

The Genus *Weissella*: A Review of its Antimicrobial Potentials, Functional Characteristics and Uses in Food Biopreservation

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Abstract

Background and Aim: Heterofermentative lactic acid bacteria belonging to the Genus *Weissella* possess unique metabolic capabilities with distinct value for natural food preservation. This review has synthesized current insights into the physiological characteristics, antibacterial pathways and antifungal mechanisms of *Weissella* species, with their practical uses within diverse food systems and safety problems restricting their commercial adoption.

Material and Methods: A comprehensive literature search was carried out using PubMed, Scopus, Web of Science and Google Scholar for peer-reviewed literature published up to June 2026. The review covered *Weissella* taxonomy, ecological distribution, functional characteristics, antimicrobial activities and mechanisms, food biopreservation and biosafety. Relevant literature was critically screened and synthesized based on strain characteristics, bioactive metabolites, mechanisms of action, food-matrix uses, preservation outcomes and safety considerations.

Results and Conclusion: The reviewed studies have indicated that *Weissella* shows strain-dependent antimicrobial activity mediated by multiple compounds, including organic acids, volatile organic compounds, bacteriocins and exopolysaccharides. These factors can inhibit foodborne and spoilage microorganisms through acidification, metabolic disruption and damage to fungal membranes and hyphae, with strains showing potentials for aflatoxin B1 adsorption. Antimicrobial effects have been reported against several bacterial and fungal pathogens, while food-model studies demonstrate decreases in microbial load and delayed visible spoilage in various food matrices. However, these effects cannot be generalized within *Weissella* because antimicrobial production, technological characteristics and safety characteristics differ within the strains. Therefore, further strain-specific safety assessment, standardized efficacy assessment, mechanistic studies and pilot and industrial-scale validation are needed before reliable use of *Weissella*-based biopreservation systems.

Conflict of interest: The authors declare no conflict of interest.

What is “already known”:

- Filamentous fungi cause degradation of sensory and nutritional quality and posing health hazards through mycotoxin production.
- Synthetic additives have been widely used but face increasing consumer concern regarding health effects and antimicrobial resistance
- LABs possess potentials as natural biopreservatives, offering eco-friendly and safe alternatives to synthetic chemicals.
- Taxonomy, ecology, fermentation, functional and antimicrobial activities of *Weissella* Sp. are reported with limited integration of strain-specific evidence within food safety considerations.

What this article adds:	• This review integrates strain-level antimicrobial activities with their reported metabolites, mechanisms of action, food-use contexts and safety considerations. • This review organizes the available evidence based on experimental level, from <i>in vitro</i> to pilot and industrial-scale contexts, making clear the limited evidence extending beyond laboratory conditions. • The review highlights specific knowledge gaps in strain-level biosafety, antimicrobial resistance, mechanistic validation, reproducibility within food matrices and validation under realistic processing and storage conditions. • By linking strain identity, antimicrobial activity, mechanisms, use context, evidence level and safety, this review provides an integrated framework for assessing the potential of <i>Weissella</i> as a food biopreservation resource.
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1. INTRODUCTION

Filamentous fungi are some of the most persistent and economically significant sources of food contamination in the global food supply chain. Fungal colonization within staple supplies such as dairy, cereals, grains and fresh produce causes progressive degradation of organoleptic characteristics, nutritional value and market acceptability [1,2]. Therefore, fungal contamination poses a significant challenge to the food industry compromising safety and quality of food products [3]. In addition to physical deterioration, filamentous fungi synthesize mycotoxins that represent critical biological hazards in foods. While ingestion of contaminated supplies triggers acute toxicosis [4], chronic dietary exposure is linked to severe long-term health consequences, including developmental delays and nutritional impairment in early childhood [5]. Foodborne diseases are still a major global public health concern, with the World Health Organization (WHO) estimating that approximately 866 million people were ill and 1.52 million died from foodborne diseases worldwide in 2021 [6]. Furthermore, fungal contamination and mycotoxin occurrence pose substantial challenges throughout the global food supply chain. Eskola *et al.* estimated that mycotoxins were detectable in up to 60–80% of food-crop samples, indicating that contamination at detectable levels is substantially more widespread than estimates based on regulatory exceedances alone [7].

Fresh fruits and vegetables are particularly susceptible to fungal deterioration during postharvest handling, storage and distribution. Despite advances in agricultural production and postharvest management, fungal diseases are still major constraints to food security because they decrease product quality, shorten storage life and decrease market value [8]. Postharvest vulnerability is further heightened in tropical and subtropical climates, where

increased ambient temperatures and relative humidity accelerate fungal proliferation. Highly perishable climacteric fruits such as mango, banana, strawberry, pineapple and papaya are susceptible to contamination by *Botrytis cinerea*, *Penicillium expansum*, *Colletotrichum* species and *Rhizopus stolonifer* during handling, transport and storage [9,10]. These pathogens typically enter host tissues via physical wounds or natural openings, rapidly colonizing plant tissues and driving severe postharvest decay and economic loss [11]. Species within *Aspergillus* and *Penicillium* represent critical postharvest threats because they merge aggressive spoilage capability with potent mycotoxin synthesis [2], positioning fungal contamination as food security and public health concern. Adequate consumption of fruits and vegetables is an important component of a healthy diet. For example, WHO links insufficient fruit and vegetable intake to nearly 19% of gastrointestinal cancer deaths, nearly 31% of ischaemic heart disease deaths and nearly 11% of stroke deaths [12]. Nevertheless, postharvest preservation is still challenging because physiological processes associated with maturation and postharvest deterioration continue after harvest [13]. Quality retention is further compromised by transpiration-driven water loss, tissue senescence and opportunistic bacterial (*Pseudomonas* spp.) or fungal infections (*Fusarium* spp., *Alternaria alternata* and *C. gloeosporioides*), which routinely damage high-value crops such as tomatoes and avocados [9,11].

To lessen postharvest deterioration, conventional food preservation depends on physical treatments, modified atmosphere packaging and chemical additives such as sorbates, benzoates, propionates, ethanol and ethylenediaminetetraacetic acid (EDTA). While these methods enhance microbial stability and extend shelf life, their long-term reliance faces increasing studies [14].



Nevertheless, exposure to chemical and biological preservatives can induce microbial stress responses and may contribute to the development of preservative-tolerant or antimicrobial-resistant microbial strains [15]. In contrast, physical preservation technologies may need substantial capital costs and potential degradation of organoleptic and nutritional quality [16]. These constraints have driven research to sustainable, natural preservation alternatives that balance broad-spectrum antimicrobial efficacy with consumer safety expectations [17].

Biopreservation uses beneficial microorganisms or their metabolites to suppress pathogens and spoilage organisms while preserving food quality. Compared with conventional chemical preservatives, this approach includes clean-label demand, although efficacy depends strongly on the food matrix, target organism and use conditions [18–20]. Lactic acid bacteria (LAB) are widely identified as promising biopreservative microorganisms due to their long-standing use in food fermentation and their ability to produce a range of antimicrobial and functional metabolites, including organic acids, bacteriocins, hydrogen peroxide and exopolysaccharides (EPS) [17,21]. Nevertheless, these characteristics are not necessarily shared by all *Weissella* strains, as their functional characteristics can vary significantly at the strain level.

Although *Weissella* has been discussed in relation to taxonomy, fermentation and probiotic potential, the evidence connecting strain-level taxonomy, metabolite-mediated antibacterial and antifungal mechanisms, practical food uses and regulatory safety is still fragmented. This review has addressed that gap by integrating recent taxonomic developments including the separation of *Periweissella* with mechanistic evidence and use outcomes within fresh produce, bakery products and cereal matrices. Its novelty depends on extending previous *Weissella*-focused reviews by providing a strain-conscious framework that links antimicrobial metabolites and mechanisms of action with matrix-specific efficacy, biosafety considerations and experimental evidence needed for further use and regulatory translation [22,23].

Accordingly, this review has assessed the taxonomy, ecological distribution, physiological and functional attributes, antimicrobial mechanisms, biopreservation performance and biosafety of *Weissella*, with particular emphasis on antifungal efficacy and the requirements for safe, scalable, clean-label food preservation. As shown in Figure 1, the food biopreservation potential of *Weissella* should be assessed through an integrated strain-level framework that addresses taxonomy, ecology, bioactive metabolites, antimicrobial activity, practical preservation outcomes and safety requirements.

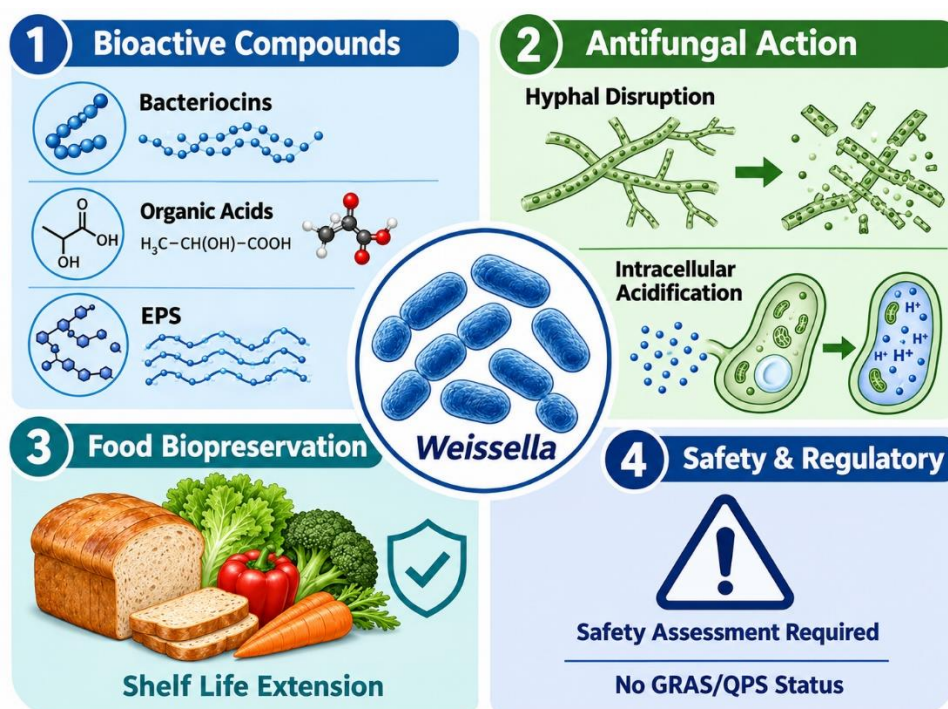


Figure1. The food biopreservation potential of *Weissella*



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2. MATERIALS AND METHODS

2.1. Literature Search Strategy

To investigate the biotechnological landscape of *Weissella*, a comprehensive literature search was carried out using PubMed, Scopus, Web of Science and Google Scholar for relevant literature published up to June 2026. The retrieval strategy targeted peer-reviewed studies investigating *Weissella* taxonomy, ecological niches, functional characteristics, antimicrobial mechanisms, food preservation trials and biosafety assessments.

