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Weissella: An Emerging Bacterium with Promising Health Benefits

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Abstract

Weissella strains have been the subject of much research over the last 5 years because of the genus' technological and probiotic potential. Certain strains have attracted the attention of the pharmaceutical, medical, and food industries because of their ability to produce antimicrobial exopolysaccharides (EPSs). Moreover, *Weissella* strains are able to keep foodborne pathogens in check because of the bacteriocins, hydrogen peroxide, and organic acids they can produce; all listed have recognized pathogen inhibitory activities. The *Weissella* genus has also shown potential for treating atopic dermatitis and certain cancers. *W. cibaria*, *W. confusa*, and *W. paramesenteroides* are particularly of note because of their probiotic potential (fermentation of prebiotic fibers) and their ability to survive in the gastrointestinal tract. It is important to note that most of the *Weissella* strains with these health-promoting properties have been shown to be save safe, due to the absence or the low occurrence of virulence or antibiotic-resistant genes. A large number of scientific studies continue to report on and to support the use of *Weissella* strains in the food and pharmaceutical industries. This review provides an overview of these studies and draws conclusions for future uses of this rich and previously unexplored genus.

Keywords *Weissella* · Technological potential · Exopolysaccharide · Probiotic

Introduction

Advances in molecular biology can be credited with the discovery of the *Weissella* genus. Collins et al. [1] reported that strains with very similar phenotypic characteristics that had previously been included in the *Leuconostoc* genus exhibited different DNA profiles from other bacteria in the group. The authors used 16S rRNA sequences to study *Weissella*. They found that certain strains demonstrated specific features that were phylogenetically closer to *Leuconostoc paramesenteroides* than others. Collins et al. [1] recommended that *L. paramesenteroides*, a new species isolated from fermented sausages, and certain heterofermentative lactobacilli be classified as a new genus, *Weissella*.

The *Weissella* genus belongs to the Firmicutes phylum, Bacilli class, the Lactobacillales order, and the *Leuconostocaceae* family. *Weissella* bacteria are closely related to other lactic acid bacteria (LAB) genera. Fusco et al. [2] reported significant difficulty in separating the *Weissella* genus from other LAB, particularly from *Leuconostoc* species. *Weissella* species morphology may differ within the genus. The short rods with rounded to tapered ends or coccoid shapes that typify *Weissella* species are similar to *Leuconostoc*, *Oenococcus*, and streptococci [1]. Nevertheless, some *Weissella* species can present pleomorphisms under stress conditions. Cells can be present alone, in pairs, or even in small chains [1, 3]. Currently, 25 *Weissella* species have been determined: *W. viridescens*, [4], *W. paramesenteroides* [5], *W. confusa* [1], *W. kandleri* [6], *W. halotolerans* [7], *W. minor* [7], *W. hellenica* [1], *W. thailandensis* [8], *W. soli* [9], *W. cibaria* [10], *W.*

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koreensis[11], *W. ghanensis* [12], *W. beninenses* [13], *W. fabaria* [14], *W. ceti* [15], *W. fabalis* [16], *W. oryzae*[17], *W. diestrammenae* [18], *W. uvarum* [19], *W. cryptocerci* [20], *W. bombi* [21], *W. jogaejeotgali* [22], *W. kimchi* [23], *W. muntiaci* [24], and *W. sagaensis* [25]. A clear, well-documented summary of *Weissella* taxonomy and ecology can be found in Fusco et al. [2]. Although *Weissella* remains a relatively new genus compared with other LAB, it has been the focus of research in the past 5 years and has attracted a strong interest from the food industry. Specific *Weissella* strains have been studied for their ability to produce (i) antimicrobial compounds like bacteriocins and hydrogen peroxide and (ii) exopolysaccharides (EPSs) and diacetyl. The latter are of major importance to dairy applications [26–29]. Different strains have been isolated from artisanally produced foods, which demonstrate the contributing role *Weissella* plays in the foods' characteristic features. Other studies have highlighted *Weissella* strains' ability to survive in the gastrointestinal tract (GIT) and even grow in the gut. Both of these attributes suggest the use of *Weissella* species as beneficial applied probiotics [30–32].

