Safety and functional significance of *Weissella cibaria* and *W. confusa* in food: a polyphasic approach

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ABSTRACT

Weissella cibaria and *Weissella confusa* are controversial species of lactic acid bacteria (LAB) found in food products. They are naturally present in many fermentation processes of vegetables and cereals, with a positive implication for the quality of food. On the other hand, *Weissella* species have been associated to possible human infections, and for this reason the strains of the species are not yet used as starter cultures and are not included in Qualified Presumption of Safety status of European Food Safety Authority (EFSA). An in-depth analysis of the physiological and genetic characteristics of *Weissella* species could help to select suitable strains for possible practical applications. A comparative genome analysis of 15 sequenced *W. cibaria* and five *W. confusa* genomes available to date was carried out, in parallel with a polyphasic study of twelve strains of *W. cibaria* and eight strains of *W. confusa* previously isolated from sourdough-like maize bran fermentation. The comparative genomic analysis resulted in an absence of severe pathogenicity factors. Although some putative virulence genes were found, these, for homology and function, were present in other LAB species/strains, considered safe by EFSA and commonly used as probiotics. The phenotypic tests carried out on our strains corroborated the genomic results. Moreover, interesting functional and pro-technological traits were highlighted in the tested strains, for both the species.

Keywords: Weissella cibaria, *Weissella confusa*, Comparative genomic analysis, Functional characteristics, Antifungal activity, Virulence traits, IS molecular typing

1. Introduction

The different species ascribed to the genus *Weissella* are known for a long time for their presence in various food matrices and in many spontaneous fermentation processes of vegetables and cereals, in particular the species *W. cibaria* and *W. confusa* (Decimo et al., 2017; Fessard & Remize, 2017; Fusco et al., 2015). Their constant presence indicates that they are part of the natural microbial population characterizing different fermented products. Nevertheless, *W cibaria* and *W. confusa* are not yet used as selected starter cultures, are not included in the list of taxonomic units proposed by the European Food Safety Authority (EFSA) for Qualified Presumption of Safety

(QPS) status, and no specific antibiotic breakpoints for these species were suggested by the Clinical and Laboratory Standards Institute (CLSI) or the EFSA.

The scientific literature on *Weissella* spp. it is not negligible, but it mainly refers to the production and characterization of (EPS) (Ahmed, Siddiqui, Arman, & Ahmed, 2012; Di Cagno et al., 2006; Hu & Ganzle, 2018; Katina et al., 2009; Wolter et al., 2014). The use of EPS synthesized by starter cultures is a common practice in the dairy industry and, in sourdough fermentation, improves texture and storage life of bread. Moreover, *W. cibaria* and *W. confusa* are able of producing *in situ* high molecular weight dextrans. These homopolysaccharides improve the softness of fresh bread, and their use is promising in gluten-free baking (Wolter et al., 2014).

Other studies on *Weissella* spp. are fragmentary. However, from these publications it is clear that, although strain-specific, other properties are of interest, both for the quality and safety of food, such as the production of bacteriocins (Masuda et al. 2011; Srionnual et al., 2007), the ability to overcome the gastric barrier (Le & Yang, 2018) and to inhibit micotoxinogenic moulds (Ndagano, Lamoureux, Dortu, Vandermote, & Thonart, 2011; Valerio et al., 2009). In this regard, a strain of *W. cibaria* has been used in probiotic yoghurt to reduce aflatoxin poisoning in Kenyan children (Nduti et al., 2016). These characteristics suggest a possible use of specific strains as potential probiotic cultures, also supported by the hypothesis that the genus may represent a common inhabitant of our intestine (Lee et al., 2012).

On the other hand, *Weissella* species have been associated to possible human infections (Kamboj, Vasquez, & Balada-Llasat, 2015), even though their clinical significance remains unclear, as they have been mainly associated to polymicrobial infection and/or to immune-compromised patients. Little information is available on the mechanism and factors related to their pathogenicity, apart from the intrinsic resistance to vancomycin and fosfomycin.

Today, highlighting possible virulence factors is easier, for the availability of genomes sequenced and deposited in public databases. However, genomic data on *Weissella* are restricted to a few publications (Abriouel et al., 2015; Figueiredo et al., 2015; Li et al. 2017). These comparative genome analyses highlighted several genes putatively involved in virulence, such as genes encoding haemolysins, collagen adhesins and antibiotic resistance-encoding genes. The role of these genes and their transferability in *Weissella* is still unknown. In fact, the presence of some adhesins, considered a virulence factor in pathogenic microorganisms, may be a desirable feature in probiotic bacteria: a fibronectin-binding protein (FbpA) in *W. cibaria* inhibits biofilm formation of *Staphylococcus aureus* (Wang, Si, Xue, & Zhao, 2017), while mucus-binding proteins may play an important role in the adhesion of the probiotic strains to the host surfaces. Comparative genomic studies on *W. cibaria* (Lynch et al., 2015) focused the attention on useful metabolic traits, such as

the bacteriocin gene cluster, dextransucrase genes and genes related to an efficient proteolytic system. No specific virulence factor genes were detected.

It follows that an in-depth study of the physiological and genetic characteristics of the species of *Weissella* could help to select suitable strains for which to assess the status of QPS and possible practical applications.

In a previous work based on the characterization of the native population of natural fermentation of maize bran (Decimo et al., 2017), different strains of *W. cibaria* and *W. confusa* were isolated. They were found mainly in the last refreshment steps, where their presence was dominant. The aim of the present work was a polyphasic study of these isolates, with particular regard to potential functional properties. In parallel, a comparative genome analysis of 15 sequenced *W. cibaria* and five *W. confusa* genomes available to date was carried out.

2. Materials and methods

2.1 Bacterial strains and growth conditions

Twelve strains of *Weissella cibaria* and eight strains of *W. confusa* previously isolated from sourdough-like maize bran fermentation (Decimo et al., 2017) were used in this study. The strains were routinely sub-cultured in MRS broth/agar (Difco Lab., Augsburg, Germany) medium for 24-48 h at 30 °C. The strains were deposited in the culture Collection of the Department of Food, Environmental and Nutritional Sciences, University of Milan, Italy, at −80 °C in MRS with 15% glycerol. Growth in milk was studied using 9% RSM (Reconstituted Skim Milk- Difco) incubated at 30 °C.

2.2 Growth at different cultural conditions

The growth performance of the strains was evaluated in MRS broth at 10 and 45 °C, at pH 9.6 and with the addition of 4.0 and 6.5% NaCl. Growth was evaluated by measuring the increase in absorbance at 600 nm (A_{600}).

2.3 Acidifying activity

Each strain was inoculated at 1% in MRS broth and in RSM. The pH was measured and recorded automatically, throughout the 24 h incubation period at 30 °C.

2.4 Redox potential

The variation in redox potential was determined according to Brasca, Morandi, Lodi, & Tamburini (2007). Strains were inoculated in MRS and incubated at 30 °C under static conditions. The oxidoreduction values were recorded every 30 min for 24 h, using a redox meter (pH302 Hanna Instruments, Villafranca Padovana, PD, Italy). The redox electrodes were standardized using two redox solutions (240 mV and 470 mV; Hanna Instruments). The Eh values were calculated according to Jacob (1970). The reduction activity was evaluated by determining the maximum difference between two measures [Dmax (mV)] over 24 h.

