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Functional Aspects and Potential Applications of *Weissella* Species in Food and Health

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Abstract

Weissella species are Gram-positive, non-spore-forming, catalase-negative, obligate heterofermentative lactic acid bacteria. They inhabit nutrient rich environments and can be isolated from a variety of sources and different fermented foods. They possess functional and technological attributes which can be of use in the safety, nutritional and sensory characteristics of foods. Among the members of this genus, *Weissella cibaria* and *Weissella confusa* have been described as high producers of exopolysaccharides and oligosaccharides, and hence exhibit texturizing properties. Several *Weissella* species isolated from food and animal sources are reported to possess various health promoting effects like cholesterol reduction, antioxidant activity, immunomodulatory effect, oral health promoting effects and anticancer effects.

Keywords: *Weissella*, Functional, Foods, Health, Lactic acid bacteria

Among the major Genera coming under the Lactic Acid Bacteria (LAB) group, the re-classification of *Lactobacillus* and *Leuconostoc* saw emergence of a new Genus called *Weissella* named after German microbiologist Norbert Weiss. It was proposed as a genus in 1993 (Fessard and Remize, 2017). Members of this genus are Gram-positive, catalase-negative, non-endospore forming cells with coccoid or rod-shaped morphology (Abrouel *et al*. 2015). Species belonging to *Weissella* are generally non motile in nature with the exception of *Weissella behninesis* which is motile due to peritrichous flagella (Padonou *et al*. 2010). *Weissella* species belong to the phylum *Firmicutes,* class *Bacilli*, order *Lactobacillales* and family *Leuconostocaceae* (Collins *et al*. 1993).

Because of the complex nutritional requirements, members of the genus *Weissella* inhabit (Table 1) nutrient rich environments and can be isolated from a variety of such sources, including vegetables, meat, fish, raw milk, sewage, blood, soil, the gastrointestinal tracts of humans and animals, as well as the oral cavity and uro-genital tract of human (Fusco *et al*. 2015). Also they are frequently detected in many fermented foods.

Weissella spp. are capable of elaborating bacteriocins, EPS (Exopolysaccharide) and hydrolytic enzymes (Abrouel *et al*. 2015). *Weissella confusa* and *Weissella cibaria* species, are being extensively studied for their ability to produce significant amounts of non-digestible oligosaccharides and extracellular polysaccharides, which can be used as prebiotics or for other applications in food, feed, clinical, and cosmetics industries (Baruah *et al*. 2019). Members

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of genus *Weisella* possess many functional and bio-technological properties, which can enhance nutritional, sensory and bio functional characteristics of foods. Several *Weissella* strains are reported to possess antimicrobial, antioxidant, anti-inflammatory, chemo-preventive and anti-obesity effects.

Table 1: Habitat of *Weissella* species

Species	Habitat or source
W. cibaria	Japanese horseradish, Tomatoes, Orange, Wheat flour, Corn stovers, Black berries, Bulgarian traditional Sour dough, Kimchi, Pickles, Greek Traditional sour dough, Camel milk, Goat milk, Human faeces & Human vagina.
W. confusa	Rhizosphere of olive trees, Soil surrounding rhizosphere, Raw red and yellow peppers, Heroin, Sugar cane, Un-pasteurised Brozoa, Masai fermented milk, Wheat sour dough, Cow milk, Human faeces & Human breast milk
W. halotolerans	Rhizosphere of olive tree, Soil surrounding rhizosphere
W. hellenica	Vegetative forage crops, Heroin, Raw milk cheese & Cow milk
W. kadleri	Dessert spring, Dessert plants & Koumiss
W. kimchi	Fluted pumpkin (*Telfairia occidentalis*) and green vegetable (*Amaranthus spinosus*), Kimchi & Human vagina
W. paramesenteriodes	Fluted pumpkin vegetable (*Telfairia occidentalis*) and green vegetable (*Amaranthus spinosus*), Semillon and Sauvignon Blanc grapes, Indian goosegrass, Mexicanpozol, Goat milk cheese & Faeces of breast-fed infants.
W. soli	Carrots & Dongchimi

Sourced from Fusco et al. 2015.