The purpose of this review is to provide an overview of the *Weissella* genus and present the latest research and findings from the past 5 years. The paper presents the most recent techniques and approaches used in *Weissella* identification and characterization. It also covers the bacteria genus' potential health benefits and its main technological features which are of interest to the food industry.

Weissella Species Identification and Characterization

The past 5 years have seen the development of new methods for identifying *Weissella* species. They now make it possible to distinguish *Weissella* species from other closely related LAB. The polyphasic identification method and sequencing and analysis of the 16S and 23S rRNA genes are two new molecular-based identification methods that allow for rapid differentiation between *Lactiplantibacillus plantarum*, *Pediococcus pentosaceus*, and *Weissella confusa* [33]. Complete genome sequence analysis using bioinformatic analysis can be used to describe metabolic routes and to predict potential uses for the identified strains [34–38]. These methods are the foundation of modern microbial taxonomy, as are matrix-assisted laser desorption ionization-time of flight method (MALDI-TOF) and database mass spectrometry [39, 40]. One recent study has shown that the colony morphology of *W. confusa* can change according to the adopted culture media and incubation conditions. *W. confusa*'s morphology can vary from rod-shaped coccobacilli—with characteristic, small, cream-colored colonies gathered in single, short or long chains—to a large, irregularly shaped, transparent morphology that lacks characteristic chain cell organization [41]. *Weissella* strains can also thrive in high-sugar niches and produce large amounts of dextran [41]. *Weissella* strains' behavior in different substrates is key to differentiating them from other LAB.

Genomic and metatranscriptomic methods have been used to assess the metabolic and fermentative traits of *W. koreensis* during the kimchi fermentation process. This has led to the characterization of metabolic pathways for certain carbohydrates fermented by *W. koreensis*. These include D-glucose, D-mannose, D-lactose, L-malate, D-xylose, L-arabinose, D-ribose, N-acetyl-glucosamine, and gluconate [42]. The determination of carbohydrate metabolic pathways makes it possible to predict how specific *Weissella* substrates will ferment, as well as pinpoint those which promote higher cell multiplication rates. It also allows for an estimation in type and quantity of products generated by the fermentation process. With this information, it is possible to predict the ecosystems where *Weissella* will survive and thus potential food and pharmaceutical applications for specific species.

Rizzello et al. [43] delved into *Weissella* genus metabolism by focusing on *W. cibaria* and *W. confusa* strains. The resulting data showed that *W. cibaria* and *W. confusa*. were able to both produce phytase and use phytic acid, thus counteracting an anti-nutritional factor in legumes. The two strains were also able to reduce the raffinose oligosaccharide (RFO) concentrations of α-galactosides raffinose, verbascose, and stachyoserafinose. RFO concentrations represent another anti-nutritional factor in vegetables, especially fava beans. When *W. cibaria* and *W. confusa* strains undergo the formation process, RFO hydrolysis enables galactose production. [43]. Legumes such as fava beans, peas, and lentils are a good source of vegetable proteins. Their anti-nutritional factors include saponins, condensed tannins, and protease inhibitors. α-Galactosides and phytic acid [44] have been shown to inhibit them. Fermentation and its subsequent reduction of these anti-nutritional factors would allow these foods to be used as new protein sources for both humans and animals. These new protein sources could in turn be used to develop new products in the food industry.

Many *Weissella* strains have been isolated from fermented products such as kimchi, pozol, jeotgal, and ogi [33, 39, 42, 45]. These strains have been characterized in terms of the carbon source they use and the compounds they can produce. While studying carbon source metabolism, López-Hernández et al. [45] observed that strains *W. cibaria*, *W. confusa*, and *W. paramesenteroides* were able to metabolize D-xylose, glucose, D-fructose, D-mannose, sucrose, and D-maltose. Some strains were also able to use ribose and esculin ferric citrate. Others were able to use galactose, metabolize cellobiose, and demonstrate β-glucosidase and β-galactosidase activity. The latter is a desirable characteristic in probiotic microorganisms, as β-glucosidase breaks down certain compounds and makes them easier for the body to absorb. Anthocyanins, which have antioxidant and anti-inflammatory properties, are among these compounds [46].