2.5 Carbohydrate fermentation assay

Weissella strains were tested for the ability to ferment glucose, xylose, L-arabinose, trehalose, sucrose, lactose, ribose and galactose. Bacterial cells, grown in MRS broth at 30 °C for 16 h, were harvested by centrifugation (5000 *g*, 15 min, 4 °C), washed twice with sterile saline solution (NaCl 0,85%) and resuspended in the same volume of diluent. The fermentation assay was performed in microtiter plates containing 200 µL of Basal Sugar Medium (BSM) broth (containing g L^{-1}: polypeptone 15, yeast extract 6, tween80 1 mL, chlorophenol-red 0.04, pH 6.4) and 1% washed cellular suspensions. Carbon sources were sterilized separately by filtration and added to the sterile BSM to obtain a final concentration of 5 g L^{-1}. The plates were incubated at 30 °C and visually examined for color change after 24 and 48 h of incubation.

2.6 FOS utilization

To assess the ability of the strains to ferment prebiotic substances, cells grown in MRS broth at 30 °C for 16 h were harvested by centrifugation (5000 *g*, 15 min, 4 °C), washed twice with saline solution and inoculated in 5 mL MRS basal medium (MRS without carbohydrates) added with fructose and fructo-oligosaccharides (FOS - Actilight, Tereos, Lille, France) to obtain a final concentration of 10 g L^{-1}. Fructose and FOS were autoclaved separately (112 °C for 30 min). After 24 h of incubation at 30 °C, growth was evaluated by measuring the increase in absorbance at 600nm (A_{600}) and the pH value.

2.7 Screening for EPS production

Exopolysaccharide (EPS) biosynthesis was evaluated as described by Bounaix et al. (2009) with slight modifications. Strains were streaked on agar plates containing modified MRS medium containing 60 g L^{-1} sucrose (namely MRS-sucrose) and incubated at 37 °C for 48 h. Mucoid growth exhibiting slime production was evaluated.

2.8 Hydrophobicity assay

Bacterial adhesion to hydrocarbons was determined according to Kos et al. (2003) with slight modifications. Bacteria grown in MRS broth at 30 °C for 24 h were harvested by centrifugation (5000 *g*, 15 min, 4 °C), washed twice in sterile saline solution and resuspended in 0.1 M KNO_3 (pH 6.2) to approximately 0.5 A_{600} (A_0). 1 mL of xylene was added to 3 mL of cell suspension. After 10-min of pre-incubation at room temperature, the two-phase system was mixed by vortexing for 2 min. The aqueous phase was removed after 20 min of incubation at room temperature, and its A_{600} (A_1) was measured. The percentage was calculated using the relation of the absorbance at 600 nm measured before and after the contact with the xylene through the following formula: Adhesion (%) = $[1 – (A_1 / A_0)] \times 100$.

2.9 Bile tolerance

Bile tolerance was measured in MRS broth containing 0.3% or 1% oxgall (Sigma–Aldrich, Steinheim, Germany), inoculated and incubated at 30 °C. The growth was evaluated by measuring the increase in absorbance at 600nm (A_{600}).

2.10 Tolerance to simulated gastric juice

The method of Charteris, Kelly, Morelli, & Collins (1998) was used with slight modifications. Overnight cultures (4 mL) were centrifuged (5000 *g*, 15 min, 4 °C), washed twice in 50 mM K_2HPO_4 (pH 6.5) and resuspended in 4 mL of the same buffer. One milliliter of washed cell suspension was harvested by centrifugation and resuspended in 10 mL simulated gastric juice (pepsin 0.3% w/v, NaCl 0.5% w/v) adjusted to pH 2.5 and 3.0. Total viable counts were performed on MRS agar before and after an incubation period of 1 and 3 h (for pH 2.5 and 3.0, respectively) at 37 °C.

182 *2.11 Antifungal activity*

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184 The *Weissella* strains were tested for their antifungal activity against *Fusarium verticillioides,*
185 *Mucor circinelloides, M. irregularis* and *Aspergillus flavus*. The moulds (from the Collection of the
186 Department of Health, Animal Science and Food Safety, University of Milan, Italy) were grown on
187 Malt Extract Agar (MEA) (Merck, Darmstadt, Germany) at 25°C for 5–7days. Then, spore
188 suspensions were harvested by adding 15 mL of sterile milli-Q water and counted by flow
189 cytometer estimation (BD Accuri C6 Flow Cytometer, BD Biosciences, Franklin Lakes, NJ USA).
190 Antifungal activity was evaluated with an overlay assay (Quattrini et al., 2018). After growth for 16
191 h in MRS broth at 30 °C, the *Weissella* strains were inoculated in 2-cm lines on MRS agar plates.
192 After incubation for 48 h at 30 °C, plates were overlaid with cooled soft (0.7%) MEA containing
193 mould spore suspension (10^4 spores mL^{-1}) and incubated for 4 days at 25 °C. The antifungal activity
194 was evaluated as clear zones of inhibition around the bacterial smears.

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196 *2.12 Antibiotic resistance*

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198 Antibiotic susceptibility tests were performed by the microdilution method in MRS broth
199 incubated at 30 °C for 24 h. The minimum inhibitory concentration (MIC) was calculated after
200 growth in MRS broth at 30 °C, using 10^5 cells mL^{-1} as initial inoculum. Interpretative criteria for
201 susceptibility status were the Clinical and Laboratory Standards Institute (CLSI) guidelines and the
202 microbiological breakpoints defined by EFSA (FEEDAP, 2012).
203 Since a breakpoint has not been indicated for the genus *Weissella*, we considered the values
204 reported for *Lactobacillus* and *Leuconostoc* together; indeed the two genera are the most
205 phylogenetically related to *Weissella* (Collins, Samelis, Metaxopoulos, & Wallbanks, 1993).

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207 *2.13 Biogenic amine production*

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209 The ability of biogenic amine production by *Weissella* strains was performed carrying out a
210 screening plate method as reported by Bover-Cid & Holzapfel (1999). The enzymatic
211 decarboxylation of histidine, lysine, ornithine and tyrosine was investigated after 24 h at 37 °C.
212 *Morganella morganii* DSMZ 30164^T was used as positive control. Positive reactions for
213 decarboxylase activity of strains were recorded when a purple colour halo occurred in response to a
214 pH shift of the bromocresol purple indicator.

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2.14 Data source for comparative genome analysis

Information of available *W. cibaria* and *W. confusa* genomes was retrieved from the National Center for Biotechnology Information (NCBI, available at http://www.ncbi.nlm.nih. gov/). Genome analysis was carried out using the Rapid Annotation using Subsystem Technology (RAST) Server (Aziz et al., 2008). The NCBI BLAST software was used for sequence similarity search (Altschul et al., 1997).

