of large yellow croaker	Food bio preservative	China
CN114449895B	2024	The methods and compositions utilize Genus Lactobacillus (Sporolactobacillus sp.), incorporated into plant seeds to improve plant growth and other characteristics.	Bio pesticide	China

The data were retrieved from the United States Patent and Trademark Office [31], European Patent Office [32], Google Patents, Global Dossier, and Justia Patents. All this evidence holds promise to elucidate further by product matrices, the biodiversity of strains, low operational costs, relatively easy follow-up treatment/solvent, and improvements in biotechnology processes, to expand the understanding of the activity of fermented products to determine their efficacy more accurately for future applications.

2. DISCUSSION

The problems associated with the use of chemical pesticides and food preservatives open a field where safer and more effective methods of control are clearly needed. This review aims to summarize the recent literature and patents regarding the use of Lactobacillus spp. strains as a natural alternative to chemicals for biocontrol. Published results from both in vitro and in vivo assays, according to different authors, show that Lactobacillus spp. have been used increasingly in food industries as natural preservatives and antimicrobial agents against foodborne pathogens, despite the pharmaceuticals that support the immune system and provide health benefits.

The market analysis, performed by searching international patents on this, evidences a global current trend, spanning from the EU to the US and China, toward the use of biocontrol strategies. Patented products were focused on fungicides, bactericides, and food preservative categories. A number of commercial formulations have been directed to: (i) the use of new active members; (ii) providing effective methods for decontaminating; (iii) improving the technology and optimizing bacteria suitable for application.

Even though this paper focuses on Lactobacillus, other species such as Streptomyces spp. have been shown to produce natural antimicrobial agents, e.g., Natamycin or ε-Polylysine, which are used as antifungal additives in the food industry to prevent the germination of fungal spores and to control fungal diseases [33]. Additionally, Bacillus strains produce lipopeptide biosurfactants, e.g., Surfactin, fengycin, which possess antimicrobial, antiviral, anti-adhesive, and insecticidal properties [34]. In conclusion, this review highlights the status of research and application by assessing the bio-control agents' activity as a suitable alternative against foodborne pathogens while also evaluating novel formulations. Nonetheless, further communication between researchers and food companies is needed. The properties of Lactobacillus serve as a key factor that stimulates the industry to develop novel formulations and encourages progress in the future. The future technique of bio-preservation as a promising alternative will be microencapsulation, as the coating may improve microbiological activity and antimicrobials may interact less with food ingredients when encapsulated, thereby avoiding their inactivation [35]. To direct future research and development efforts toward strains with the highest likelihood of commercial success, a practical bioassay that emulates commercial production and application environments must be created.

Funding: This research is supported by Albanian University, Albania (Grant number: 2025/11).
Institutional Review Board Statement: Not applicable.
Transparency: The authors state that the manuscript is honest, truthful, and transparent, that no key aspects of the investigation have been omitted, and that any differences from the study as planned have been clarified. This study followed all writing ethics.
Competing Interests: The authors declare that they have no competing interests.
Authors' Contributions: All authors contributed equally to the conception and design of the study. All authors have read and agreed to the published version of the manuscript.

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