The retrieval strategy combined controlled vocabulary and keyword terms, including *Weissella*, *Weissella cibaria*, *Weissella confusa*, "bacteriocins", "weissellicins", "antimicrobial activity", "antifungal activity", "biopreservation", "lactic acid bacteria", "EPS", "postharvest diseases", "volatile organic compounds" and "mycotoxins". Terms were linked using Boolean logic (AND/OR) and reference lists of retrieved articles were manually screened to capture additional eligible studies.

2.2. Study Selection

Relevant peer-reviewed research articles, review articles and authoritative taxonomic resources were addressed. Non-peer-reviewed reports, conference abstracts, editorials and duplicate publications were excluded. Relevant studies addressed key facets of *Weissella* biology, including taxonomy, ecological distribution, physiological traits, antimicrobial modes of action, food preservation trials, probiotic attributes and biosafety assessments. Non-peer-reviewed reports, conference abstracts, editorials and duplicate datasets were excluded.

2.3. Data Collection and Synthesis

Data extraction focused on strain identification, ecological origins, synthesized bioactive metabolites, target microbial spectrums, mechanistic pathways, food matrix uses, shelf-life outcomes, technological functionality and safety parameters. Studies addressing antifungal activity against postharvest phytopathogens and food spoilage molds were prioritized with trials testing *Weissella* strains in practical biopreservation systems.

Current bacterial nomenclature and taxonomic reclassifications were verified using the "List of Prokaryotic Names with Standing in Nomenclature"

(LPSN) to ensure consistency with the latest accepted classification of the genus. Information achieved from various studies were critically compared and synthesized to provide a comprehensive overview of the current state of knowledge, while identifying major advances, existing limitations and further research priorities [24].

3. RESULTS AND DISCUSSION

3.1. Taxonomy and Diversity of the Genus *Weissella*

3.1.1. History and Current Taxonomy of *Weissella* and *Periweissella*

The *Weissella* Sp. was introduced by Collins *et al.* to classify Leuconostoc-like LAB isolated from naturally fermented Greek sausage. It initially contained seven species of *W. confusa*, *W. halotolerans*, *W. kandleri*, *W. minor*, *W. paramesenteroides*, *W. viridescens* and *W. hellenica*, with *W. viridescens* serving as the type species [25]. The genus was originally addressed non-motile, but this description was changed after the discovery of motile *W. beninensis* in fermented cassava [26].

The number of identified species increased as *Weissella* strains were isolated from fermented fish, kimchi, soil, cocoa, rice, grapes, dairy products, insects, animals and marine mammals. Important latest species included *W. thailandensis* [27], *W. cibaria* [28], *W. soli* [29], *W. koreensis* [30], *W. fabaria* [31], *W. fabalis* [32], *W. diestrammenae* [33], *W. oryzae* [34], *W. uvarum* [35], *W. bombi* [36], *W. ceti* [37], *W. cryptocerci* [38], *W. muntiacci* [39], *W. sagaensis* [40], *W. coleopterorum* [41] and *W. fangxianensis* [42]. However, *W. jogaejeotgali* was later addressed a heterotypic synonym of *W. thailandensis*, while *W. kimchi* was regarded as a synonym of *W. cibaria* [43,44]. Therefore, reported species numbers for *Weissella* may differ depending on the taxonomic database consulted, date of consultation and if synonyms are included. Based on the current LPSN classification consulted for this review, *Weissella* contains 21 names with the taxonomic status "correct name," whereas 28 validly published names are listed when synonyms are included [24].

A major taxonomic revision was suggested in 2022. Phylogenetic analyses based on 498 core proteins and 16S ribosomal RNA gene sequences showed that five species formerly classified within *Weissella* formed a distinct evolutionary lineage. These species were transferred to the



novel Genus *Periweissella* with *P. beninensis*, *P. fabalis*, *P. fabaria*, *P. ghanensis* and *P. cryptocerci* [45]. This separation was supported by genome-based phylogenomic analysis, which grouped *Weissella* species into six major clusters and identified the same five-species group [45]. The two genera belonged to the Family Lactobacillaceae within the Order Lactobacillales [46]. Their classification now depends on polyphasic evidence, including genome-wide phylogeny, average nucleotide identity, DNA-DNA hybridization, *pheS* gene analysis, biochemical characteristics and carbohydrate metabolism, because 16S ribosomal RNA comparisons alone cannot reliably distinguish all closely linked species (Table 1).

3.1.2. Ecological Distribution and Sources of Isolation

The broad ecological distribution of *Weissella* is one of its most distinctive biological characteristics and provides important insights into its significant physiological versatility. Whereas several LABs inhabit restricted ecological niches, *Weissella* species show broad environmental distribution. Strains have been isolated within nutrient-dense substrates, including fermented matrices, fresh produce, unpasteurized milk, meat, aquatic products, soil and wastewater [50]. This broad occurrence highlights versatile metabolic and stress-response pathways that support survival under fluctuating physicochemical conditions, enabling persistence within natural habitats and industrial food environments.

Isolating *Weissella* species from insect digestive tracts highlights their capacity to establish stable host associations within diverse animal taxa, including humans [33]. These host-associated strains likely act as functional commensals within gut ecosystems rather than transient colonizers. This host adaptation has prompted research into their probiotic attributes such as gut microbiota modulation, pathogen exclusion and mucosal health maintenance [51]. However, probiotic traits are still strictly

strain-specific, with documented variations in epithelial adherence, metabolite yield and host immunomodulation.

In addition, these bacteria play a key role in the spontaneous fermentation of several traditional foods such as kimchi, doenjang and various fermented dairy and seafood products [30,52]. Their consistent occurrence within geographically diverse fermentations indicates evolutionary adaptation to food matrices, where rapid sugar metabolism and organic acid production offer a selective advantage. Accordingly, their presence reflects functional participation in fermentation ecology rather than transient environmental contamination.

The technological effect of *Weissella* extends beyond microbial succession during fermentation. By secreting short-chain fatty acids, EPSs and volatile aroma compounds, *Weissella* strains shape rheological, sensory and flavor profiles of the fermented foods [52]. Simultaneously, these metabolic end products suppress spoilage microorganisms and alter matrix pH, linking ecological adaptation directly to biopreservative functionality. Therefore, a strain's isolation niche may provide useful ecological context for subsequent technological screening and strain selection.

The niche diversity of *Weissella* offers a strategic guide for strain screening and bioprospecting. While fermented food isolates routinely demonstrate targeted processing traits such as rapid acidification, bacteriocin synthesis and antifungal potency, environmental and host-associated strains harbor novel, poorly characterized metabolic pathways. Source ecology thus provides predictive value rather than mere metadata, streamlining targeted strain discovery for specific food biotechnology uses. This ecological diversity provides an important context for the strain-level interpretation of *Weissella* characteristics, antimicrobial activities and food-biopreservation potentials presented in this review (Figure 2).



Table 1. Current accepted *Weissella* and *Periweissella* taxa, type strains, standardized isolation sources, taxonomic status and 16S ribosomal RNA accession numbers.

Species (current name)	accepted	Type strain	Standardized isolation source	Taxonomic status (LPSN)	16S rRNA accession	Ref.
<i>Weissella bombi</i>		DSM 28794T	Animal — bumble bee gut, Belgium	Correct name	LK054487	[36]
<i>Weissella ceti</i>		CCUG 59653T	Animal — beaked whale (<i>Mesoplodon bidens</i>), USA	Correct name	FN813251	[37]
<i>Weissella cibaria</i>		CCUG 41967T	Food — fermented chili (Chili Bo), Malaysia	Correct name	AJ295989	[47]
<i>Weissella coleopterorum</i>		HDW19T	Animal — intestine of diving beetle (<i>Cybister lewisianus</i>), South Korea	Correct name	MN099422	[41]
<i>Weissella confusa</i>		ATCC 10881T	Plant/food — cabbage, India	Correct name	AB682449	[25]
<i>Weissella diestrammenae</i>		DSM 27940T	Animal — camel cricket gut (<i>Diestrammena coreana</i>), South Korea	Correct name	JQ646523	[33]
<i>Weissella fangxianensis</i>		HBUAS51963T	Food — Chinese rice-wine starter, China	Correct name	OM943160	[42]
<i>Weissella fermenti</i>		BK2 / JCM 35750	Food — kimchi, South Korea	Correct name	OP376746	[48]
<i>Weissella halotolerans</i>		ATCC 35410T	Food — Greek fermented sausage	Correct name	AB022926	[25]
<i>Weissella hellenica</i>		ATCC 51523T	Food — Greek fermented sausage	Correct name	S67831	[25]
<i>Weissella kandleri</i>		ATCC 51149T	Environmental — spring in the Namib Desert, Namibia	Correct name	M23038	[25]
<i>Weissella koreensis</i>		CCUG 47134T	Food — kimchi, South Korea	Correct name	AY035891	[30]
<i>Weissella minor</i>		ATCC 35412T	Animal-associated/food equipment — milking-machine slime	Correct name	AB022920	[25]
<i>Weissella muntiaci</i>		8H-2T	Animal — feces of Formosan barking deer (<i>Muntiacus reevesi</i>), South Korea	Correct name	MK774696	[39]
<i>Weissella oryzae</i>		DSM 25784T	Food — fermented rice grain, Japan	Correct name	AB690345	[34]
<i>Weissella paramesenteroides</i>		ATCC 33313T	Animal — human intestinal microbiota	Correct name	X95982	[25]
<i>Weissella sagaensis</i>		CCM 8924T	Food — traditional Chinese yogurt	Correct name	LC438526	[40]
<i>Weissella soli</i>		CCUG 46608T	Environmental — garden soil, Sweden	Correct name	AY028260	[29]
<i>Weissella thailandensis</i>		CCUG 46557T	Food — fermented fish, Thailand	Correct name	MT760016	[27]
<i>Weissella uvarum</i>		B18NM42T	Plant/food — wine grapes, Greece	Correct name	KF999666	[35]
<i>Weissella viridescens</i>		ATCC 12706T	Food — cured meat product	Correct name	X52568	[25]
<i>Periweissella beninensis</i>		DSM 22752T	Food — submerged fermenting cassava, Benin	Correct name; transferred from <i>Weissella</i>	EU439435	[26]
<i>Periweissella cryptocerci</i>		26KH-42T	Animal — gut of <i>Cryptocercus kyebangensis</i> , South Korea	Correct name; transferred from <i>Weissella</i>	MK395366	[38]
<i>Periweissella fabalis</i>		LMG 26217T	Food — fermented cocoa beans, Brazil	Correct name; transferred from <i>Weissella</i>	HE576795	[32]
<i>Periweissella fabaria</i>		LMG 24289T	Food — fermented cocoa beans, Ghana	Correct name; transferred from <i>Weissella</i>	FM179678	[31]
<i>Periweissella ghanensis</i>		DSM 19935T	Food — Ghanaian cocoa fermentation	Correct name; transferred from <i>Weissella</i>	AM882997	[49]