2.15 Insertion Sequences

The search of Insertion Sequences (IS) along the genomes of *Weissella* strains was performed using the NCBI BLAST and ISfinder (http://www-is.biotoul.fr) (Siguier, Perochom, Lestrade, Mahillon, & Chandler, 2006). Positive matches for transposase were confirmed manually to determine which family they belong. For new elements, IS names were kindly provided by ISfinder. Genomic copy number and distribution of ISs were determined by digestion of the total DNA from the 20 strains tested with *Hind* III restriction enzyme. The resulting fragments, separated on a 0.8% w/v agarose gel were transferred to a nylon membrane by Southern blotting. The primers used for the production of IS-specific probes are listed in Table 1. The primers were obtained from Eurofins Genomics GmbH (Ebersberg, Germany). The PCR amplification procedure was performed as described previously (Ricci & Fortina, 2006). The DIG DNA Labelling and Detection Kit (Roche Diagnostic GmbH, Mnnheim, Germany) was used for digoxigenin labelling of the probes. Prehybridization and hybridization were performed in 50% (w/v) formammide at 42 °C. The probes were detected by chemio-luminescent detection using CSPD (Roche) and the signals were visualized by exposure to X-ray film for 2 h.

2.16 Statistical analysis

Three independent replicates of all experiments were done, and data are reported as mean values ± standard deviation. When necessary, the data were compared through one-way ANOVA, followed by Tukey's test ($p < 0.05$).

3. Results and discussion

3.1 Comparative genomic analysis

A comparative genomic analysis on 15 sequenced *W. cibaria* and 5 *W. confusa* genomes was carried out. Among the 20 genomes available in GenBank, four were related to strains coming from healthy infant saliva, two from kimchi, three from sourdough, two from insects and other sources.

The search of hypothetical virulence factors showed the presence of genes that could be involved in pathogenicity. In particular, the search for annotated genes resulted in three different haemolysins: α-haemolysin (*hly*A), haemolysin C (*hlyC*) and haemolysin III.

Haemolysin III is described as predicted membrane channel-forming protein YqFA, previously described in *Bacillus cereus* (NP_835109.1). The related gene was present in all genomes tested and its nucleotide sequence seemed highly conserved among the genomes (99 %). The multiple alignments showed that YqFA, largely distributed among Gram positive bacteria, showed similarity with haemolysin III of several genera as *Leuconostoc* (WP_004911898.1) (64%), *Lactobacillus* (WP_107739861.1) (45%), *Enterococcus* (OJG68906.1) (43%), *Bacillus* (RBJ50015.1) (43%), *Listeria* (WP_036096723.1) (41%).

The putative α-haemolysin is described as RNA methyltransferase. The α-haemolysin, also known as α-toxin, has been well-characterized in *Staphylococcus aureus* In *S. aureus*, the level of expression of the related gene (*hla)* is tightly controlled by the accessory gene regulator (*agr*), a quorum-sensing (QS) regulator that controls the expression of specific virulence genes. Among *Weissella* species, only the aquaculture pathogenic *W. ceti* contains genes encoding the two-component system regulator, *agrA* (WS105_0510)) and *agrC* (WS105_0511); these genes are not present in any other *Weissella* species (Figueiredo et al., 2015) and should be considered species-specific genes. Consequently, the only presence of *hlyA* gene has not to be seen as a virulence factor in *W. cibaria*/*confusa*.

The haemolysin C gene is annotated as *hlyC*/*CorC* (CP012873.1)*;* analysing the conserved domain of the protein, the main function predicted seems to be related to magnesium and cobalt transporter, rather than haemolytic activity.

Among the candidate genes encoding cell surface adhesins, we only found two annotated genes encoding a fibronectin binding protein (WP_010373731.1) and a mucus binding protein (NZ_CP012873.1). The gene encoding the fibronectin binding protein was detected in all *W. cibaria* and *W. confusa* genomes analysed and the amino acid identity of the protein was very high (94%). The alignments resulted in high identities in closer genera as *Leuconostoc* (WP_036068220.1) (60%), *Lactobacillus* (WP_017261841.1) (56%), *Pediococcus* (WP_057748137.1) (54%). In the last decade, several studies have revealed that a wide range of bacteria possess adhesin-like proteins, able to bind to fibronectin, that could play a direct role in

bacterial colonization and in bacteria–host interactions (Henderson, Nair, Pallas, & Williams, 2010). Their presence in pathogenic strains can be considered the first step of infection; on the contrary, in strains with probiotic potential this characteristic should be regarded as a key factor for the attachment of probiotic bacterial cells to the human host. In this context, Wang et al. (2017) demonstrated that a fibronectin-binding protein of *W. cibaria* isolates was able to inhibit *Staphylococcus aureus* colonization on host tissues.

In *W. cibaria* genomes there is an annotated gene codifying for a "mucus binding protein" (NZ_CP012873.1), which is a huge complex of about 6000 amino acids, with multiple conserved domains mainly correlated to external viral teguments. The functionality of this atypical gene could be controversial and further studies are necessary to know its potential role in pathogenicity.

Regarding antibiotic resistance determinants, *Weissella* spp., like many lactic acid bacteria, possess an intrinsic resistance to vancomycin. This antibiotic interferes with precursors of peptidoglycan synthesis, binding D-Ala/D-Ala dipeptide, inhibiting the polymerization. In *Weissella*, the terminal D-Ala is substituted with a D-lactate or D-Ser, avoiding the antibiotic to bind to that site, and conferring the resistance phenotype (Gueimonde, Sánchez, Reyes-Gavilán, & Margolles, 2013).

A multidrug efflux pump related to fosfomycin resistance was found in all genomes analysed (CP012873.1). Further analysis showed that the efflux pump is widespread in different Gram-positive and Gram negative genera, *Leuconostoc* (WP_036067854.1) (66% amino acid identity), *Lactobacillus* (WP_010622689.1) (58%), *Listeria* (WP_096926801.1) (50%), *Staphylococcus* (WP_000610059.1) (42%), *Salmonella* (WP_050189798.1) (34%) *Escherichia* (WP_001612799.1) (29%). As for vancomycin, also fosfomycin has to be considered an intrinsic resistance.

No genes related to tetracycline resistance were found in the genomes of *W. cibaria* and *W. confusa*. In *W. cibaria* genomes one gene present in two copies (CP012873.1) was annotated as a methicillin resistance protein. Two genes exhibiting high level of similarity were also found in the *W. confusa* genomes. Methicillin is a ß-lactamic antibiotic targeting the enzymes responsible for peptidoglycan synthesis. A search on NCBI-CDD indicates that the gene found in *Weissella* genomes encodes a protein having a catalytic domain related to enzymes involved in cell wall peptidoglycan synthesis, specifically a transpeptidase involved in pentaglycin bridge formation. This protein is present in all-related Gram-positive genera (*Leuconostoc*, *Lactobacillus*, *Pediococcus*) with high identities (>80%) and supposedly with the same function. The nucleotide sequence was aligned with the known *mecA* gene (Katayama & Hiramatsu, 2000), responsible of the methicillin resistance in *S. aureus* (KC243783.1), showing no homology. Moreover, the two copies of the gene in *Weissella cibaria* are located in two different chromosomal loci and they

appear not to be into a transferable cassette, as previously described for the methicillin resistance gene cassette *mecA* in *S. aureus*.

No genes encoding decarboxylases, related to biogenic amines production were detected in any of the genomes analysed.