History and Taxonomy

Collins and colleagues were the first to designate the genus *Weissella* in 1993 after taxonomic studies on atypical *Leuconostoc*-like microorganisms which came from fermented sausages produced in Greece. Further molecular systematic investigations suggested that *Leuconostocs* could be separated into three distinct genetic lineages. Study based on phenotypic, biochemical and 16S rRNA gene analyses allowed the differentiation of the new genus *Weissella* and reassignment of the species previously grouped in the genus *Lactobacillus* as *W. confusa*, *W. halotolerans*, *W. kandleri, W. minor*, and *W. viridescens* (Abriouel *et al*. 2015).

Biochemical Characteristics

Weissella bacteria are facultatively anaerobic chemoorganotrophs with an obligately fermentative metabolism. They do not possess cytochromes and ferment glucose heterofermentatively *via* the hexose-monophosphate and phosphoketolase pathways. End products of glucose heterofermentation include lactic acid (with some species producing only D(−) and others both D(−) and L(+) lactic acid enantiomers), gas (CO_2) and ethanol and/or acetate (Bjrkroth *et al*. 2014). Biochemical and sugar fermentation characteristics of different Weissella species are shown in Table 2 and Table 3.

Molecular Characteristics

DNA: DNA hybridization analyses, together with phenotypic data in a polyphasic taxonomical approach grouped *Weissella* in five phylogenetic branches based on 16S phylogeny. The genome sequence of 28 strains belonging to 12 species has been published. The smallest genomes belonged to *Weissella halotolerans* (1.36 Mb) and *W. ceti* (1.35 to 1.39 Mb), which share the same taxonomic branch. The largest genomes belonged to *W. cibaria*, *W. confusa*, *W.jogaejeotgali* and *W. oryzae.* The core-proteome of *Weissella* represented 729 COGs (Clusters of Orthologous Genes), over a pan-proteome of 4712 COGs (Abriouel *et al*. 2015).

Bacteriocins elaborated by *Weissella spp.*

Several *Weissella* species produce bacteriocins (Table 4) and shows broad spectrum antimicrobial activity against different food borne pathogens. The first bacteriocin produced by *Weissella* strains to be discovered was Weissellicin 110 in the year 2007. This bacteriocin has antimicrobial activity against some

Table 2: Biochemical characteristics of Different *Weissella* species

Characteristics	W. cibaria	W. confusa	W. paramesentriods	W. hellenica	W. koreensis	W. halotolerence
Esculin hydrolysis	+	+	V	ND	-	-
Ammonia from Araginine	+	+	-	-	+	+
Dextran formation	+	+	-	-	+	ND
Lactic acid configuration	DL	DL	D	D	D	DL
Mol % G+C content	44-45	45-47	40.6	39-40	37	44

(+ 90% or more strains are positive), (- 90 or more strains are negative (d-11-89% of strains are positive, (ND-No data available), (V-Variable) Sourced from Fusco et al. (2015).

Table 3: Sugar fermentation characteristics of *Weissella* species

Acid Produced from	W. cibaria	W. confusa	W. paramesenteroides	W. hellenica	W. koreensis	W. halotolerans
Arabinose	+	-	D	+	+	-
Fructose	+	+	+	+	ND	+
Galactose	-	+	+	-	-	-
Maltose	+	+	+	+	-	+
Raffinose	-	-	D	-	-	-
Ribose	-	+	D	+	+	+
Sucrose	+	+	+	-	-	-
Xylose	+	+	D	-	+	-

(+ 90% or more strains are positive), (- 90 or more strains are negative, (d-11-89% of strains are positive, (ND-No data available) Fusco et al. 2015.

Table 4: Bacteriocins produced by *Weissella* species

Name of Bacteriocin	Producing organism	Class	Reference
Weissellicin 110	*W. cibaria* 110	Unclassified	(Srionnual *et al*. 2007)
Weissellin A	*W. parameseteriods* Dx	Class IIA	(Papggianni *et al*. 2011)
Weissellicin L	*W. hellenica* 4-7	Unclassified	(Leong *et al*. 2013)
Weissellicin D	*W. hellenica* D1501	Unclassified	(Chen *et al*. 2014)
Weissellicin M	*W. hellenica* QU13	Unclassified	(Masuda *et al*. 2012)
Weissellicin Y	*W. hellenica* QU13	Unclassified	(Masuda *et al*. 2012)

Gram-positive microorganisms and it is resistant to high temperatures and catalase (Srionnual *et al*. 2007). The listericidal bacteriocin weissellin A was further investigated for its technological application in fermented sausages (Fusco *et al*. 2017). Bacteriocinogenic strain *W. hellenica* D1501 was successfully used to enhance the shelf-life of tofu (Chen *et al*. 2014). Weissellicin MBF is encoded by a large transferable plasmid of 17.6 kbp, pWcMBF8-1. It was the first study which reported plasmid-associated bacteriocin activity in the genus *Weissella* (Malik *et al*. 2016).