Abbreviations: ATCC, American Type Culture Collection; CCM, Czech Collection of Microorganisms; CCUG, Culture Collection University of Gothenburg; DSM, German Collection of Microorganisms and Cell Cultures (DSMZ); HBUAS, Hubei University of Arts and Science; JCM, Japan Collection of Microorganisms; LMG, Laboratory of Microbiology, Ghent University; LPSN, List of Prokaryotic names with Standing in Nomenclature; rRNA, Ribosomal ribonucleic acid; ^T, Type strain; Ref., Reference.



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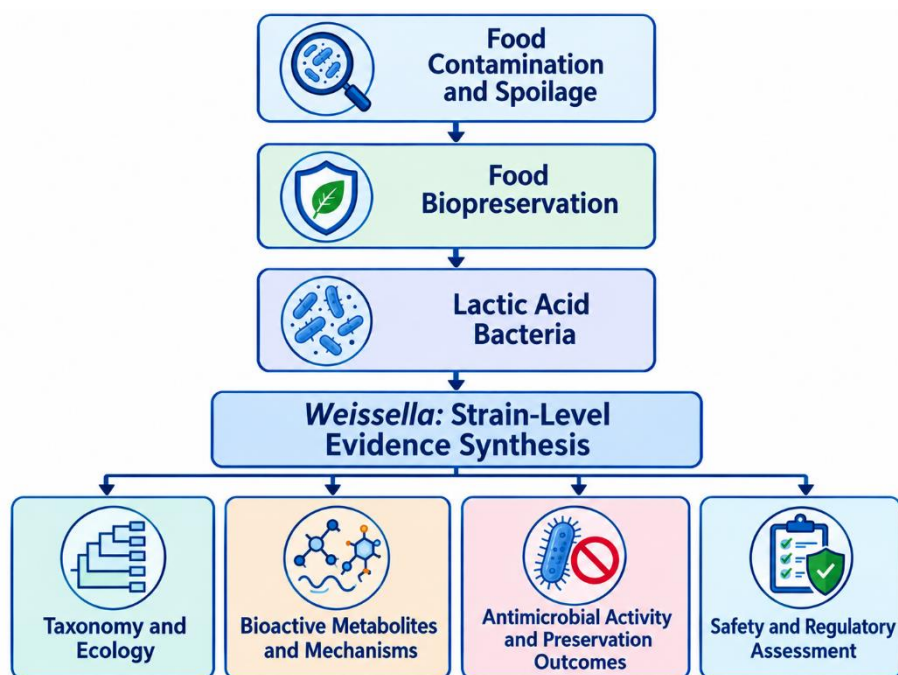


Figure 2. *Weissella* characteristics, antimicrobial activities and food-biopreservation

3.1.3. Identification Methods

Accurate identification of *Weissella* species is still one of the principal challenges in LAB taxonomy because of the substantial phenotypic and biochemical similarities shared with closely linked genera, particularly *Leuconostoc* and members of the former *Lactobacillus* complex. Conventional identification approaches based on colony morphology, carbohydrate fermentation profiles and routine biochemical assays frequently lack sufficient discriminatory power for reliable species-level classification, increasing the likelihood of taxonomic misidentification [23]. Therefore, reliance on phenotypic characteristics alone is no longer addressed adequate for studies needing precise taxonomic assignment or strain selection for food biotechnology.

Advances in molecular and proteomic methods have improved *Weissella* identification. Polymerase chain reaction (PCR)-based assays enabled identification of six *Weissella* strains and ten type strains [53]. An aptamer-based fluorescence polarization biosensor detected *W. viridescens* at 10^2 – 10^6 colony-forming units (CFU) per millilitre, with 90.6–107.7% recovery in smoked hams [54]. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) with an expanded database identified 83.7% of *Weissella* isolates to the

species level and 16.3% to genus level [55]. These methods provide complementary approaches for accurate *Weissella* identification. Although 16S ribosomal RNA gene sequencing is still a standard taxonomic marker, its discriminatory resolution for *Weissella* species is limited. High gene sequence conservation within closely linked taxa obscures species-level boundaries, particularly within recently diverged lineages [23]. Relying solely on 16S ribosomal RNA loci can hence undercount species diversity and introduce ambiguity into comparative genomic analyses.

To address these diagnostic constraints, pan-genome mining has informed the design of high-resolution molecular assays. For example, species-specific real-time PCR primers targeting pan-genomic locus tags now enable clear differentiation of 11 food-associated *Weissella* species that previously resisted conventional PCR resolution [56]. These targeted assays enhance diagnostic specificity while streamlining real-time monitoring of key starter strains during industrial fermentations. Whole-genome sequencing (WGS) provides a high-resolution approach for *Weissella* characterization. In addition to taxonomic classification, WGS maps genome architecture, biosynthetic gene clusters for antimicrobials, metabolic pathways, probiotic locus tags and mobile resistome or



virulome elements [45]. This genomic resolution shifts strain assessment from basic taxonomy to predictive modelling of technological efficiency and biosafety.

Comparative genomics resolves persistent taxonomic ambiguities in *Weissella*. High-throughput phylogenomic pipelines differentiate closely linked isolates beyond the resolution of single-locus markers while simultaneously profiling functional operons such as bacteriocin gene clusters, EPS synthases, carbohydrate active enzymes, stress response systems and antibiotic resistance genes. Comparative genomic analyses therefore provide a useful framework for addressing strain-specific functional and safety traits relevant to biopreservation and probiotic uses [57].

3.2. Physiological and Metabolic Characteristics

The physiological and metabolic traits of *Weissella* drive its use in food biotechnology and biopreservation. As LAB, *Weissella* species ferment carbohydrates into short-chain organic acids (SCOA) while contributing to matrix preservation, microbial community stability and host health [23]. In addition to basic acid production, these organisms produce diverse bioactive end-products that shape food quality, rheology and microbial interactions. Morphologically and physiologically, *Weissella* species are Gram-positive, catalase-negative non-spore-forming cocci or short rods depending on culture conditions [25]. While motility is rare within the genus, *P. beninensis* is a distinct exception due to peritrichous flagellation [26]. Such phenotypic variations demonstrate that functional traits diverge within species.

Central to *Weissella* physiology is an obligatory heterofermentative metabolism. Using 6-phosphogluconate/phosphoketolase pathway, *Weissella* species ferment hexoses into D/L-lactic acid, CO₂, ethanol and acetate [23]. This coproduction of organic acids, volatile compounds and gas enhances fermentation dynamics by driving matrix acidification, suppressing spoilage agents and generating complex flavor and aroma profiles. The industrial use of *Weissella* thus depends on multifaceted biochemical activity rather than simple acid accumulation. The energy metabolism of *Weissella* highlights its adaptation to fermentative niches. Since chemoorganotrophs lacking heme synthesis pathways, these bacteria depend entirely on substrate-level

phosphorylation, propagating under anaerobic or microaerophilic conditions [23]. This physiological dependence explains their frequent dominance during spontaneous food fermentation, where oxygen availability is limited and fermentable carbohydrates are abundant. Their metabolic strategy therefore provides a competitive ecological advantage over several aerobic microorganisms, while simultaneously promoting rapid acidification of the food matrix.

Temperature tolerance contributes significantly to the industrial relevance of this genus. Although all identified *Weissella* species are capable of growth at approximately 15 °C, several species tolerate temperatures between 42 and 45 °C [23], whereas optimal growth generally occurs between 20 and 30 °C [58]. Such physiological thermal tolerance enables *Weissella* strains to adapt within varied food matrices—from warm-incubated dairy fermentations to moderately conditioned meat processing. This thermal range broadens the industrial use of *Weissella* within diverse food processing environments rather than limiting it to a single production niche. In addition to core fermentative pathways, *W. cibaria* strains harbor key biochemical traits significantly dextran biosynthesis, esculin hydrolysis and arginine deamination [59]. Dextran synthesis is of important industrial interest; as a high-molecular-weight (HMW) glucan, it increases apparent viscosity, water-binding capacity and rheological stability in fermented dairy matrices. By improving mouthfeel and syneresis resistance without synthetic hydrocolloids, EPS production serves as a hydrocolloid-replacing functional trait for clean-label food design.