From the data obtained *W. cibaria* and *W. confusa* seem associated to low virulence profiles and their presence in food could be considered not only a low health risk, but also an adjunct advantage. Indeed several functional traits were detected. The first functional trait investigated was the arabinoxylan catabolism, a trait not yet studied in the species. The arabinoxylan degradation is related to the activity of several enzymes, such as endo-1,4-β-xylanases, α-l-arabinofuranosidase, β-xylosidase, α-glucuronidase and feruloyl esterase. Their combined action allows the obtainment of oligosaccharides with prebiotic properties, an increase of soluble fiber and, with the action of feruloyl esterase, the increase of free ferulic acid. All *W. cibaria* genomes harbour the gene encoding a β-xylosidase (WP_010373933.1). This gene is also present in all *W. confusa* genomes tested (nucleotide similarity 95%). The protein exhibited significant similarity to the known β-xylosidases of *Leuconostoc* spp. (WP_029509980.1) (84% amino acidic identity) and *Lactobacillus oligofermentans* (WP_057890071.1) (78%). The gene encoding the feruloyl esterase was no detected. The other genes related to arabinoxylans degradation were differently distributed. The α-N-arabinofuranosidase gene (CP012873.1) was only found in *W. cibaria* genomes, with predicted double function of both β-xylosidase/α-N-arabinofuranosidase activity. The alignment resulted in high amino acidic identity with β-xylosidase of *Weissella bombi* (WP_092461590.1) (80%), *Lactococcus lactis* (WP_058219862.1) (76%), *Lactobacillus brevis* (WP_021741280.1) (75%), *Pediococcus acidilactici* (WP_063504605.1) (75%). On the other hand, the endo-1,4-β-xylanase gene was only present in *W. confusa* genomes. *W. confusa* β-xylanase exhibited similarity to endo-1,4-β-xylanases of *Enterococcus timonensis* (WP_071130632.1) (44%identity) and *Bifidobacterium adolescentis* (WP_107646029.1) (40%). Therefore, it is possible to hypothesize that the degradation of arabinoxylans could be obtained by a potential synergistic action of selected strains of the two species, which are isolated most of the times from the same fermented cereal products (Bjorkroth et al., 2002).

Another functional trait, well documented in *Weissella* species, is the dextran production, mainly correlated to dextransucrase activity (Galle S, Schwab, Arendt, & Gänzle, 2010). In the genomes analysed, dextransucrase gene was found in all *W. cibaria* (GU237484.3) *and W. confusa* (KP729387.1) strains, with an amino acid identity of 75%. In addition, an EPS gene cluster, encoding different enzymes (tyrosine-protein kinase transmembrane modulator, undecaprenyl-phosphate galactose phosphotransferase, glycosyl transferase, tyrosine-protein kinase EpsD) was

found in both species. These genes, not deeply investigated, could be related to the production of other EPS, as glucans and fructans (Di Cagno et al., 2006; Malik, Radji, Kralj, & Dijkhuizen, 2009).

Finally, the published *W. confusa* and *W. cibaria* genomes were searched for the presence of Insertion Sequence (IS) elements. To date, no insertion elements have been described for the two species in the IS element database (http://www-is.biotoul.fr). Studies regarding the occurrence and distribution of these Mobile Genetic Elements (MGE) can represent an interesting approach to evaluate the genome plasticity related to the ability of adaptation of the strains to different ecological niches. Analysis of the genomes revealed the presence of three IS elements. IS names were kindly provided by ISfinder and designated IS*Wci1* (CP012873.1), IS*Wci2* (CP012873) and IS*Wco1* (CAGH01000055) respectively. These IS elements are members of the IS*3* family. IS*Wci1* is 56% aa similar to IS*Bce19* found in *Bacillus cereus* and was present in all genomes of *W. cibaria* and in one of *W. confusa*. IS*Wci2* is 66% aa similar to IS*1520* in *Lactobacillus sakei* and to IS*981* identified in several lactococci. The sequence was found in all tested genomes. IS*Wco1* is 60% aa similar to IS*Lsa2*, another IS element found in *L. sakei*. This IS element was found in all *W. confusa* genomes and in one of *W. cibaria*. Given the draft nature of the genomes analysed it is possible to suppose a higher number of these MGE. However, the estimable number remains low, if compared with the high number of transposable elements found in other LAB strains (Eraclio, Ricci, & Fortina, 2015; Vogel et al., 2011).

3.2 Characterization of W. cibaria *and* W. confusa *strains isolated from sourdough-like maize bran fermentation*

3.2.1 Physiological and technological properties

The strains tested showed a good adaptability towards cultural stresses, such as temperature, NaCl concentration and pH value. All strains were able to grow at 10°C, in presence of 4% NaCl and a pH 9.6. The growth at 45°C was more evident for *W. confusa* strains.

All strains were able to utilize glucose, xylose and sucrose as sole carbon sources. Lactose and trehalose were not fermented. Galactose and ribose were only fermented by strains belonging to *W. confusa*. This ability, which occurred after a longer incubation period (48-72 h), could be due to a selection inside the population and, for galactose, to the activation of an inducible Leloir pathway (Frey, 1996). The fermentation of L-arabinose also allowed to discriminate between the two species: all *W. cibaria* strains were able to utilize this carbon source, contrarily to *W. confusa*

386 strains. This is in accordance with the exclusive presence of the gene-cluster for using L-arabinose
387 in *W. cibaria* genomes.
388 As reported in Table 2, all tested strains showed a high acidification rate in MRS medium, with a
389 pH value, after 24 h of incubation at 30°C, ranging from 3.5 to 4.9. On the contrary, a limited
390 capacity of growing and acidifying in milk was observed, probably due to the inability to ferment
391 lactose, and for the obligate heterofermentative metabolism.
392 The 20 *Weissella* strains were also screened to evaluate the changing in the redox potential
393 during the growth in MRS liquid medium, a parameter not yet investigated in these species. All
394 strains had high reducing ability (Table 2), in particular the strains CM18, CM10 and CR36, which
395 could reach values close to -400 mV. In sourdough environment, this parameter can be coupled
396 with the traditional pH measurement, to successfully control the baking process (Capuani, Behr, &
397 Vogel, 2012). It allows to control that the fermentation process is going in the prefigured direction,
398 ensuring the desired quality of the product and monitoring possible contaminations. Moreover, the
399 redox potential has been described to influence the aromatic profile, as well as protein structure and
400 texture of baked products (Kieronczyk, Cachon, Feron, & Yvon, 2006).
401 The ability to synthesize exopolysaccharides (EPS) by *W. confusa* and *W. cibaria* is well
402 documented by many authors. In this work we qualitatively screened the ability of EPS production
403 in solid medium added with high percentage of sucrose, resulting in sticky and viscous colonies. All
404 strains were able to produce EPS, as shown in Figure 1. This technological trait is highly requested
405 for sourdough fermented products, such as gluten free doughs, where hydrocolloids are crucial to
406 improve the texture and the specific volume of the bread. EPS are also favourable in yogurt to reach
407 a creamy texture.
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409 *3.2.2 Functional traits*
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411 The ability of the tested strains to grow in presence of FOS, is shown in Table 3. All *W. cibaria*
412 and *W. confusa* strains were able to ferment fructose; FOS were used as carbohydrate source by all
413 *W. confusa* strains and by 10/12 *W. cibaria*. The utilization of prebiotic compounds is one of the
414 indicator of probiotic potential of a bacterial strain (Kaplan & Hutkins, 2000). These data highlight
415 that, generally, these species possessed a high attitude to use prebiotics as growth source, in a future
416 probiotic perspective.
417 An essential trait of a probiotic microorganism is its ability to reach and survive in the large
418 intestine, overcoming the intestinal barrier. The strains tested showed a moderate tolerance to
419 simulated gastric juice, many strains decreasing their viability of 3 log cycles at pH 3 (Table 4). In

simulated gastric juice at pH 2.5 a progressive reduction in viability was observed, but recovered viability after 1 h was about 10^2-10^4 CFU mL^{-1} for all strains. Moreover, all strains showed a noticeable ability to resist to bile salts, reaching values between 30% and 60% of residual growth even when the highest concentration of bile salts (1%) was added to the medium (Table 5). At the concentration of 0.3%, the inhibition was minimal, with residual growth ranging from 48% to 86%.