FUNCTIONAL ASPECTS

Antimicrobial activity

Several *Weissella* strains produce a variety of antagonistic substances, including organic acids and bacteriocins. *Weissella cibaria* TM128 strain showed inhibition against phyto-pathogens *Monilinia laxa*, *Erwinia carotovora* and *Xanthomonas campestris* by production of organic acids and hydrogen peroxide (Trias *et al*., 2008). Singh *et al.* (2015) explored bacterial strain, *Weissella oryzae* DC6, isolated from mountain ginseng, for the synthesis of silver nanoparticles. The synthesized nanoparticles were evaluated for their antimicrobial activity against clinical pathogens including *Vibrio parahaemolyticus, Bacillus cereus, Bacillus anthracis, Staphylococcus aureus, Escherichia coli*, and *Candida albicans*. Further the potential of nanoparticles has been observed for biofilm inhibition against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Their study concluded that the synthesis of silver nanoparticles by the strain *W. oryzae* DC6 may serve as a simple, green, cost-effective, consistent, and harmless method to produce antimicrobial silver nanoparticles. *W. confusa* K75KT361205 strain isolated from vaginal swab showed highest inhibition against *P. aeruginosa* (MTCC3541) followed by *Proteus mirabilis* (MTCC428).The results indicated that this particular strain can be used for treating vaginal infections (Purkhayastha *et al*. 2017).

Yu *et al*. (2019) reported that antimicrobial activity of *W. cibaria* JW15 is stronger than probiotic strain *Lactobacillus rhamnosus* GG (LGG*). Weissella* strain showed higher inhibition against *Listeria monocytogenes* and *Salmonella enteritidis* and also showed higher lactic acid and acetic acid production than LGG (Yu *et al*. 2017)*.* Huy *et al.* (2020) reported that *W. cibaria* HN05 isolated from white pacific shrimp exhibited strongest antagonistic activity against *Vibrio parahaeamolyticus with* antagonistic activity 220 AU/ml to 500 AU /ml. *W. cibaria* HN05 inhibited *E. coli* growth with antagonistic activity of 240 AU/ml. Fhoula *et al*. (2018) reported that *W. halotolerans* F99 isolated from camel faeces showed highest zone of inhibition against *P. aeruginosa* ATCC27853 (24.6 mm) followed by *S. typhimurium* IPT13 (21.4 mm).

Antioxidant activity

The cell free extracts (CFS) and EPS from several *Weissella* strains isolated from fermented foods shows high antioxidant activity. They produce several antioxidant enzymes including superoxide dismutase, Glutathione reductase and NADH oxidase. Sharma *et al.* (2018) studied the anti-oxidative activity of *W. confusa* KR780676, isolated from an Indian traditional fermented food (*Idli* batter). Cell-free extract showed prominent hydroxyl radical scavenging activity, while intact cells exhibited significant DPPH, superoxide anion radical scavenging potential and lipid peroxidation inhibition. *W. cibaria* GA44 isolated from *Gari* showed antioxidant activity. The *in vitro* antioxidant activities of the EPS showed good scavenging effects on superoxide anion radical and hydroxyl radical (Dahnusi *et al*. 2018). Yu *et al*. (2019) studied the antioxidant property of *W. cibaria* JW15. They have found that intact JW15 cells showed radical scavenging effect ranging from 23.53 to 60.85% and inhibited lipid peroxidation of 33.31% in the tested assays. Additionally, JW15 exhibited a statistical difference on the antioxidant effect compared to *L. rhamnosus* GG.