Weissella strains show a versatile carbohydrate fermentative profile, using cellobiose, fructose, galactose, maltose, raffinose, ribose, trehalose and xylose [23]. This metabolic range supports growth within diverse substrates, accounting for the prevalence of *Weissella* in fermented vegetables, dairy, cereals and meats. Such carbohydrate adaptability facilitates the design of matrix-tailored starter cultures for specialized industrial fermentations. Collectively, these physiological and metabolic characteristics demonstrate that the technological value of *Weissella* is set by the integration of multiple functional traits, rather than by a single metabolic capability. Efficient carbohydrate fermentation, heterofermentative metabolism, broad substrate utilization, environmental adaptability and EPS production have collectively



contributed to the increasing interest in this genus as a multifunctional platform for food fermentation, natural preservation and probiotic development. Nevertheless, accumulating evidence indicates significant strain-dependent variability in these physiological characteristics, highlighting the importance of comprehensive phenotypic and genomic characterization before selecting candidate strains for commercial food biotechnology.

3.3. Functional Characteristics and Technological Uses

3.3.1. Exopolysaccharide Production

The EPS biosynthesis represents a major functional attribute expanding use of *Weissella* in food biotechnology. As natural biopolymers, EPS improve rheological characteristics, moisture retention and sensory quality in fermented matrices [60]. The EPS-yielding *Weissella* strains are thus increasingly assessed as functional starter cultures and cell factories for clean-label food formulations. Within the Leuconostocaceae family, *Leuconostoc* and *Weissella* are prime EPS producers, with *Weissella* showing high yields of dextran-type homopolysaccharides [61,62]. The *W. cibaria*, *W. confusa* and *W. hellenica* synthesize structurally varied EPS that differ in monosaccharide composition, glycosidic linkage patterns, molecular weight distribution and branching degree [63]. This structural diversity dictates polymer physicochemical traits, governing solubility, rheological behavior and hydrocolloid functionality in food systems.

The industrial use of EPS produced by selected *Weissella* strains extends into functional food formulation. These polymers serve as bioemulsifiers, thickeners, gelling agents and hydrocolloids, increasing apparent viscosity, water-holding capacity and matrix stability in fermented foods [64,65]. These rheological enhancements improve mouthfeel, texture and syneresis resistance while decreasing dependence on synthetic food additives. Therefore, EPS yield represents a key selection benchmark during industrial strain screening.

However, EPS functionality cannot be inferred from taxonomy alone due to pronounced strain-level divergence. Variations in polymer yield, monomeric composition, molecular weight and branching architecture cause significant differences in rheological behavior and hydrocolloid efficacy. Taxonomic classification is therefore inadequate for predicting technological performance, needing comprehensive strain-level phenotyping and genomic profiling to identify superior EPS-producing isolates [66]. As summarized in Figure 3, selected *Weissella* strains may inhibit fungi through the contributions of various metabolite classes, including organic acids, volatile compounds and bacteriocins, as well as EPS, although the relative contribution of each factor varies within strains and experimental conditions.

3.3.2. Nutritional Enhancement and Probiotic Potential

In addition to processing use, experimental studies indicate that the functional characteristics of *Weissella* are strongly strain-dependent and should not be generalized at the species level. For example, *W. cibaria* YB-1 produced a purified dextran with a molecular mass of 3.89×10^6 Da and predominantly $\alpha(1 \rightarrow 6)$ glycosidic linkages, with 4.3% $\alpha(1 \rightarrow 3)$ -linked branches. The purified EPS showed a water solubility index of $95.23\% \pm 4.45$, water-holding capacity of $287.84\% \pm 16.23$ and emulsifying activity of $84.43\% \pm 3.65$ and further showed *in vitro* scavenging activity against superoxide anion and hydroxyl radicals [67]. Similarly, EPS production by *W. cibaria* GA44 reached an experimentally measured yield of 4.80 g l^{-1} under optimized cultivation conditions and the purified polymer demonstrated *in vitro* antioxidant activity against superoxide anion and hydroxyl radicals [68]. These findings demonstrate that EPS-associated functionality depends on the characteristics of individual strains and their polymers rather than on species identity alone.



Ecological Diversity and Sources of *Weissella* Species

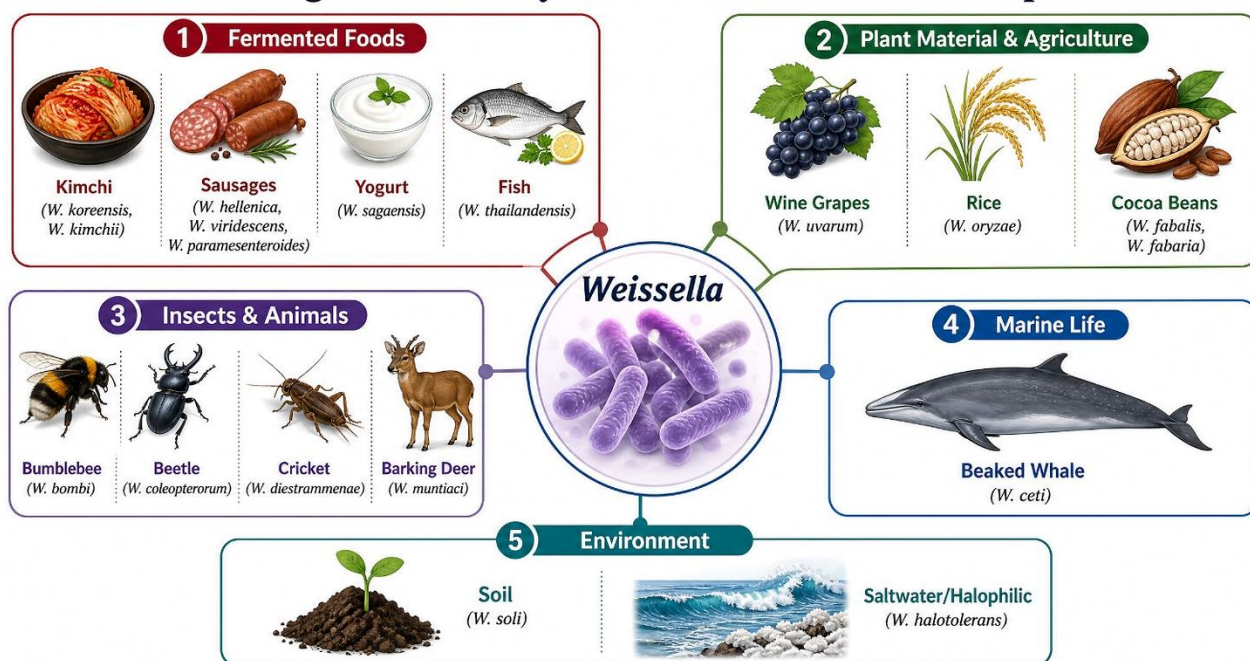


Figure 3. Inhibition of fungi by selected *Weissella* strains through antimicrobial metabolites

Experimental evidence supports strain-specific bioactivity in addition to EPS production. Cell-free supernatant (CFS) from the oral probiotic strain *W. cibaria* CMU inhibited periodontal pathogens in a dose-dependent manner, with the antibacterial activity being predominantly associated with organic acids and hydrogen peroxide. Lactic acid, acetic acid and citric acid were the principal organic acids detected, reaching concentrations of 6.42, 2.78 and 0.88 mg ml⁻¹, respectively. Proteomic analysis additionally identified 19 secreted proteins, including N-acetylmuramidase, providing experimental evidence for multiple antimicrobial components in this strain [69]. Thus, the functional potential of *Weissella* is better represented by experimentally characterized strain-metabolite relationships than genus-level claims.

Selected *Weissella* strains can produce biogenic amines, although this trait is strain-dependent. For example, *W. halotolerans* FAS29 and FAS30 showed tyrosine decarboxylase activity and produced tyramine, whereas *W. confusa* FS77 produced tyramine and histamine; putrescine production was detected in *W. confusa* FS52, FS53 and FJ2 [70]. Therefore, selection of strains for functional-food uses should combine technological screening with metabolite profiling and appropriate biosafety assessment

to distinguish beneficial functional characteristics from potential safety risks [70].

3.4. Antimicrobial Potential and Mechanisms

3.4.1. Antibacterial Activity

The antibacterial activity reported for selected *Weissella* strains is primarily associated with the production of organic acids and other inhibitory metabolites. Lactic and acetic acids can decrease the environmental pH and, particularly in their undissociated forms, diffuse within bacterial membranes. Subsequent intracellular dissociation contributes to cytoplasmic acidification, disruption of proton gradients and impairment of essential metabolic processes; thereby, restricting the growth of susceptible bacteria [23,71]. However, these effects should be interpreted at the strain level because antibacterial activity varies based on the *Weissella* strain, target organism, culture conditions and composition of the cell-free preparation. For example, *W. cibaria* TM128 showed antibacterial activity against selected foodborne and spoilage bacteria, supporting its potential as an antagonist in food-associated environments [71]. Similarly, *W. cibaria* BYL4.2 and its CFS inhibited several bacterial



species, including *Escherichia coli*, *Staphylococcus aureus*, *P. aeruginosa* and *Listeria monocytogenes* [72]. Other *W. cibaria* strains have demonstrated inhibitory activity against respiratory pathogens, including *Streptococcus pyogenes*, *S. aureus* and *S. pneumoniae* [73]. In foods, *W. cibaria* D30 showed antilisterial activity, when used in cottage cheese, indicating that antibacterial activity can be reserved within a food matrix [74].

Antibacterial effects have been documented for selected *W. confusa* strains. The *W. confusa* PL9001 decreased the adherence and growth of *Helicobacter pylori* [75], whereas *W. confusa* DD_A7 demonstrated antibacterial activity against an extended-spectrum β -lactamase-producing *E. coli* strain [76]. These findings reveal that antibacterial activity is not a uniform characteristic of the genus but rather a strain-dependent characteristic that should be established experimentally for each candidate strain.