Hydrophobicity properties of the strains were evaluated as percentage of adhesion to a hydrophobic solvent, the xylene. This bacterial trait could be predictive of adhesiveness of probiotic bacteria, the first step of the colonization of the epithelium. The results are shown in Table 5. Generally, the data obtained indicate a low potentiality of the strains to adhere to the solvent, even though this ability could be considered strain-specific: high values (55.6, 48.0 and 44.3%) were obtained for strains CM9, CM27 (*W. cibaria*) and CR21 (*W. confusa*), respectively.

3.2.3 Antifungal activity

Antifungal activity is an appreciated additional feature of starter and adjunct cultures, in several food sectors, such as cereal sector, where moulds are widely present and can represent an extra risk for the possible production and accumulation of mycotoxins. Several publications have highlighted the positive use of LAB strains as agents of biocontrol, due to their ability to secrete compounds such as organic acids, phenyllactic acid, cyclic dipeptides, hydroxy-fatty acids, able to inhibit mould development, limiting the mycotoxin production (Lavermicocca et al., 2000; Quattrini et al., 2018). Little information is available regarding the antifungal potential of *Weissella* strains.

Interestingly, all *Weissella* strains tested were able to inhibit the growth of *Fusarium verticillioides*, the most prevalent fungus infecting the maize crops, producing a wide range of mycotoxins, including fumonisin B1 (Deepa & Sreenivasa, 2017), considered the most toxic one (Table 6- Figure 2). Moreover, most of the strains analysed (17/20) exerted a strong inhibition against the aflatoxigenic *Aspergillus flavus*. Approximately 55 and 45% of the strains inhibited *Mucor irregularis* and *M. circinelloides* respectively. Also, these data are of interest, because *Mucor* species are frequently isolated from food matrices and *M. circinelloides* is considered one of the causal agents of the fungal infection mucormycosis (Lee et al., 2014). Further analysis will allow understanding the mechanism of action of this fungal inhibition.

3.2.4 Antibiotic resistance and virulence traits

Antibiotic resistance is regarded with increasing attention from EFSA and OMS, for the spread of microbial resistances. To test antibiotic resistance profiles of the strains, we considered the breakpoints established by EFSA (FEEDAP, 2012) for *Leuconostoc* and *Lactobacillus*, which are the closest genera to *Weissella*. Indeed, up to now no specific antibiotic breakpoints for these species have been suggested by the CLSI or the EFSA. According to the breakpoints listed in Table 7, the strains were identified as either sensitive (S, MIC ≤ breakpoint) or resistant (R, MIC > breakpoint). Since intrinsic resistance to vancomycin and fosfomycin are known, these antibiotics were not tested.

Results showed that all tested strains were susceptible to tetracycline, ampicillin and chloramphenicol. Lincomycin had effect on 12/20 strains tested. On the other hand, all the strains were resistant to aminoglycosides (AG; gentamycin, kanamycin and streptomycin). These data were in agreement with previous findings (Hummel, Hertel, Holzapfel, & Franz, 2007; Katla, Kruse, Johnsen, & Herikstad, 2001), showing several LAB starters and not-starters with 70-80% of resistant phenotype. The most common mechanism of AG resistance is a chemical modification by aminoglycoside-modifying enzymes (AMEs) (Garneau-Tsodikova & Labby, 2016). However, along the tested genomes available in databases no genes encoding AG-acetyltransferases, AG-nucleotidyltransferases and AG-phosphotransferases were found. For these reasons, it is possible to hypothesize two other potential, acquired mechanisms of resistance: mutations of the ribosome or enzymatic modifications of the ribosome (Garneau-Tsodikova & Labby, 2016). In this case, AG resistance should be considered intrinsic and not transferable. The sulphonamide resistance can also be due to intrinsic modification of the dihydropteroate synthetase enzyme. As for aminoglycosides, many LAB show a natural reduced sensibility towards these antimicrobials since most of them lack the complete pathway of ex-novo folic acid biosynthesis (the target of the sulphonamides) (Katla et al., 2001). Regarding methicillin, we evaluated the MIC values for oxacillin, according to break point related to methicillin resistant bacteria (4 μg mL^{-1}), in comparison with three *Lactobacillus* strains, commercially used as probiotic cultures, *Lactobacillus rhamnosus* GG, *Lactobacillus paracasei* ATCC 5622, *Lactobacillus plantarum* ATCC 4008. All *Weissella* and *Lactobacillus* strains tested showed MIC values ranging from 4 to 8 μg mL^{-1}. These values seem to indicate a methicillin resistance, which, if related to transpeptidase enzyme or to a modification of the protein (as specified above, in 3.1 section), could be considered an intrinsic resistance not easily horizontally transferable. Nevertheless, further studies are needed to fully disclose the mechanism of resistance towards this antimicrobial.

Finally, in accordance with genotypic traits, none of the *W. cibaria* and *W. confusa* strains tested showed the ability to produce biogenic amines.

3.2.5 IS: molecular typing

IS*Wci1,* IS*Wci2* and IS*Wco1,* previously found along the available genomes of the two species, were tested for frequency and distribution on the genomic DNA of the strains studied.
The IS*Wci2* seemed the most representative, with several copies distributed in all genomes of *W. cibaria* and *W. confusa* strains (Figure 3A). The results indicate a copy number ranging from 2 to at least 8. Among the 12 *W. cibaria* strains tested, almost 7 different restriction patterns were identified; at least 4 different profiles were distinguishable among the 8 *W. confusa* strains. IS*WCi1* and IS*Wco1* (Figure 3B-C) were present in a minor copy number and did not highly differentiate the strains. Further studies are in progress to understand the contribution of these IS on the genome plasticity and on the adaptive response of their host.

4. Conclusions

Our results support the idea that selected strains of *W.cibaria* and *W. confusa* could represent interesting adjunct culture to be exploited in food sector and in probiotic formulations. The comparative genome analysis carried out in parallel with a polyphasic study on 20 strains previously isolated from maize bran natural fermentation, seemed to indicate the absence of severe virulence factors. Moreover, even though antibiotic resistance studies deserve to be further investigated, it was possible to hypothesize an intrinsic resistance to many antibiotics, trait present in other LAB commonly used as probiotics, and not easily transferable to other bacteria strains.

Finally, interesting functional and pro-technological traits were highlighted in the tested strains, for both species. For these reasons, further studies are in progress on selected strains to obtain the QPS status required for food applications.

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Conflicts of interest

The authors declare no conflict of interest.

Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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683

684 **Table 1.**

685 PCR primers and conditions used for the detection of IS in *Weissella cibaria* and *W. confusa*
686 strains.

687

Gene product	Accession number	Primer pair (5′-3′)	Thermal conditions	Amplicon (bp)
IS*Wci2*	CP012873	F: TGCATCTCGACAAGAGATTG R: GAGAGCTTCCATTCGCTCAT	94 °C × 1’ 58 °C × 1’× 35 cycles 72°C x 1’	946
IS*Wci1*	CP012873.1	F: TCCAGGATTGCCTCTTGTTT R: CACCGTCGTTTCAAGACTGA	94 °C × 1’ 58 °C × 1’× 35 cycles 72°C x 1’	841
IS*Wco1*	CAGH01000055	F: TTCTTGATCTTGTCGTGTTC R: GATCGACCATATCAGAAGGT	94 °C × 1’ 58 °C × 1’× 35 cycles 72°C x 1’	502

688

689

690 **Table 2.**

691 pH values in MRS and RSM media and maxim redox potential difference in MRS ΔEmax (mV)
692 after 24 h of incubation at 30°C. Data are shown as mean ± standard deviations of triplicates.
693 Values differ if they do not share a common superscript ($p < 0.05$).
694

Strains		pH value		$\Delta E_{max\ (mV)}$
		MRS	RSM	
W. cibaria	CM1	4.37 ± 0.4^{b}	$6,01\pm0.08^{a}$	-334 ± 13^{d}
	CM6	4.92 ± 0.39^{b}	$5,96\pm0.10^{a}$	-378 ± 55^{d}
	CM10	4.59 ± 0.19^{b}	$6,06\pm0.19^{a}$	-355 ± 38^{d}
	CM18	4.7 ± 0.4^{b}	$6,05\pm0.32^{a}$	-385 ± 29^{d}
	CM34	4.61 ± 0.34^{b}	$6,05\pm0.25^{a}$	-87 ± 24^{a}
	CM23	4.21 ± 0.17^{ab}	$6,06\pm0.04^{a}$	-182 ± 25^{b}
	CM32	4.09 ± 0.28^{ab}	$6,11\pm0.28^{a}$	-365 ± 42^{d}
	CM9	3.49 ± 0.28^{a}	$6,07\pm0.05^{a}$	-148 ± 18^{b}
	CM19	3.54 ± 0.12^{a}	$6,02\pm0.15^{a}$	-157 ± 1^{b}
	CM27	4.09 ± 0.26^{ab}	$6,07\pm0.14^{a}$	-114 ± 16^{b}
	CR23	4.40 ± 0.13^{b}	$6,04\pm0.05^{a}$	-182 ± 32^{b}
	CR24	3.93 ± 0.23^{ab}	$6,02\pm0.12^{a}$	-187 ± 16^{b}
W. confusa	CR21	4.47 ± 0.30^{b}	$5,90\pm0.03^{a}$	-224 ± 31^{c}
	CR31	3.70 ± 0.11^{a}	$5,95\pm0.24^{a}$	-260 ± 6^{c}
	CR36	4.06 ± 0.14^{ab}	$5,98\pm0.28^{a}$	-376 ± 33^{d}
	CR39	4.45 ± 0.08^{b}	$5,95\pm0.35^{a}$	-192 ± 35^{b}
	CR48	4.12 ± 0.29^{ab}	$5,96\pm0.37^{a}$	-258 ± 15^{c}
	CR49	3.83 ± 0.32^{ab}	$6,03\pm0.38^{a}$	-242 ± 32^{c}
	CR51	4.03 ± 0.28^{ab}	$5,96\pm0.35^{a}$	-255 ± 32^{c}
	CR55	4.15 ± 0.1^{ab}	$5,98\pm0.05^{a}$	-232 ± 2^{c}

695
696

Table 3.

Prebiotics utilization. Data are shown as mean ± standard deviations of triplicates. Values differ if they do not share a common superscript ($p < 0.05$).

Strains		FOS utilization					
		Basal MRS medium		MRS + FOS		MRS + fructose	
		A_{600}	pH	A_{600}	pH	A_{600}	pH
W. cibaria	CM1	0.57±0.12^{a}	6.74±0.19^{a}	1.4±0.09^{b}	5.65±0.05^{a}	1.70±0.05^{b}	5.1±0.20^{a}
	CM6	0.47±0.16^{a}	6.84±0.29^{a}	1.44±0.12^{b}	5.66±0.12^{a}	1.76±0.02^{b}	5.09±0.11^{a}
	CM10	057±0.13^{a}	6.78±0.23^{a}	1.44±0.16^{b}	5.65±0.12^{a}	1.76±0.04^{b}	5.08±0.09^{a}
	CM18	0.58±0.10^{a}	6.81±0.14^{a}	1.50±0.05^{b}	5.6±0.10^{a}	1.76±0.19^{b}	5.12±0.22^{a}
	CM34	0.61±0.09^{a}	6.68±0.23^{a}	1.45±0.03^{b}	5.66±0.01^{a}	1.76±0.01^{b}	5.11±0.15^{a}
	CM23	0.37±0.00^{a}	6.83±0.27^{a}	0.49±0.11^{a}	6.69±0.11^{b}	1.62±0.18^{b}	5.22±0.10^{a}
	CM32	0.40±0.14^{a}	6.82±0.09^{a}	0.46±0.00^{a}	6.76±0.06^{b}	1.62±0.00^{b}	5.24±0.16^{ab}
	CM9	0.32±0.18^{a}	6.79±0.14^{a}	1.17±0.08^{b}	5.61±0.10^{a}	1.57±0.01^{b}	5.04±0.18^{a}
	CM19	0.41±0.12^{a}	6.72±0.29^{a}	1.22±0.09^{b}	5.65±0.15^{a}	1.62±0.09^{b}	5.01±0.07^{a}
	CM27	0.37±0.10^{a}	6.84±0.07^{a}	1.13±0.19^{b}	5.55±0.16^{a}	1.50±0.18^{b}	5.11±0.08^{a}
	CR23	0.37±0.20^{a}	6.83±0.16^{a}	1.22±0.05^{b}	5.63±0.03^{a}	1.68±0.03^{b}	4.95±0.23^{a}
	CR24	0.37±0.04^{a}	6.85±0.22^{a}	1.19±0.02^{b}	5.58±0.08^{a}	1.84±0.07^{b}	4.82±0.25^{a}
W. confusa	CR21	0.31±0.00^{a}	6.87±0.13^{a}	1.38±0.11^{b}	5.73±0.16^{a}	1.88±0.17^{b}	4.89±0.04^{a}
	CR31	0.35±0.20^{a}	6.87±0.16^{a}	1.14±0.08^{b}	5.85±0.17^{a}	0.99±0.12^{a}	5.94±0.09^{c}
	CR36	0.34±0.18^{a}	6.89±0.17^{a}	1.33±0.14^{b}	5.82±0.04^{a}	1.42±0.19^{b}	5.63±0.14^{bc}
	CR39	0.31±0.03^{a}	6.84±0.18^{a}	1.24±0.13^{b}	5.62±0.11^{a}	1.07±0.03^{a}	5.54±0.02^{b}
	CR48	0.41±0.14^{a}	6.94±0.07^{a}	1.38±0.17^{b}	5.67±0.18^{a}	1.51±0.05^{b}	5.26±0.09^{ab}
	CR49	0.48±0.10^{a}	6.97±0.17^{a}	1.51±0.18^{b}	5.75±0.08^{a}	1.31±0.03^{ab}	5.78±0.10^{bc}
	CR51	0.45±0.00^{a}	6.94±0.22^{a}	1.51±0.16^{b}	5.74±0.01^{a}	1.36±0.05^{b}	5.78±0.02^{bc}
	CR55	0.38±0.01^{a}	6.89±0.05^{a}	1.29±0.0^{b}	5.73±0.0^{a}	1.13±0.17^{a}	5.78±0.01^{bc}

Table 4.