Amrutha *et al*. (2019) studied the anti-oxidant activity of *Weissella* strains isolated from tender coconut water. They have found that the EPS produced by *W. cibaria* DMA 18 displayed remarkable antioxidant activity. DPPH assay revealed antioxidant activity of 27% and 33.55% respectively with sample volumes as low as 10 μl and 6μl respectively. The results confirm the potential of *W. cibaria* to be used as a functional culture for designing foods to ameliorate disorders induced by oxidative stress. Mercha *et al*. (2020) reported that *Weissella* strains isolated from camel milk showed higher anti-oxidant activity. *W. confusa* strain showed 59.01 to 63.31% and for *W. cibaria* 52.68 to 62.41% free radical scavenging activity was recorded. The *W. confusa* I17 recorded the highest scavenging ability (63.31%).

Exopolysaccharide production

EPS produced by strains of the genus *Weissella* (Table 5) are homopolysaccharides (HoPS) such as dextran, fructan, galactan, levan and inulin. Strains of the genus *Weissella* use sucrose as the obligatory substrate for the extracellular synthesis of HoPS. The enzymes

Table 5: EPS Produced by different *Weissella* strains

Species	Substrate	EPS	Reference
W. cibaria RBA12	Sour dough	Dextran	(Baruah *et al*. 2016)
W. confuse	Rye bran	Dextran	(Kajala *et al*. 2016)
W. confusa KR780676	2% sucrose supplemented MRS agar	Linear exopolysaccharide, Galactan	(Devi *et al.* 2016)
W. confusa & W. cibaria	MRS agar supplemented 10% sucrose	Dextran, Fructan, Leavan, Inulin	Malang (*et al*. 2015)

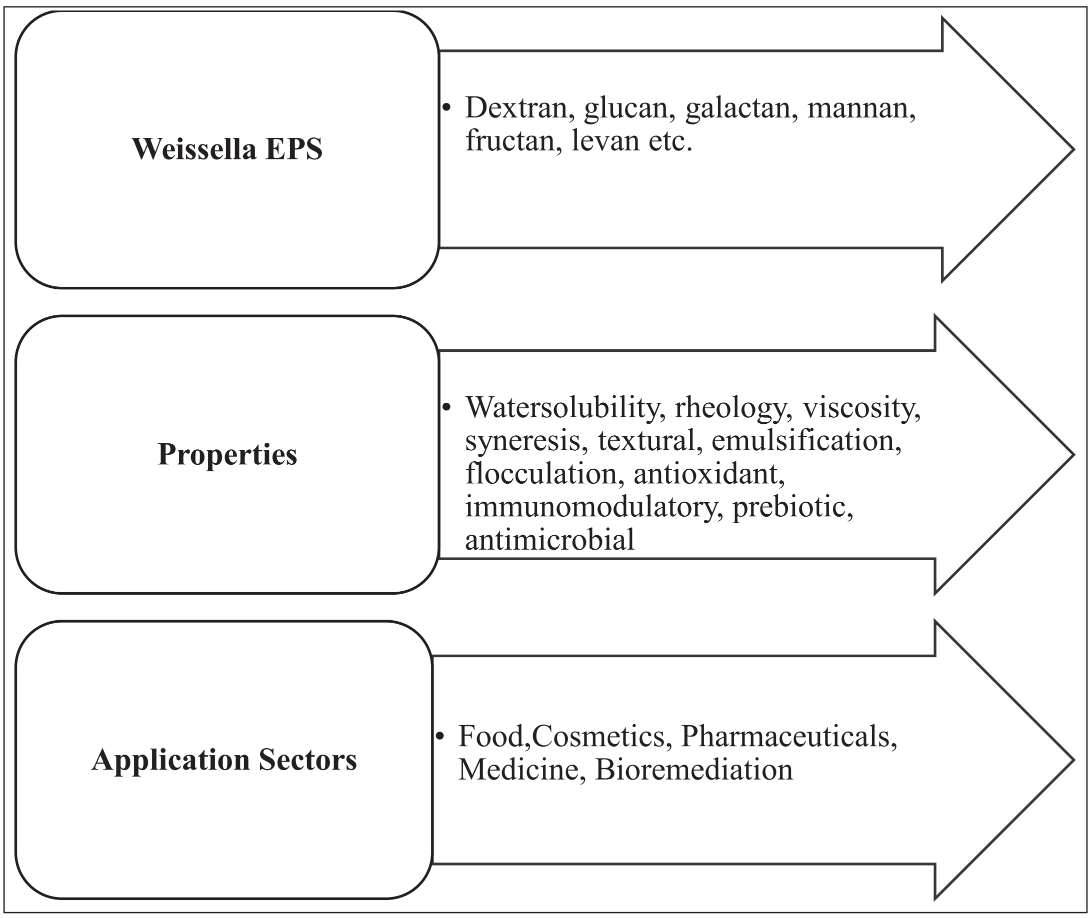

Fig. 1: Types of EPS, Properties and Application Sector
(Sourced from Baruah *et al*., 2019)