Bacteriocins represent an additional class of antibacterial compounds in selected *Weissella* strains. Weissellicins and other bacteriocin-like peptides have been characterized in *W. paramesenteroides* and *W. hellenica*, with activity directed against susceptible bacterial organisms [77]. For example, weissellin A production has been investigated in *W. paramesenteroides* DX [78], while a bacteriocin produced by *W. hellenica* D1501 was characterized from a Chinese fermented meat product [79]. Therefore, bacteriocin-mediated effects should be described specifically as antibacterial unless direct antifungal activity has experimentally been demonstrated.

3.4.2. Antifungal Mechanisms

The antifungal activity of *Weissella* is associated with the production of multiple metabolites, although their relative contributions seem as strain and condition dependent. Experimental evidence has demonstrated that organic acids can contribute significantly to the antifungal activity of the selected *Weissella* strains. For example, metabolites from *W. cibaria* KM14 showed 94.3–99.4% inhibition against *A. fumigatus*, *P. expansum* and *P. oxalicum* and organic acids were identified as the most effective antifungal components, with lactic and acetic acids were within the predominant organic acids detected [80]. Scanning electron microscopy (SEM) revealed morphological changes in fungal hyphae after treatment with the CFS, although the precise molecular basis of these effects must fully be established [80]. Similarly, *W. cibaria*

BYL4.2 and its CFS showed antifungal activity against *P. chrysogenum*. The supernatant presented pH-dependent activity, while metabolomic analysis indicated alterations in fungal metabolic pathways, including pathways linked to ATP-binding cassette transporters. These findings suggest possible metabolic mechanisms underlying fungal inhibition; however, they do not establish direct molecular targets [72].

Organic acids are therefore within the major antifungal metabolites reported for the selected *Weissella* strains [80,81]. Their antifungal effects are generally associated with extracellular acidification and, for undissociated organic acids, diffusion within the fungal plasma membrane followed by intracellular dissociation and proton accumulation. These processes have been suggested to disturb intracellular pH homeostasis and cellular metabolism; however, the relative contribution of each process may vary based on the strain, metabolite concentration, fungal species and environmental conditions [81]. The findings achieved with *W. cibaria* KM14 provide experimental support for the contribution of organic acids to antifungal activity; however, they do not establish that intracellular acidification is the unique or universal mechanism of fungal inhibition [80].

The effects observed with *W. cibaria* BYL4.2 further suggest that metabolic alterations may accompany *Weissella*-associated fungal inhibition. The CFS produced pH-dependent antifungal activity against *P. chrysogenum*, while metabolomic analysis revealed changes in fungal metabolic pathways, including pathways linked to ATP-binding cassette transporters [72]. These observations indicate that alterations in transport and cellular metabolism may contribute to fungal inhibition. However, direct inhibition of ATP-binding cassette transporters by a specific *Weissella* metabolite has not experimentally been demonstrated. Thus, these metabolic changes should be addressed potential mechanisms rather than verified molecular targets [72].

Volatile organic compounds (VOCs) have been experimentally implicated in the antifungal activity of selected *Weissella* strains. In *W. paramesenteroides* R2, gas chromatography-mass spectrometry (GC-MS) analysis identified 84 VOCs, with pyrazines accounting for approximately 51% of the detected VOC profile. Three major compounds—2,5-dimethylpyrazine, 2,4-di-tert-butylphenol and 2-furanmethanol—were individually



assessed for antifungal activity. At 150 μ l, 2,5-dimethylpyrazine produced maximum inhibition of approximately 83, 25 and 49% against *B. cinerea*, *C. gloeosporioides* and *F. oxysporum*, respectively. Use of R2-derived VOCs decreased disease incidence and severity in stored strawberries [82]. These findings provide experimental support for VOC-associated antifungal activity in this strain, whereas the precise cellular mechanisms underlying these effects are still incompletely characterized.

Bacteriocins and EPS have been investigated as biologically active metabolites of *Weissella* [77,83]. Bacteriocins produced by *Weissella* have been characterized primarily for their antibacterial activities, whereas evidence for their direct antifungal effects is still limited [77]. Similarly, galactan EPS produced by *W. confusa* KR780676 has demonstrated aflatoxin B1-binding activity, which is relevant to mycotoxin modification but represents toxin sequestration rather than direct inhibition of fungal growth [83]. Accordingly, bacteriocins and EPS should not currently be addressed established components of a direct antifungal mechanism.

Overall, the available evidence supports the contribution of specific metabolites, particularly organic acids and VOCs, to antifungal activity in selected *Weissella* strains. However, the available studies do not provide sufficient quantitative evidence to establish synergistic interactions within organic acids, VOCs, bacteriocins and EPSs. The observed effects should therefore be interpreted as strain and condition-dependent activities of individual or combined metabolites rather than evidence of a universally established multi-metabolite synergy. Further controlled combination studies using defined metabolite concentrations and appropriate interaction models are needed to investigate if these compounds act synergistically, additively or independently.

3.4.3. Physical and Cellular Effects on Fungi

The inhibitory effects of the selected *Weissella* strains and metabolites may be accompanied by observable morphological changes in susceptible fungi. In a study of *W. cibaria* KM14, exposure to its metabolites was associated with alterations in fungal morphology and cellular structure, including changes in hyphal appearance and structural integrity. These observations provide morphological evidence of cellular or structural

disturbance associated with treatment and complement the reported quantitative inhibition of fungal growth. However, the morphological changes reported using SEM cannot establish specific antifungal mechanisms or identify metabolites responsible for the observed effects [80].

The relationship between the exposure to the cell-free preparation and the observed morphological changes therefore is insufficiently resolved. Although organic acids were identified as important contributors to the antifungal activity of *W. cibaria* KM14, the available morphological observations do not demonstrate that the ultrastructural changes were specifically caused by organic acids or other individual metabolites [80]. Establishing such a causal relationship needs component-specific experiments such as assessing purified or individually characterized metabolites at defined concentrations and comparing their effects with those of the complete cell-free preparation. Accordingly, the SEM findings should be interpreted as evidence of morphological or structural alterations rather than direct evidence of a particular molecular mechanism.

Overall, the available evidence indicates that the selected *Weissella* strains can show antibacterial or antifungal activity; however, mechanisms responsible for these effects are still strain and metabolite dependent. Quantitative inhibition data, metabolite analyses and morphological observations provide various levels of evidence and should not be treated similarly. In particular, morphological changes should be distinguished from mechanistic evidence and specific molecular mechanisms should be suggested only when supported by appropriate component-specific or mechanistic experiments. Furthermore, antibacterial activity, antifungal activity and toxin-binding activity represent distinct biological outcomes and should be described separately when interpreting the available evidence.

3.5. Biopreservation in Food Systems

3.5.1. Spectrum of Antifungal Activity

The biopreservative potential of *Weissella* depends on broad-spectrum activity against key postharvest pathogens and food spoilage molds [80,82,84]. Isolates inhibit fungal species responsible for raw commodity decay and processed food spoilage, offering a bio-based alternative to synthetic fungicides. The observed inhibition may involve one or more extracellular metabolites, including organic acids and VOCs, although their relative contributions vary



within strains and experimental conditions. Multiple investigations verify *Weissella* activity against major phytopathogenic fungi, significantly *B. cinerea*, *C. gloeosporioides*, *F. oxysporum* and *P. expansum* [80,82,84].

Inhibition within distinct fungal genera demonstrates that *Weissella* metabolites target conserved physiological pathways in filamentous fungi rather than species-specific loci. This cross-genus efficacy highlights the use of *Weissella* for pre and postharvest biocontrol. For example, *W. confusa* and *Limosilactobacillus fermentum* strains isolated from traditional attieke fermentations show strong antagonism against *Fusarium* species, *C. gloeosporioides*, *Botryodiplodia theobromae*, *A. carbonarius* and *A. niger* [84]. These findings support use of traditional fermented foods as potential sources for bioprospecting antifungal LAB. Efficacy within diverse fruit and vegetable pathogens highlights *Weissella* as a bio-based intervention to limit postharvest loss and decrease chemical fungicide reliance. In addition to raw production, *Weissella* controls spoilage fungi in processed food matrices. In rice cake preservation, *W. confusa* inhibited *Cladosporium* species, *Neurospora* species and *P. crustosum*, significantly delaying fungal spoilage during storage [85]. Similarly, *W. cibaria* KM14 suppresses *A. fumigatus*, *P. expansum* and *P. oxalicum* common spoilage molds within diverse food supplies [80]. These findings indicate that metabolites produced by the selected *Weissella* strains can inhibit multiple spoilage fungi under the assessed conditions.

3.5.2. Practical Uses and Efficacy in Food Biopreservation

Translational studies verify that *Weissella* antifungal efficacy extends beyond *in vitro* assays into food preservation systems. Within diverse food matrices, *Weissella* inoculants decrease fungal spoilage, extend shelf life and preserve organoleptic quality, supporting their development as sustainable alternatives to chemical preservatives [82,84–86]. These uses illustrate that the effectiveness of *Weissella* is closely associated with strain-specific metabolic activity and characteristics of the target food matrix, emphasizing the importance of selecting appropriate strains for individual preservation strategies.

A representative experimental example of practical applicability is the use of *W. paramesenteroides* R2 isolated from mango for the biological control of postharvest grey mold caused by *B. cinerea* in strawberries

[82]. This strain significantly decreased disease development while extending fruit shelf life, with the protective effect primarily attributed to the production of VOCs. Unlike conventional antimicrobial treatments that need direct contact with food surfaces, VOC-mediated inhibition provides an important technological advantage by enabling the remote suppression of fungal pathogens. Such contact-independent activity is particularly valuable for delicate fresh produce, where minimizing physical handling is essential to preserve product quality.