Viability of *Weissella* cibaria and *W. confusa* strains under influence of simulate gastric juice. Data are shown as mean ± standard deviations of triplicates. Values differ if they do not share a common superscript ($p < 0.05$).

Strains		Viability (log CFU mL^{-1})[1]		
		T_0	T_3 pH 3	T_1 pH 2.5
W. cibaria	CM1	9.00±0.08^{a}	4.00±0.08^{b}	3.49±0.05^{b}
	CM6	8.98±0.16^{a}	5.80±0.29^{c}	4.18±0.17^{c}
	CM10	9.23±0.11^{a}	6.26±0.41^{c}	2.00±0.15^{a}
	CM18	9.11±0.10^{a}	3.78±0.14^{ab}	3.23±0.75^{b}
	CM34	9.20±0.23^{a}	4.15±0.20^{b}	2.00±0.79^{a}
	CM23	9.04±0.31^{a}	6.04±0.46^{c}	3.43±0.08^{b}
	CM32	8.98±0.02^{a}	6.61±0.16^{c}	3.04±0.06^{b}
	CM9	9.08±0.04^{a}	6.04±0.35^{c}	2.70±0.46^{b}
	CM19	8.98±0.18^{a}	6.18±0.18^{c}	2.30±0.25^{a}
	CM27	9.11±0.03^{a}	5.79±0.13^{c}	2.95±0.53^{b}
	CR23	9.11±0.20^{a}	6.34±0.12^{c}	2.00±0.13^{a}
	CR24	9.20±0.04^{a}	6.23±0.02^{c}	1.70±0.41^{a}
W. confusa	CR21	9.18±0.00^{a}	6.40±0.16^{c}	4.18±0.00^{c}
	CR31	9.00±0.00^{a}	3.00±0.42^{a}	2.60±0.07^{ab}
	CR36	8.85±0.10^{a}	4.43±0.09^{b}	2.00±0.09^{a}
	CR39	9.26±0.05^{a}	4.32±0.12^{b}	1.70±0.27^{a}
	CR48	9.08±0.10^{a}	6.26±0.06^{c}	2.48±0.18^{ab}
	CR49	9.04±0.00^{a}	5.09±0.47^{b}	3.53±0.87^{c}
	CR51	8.30±0.40^{a}	4.70±0.28^{bc}	3.76±0.30^{c}
	CR55	9.00±0.02^{a}	4.32±0.24^{b}	3.93±0.09^{c}

[1] T_0= control; T_3 pH 3= viability after 3 h at pH 3; T_1 pH 2.5= viability after 1 h at pH 2.5

745 **Table 5.**

746 Bile tolerance and hydrophobicity of the tested strains. Data are shown as mean ± standard
747 deviations of triplicates. Values differ if they do not share a common superscript ($p < 0.05$).
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Strains		Bile tolerance					Hydrophobicity (adhesion %)
		Control	Bile 0.3%		Bile 1%		
		(A_{600})	(A_{600})	% Growth	(A_{600})	%Growth	
W. cibaria	CM1	4.37±0.05^{b}	2.23±0.43^{a}	51.03	1.40±0.33^{a}	32.04	7.4
	CM6	4.92±0.10^{b}	3.25±0.02^{a}	66.06	1.96±0.19^{b}	39.84	14.3
	CM10	4.59±0.20^{b}	2.65±0.02^{a}	57.73	1.9±0.12^{b}	41.39	6.4
	CM18	4.7±0.36^{b}	2.27±0.74^{a}	48.30	1.49±0.08^{a}	31.70	5.9
	CM34	4.61±0.22^{b}	2.37±0.04^{a}	51.41	1.69±0.11^{ab}	36.66	3.7
	CM23	4.21±0.05^{ab}	2.19±0.72^{a}	52.02	1.74±0.02^{ab}	41.33	30.8
	CM32	4.09±0.12^{ab}	2.51±0.55^{a}	61.37	1.58±0.39^{ab}	38.63	27.8
	CM9	3.49±0.35^{a}	3.01±0.17^{a}	86.25	2.08±0.2^{b}	59.60	55.6
	CM19	3.54±0.18^{a}	2.69±0.55^{a}	75.99	1.64±0.27^{ab}	46.33	41.7
	CM27	4.09±0.40^{ab}	2.82±0.41^{a}	68.95	2.15±0.28^{b}	52.57	48.0
	CR23	4.40±0.01^{b}	3.32±0.42^{a}	75.45	2.00±0.17^{b}	45.45	0.5
	CR24	3.93±0.60^{ab}	3.35±0.7^{a}	85.24	2.04±0.33^{b}	51.91	0.2
W. confusa	CR21	4.47±0.25^{b}	2.92±0.45^{a}	65.32	1.8±0.27^{ab}	40.27	44.3
	CR31	3.70±0.10^{a}	2.51±0.86^{a}	67.84	1.64±0.05^{ab}	44.32	1.9
	CR36	4.06±0.23^{ab}	3.01±0.87^{a}	74.14	1.73±0.3^{ab}	42.61	15.8
	CR39	4.45±0.01^{b}	3.21±0.44^{a}	72.13	2.46±0.09^{b}	55.28	2.6
	CR48	4.12±0.22^{ab}	2.73±0.22^{a}	66.26	2.18±0.14^{b}	52.91	19.1
	CR49	3.83±017^{ab}	3.02±0.01^{a}	78.85	1.78±0.04^{ab}	46.48	11.2
	CR51	4.03±0.15^{ab}	2.86±0.13^{a}	70.97	1.95±0.14^{b}	48.39	3.8
	CR55	4.15±0.05^{ab}	2.81±0.15^{a}	67.71	1.84±0.13^{ab}	44.34	15.1

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Table 6.

Antifungal activity of *Weissella* strains against *Mucor irregularis*, *M. circinelloides*, *Fusarium verticillioides* and *Aspergillus flavus*. Strains were classified as no (-) or strong (+) inhibitors by using the overlay method.

		Antifungal activity			
Strains		*Mucor irregularis*	*Mucor circinelloides*	*Fusarium verticillioides*	*Aspergillus. flavus*
W. cibaria	CM1	+	+	+	+
	CM6	+	+	+	+
	CM10	+	+	+	+
	CM18	+	+	+	+
	CM34	-	-	+	+
	CM23	-	-	+	+
	CM32	-	+	+	+
	CM9	-	-	+	-
	CM19	-	-	+	+
	CM27	-	-	+	+
W. confusa	CR23	-	-	+	-
	CR24	-	-	+	+
	CR21	-	-	+	+
	CR31	-	-	+	+
	CR36	+	-	+	-
	CR39	+	+	+	+
	CR48	+	+	+	+
	CR49	+	+	+	+
	CR51	-	-	+	+
	CR55	+	+	+	+

Table 7.