used to hydrolyze sucrose are glycosyltransferase, glucan-sucrase (GS) or fructan-sucrase (FS) (Teixeira *et al*. 2018). *W. confusa* and *W. cibaria* have ability to produce significant amounts of dextran. Dextran produced by *Weissella* spp. have similar structures with mainly α-(1-6) linkages and only α-(1-3) linkages (Malang *et al*. 2016). The EPS produced by *Weissella* species possess different properties like water solubility, rheology, viscosity, textural, emulsification, flocculation, antioxidant, immunomodulatory, prebiotic, antimicrobial properties (Fig. 1).

Galle *et al*. (2011) screened EPS producing *Weissella* strains for use as starter in sorghum and wheat sourdough. *W. cibaria* MG1 produced considerable amount of dextran (0.6 to 8.0 g/kg sourdough) which were supplemented with 15% sucrose. Incorporation of dextran enriched sourdough (10% and 20%) provided mildly acidic wheat bread with improved bread quality.

APPLICATION IN FOOD INDUSTRY

In Bread baking

W. cibaria MG1 was reported as a suitable starter culture for sourdough fermentation of buckwheat, quinoa and teff flour (Wolter, 2012). Galle *et al*. (2011) screened the EPS-forming *Weissella* strains for their potential use as starter strains in sorghum and wheat sourdoughs strains *W. kimchii* and *W. cibaria* MG1 produced dextrans in concentrations high enough to be used as potential replacers of non-bacteria hydrocolloids, such as guar gum and Hydroxyl poly methyl cellulose (HPMC) in gluten-free sourdoughs bread. Malang *et al*. (2015). reported that the EPS dextran, levan and ropy capsular polysaccharide, produced by *W. confusa* were evaluated in breads and the delaying of the deterioration by fungus, as well as improving of the texture. Kajala *et al*. (2015) characterized, and cloned the potential of dextransucrase obtained from *W. confusa* VTT E-90392 in preparing bran for use as a baking aid in high fiber baking. They have concluded that presence of dextran improved bread softness and neutralized bran-induced volume loss.

As Texturizing Agent

Use of EPS dextran produced by *W. cibaria* TN610 to improve textural properties of semi-skimmed milk supplemented with sucrose was reported by Bejar *et al.* (2013). This strain has potential in the application as a safe additive in food to improve the texture of dairy products. *W. cibaria* MG1 is capable of producing dextran & glucooligosaccharides. During sucrose-supplemented barley-malt-derived wort fermentation up to 36.4 g/l of dextran was produced in an optimized system, which improved the rheological profile of the resulting fermentate (Zanini *et al*. 2013). Quinoa-based yoghurt fermented with dextran producer *W. cibaria* MG1 was developed by Zanini *et al*. (2015). Concentration of EPS (40 mg/L) guaranteed the high water retention capacity and viscosity (0.57 mPa s) of the final product. Dextran from *W. confusa* was able to improve the texture and sensory properties of pureed carrots with pleasant odor and flavor (Juvonen *et al*. 2015).

As an adjunct culture in Cheddar cheese making

Lynch *et al.* (2013) examined the potential of dextran producing *Weissella* MG1 for use in Cheddar cheese manufacture as an adjunct starter. The strain survived in cheese with levels increased by 1.5 log cycles over the ripening period. It also increased the moisture retention of cheese without significantly affecting cheese proteolysis.