New identification methods have allowed for rapid and accurate identification of *Weissella* strains and reduced the risk of misidentification. Characterizing *Weissella* strains based on their metabolic pathways opens up the possibilities for their use in the medical and food production sectors.

Beneficial Properties of *Weissella*

Once the metabolic pathways of *Weissella* have been characterized, isolated strains can be studied for use in the pharmaceutical and food industries. *Weissella* isolates have beneficial, probiotic features and are able to produce EPS. These make them an important bacteria in both pharmaceutical and food production sectors. Abriouel et al. [47] described the technological, functional, and pathogenic potential of *Weissella* genus applications in the food industry, but their review only covered research carried out before 2015. Below, our review presents and discusses the primary benefits of *Weissella* based on these areas of study.

Health Benefits of *Weissella*

Few studies in the past 5 years have examined the effects of *Weissella* strains on human health [48–50]. Some research has evaluated their effects on mice [51–55], gerbils [56], and beagles [57]. Most studies have demonstrated how *Weissella*'s probiotic characteristics and antimicrobial properties may offer health benefits. Only two strains of *W. cibaria* have been studied for their probiotic potential for humans. *W. cibaria* JW15 was studied because of how it affects cytokine and immunoglobulin natural killer cell activity, and *W. cibaria* CMU has been the subject of research because of its oral epithelial cell adhesion and oral colonization; *W. cibaria* CMU's antibacterial activity against *Fusobacterium nucleatum* and *Streptococcus mutans* [48–50] represents another important species feature. A recent study found that immune functions were enhanced by an increase in natural killer cell activity for subjects who consumed probiotic *W. cibaria* capsules [50]. Moreover, oral ingestion of *W. cibaria* CMU can help to reduce halitosis and microbiota numbers in the gingival sulcus, thus improving oral health [48, 49].

Fonseca et al. [56] tested strains of *Bifidobacterium longum* and *W. paramesenteroides* to assess their probiotic potential and their impact on parasite load in gerbils. They observed the positive effect these two strains had in reducing the parasitic load of animals infected with *Giardia*. Strains of *W. cibaria* have been studied as a way to reduce cancer treatment drug side effects in mice [55]. *W. cibaria* strains were shown to assist in the recovery of lymphocytes, hemoglobins, and platelet levels that drop when cyclosporamide (an anti-cancer agent) is administered. Cyclosporamide is widely used in the treatment of acute and chronic leukemia, lymphoma, and a number of autoimmune diseases [55]. *W. cibaria* strains also proved to be efficient in treating atopic dermatitis when administered orally to mice. *W. cibaria* improved the clinical symptoms of lesions, such as erythema/hemorrhage, edema/excoriation, erosion, scarring/dryness, and lichenification [54]. In yet another study, *W. cibaria* was shown to reduce the destruction of periodontal tissue in mice and also protected against alveolar bone destruction in mice. *W. cibaria* co-aggregates with periodontal bacteria to produce hydrogen peroxide and bacteriocins that inhibit the production of pro-inflammatory cytokines [58]. Although clinical studies using mice are an effective research technique, these studies cannot reliably predict the outcome of human studies [59]. Further study is needed to determine the health benefits of *Weissella* strains for humans.

Although there have been few human or animal studies using *Weissella* strains in vitro research indicates a potential for future use as antimicrobial agents (Table 1)

Table 1 *Weissella* species with antimicrobial effects and the respective pathogens that each one inhibits

Species	Pathogen	Reference
W. viridescens	*L. monocytogenes*	Stratakos et al. [60]
W. paramesenteroides	*Giardia lamblia*	Fonseca et al. [56]
	E. coli *S. aureus*	Pabari et al. [62]
W. cibaria	*Porphyromonas gingivalis* *Prevotella intermedia* *Fusobacterium nucleatum*	Do et al. [57]
	S. enterica subsp. enterica *Escherichia coli*	Tenea et al. [108]
W. confusa	*S. enterica* Typhi	Pelyuntha et al. [109]
	E. coli	Dey et al. [29]
	Candida albicans	Rosca et al. [80]
	S. enterica *S. enterica* Typhi *L. monocytogenes* *S. aureus*	Lakra et al. [61]
	L. monocytogenes *S. aureus* *S. enterica* Typhimurium *B. cereus* *E. coli*	Dey et al. [27]