Similar preservation outcomes occur in tomato postharvest trials using *W. confusa* B35 live-cell coating or CFS preparations. The bacterial culture was prepared at approximately 10^7 CFU ml⁻¹ before separation of cells and supernatant; the cells were used as a surface coating, whereas the CFS was used by immersion for 10 min, with untreated tomatoes serving as the negative control. The two treatments decreased visible spoilage, while CFS treatment showed the better preservation outcome, with treated tomatoes being visually intact over the 2-w experimental time at room temperature (RT). These findings indicate the potential contribution of secreted extracellular metabolites to tomato preservation; however, they should not be generalized as evidence of industrial-scale shelf-life extension without further validation under defined commercial processing and storage conditions [84]. This outcome demonstrates that secreted extracellular metabolites, rather than live cell colonization, drive antifungal efficacy. Use of cell-free preparations may offer potential formulation and processing advantages; however, their stability, standardization and regulatory requirements need dedicated assessments.

Weissella demonstrates biopreservative use in cereal matrices. In rice cake preservation trials, sourdough fermentation with *W. confusa* suppressed common spoilage molds and extended shelf life [85]. Under the conditions of the reported rice-cake study, *W. confusa* showed antifungal activity, similar to or greater than that observed with 0.3% calcium propionate. This finding indicates potential for further investigation of *Weissella* as a biological preservation approach in bakery products. Cereal and grain biopreservation represents an important use for *Weissella* due to its inhibition of mycotoxigenic *Fusarium* species [82,84]. Such antifungal activity may decrease the risk of subsequent mycotoxin accumulation by limiting fungal growth; however, inhibition of fungal



growth should not be interpreted as direct mycotoxin degradation or detoxification. Where mycotoxin binding or sequestration has been demonstrated, this should be addressed a distinct mechanism from antifungal activity. The *W. confusa* isolates specifically inhibit spore germination in *F. culmorum*, verifying that antagonism operates at early stages of fungal development [87].

Nevertheless, comparative investigations have clearly demonstrated that antifungal efficacy is highly strain dependent. Although *W. confusa* ML2 showed antagonistic activity similar to that of BF2 against several *Fusarium* isolates, important differences were evident when individual fungal strains were assessed [86]. In peanut biocontrol experiments, BF2 successfully suppressed *F. verticillioides* NCIM 1099, *F. graminearum* MTCC 2089 and *F. oxysporum* MTCC 284, decreasing disease incidence from 100% in untreated controls to zero after bacterial treatment. In contrast, ML2 showed limited activity against *F. verticillioides* NCIM 1100, where disease incidence was 100%, although it effectively controlled *F. graminearum* MTCC 2089 and significantly decreased disease caused by *F. oxysporum* MTCC 284 to 10.52% [86]. These findings demonstrate that antifungal performance cannot be generalized within strains, even within the same species and strongly support the need for strain-level selection before commercial uses.

Additional evidence for this strain-dependent variability was achieved using surface-disinfected wheat grains artificially infected with *Fusarium* species [86]. Interestingly, the response observed in wheat differed from that observed in peanuts. Under these conditions, *F. verticillioides* NCIM 1100 was susceptible to *W. confusa* ML2 and BF2, whereas *F. graminearum* MTCC 2089 was further sensitive to BF2. In contrast, *F. oxysporum* MTCC 284 showed little or no response to CFS. These divergent outcomes demonstrate that antifungal efficacy depends on strain-specific metabolic traits and matrix-dependent physicochemical factors. Biopreservative performance must therefore be validated under specific food processing and storage conditions rather than generalized from simplified *in vitro* or single-matrix models.

Collectively, these findings establish *Weissella* as a versatile microbial platform for food biopreservation within fresh productions, bakery goods and cereal supplies. However, performance disparities within strains and food matrices necessitate rigorous use-specific screening and pilot validation under realistic processing parameters. Systematic strain selection optimizes *Weissella*-based biopreservation, advancing sustainable, clean-label food preservation strategies (Table 2).



Table 2. Experimental conditions and reported antifungal outcomes of *Weissella* strains evaluated against food-associated filamentous fungi

Strain	Target organism	Food matrix / assay	Metabolite	Mechanism	Inoculum concentration	Treatment format	Storage / assay temperature	Storage / assay duration	Control	Replication	Quantitative efficacy outcome	Safety assessment	Experimental level	Ref.
<i>W. paramesenteroides</i> R2	<i>B. cinerea</i>	PDA; strawberry postharvest model	VOCs: 2,5-dimethylpyrazine, 2,4-di-tert-butylphenol, 2-furanmethanol	VOC-mediated gas-phase inhibition without direct contact	R2: 3 inoculated MRS plates per ~5,500-mL box; exact CFU/mL NR	VOC exposure in sealed box; individual VOCs 50–150 µL	28°C assay; 25°C strawberry storage, 90% RH	5 d assay; 5 d strawberry storage	Uninoculated MRS; ddH ₂ O/ethanol for individual compounds	NR	Significant reduction in postharvest disease incidence/severity; individual VOCs showed antifungal activity	Described as food-safe; no dedicated strain-level safety testing	<i>In vitro</i> assay + Cell-free/VOC preparation + Food-model + Postharvest/storage study	[82]
<i>W. paramesenteroides</i> R2	<i>C. gloeosporioides</i>	PDA	VOCs: 2,5-dimethylpyrazine, 2,4-di-tert-butylphenol, 2-furanmethanol	VOC-mediated gas-phase inhibition	Bacterial inoculum as above; exact CFU/mL NR	Individual VOCs 50–150 µL	28°C	5 d	Uninoculated MRS; ddH ₂ O/ethanol	NR	Strong VOC-mediated inhibition; individual compounds validated	No dedicated strain-level safety assessment	<i>In vitro</i> assay + Cell-free/VOC preparation	[82]
<i>W. paramesenteroides</i> R2	<i>F. oxysporum</i>	PDA	VOCs: 2,5-dimethylpyrazine, 2,4-di-tert-butylphenol, 2-furanmethanol	VOC-mediated gas-phase inhibition	Bacterial inoculum as above; exact CFU/mL NR	Individual VOCs 50–150 µL	28°C	5 d	Uninoculated MRS; ddH ₂ O/ethanol	NR	Strong VOC-mediated inhibition; individual compounds validated	No dedicated strain-level safety assessment	<i>In vitro</i> assay + Cell-free/VOC preparation	[82]
<i>W. confusa</i> B10	<i>Fusarium</i> sp.; <i>C. gloeosporioides</i> ; <i>B. theobromae</i> ; <i>A. carbonarius</i> ; <i>A. niger</i>	PDA agar-well assay	CFS; specific antifungal metabolite not identified	CFS-mediated antifungal activity	CFS; live-cell inoculum not applicable	100 µL CFS	37°C assay	24 h	Untreated/negative control	NR	Strong broad-spectrum <i>in vitro</i> antifungal activity (qualitative in reviewed source)	Antibiotic susceptibility assessed; no comprehensive strain-level safety assessment	<i>In vitro</i> assay + Cell-free preparation	[84]
<i>W. confusa</i> B17	Same targets as B10	PDA agar-well assay	CFS; specific antifungal metabolite not identified	CFS-mediated antifungal activity	CFS; live-cell inoculum not applicable	100 µL CFS	37°C assay	24 h	Untreated/negative control	NR	Antifungal activity detected in agar-well assay	Antibiotic susceptibility assessed; no comprehensive strain-level safety assessment	<i>In vitro</i> assay + Cell-free preparation	[84]
<i>W. confusa</i> B22	Same targets as B10	PDA agar-well assay	CFS; specific antifungal metabolite not identified	CFS-mediated antifungal activity	CFS; live-cell inoculum not applicable	100 µL CFS	37°C assay	24 h	Untreated/negative control	NR	Antifungal activity detected in agar-well assay	Antibiotic susceptibility assessed; no comprehensive strain-level safety assessment	<i>In vitro</i> assay + Cell-free preparation	[84]



Strain	Target organism	Food matrix / assay	Metabolite	Mechanism	Inoculum concentration	Treatment format	Storage / assay temperature	Storage / assay duration	Control	Replication	Quantitative efficacy outcome	Safety assessment	Experimental level	Ref.
<i>W. confusa</i> B35	Same targets as B10	PDA agar-well assay	CFS; specific antifungal metabolite not identified	CFS-mediated antifungal activity	CFS; live-cell inoculum not applicable	100 µL CFS	37°C assay	24 h	Untreated/negative control	NR	Strong activity against most tested molds; weaker against <i>A. niger</i>	Antibiotic susceptibility assessed; no comprehensive safety assessment	<i>In vitro</i> assay + Cell-free preparation	[84]
<i>W. confusa</i> B35	<i>Penicillium</i> sp.	Tomato	CFS and viable cells; specific active metabolite not identified	Antifungal biopreservation /inhibition of fungal spoilage	~10 ⁷ CFU/mL for viable-cell treatment	Live-cell surface coating or CFS after 10-min immersion	Room temperature	2 weeks	Untreated tomatoes	NR	Reduced spoilage; CFS was more effective than viable cells	Antibiotic susceptibility assessed; no dedicated hemolysis/cytotoxicity/genomic safety assessment	<i>In vitro</i> assay + Cell-free preparation + Food-model study + Storage study	[84]
<i>W. confusa</i>	<i>Cladosporium</i> sp. YS1	Rice cake/rice dough	Lactic acid and acetic acid	Organic-acid-mediated antifungal activity	Fermentation culture; exact CFU/mL NR	Fermentation ; lactic/acetic acids individually tested at 35 mM	Storage temperature NR; fungal growth monitored during fermentation /storage	Fermentation + storage; exact duration NR in current table	0.3% calcium propionate and non-fermented control	NR	At 35 mM: acetic acid ~80.5% inhibition; lactic acid ~80% inhibition	No dedicated strain-level safety assessment	<i>In vitro</i> assay + Food-model study + Storage study	[85]
<i>W. confusa</i>	<i>Neurospora</i> sp. YS3	Rice cake/rice dough	Lactic acid and acetic acid	Organic-acid-mediated antifungal activity	Fermentation culture; exact CFU/mL NR	Fermentation ; lactic/acetic acids individually tested at 35 mM	Storage temperature NR	Fermentation + storage; exact duration NR in current table	0.3% calcium propionate and non-fermented control	NR	At 35 mM: acetic acid ~85% inhibition; lactic acid ~62% inhibition	No dedicated strain-level safety assessment	<i>In vitro</i> assay + Food-model study + Storage study	[85]
<i>W. confusa</i>	<i>Penicillium crustosum</i> YS2	Rice cake/rice dough	Lactic acid and acetic acid	Organic-acid-mediated antifungal activity	Fermentation culture; exact CFU/mL NR	Fermentation ; lactic/acetic acids individually tested at 35 mM	Storage temperature NR	Fermentation + storage; exact duration NR in current table	0.3% calcium propionate and non-fermented control	NR	At 35 mM: acetic acid ~85% inhibition; lactic acid ~79% inhibition	No dedicated strain-level safety assessment	<i>In vitro</i> assay + Food-model study + Storage study	[85]