Application in food packaging

Woraprayote *et al*. (2018) developed a biodegradable food packaging with antimicrobial properties by incorporating the Bacteriocin 7293 produced by the strain *W. hellenica* BCC 7293. They reported that use of this controlled pathogenic bacteria in fillets of pangasius fish. The film produced inhibited the multiplication of both Gram-positive bacteria such as *L. monocytogenes* and *S. aureus* as well as Gram-negative bacteria such as *P. aeruginosa*, *Aeromonas hydrophila*, *E. coli* and *Salmonella typhimurium*

Vitamin production

Some strains of *W. cibaria* and *W. confusa* also have the capacity to produce folate (vitamin B9), which allows the nutritional improvement of fermented products that use these strains in the fermentation process. Divya *et al*. (2012) studied the folate production of *Weissella* strains. *W cibaria* strain isolated from pickle produced 11.2 ng/ml of folate in the medium. They have concluded that further development of the bioprocess using the strain *W. cibaria* can enhance the bioavailability of this vitamin.

The use of glutaminase producing *W. cibaria* MSS2 as starter culture for the production of *Nham* fermented sausage and for kimchi was reported by Abriouel *et al.* (2015).

HEALTH APPLICATIONS

Probiotic Potential

Aiming at developing novel probiotic foods or probiotic animal feeds, many researchers have isolated and screened *Weissella* strains from humans. However, only few studies investigated the probiotic potential of *Weissella* strains using *in vivo* studies. Patel *et al*. (2012) reported that *W. confusa* AI10 was the most resistant strain to bile salts (0.3%), with 72% of survival after 24 h at 37◦C. On the other hand, *Lb. plantarum* AD29 was the less resistant, with 14% of survival. In the same study, *W. cibaria* 142 showed 31% of survival after 2.5 h at 37◦C in MRS broth adjusted to pH 3, which demonstrated the ability of this strain to grow in acidic conditions. Anandharaj *et al*. (2015) reported that *W. koreensis* FKI21 was the best resistant strain to pH 1.0, with 29.8% of survival, while *Lb. crispatus* GI6 showed lower resistance with 18.3% of survival. In this same study, *W. koreensis* and *Lb. crispatus* strains showed approximately the same resistance profile to 0.3% and 0.5% of bile salts.

Elavarsi *et al*. (2015) reported that *W. cibaria* KTSMBNL 28 isolated from goat milk exhibited high potential probiotic properties. This strain was able to tolerate pH3.0 maintained in gastric fluid and resist upto 1% of bile salt retained in intestinal fluid. Moreover, KTSMBNL28 recorded highest level of hydrophobicity (35-70%) and its non-hemolytic property ensured it's safety.

Wang *et al.* (2020) isolated *W. confusa* from human fecal samples and evaluated its probiotic properties. Similar to probiotics, *W. confusa* could inhibit pathogen growth and could tolerate high bile salt concentration and low pH. The survival rate in 0.3% concentration bile salt of *W. confusa* strains isolated from human ranged from 2.2 to 128.8%, and its acid tolerance ranged from 2.4 to 20.2%. Mercha *et al.* (2020) reported that *W. confusa*, *W.cibaria* isolated from camel milk showed great probiotic potential. These isolates were resistant to simulated gastrointestinal tract conditions, presented noticeable hydrophobicity and auot-aggregation percentages, high EPS and diacetyl production abilities, as well as significant acidifying, proteolysis and autolysis activities. These isolates also exhibited antibacterial activity against a wide range of pathogenic bacteria and did not present hemolytic and DNase activities.

Oral health

W. cibaria strain CMU (Chonnam Medical University) has shown oral colonizing ability and inhibitory effects against the formation of volatile sulphur compounds (VSC). *Weissella cibaria* strains showed inhibitory effects against biofilm formation.

W. cibaria isolates from children's saliva were shown to inhibit *in vitro* biofilm formation and proliferation of *S. mutans*, causative agent of early childhood dental caries, by production of polymers from sucrose (Kang *et al*. 2005). In an *In vivo* study on 72 volunteers who rinsed their teeth after brushing in the morning, afternoon and evening, with a rinse that contained the probiotic *W. cibaria* CMU significantly reduced plaque index by 20% (Kang *et al*. 2005). Gargling with oral probiotic solution containg *W. cibaria* CMS1 and CMS2 was resulted in reduction in hydrogen sulphide (H2S) (48.2%) and methanethiol (CH_3SH) (59.2%) and inhibition of *Fusobacterium nucleatum* by production of hydrogen peroxide was observed (Kang *et al*. 2006).