Table 2 In vitro and in vivo evaluation of *Weissella* species as a probiotic candidate

Species	Probiotic property	References
Weissella spp.	Cholesterol-reducing potential	Anandharaj et al. [30]
W. cibaria	Enhance in immune functions	Lee et al. [50]
	Reduce the side effects of drugs	Park and Lee [55]
	Improve atopic dermatitis	Lim et al. [54]
	Protective effects against alveolar bone destruction	Kim et al. [58]
	Short-chain fatty acids production and adhesion to HT-29 cells	Silva et al. [64]
W. confusa	inhibition of –α-amylase activity	Xia et al. [32]
	Thermostability, cholesterol removal, β-galactosidase production and proteolytic activity	Sharma et al. [63]
W. confusa and *W. cibaria*	reduction in cholesterol, DPPH free radical and inhibition of linoleic acid peroxidation	Xia et al. [32] Lakra et al. [61]
W. paramesenteroides	Formation of short-chain fatty acids and antimicrobial compounds	Pabari et al. [62]

and probiotics (Table 2). Further study on animals and/or humans is necessary to prove their efficiency as a functional species. The *W. confusa* DD_A7 strain has been shown to be a natural alternative prophylactic agent against multidrug-resistant (MDR) ESBL (extended-spectrum β-lactamase) positive *E. coli* bacteria [27, 29]. *W. confusa* has also been shown to be effective destroying *Listeria monocytogenes* (ATCC 7644), *Staphylococcus aureus* (ATCC 12600), *Salmonella* Typhimurium (ATCC 43174), *Bacillus cereus* (ATCC 13061), and *E. coli* O157: H7 (ATCC 43889) pathogens. Several studies have shown that the supernatant from of *W. confusa* culture weakened the membrane of the pathogen *E. coli* O157: H7 (ATCC 43889) and damaged the integrity of its DNA [27, 29]. Another study which paired *W. viridescens* with high-pressure processing showed strong results when it came to protecting ready-to-eat salads from *L. monocytogenes*. The research showed that high pressure applied to the weakened pathogen membranes which then facilitated the antimicrobial activity produced by the *W. viridescens* strain [60].

Recent in vitro studies have shown that *W. cibaria*, *W. koreensis*, *W. confusa*, and *W. paramesenteroides* strains are strong candidates for probiotic applications [30, 32, 61–64]. The survival of these strains in the GIT and their tolerance levels when in contact with bile and acids are important factors for intestinal colonization and pathogen protection. *W. paramesenteroides* has also been shown to form a biofilm which facilitates mucin adherence. It uses fructooligosaccharides (FOS) and galactooligosaccharides (GOS) as prebiotic fibers to the form of compounds that can survive the gut microbiome. *W. paramesenteroides* has also demonstrated antimicrobial activity against *E. coli* and *S. aureus* [62]. In another study, Anandharaj et al. [30] demonstrated that *Weissella* species may help reduce cholesterol due to its ability to use cholesterol from de Man, Rogosa and Sharpe (MRS) agar with added Oxgall and water-soluble cholesterol. Cholesterol reduction is common in probiotic microorganisms isolated from the intestine [65–67]. Interestingly, Kim et al. [58] found that using the *W. cibaria* D30 probiotic strain in fermented *Inula britannica* created a product with improved anti-inflammatory capacity.

W. confusa and *W. cibaria*—isolated from Plaa Som Fug—have also been shown to produce folate. Folate, or Vitamin B9, plays an important role in DNA replication, repair, and methylation. It can also act as an antioxidant [68]. Folate is important for immune and nervous system functions because it plays an integral part in the synthesis of neurotransmitters in the central nervous system [69]. This is especially the case during the early stages of uterine growth and development.