Antibiotic susceptibility test. The antimicrobials were used at break-point concentrations for related *Leuconostoc*/*Lactobacillus* genera (FEEDAP, 2012). (S, sensible, MIC ≤ breakpoint; R, resistant, MIC > breakpoint).

Strains		Antibiotic resistance MIC (μg mL $^{-1}$)[1]								
		Tet (8)	Str (64)	Kan (64)	Gen (16)	Lin (8)	Cm (4)	Tmp+ Sul (8+160)	Oxa (4)	Amp (4)
W. cibaria	CM1	S	R	R	R	R	S	R	R	S
	CM6	S	R	R	R	R	S	R	R	S
	CM10	S	R	R	R	S	S	R	R	S
	CM18	S	R	R	R	S	S	R	R	S
	CM34	S	R	R	R	R	S	R	R	S
	CM23	S	R	R	R	R	S	R	R	S
	CM32	S	R	R	R	R	S	R	R	S
	CM9	S	R	R	R	S	S	R	R	S
	CM19	S	R	R	R	S	S	R	R	S
	CM27	S	R	R	R	S	S	R	R	S
W. confusa	CR23	S	R	R	R	S	S	R	R	S
	CR24	S	R	R	R	S	S	R	R	S
	CR21	S	R	R	R	R	S	R	R	S
	CR31	S	R	R	R	S	S	R	R	S
	CR36	S	R	R	R	S	S	R	R	S
	CR39	S	R	R	R	R	S	R	R	S
	CR48	S	R	R	R	R	S	R	R	S
	CR49	S	R	R	R	S	S	R	R	S
	CR51	S	R	R	R	S	S	R	R	S
	CR55	S	R	R	R	S	S	R	R	S

[1] Abbreviations: Tet, tetracycline; Str, Streptomycin; Kan, Kanamycin; Gen, gentamicin; Lin, lincomycin; Cm, chloramphenicol; Tmp–Sul: trimethoprim–sulfamethoxazole; Oxa, oxacillin; Amp, ampicillin

Fig. 1. Sticky and viscous colonies of *W. cibaria/confusa* in MRS-sucrose agar, after 24 h at 30°C.

Fig. 2. Antifungal effect of *Weissella* strains against *Fusarium verticillioides*

Fig. 3. IS fingerprints of *W. cibaria* and *W. confusa* strains. A) IS*WCi2* patterns; B) IS*WCi1* patterns; C) IS*WCo1* patterns

Fig. 1

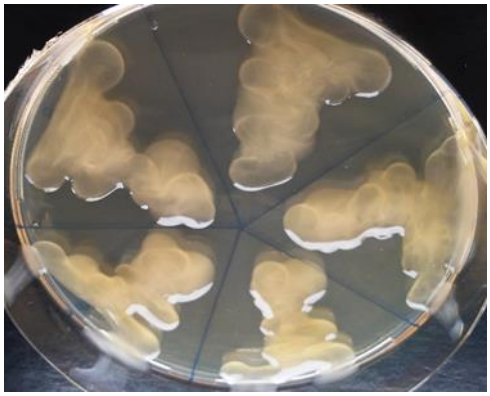


Fig. 2

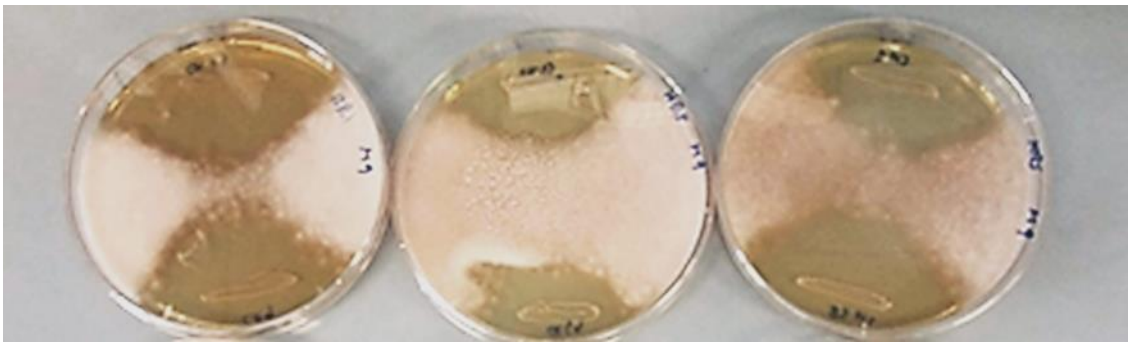
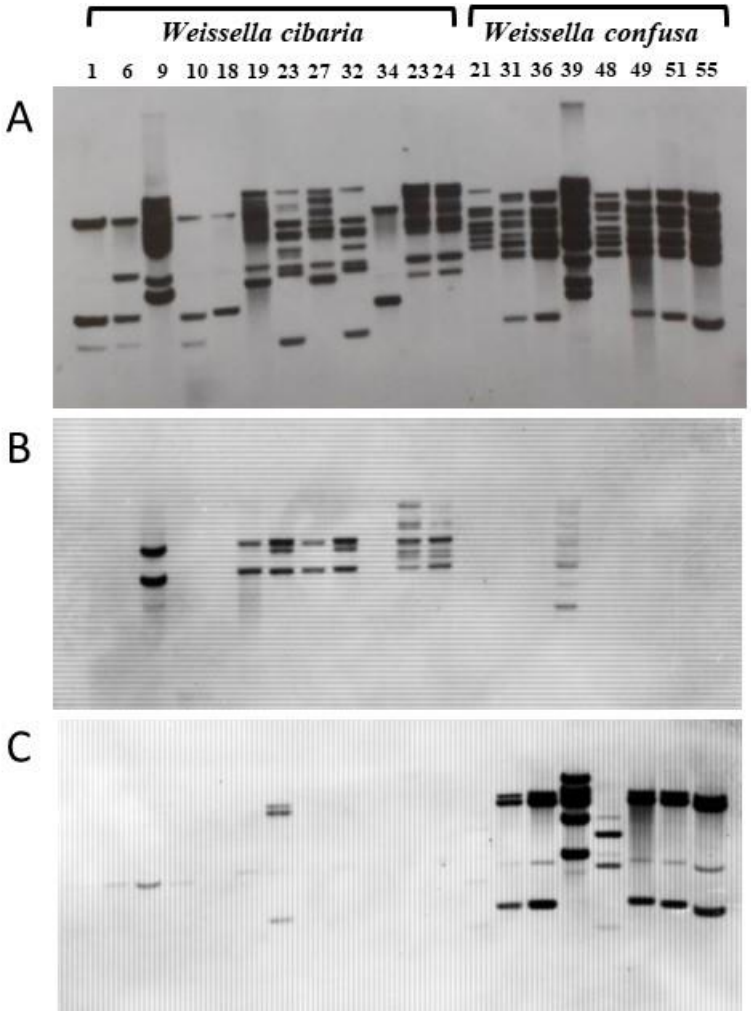


Fig. 3


Weissella cibaria
Weissella confusa
1 6 9 10 18 19 23 27 32 34 23 24 21 31 36 39 48 49 51 55
A
B
C