Jang *et al*. (2016) conducted a study to compare the characteristics of oral care probiotics. *W. cibaria* CMU and 4 commercial probiotic strains were used in the study. *W. cibaria* produced less acid and more hydrogen peroxide than other four strains during the study. They have also observed that *W. cibaria* inhibited the biofilm formation by *Streptococcus mutans* (97% inhibition) at lower level concentration (5×10^8 cells/ml). They have concluded that *W. cibaria* CMU can be used as an oral care probiotic.

Park *et al*. (2018) studied the effect of diet containing *W. cibaria* CMU on *S. mutans* causing dental caries. The participants were divided randomly in to two groups and they have been instructed to take the tablets daily. The results revealed that *W. cibaria* CMU diet significantly supressed the biofilm formation on the surface enamel. Oral colonization of *W. cibaria* was also observed which prevented the proliferation of *S. mutans* and prevented the formation of dental caries.

Lim *et al.* (2018) reported that the cell free supernatant (CFS) from *W. cibaria* CMU showed inhibitory effect on *F. nucleatum*. They have identified several antimicrobial compounds like hydrogen peroxide, organic acids and free fatty acids. The CFS was also detected for the secretory protein N-actyalmuramidase having antimicrobial activity against *Prophyromonas hydrophilla.*

Kim *et al*. (2019) reported that CFS of *W. cibaria* CMU (OraCMU) suppressed the production of volatile sulphur compounds not only through its bactericidal effects on *P. gingivalis*, but also via its inactivating effects on bacterial METase. Do *et al*. (2019) conducted a study to analyse the effects of *W. cibaria* CMU on canine oral health. There was a significant reduction in methyl mercaptan, plaque index in the CMU treated groups, and also these groups showed significant decrease in *F. nucleatum*, *Prophromonas gingivalis*, *Prevotella intermedia* and *Tannerella forsythia*. They have concluded that *W. cibaria* have the ability to colonize in the canine oral cavity and it supressed the halitosis and inhibited the proliferation of mal odour causing oral bacteria in beagles.

Kang *et al*. (2019) evaluated the safety of *W. cibaria* CMU and CMSI through genotypic and phenotypic analysis. These strains have been registered as safe raw materials by the Korea Food and Drug Administration (KFDA) and are actively commercialized as oral care probiotics in Korea. Both strains tested negative for haemolytic activity, mucin degradation, platelet aggregation and toxin production. They have observed that there was no antibiotic gene transferability and virulence gene presence in both strains.

Kang *et al*. (2020) reported that oral administration of *W. cibaria* CMU (OraCMU) containing tablets can improve periodontal health and oral microbiota. They have conducted a trial on double blind, placebocontrolled groups in 92 adults without periodontitis. The tablets were administrated once daily basis for 8 weeks. They have observed periodontal clinical parameters including bleeding on probing (BOP), probing dept (PD), gingival index (GI) and plaque index They have found that BOP improved more in probiotic group over 8 weeks.

Anticancer activity

Cha *et al.* (2008) studied the anticancer activity of *W. cibaria* in colorectal cancer. *W. cibaria* was incubated for 24 hours in MRS (Deman Rogosa Sharpe) badge, diluted with phosphate-buffered saline, and 10% concentration of bacteria samples were provided to normal cell strains and colorectal cancer cells strains for 72 hours. After incubation, the suspension of cell growth was measured using the MTT assay. Cell growth was suppressed by treatment of *W. cibaria* in colorectal cancer cells but not in normal cells.

Cholesterol reduction

Use of *W. cibaria* as a starter for food fermentation promoted the formation of ornithine from arginine, which in turn provide health beneficial effects, such as anti-obesity effects due to high levels of ornithine in fermented food (Kwak *et al*. 2014). Kim *et al*. (2006) reported that *W. kimchi* KCTC3746 and *W. confusa* KCTC 3499 showed cholesterol lowering effect of about 55%. Moon *et al.* (2012) in an *in vitro* study demonstrated that intracellular lipid accumulation in 3T3-L1 cells could be inhibited by the ornithine rich cytoplasmic extract of *W. koreensis* OK1-6. Also, cytoplasmic fraction of *W. koreensis* OK1-6 reduced the expression level of lipogenic gene resulting in the reduced triglyceride level in treated 3T3-L1 cells compared with control groups. *W. halotolerans* F99 isolated from camel faeces was found to reduce cholesterol *in vitro* by 49% also the same strain was evaluated for the serum lipid metabolism in Wistar rats fed with a high cholesterol diet. Study results revealed that compared with rats fed a high-fat (HF) diet without *Weissella* administration, total serum cholesterol, low-density lipoprotein cholesterol, and triglycerides levels were significantly reduced in *W. halotolerans* F99-treated HF rats, with no significant change in high-density lipoprotein cholesterol