Strain	Target organism	Food matrix / assay	Metabolite	Mechanism	Inoculum concentration	Treatment format	Storage / assay temperature	Storage / assay duration	Control	Replication	Quantitative efficacy outcome	Safety assessment	Experimental level	Ref.
<i>W. confusa</i> BF2	<i>F. verticillioides</i> NCIM 1100/1099; <i>F. graminearum</i> MTCC 2089; <i>F. oxysporum</i> MTCC 284	MRS; peanut and wheat food models	CFS; antifungal compounds not individually identified	Cell-associated and extracellular metabolite-mediated inhibition	CFC: 10× concentrated; 2 mL/10 g peanut or 5 mL/40 g wheat; fungal challenge separately	CFS/ethyl-acetate CFC extract; 3-h pretreatment in food model	30°C	7 d fungal growth; 3 h pretreatment before challenge	Fusarium alone; sterile MRS-treated food matrix	NR in review table; statistical testing reported in source	Peanuts: disease incidence 0% for BF2 against all three tested <i>Fusarium</i> strains; controls 100%. Wheat: reduced contamination, with strain-specific effects.	Safety concerns discussed; no comprehensive toxicological/genomic validation	<i>In vitro</i> assay + Cell-free preparation + Food-model study	[86]
<i>W. confusa</i> ML2	<i>F. verticillioides</i> NCIM 1100/1099; <i>F. graminearum</i> MTCC 2089; <i>F. oxysporum</i> MTCC 284	MRS; peanut and wheat food models	CFS; antifungal compounds not individually identified	Cell-associated and extracellular metabolite-mediated inhibition	CFC: 10× concentrated; 2 mL/10 g peanut or 5 mL/40 g wheat	CFS/ethyl-acetate CFC extract; 3-h pretreatment	30°C	7 d fungal growth; 3 h pretreatment	Fusarium alone; sterile MRS-treated food matrix	NR in review table; statistical testing reported in source	Peanuts: disease incidence 100% for NCIM 1100, 0% for MTCC 2089, and 10.52% for MTCC 284. Wheat response was variable.	Safety concerns discussed; no comprehensive toxicological/genomic validation	<i>In vitro</i> assay + Cell-free preparation + Food-model study	[86]
<i>W. confusa</i> FST 1.31	<i>F. culmorum</i> TMW 4.0754	Barley/ malt	Lactic acid/organic acids; specific antifungal metabolite not individually identified	Acidification-associated inhibition of fungal spore proliferation	Starter culture added through steeping water; exact inoculum NR	Starter-culture treatment during simulated industrial malting	Process-dependent; exact storage temperature not specified	Simulated industrial malting process	Non-acidified/ disinfected barley; chemically acidified and MRS controls	NR	Inhibited <i>F. culmorum</i> spore proliferation, but fungal contamination and acidification adversely affected malt/wort quality	No dedicated strain-level safety assessment	<i>In vitro</i> / fermentation assay + Food-model study + Pilot/ simulated industrial-scale processing	[87]

Abbreviations: A., *Aspergillus*; B., *Botrytis* / *Botryodiplodia*; C., *Colletotrichum*; CFC, cell-free concentrate; CFS, cell-free supernatant; CFU, colony-forming unit; d, day(s); ddH₂O, double-distilled water; *F.*, *Fusarium*; h, hour(s); min, minute(s); mM, millimolar; MRS, de Man, Rogosa and Sharpe medium; MTCC, Microbial Type Culture Collection; NCIM, National Collection of Industrial Microorganisms; NR, not reported; PDA, potato dextrose agar; Ref., reference; RH, relative humidity; sp., species; TMW, Technische Mikrobiologie Weihenstephan; VOCs, volatile organic compounds; *W.*, *Weissella*; µL, microliter.



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3.5.3. Classification of Evidence by Experimental Level

The available evidence for *Weissella*-mediated biopreservation can be classified based on the experimental level; at which, antimicrobial efficacy has been demonstrated. At the *in vitro* level, studies have primarily assessed the inhibitory activity of *Weissella* strains or their metabolites against foodborne and phytopathogenic microorganisms under controlled laboratory conditions. These assays provide essential evidence of antimicrobial potential but do not fully reproduce the physicochemical complexity of food matrices. A second level comprises cell-free preparations, particularly CFS and metabolite-containing fractions. Studies using CFS from *Weissella* strains, including *W. cibaria* KM14 and BYL4.2, have demonstrated antifungal activity against several spoilage molds, supporting the contribution of extracellular metabolites to fungal inhibition.

The third level involves food-model studies; in which, *Weissella* strains or their metabolites are assessed directly in food matrices. Examples include the use of *W. confusa* in rice cakes and tomato preservation, demonstrating that antimicrobial activity observed under laboratory conditions can translate into measurable effects within food systems. The fourth level comprises storage and postharvest studies, which provide a higher degree of use relevance by addressing a performance under realistic storage conditions. Examples include *W. paramesenteroides* R2 for the control of *B. cinerea* on strawberries, *W. confusa* treatments in postharvest tomatoes and biocontrol studies involving peanuts and wheat grains. These studies demonstrate that efficacy is strongly affected by the strain characteristics and target food matrices.

Ultimately, pilot and industrial-scale validation represents the highest level of translational evidence. However, this level is still insufficiently represented in the current *Weissella* literature. Most available studies are still carried out under controlled laboratory or small-scale food-model conditions, while systematic validation under commercial processing, storage, supply-chain and economic constraints is still limited (Table 2). Therefore, further studies should progressively move from laboratory screening to pilot-scale and industrial validation to establish reproducibility, operational stability, safety and

cost-effectiveness before commercial implementation (Figure 4).

3.6. Biosafety and Regulatory Considerations

3.6.1. Safety Discussion

The safety of *Weissella* strains intended for food biopreservation should be assessed at the strain level rather than inferred from species or genus identity alone. A comprehensive assessment should include phenotypic antimicrobial susceptibility testing (AST) and genomic screening for acquired antimicrobial-resistance determinants, particularly genes associated with clinically relevant antimicrobials and their potential association with mobile genetic elements (MGE). Additional safety parameters should include the assessment of biogenic amine production, hemolytic activity, cytotoxicity, virulence-associated traits and other potentially harmful metabolic activities. The WGS can further support safety assessment by enabling characterization of the resistome, virulome, plasmid-associated genes, MGEs and other genomic determinants potentially relevant to safety. However, the presence of a putative gene identified by *in silico* analysis should not be interpreted as evidence of pathogenicity; genomic findings should be complemented by appropriate phenotypic validation. This approach is particularly important for *Weissella* because antimicrobial susceptibility, metabolic characteristics and safety-linked traits can vary significantly within individual strains.

Fanelli *et al.* [88] assessed the probiotic and safety-linked characteristics of six type strains representing *Weissella* and *Periweissella*, including *P. beninensis* LMG 25373T (LMG 25373T), *P. fabalis* LMG 26217T, *P. fabaria* LMG 24289T, *P. ghanensis* DSM 19935T, *W. diestrammenae* DSM 27940T and *W. uvarum* B18NM42T (DSM 28060T). All strains were non-hemolytic, whereas differences in antibiotic susceptibility profiles were observed within the strains. These findings demonstrate that safety-linked characteristics should be interpreted at the strain level rather than generalized within the genus. Accordingly, candidate strains for food or probiotic uses need further assessment using appropriate phenotypic, genomic and use-specific safety assessments [88].





Regulatory status should be described using current authoritative sources and should not be generalized to the entire genus. According to the U.S. Food and Drug Administration (FDA), Generally Identified as Safe (GRAS) notice no. 1063 received a “no questions” letter for *W. cibaria* CMU in 2023. This is specifically used to the strain and the intended uses described in the GRAS notice and should not be generalized to other *Weissella* species or strains [89].

Similarly, qualified presumption of safety (QPS) status should be interpreted based on the current European Food Safety Authority (EFSA) framework. The EFSA investigates microorganisms within defined taxonomic units and addresses taxonomic identity, the available body of knowledge and relevant safety concerns, including acquired antimicrobial resistance in bacteria, when maintaining and updating the QPS framework [90]. The QPS status should not therefore be interpreted as a general



safety designation for the entire genus or blanket authorization of a specific food use. Regulatory status should be assessed based on the applicable taxonomic unit, individual strain and intended use rather than generalized within *Weissella*.

Accordingly, further development of *Weissella*-based biopreservatives should adopt a standardized strain-level safety framework integrating AST, screening for transferable resistance genes and MGEs, biogenic amine assessment, hemolysis and cytotoxicity testing, characterization of virulence-associated traits and WGS. Such an integrated approach provides a stronger scientific basis for addressing strains with demonstrated antimicrobial or biopreservative activity and supporting later regulatory assessment.