Extensive research has been carried out on *Weissella* genus antimicrobial activity and its potential use of strains as probiotics. However, other beneficial compounds produced by *Weissella*, including vitamins, have only been touched on in research thus far.

EPS Production by *Weissella*

EPSs are extracellular macromolecules that have potential applications in the food, medical, and pharmaceutical industries. It has been shown that *Weissella* strains can produce these molecules, and EPS production has been the focus of several *Weissella* studies (Table 3). EPS production has been primarily documented for *W. confusa* and *W. cibaria* strains, with dextran as the dominant EPS produced by the strains. Structurally, dextran molecules are highly linear. They are formed by α-1,6-linked glucose residues with branches linked to α-1,2, α-1,3, or α-1,4. The α-1,2 bonds are representative of prebiotic properties because they are resistant to adverse GIT conditions and can help to modulate the intestinal microbiota [41, 70–72]. EPS produced by LAB are generally recognized as safe (GRAS), which means they can be used in foods, prebiotics, and medical applications.

Table 3 *Weissella* species and its EPS products

Species	EPS produced	References
W. confusa	EPS	Benhouna et al. [28]
	Dextran	Heperkan et al. [41]
		Rosca et al. [80]
		Tang et al. [86]
	Galactan	Devi et al. [73]
		Kavitake et al. [81]
	Mannan	Lakra et al. [110]
	Lactose- and cellobiose-derived branched trisaccharides Isomelezitose	Shi et al. [78]
	Homopolysaccharide with glucose monomers	Adesulu-Dahunsi et al. [33]
Wild and mutant *W. confusa*	EPS with eight sugar moieties	Adebayo-Tayo et al. [51]
W. cibaria	Dextran	Xu et al. [87]
		Zannini et al. [84]
		Baruah et al. [77, 82]
		Kanimozhi et al. [72]
		Yu et al. [75]
	Linear dextran	Ye et al. [74]
	Dextran Oligosaccharides	Hu, Gänzle [71]
	Heteropolysaccharides	Zhu et al. [76]
	Isomalto-oligosaccharide	Baruah et al. [77, 82]
		Rolim et al. [85]
	EPS mainly composed of glucose and rhamnose sugar units	Adesulu-Dahunsi et al. [26]

Of the LAB *Weissella* species, *W. cibaria* and *W. confusa* are the most important EPS producers, which is why research has focused on them and their production, purification, and characterization [41, 71–76]. Some studies have proposed ways to optimize EPS production and to test different parameters, such as the effects of different temperatures and substrates. Hu and Gänzel [71] showed that *W. cibaria* strains grew faster at 30 °C, but oligosaccharide production was higher at temperatures at or below 20 °C. The greatest dextran production occurred at 15 °C.

The physicochemical properties of bacteria-produced EPS are an important factor for functionality and use. Devi et al. [73] carried out physicochemical characterizations of galactan EPS produced by a *W. confusa* strain. They showed that the EPS displayed a high oil absorption capacity, strong emulsifying activity, and an emulsion kinetic stability of up to 15 days. These results supported the theory that galactan EPS may be a good candidate as an emulsifier in forming long-term colloidal systems in food, pharmaceutical, and cosmetic products. Other studies have shown that several *Weissella* strains use a dextransucrase enzyme to produce dextran. This enzyme has been the focus of isolation and purification studies for the production of EPS [77, 78] and caffeic acids [79]. Dextransucrase produced by a *W. confusa* strain has been shown to synthesize a rare oligosaccharide called isomelezitose. This oligosaccharide has a potent nutraceutical effect due to its ability to promote bifidobacteria growth in the colon [78].