HDL-C levels. *W. koreensis* FK121 isolated from traditionally fermented *Koozh* was able to assimilate more cholesterol from growth medium than other tested strain. Cholesterol assimilation (µg/ml) for *W. koreensis* FK121 was about 56.25% with bile salts.

Anti-inflammatory and immunomodulatory activity

Ahn *et al.* (2013) reported that the immune control effect of *W. cibaria* was stronger than the well-known probiotic bacterium, *L. rhammosus* GG (LGG). *W. cibaria* produced higher levels of nitric oxide, nuclear factor (NF)-κ B, cytokines (e.g., interleukin-1β and tumor necrosis factor-α) than LGG, suggesting that *W. cibaria* is more effective in immune control compared to LGG. Lim *et al.* (2018) applied *W. cibaria* WIKIM28 isolated from *gatkimchi* in a mouse atopic dermatitis (AD) model and found that this strain can ameliorate AD-like symptoms by suppressing allergic Th2 responses. These results suggest a potential application of *W. cibaria* WIKIM28 as a dietary supplement or a therapeutic agent to ameliorate AD. Lee *et al*. (2018) investigated the impact of consuming *W. cibaria* JW15 on natural killer (NK) cell activity, cytokines and immunoglobulins (Igs) in 100 non-diabetic participants. Administration of *W. cibaria* JW15 for 8 weeks improved the overall immunity in probiotic group by stimulating the natural killer cells activity. Administration of *W. cibaria* JWI5 in an immunosuppressed mouse by cyclophosphamide increased the number of WBC and splenocyte cells and stimulated the production of cytokines (TNF- α and IL-6) (Park *et al*. 2018).

Weissella strains isolated from *idli* batter and infant faeces were tested for cholesterol reduction, adhesion to Caco-2 cells and mucin and their ability to prevent LPS-induced nitric oxide and pro-inflammatory cytokines. All the strains suppressed the production of TNFα (Tumor necrosis factor alpha), IL-6 (Interleukin-6) and IL-8 (Interleukin-8) cytokines in murine macrophage model and suppressed the production of IL-8 in Caco-2 cell model (Singh *et al*. 2018). Yu *et al*. (2019) evaluated the anti-inflammatory potential of *W. cibaria* JW15 against lipopolysaccharide (LPS) stimulation. Heat-killed JW15 displayed anti-inflammatory potential by alleviating the pro-inflammatory features in LPS-induced RAW 264.7 cells through suppressing NF-κB activation. The cellular signaling pathways of its anti-inflammatory effect were involved in inhibition of MAPKs activation.

SAFETY ASPECTS

Clinical Infections Associated with *Weissella* Species

Weissella strains have been isolated from clinical specimens such as blood, skin, infected wounds and feces of both humans and animals. *W. cibaria*, the only species of *Weissella,* which have been described as opportunistic pathogens of humans or as emerging pathogen for farmed rainbow trouts. *W. ceti* has been recognized as etiological agent of weissellosis, an emergent disease occurring in farmed rainbow trout (*Oncorhynchus mykiss*) which cause septicemia with a high mortality rate (Fusco *et al*. 2015).

The presence of several virulence determinants such as collagen adhesins, aggregation substances, hemolysin, mucus binding proteins and staphylococcal surface protein are reported in strains of *W. ceti, W. cibaria, W. confusa, W. halotolerans, W. hellenica, W. koreensis, W. oryzae, W. paramesenteroides* and *W. thailandensis* (Abriouel *et al.* 2015).

CONCLUSION

Weissella is now a well delineated genus in the lactic acid bacteria and the members of this genus occur in a variety of habitats. *Weissella* species produce functional biomolecules such as bacteriocins, EPS and hydrolytic enzymes having potential applications in food and health. *W. cibaria* strains are well known for oral health applications. However, the safety aspects of *Weissella* should be taken into consideration prior to its application in the food and health industry.

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