3.6.2. Virulence Factors

WGS clarifies the distribution of putative virulence factors in *Weissella*. Comparative genomic mining reveals loci encoding hemolysins, collagen-binding domain proteins and cell-surface adhesins [50]. However, *in silico* gene annotation does not equate to active pathogenicity, as gene presence alone does not guarantee transcription, translation or functional phenotype expression. Specifically, *W. cibaria* JW15 showed low-homology matches to the endocarditis-associated antigen gene (*efaA*) and hemolysin A locus (*CVF417*) [91], while *W. hellenica* 0916-4-2 carries putative virulence-associated loci including *hasC*, *cpsI*, *gnd*, *cpsF* and *clpE* [92]. Although these genomic determinants trigger regulatory studies, their expression profiles, phenotypic contributions to virulence and potentials for horizontal gene transfer are still unverified. Furthermore, several sequence homologs annotated as putative virulence factors occur within food-grade LAB with long histories of safe fermentation uses. For example, hemolysin-like genes are common within traditional LAB genera without conferring clinical toxicity or phenotypic hemolysis. *In silico* homology alone should not therefore be treated as a definitive marker of pathogenic risk without phenotypic validation.

Paradoxically, several surface loci annotated as virulence factors mediate probiotic interactions in gut environments. Surface adhesins facilitate epithelial attachment, promoting mucosal colonization, ecological persistence and pathogen exclusion. For example, the fibronectin-binding protein gene (*fbpA*) in *Weissella*

strains inhibits *S. aureus* biofilm formation, functioning as a protective factor rather than a virulence determinant [93,50]. These dual-function traits highlight the limitation of relying alone on genomic screening for safety assessments. Gene presence does not infer biological activity, as phenotypic expression depends on transcriptional regulation, environmental cues and functional context. Automated genome annotation must therefore be paired with phenotypic assays, including AST, phenotypic hemolysis screening and cell toxicity models to establish strain biosafety.

Despite advances in genome sequencing, genomic diversity and strain-level variation are still important considerations in assessing the safety and functional potential of *Weissella*. Comparative genomic studies, including analysis of *W. hellenica* 0916-4-2, have demonstrated significant genomic variation and presence of safety-linked genomic characteristics [92]. More recently, analysis of 347 high-quality *Weissella* genomes representing 16 species revealed extensive genomic diversity and species and strain-level variations in functional and safety-linked traits [94]. In combination with previous genome-based assessments of putative virulence and antimicrobial-resistance determinants [50], these findings support integrating comparative phylogenomics with functional genomics and phenotypic characterization to enable strain-specific safety assessment and support further regulatory assessment of the candidate strains for food uses.

4. CONCLUSION AND FUTURE PERSPECTIVE

4.1. Conclusion

This review indicates that the selected *Weissella* strains possess antimicrobial (antibacterial and antifungal) activities associated with metabolites such as organic acids, bacteriocins, volatile organic compounds and EPSs. These effects may involve acidification, cellular or membrane disruption, metabolic disturbance and modification of the surrounding microbial environment, although the contribution of individual components and specific molecular mechanisms is still incompletely resolved. Separately, aflatoxin B1-binding activity reported for extracellular polysaccharides represents toxin sequestration rather than a mechanism of fungal-growth



inhibition and should not be conflated with the antifungal effects described previously.

The reviewed evidence demonstrates substantial strain, target, matrix and condition-dependent variations in antimicrobial activity and technological performance. Experimental evidence ranges from laboratory assays and cell-free preparations to food-model and storage studies, whereas evidence under pilot-scale and industrially relevant conditions is still limited. Overall, the current evidence supports further investigation of selected *Weissella* strains for food biopreservation but does not support generalizing efficacy or technological performance within the genus.

4.2. Limitations of the Current Evidence

The reviewed literature has several limitations that restrict the translation of *Weissella* research into broader food uses. Evidence ranges from *in vitro* assays and cell-free preparations to selected food-model and storage studies, with outcomes varying based on strain, target organism and food matrix [80,82,86]. Methodological differences in treatment conditions, controls, food matrices and outcome measurements limit direct comparison within studies. In addition, some studies have not fully established the contribution of individual metabolites or clearly distinguished antifungal activity from other biological effects [80]. Although strain-specific safety and genomic assessments have been reported [88,94], broader validation under realistic food-processing conditions is still limited. Evidence from pilot-scale and industrial uses is less developed than that from laboratory and food-model studies. These gaps highlight the need of standardized methods, consistent reporting and progressive validation under realistic processing and storage conditions.

4.3. Future Perspective

Key research gaps include the lack of standardized comparative studies linking strain characteristics with antimicrobial efficacy, technological performance and safety. Integrating WGS with transcriptomics, proteomics, metabolomics and phenotypic characterization could help identify determinants associated with antimicrobial production and technological stability. Mechanistic studies should distinguish experimentally demonstrated effects from suggested mechanisms and investigate the individual

and combined contributions of organic acids, bacteriocins, volatile compounds, EPSs and cell-associated factors.

Another important gap is the limited evidence distinguishing inhibition of fungal growth from mycotoxin degradation, detoxification or adsorption. Selected strains and their metabolites should therefore be assessed using standardized assays and progressively validated in realistic food matrices, storage conditions and pilot-scale systems. Such studies should assess sensory quality, nutritional characteristics, processing compatibility, stability and economic feasibility.

Ultimately, strain-specific safety and regulatory assessments are still essential, including antimicrobial susceptibility, transferable resistance determinants, MGEs, virulence-associated traits, hemolytic and cytotoxic activities and biogenic amine production. Coordinated experimental and reporting standards, with accessible strain-level datasets, improve reproducibility and facilitate comparison within various studies. Addressing these gaps helps investigate if laboratory findings can be translated into safe and reproducible *Weissella*-based food-biopreservation uses.

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5.2. Ethics and Consent to Participate

Not applicable. This article is a review of previously published literature and involved no human participants, animals, or identifiable personal data.

5.3. Data Availability

No new datasets were generated or analysed. The literature sources supporting this review are cited in the reference list.

5.4. Competing Interest

The authors declare that they have no competing interests.

5.5. Authors' Contribution

Gh.A.A.N. and A.A.M.H. conceived and designed the study. Gh.A.A.N. conducted the literature search, screened the retrieved records, and curated the data. Gh.A.A.N. prepared the original draft of the manuscript. A.A.M.H.



developed the search strategy, supervised the study, managed project administration, reviewed and edited the manuscript, and contributed to the visualization and presentation of the figures and tables. All authors read and approved the final manuscript.

5.6. Declaration of Generative AI in Figure Preparation

The authors confirm that all figures presented in this manuscript were originally created. Figure 1 and 4 included in this manuscript was created by the authors using ChatGPT (OpenAI) as an image-generation and illustration tool based on author-provided scientific content and design specifications. The authors reviewed, verified, and edited all figure elements for scientific accuracy. No copyrighted third-party figures or images were reproduced or adapted in this work.

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جنس ویسلا: مروری بر توانمندی‌های ضد میکروبی، ویژگی‌های عملکردی و کاربردهای آن در زیست‌حفاظت مواد غذایی

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چکیده

سابقه و هدف: باکتری‌های اسیدلاکتیک هتروفرمنتاتیو متعلق به جنس ویسلا دارای توانمندی‌های متابولیکی منحصربه‌فردی هستند که از ارزش ویژه‌ای در نگهداری طبیعی مواد غذایی برخوردارند. در این مقاله مروری، یافته‌های کنونی درباره ویژگی‌های فیزیولوژیک، مسیرهای ضد میکروبی و سازوکارهای ضد قارچی گونه‌های ویسلا، همچنین کاربردهای عملی آن‌ها در سامانه‌های مختلف غذایی و مسائل ایمنی محدودکننده استفاده تجاری از این باکتری‌ها، بررسی و یکپارچه‌سازی شده است.

مواد و روش‌ها: جست‌وجوی جامع منابع در پایگاه‌های پابمد، اسکوپوس، وب آو ساینس و گوگل اسکالر برای مقالات داوری شده منتشر شده تا اوت ۲۰۲۶ انجام شد. این مرور، رده‌بندی، پراکنش بوم‌شناختی، ویژگی‌های عملکردی، فعالیت‌ها و سازوکارهای ضد میکروبی، زیست‌حفاظت مواد غذایی و زیست‌ایمنی جنس ویسلا را دربر گرفت. منابع مرتبط بر اساس ویژگی‌های سویه، متابولیت‌های زیست‌فعال، سازوکار اثر، کاربرد در ماتریس‌های غذایی، پیامدهای نگهداری و ملاحظات ایمنی، به‌صورت نقادانه غربال و تلفیق شدند.

یافته‌ها و نتیجه‌گیری: مطالعات بررسی شده نشان دادند که ویسلا دارای فعالیت ضد میکروبی وابسته به سویه است که توسط ترکیبات متعددی از جمله اسیدهای آلی، ترکیبات آلی فرار، باکتریوسین‌ها و آگزوپلی‌ساکاریدها میانجی‌گری می‌شود. این عوامل می‌توانند از طریق اسیدی‌سازی، اختلال در فرایندهای متابولیکی و آسیب به غشا و هیف قارچ‌ها، رشد میکروارگانیسم‌های بیماری‌زا و عامل فساد مواد غذایی را مهار کنند. همچنین، برخی سویه‌ها قابلیت جذب آفلاتوکسین B1 را نشان داده‌اند. اثرات ضد میکروبی علیه چندین باکتری و قارچ بیماری‌زا گزارش شده است؛ در حالی که مطالعات انجام‌شده در مدل‌های غذایی، کاهش بار میکروبی و به‌تعویق افتادن فساد قابل مشاهده را در ماتریس‌های غذایی مختلف نشان داده‌اند. بالاین‌حال، این اثرات را نمی‌توان به‌طور کلی به تمام اعضای جنس ویسلا تعمیم داد، زیرا تولید ترکیبات ضد میکروبی، ویژگی‌های فناورانه و ویژگی‌های ایمنی در سویه‌های مختلف متفاوت است. بنابراین، پیش از استفاده قابل اعتماد از سامانه‌های زیست‌حفاظت مبتنی بر ویسلا، انجام ارزیابی‌های ایمنی اختصاصی سویه، ارزیابی‌های استاندارد شده اثربخشی، مطالعات مکانیسمی و اعتبارسنجی در مقیاس پایلوت و صنعتی ضروری است.

واژگان کلیدی: فعالیت ضد میکروبی، زیست‌حفاظت مواد غذایی، ایمنی مواد غذایی، باکتری‌های اسیدلاکتیک، ویسلا