The EPS produced by LAB have antifungal [80], anti-inflammatory, and immunomodulatory properties [51] that may be of great interest to the medical sector. EPS can also act as antioxidants [26, 28, 51, 76] and help probiotics survive passage through the GIT [81]. The linear EPS galactan produced by the *W. confusa* strain shows strong potential for encapsulation technology that is used to deliver bioactive compounds, probiotics, and drugs. The EPS produced by a *W. confusa* strain was shown to inhibit the multiplication of *Candida albicans* and was able to destroy up to 70% of the biofilm formed by this pathogen [80]. This suggests that it could be a promising candidate for use in antifungal treatments. Another recent study showed that *W. confusa* can produce mannan, an EPS made up of only monomeric units of mannose. This EPS displayed antibiofilm activity against pathogenic bacteria such as *S. aureus*, *L. monocytogenes*, *S. enterica*, and *S. typhi*. It also demonstrated potential as an antioxidant compound [61].

EPS may also have prebiotic properties. This is the case for the EPS produced by the *W. cibaria* strain isolated from pomelos (*Citrus maximum*) grown in India [82]. To be categorized as a prebiotic compound, the EPS carbohydrates must demonstrate higher metabolization levels from probiotic bacteria than from enteric bacteria present in the intestine. Dextran produced by *W. cibaria* was shown to stimulate the growth of *L. plantarum*, *L. acidophilus*, *B. animalis*, *B. bifidum*, and *B. infantis* more than *E. coli* and *E. aerogenes* [82]. This activity confirmed its classification as a prebiotic compound.

In the food industry, EPS can be used to improve the rheological characteristics and texture of fermented products. EPS can also play a role as an emulsifier and stabilizer [28, 74, 81]. EPS-producing LABs can be used as starter or secondary cultures in the production of fermented products such as yogurt [83]. The use of these cultures leads to the production of EPS in situ, where they act as natural thickeners and can eliminate the need for artificial additives [76]. *Weissella* strains have been used to ferment quinoa-based yogurt [84] and fruit juices [85, 86]. The EPS produced can improve the sensory characteristics of such products. One example: Orange juice concentrate was used to produce oligosaccharides. Then, after a fermentation process involving the sucrose present in the juice itself, a drink was obtained with reduced a sugar content and specific acidity and sweetness levels [85, 86].

EPS are also of interest to the frozen dough industry, as they can modify the texture and improve the end product characteristics of frozen and thawed doughs. Studies have shown that dextrans produced by different microorganisms have different functionalities. For instance, dextran produced by the *W. cibaria* strain demonstrated a greater ability to reinforce gelling properties compared with dextran produced by *L. pseudomesenteroides* when the strains were mixed with fava bean (*Vicia faba L.*) protein isolate [87, 88]. *Weissella* EPS has also been shown to improve the characteristics of wheat gluten during dough freezing and thawing. In general, the freezing-thawing process leads to an increase in water loss, a decrease in gluten viscoelasticity, and changes in water mobility and distribution. During freezing and thawing, the continuous structure of gluten is destroyed by mechanical damage caused when ice recrystallizes. An addition of *W. confuse*-produced EPS to doughs helped the gluten to maintain its qualities and structural integrity during the freezing-thawing process. This makes *Weissella* EPS promising cryoprotectant candidates for the frozen dough industry [86].

Weissella as an Emerging Pathogen

Weissella isolates with potential health benefits must also be assessed for any adverse effects. Testing is standard procedure for isolates to be used as food supplements and starter cultures to determine if they harbor virulent or antibiotic-resistant genes, even when the negative effects are not active or apparent. [89]. If or when any noxious genetic properties are present in the isolate genome, they represent a health risk because they may be transferred to other bacteria present in the food matrix or gut [90–92].

Although many LAB cultures found in foods and are considered safe, the biosafety of new cultures must be established before they can be used in food production [93]. Most of the studies carried out to determine *Weissella* strains' probiotic potential also evaluate the strains' health risks. These assessments include tests for hemolytic capacity and resistance to antibiotics, PCR detection of virulence genes, and biogenic amine production [94–96]. Kang et al. [97] assessed the safety of *W. cibaria* CMU and CMS1 when used for oral care probiotics. Their results confirmed the strain is resistant to vancomycin and kanamycin (antibiotics) and that the resistance is intrinsic to the genus. They also demonstrated that the antibiotic resistance of *W. cibaria* CMU was not transferred to recipient strains. This was confirmed by the fact that no homology for a conjugative transposon integron-specific gene was present, i.e., there was no trace of antibiotic resistance genes in either chromosomal or plasmid DNA samples. They found that the strain had negative results for β-hemolysis, mucin degradation, and platelet aggregation tests. The strain also tested negative for various toxic secondary metabolites [97].

Until now, *Weissella* strains have only been associated with "weissellosis," a disease that affects rainbow trout (*Oncorhynchus mykiss*). The disease leads to the septicemia the fish and can cause important economic losses on fish farms. "Weissellosis" is caused by a specific species, the *W. ceti* [98–100]. Because certain *Weissella* strains are resistant to antibiotics such as vancomycin, teicoplanin, ceftazidime, and sulfamethoxazole, human infections remain a possibility. These would primarily affect immunocompromised patients [3, 101]. It should be noted, however, that even generally recognized probiotic strains may cause health complications. *Lacticaseibacillus rhamnosus* GG and *L. helveticus* are two examples of this [102, 103].

Even though there are few cases of this genus causing disease in humans or animals, recent studies have evaluated the safety of *Weissella* use in foods. Any strain considered for use in fermented foods must be approved by government regulatory commissions. Although Brazil, the USA, and European Union countries do not have specific legislation for the production of new types of yeast, they do have recommendations. In the USA, for example, some cultures are classified as safe and suitable for human consumption while others are classified as GRAS and must apply for approval from the FDA (Food and Drug Administration) [104].

In 2011, some *Weissella* species, including *W. confusa*, were added to the International Dairy Federation (IDF) inventory, a list of microorganisms allowed in food fermentation processes. The German Committee for Biological Agents has also placed *W. confusa* in Risk Group 1 for microorganism that are unlikely to cause human diseases [105]. *W. confusa*'s important technological potential as a source of probiotics and other health benefits have led to two studies that address the microorganism's safety [105, 106]. Cupi, Elvig-Jørgensen [106] carried out a series of toxicity tests which included genotoxicity, skin irritation, eye irritation, and sub-chronic oral toxicity in rats. They found that the *W. confusa* strain did not show any toxic effects and therefore can be used as a safe, direct-feed microbial product for animals. Kang et al. [97] showed that *W. cibaria* strains could be granted GRAS status in the future because: (i) they do not have antibiotic-resistant genes, (ii) they lack antibiotic resistance transferability, (iii) their genomic sequences do not include virulent genes related to pathogenic bacteria, and (iv) they tested negative for most virulent factors (β-hemolysis, mucin degradation, etc.) and for toxic metabolic production (ammonia production, β-glucuronidase activity, etc.).

Although many studies have demonstrated the safety of *Weissella*, the genus has not yet been included in the Qualified Presumption of Safety (QPS) list published by the European Food Safety Authority (EFSA) [107]. Research on *W. cibaria* and *W. confusa* has been carried out to this end [96]. To be granted the QPS status, a microorganism must meet the following criteria: (i) its taxonomic identity must be well defined, (ii) the available body of knowledge must be enough to establish its safety, (iii) the lack of pathogenic properties must be established and substantiated, and (iv) its intended use must be clearly described [107].

Conclusion

Over the past 5 years, the *Weissella* genus has been the focus of many studies due to its strong potential as a probiotic that could be used in both the food and pharmaceutical industries. *Weissella* strains' probiotic potential is attributed to its remarkable ability to survive passage through the GIT, produce antimicrobial substances for a variety of pathogens, and promote the formation of compounds that stimulate the gut microbiome. The ability of some *Weissella* strains to produce large amounts of EPS represents another major attribute, as EPS have prebiotic properties. *Weissella* EPS production also demonstrates the strains' potential as a natural thickener for foods, among other possibilities.

Moreover, recent studies have demonstrated that *Weissella* strains do not have antibiotic-resistant genes and they generally test negative for virulent factors. Thus, there is a low probability that the genus could cause foodborne diseases or carry on virulent genes that may be transferred to other bacteria pathogens.

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Declarations

Conflict of Interest The authors declare that they have no competing interest.

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