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Identification, safety, and technological characteristics of *Weissella* strains from traditional Southwestern Algerian *kaddid*

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Abstract

Kaddid is a dry-fermented meat product traditionally produced in North Africa by spontaneous fermentation. As a reservoir of natural biodiversity, the identification and relevant traits of lactic acid bacteria (LAB) were carried out from South-western Algeria homemade samples. After a preliminary physiological and biochemical screening, 19 presumptive LAB isolates were selected on the basis of antimicrobial compounds production. The isolates were identified by 16S rRNA gene sequencing as *Weissella cibaria*, *W. confusa*, *W. paramesenteroides*, *Pediococcusacidilactici*, and *Enterococcus hirae*. As predominant, the safety and technological characterization of *Weissella* strains were performed. The production of antimicrobial and antifungal compounds was observed, while neither H_2S, biogenic amines nor hemolytic activity were detected; antibiotic resistance was exhibited, however several strains were more susceptible to assayed antibiotics. Technological characterization of *Weissella* strains showed high acidification rates even in the presence of up to 10 % NaCl, autolytic and proteolytic capacity however, no EPS production and lipolytic activity were observed. Strains characterization led to the selection of *W. cibaria* BK2, *W. confusa* BK6 and BK11 as well as *W. paramesenteroides* BK8 to be considered as possible candidates for use as starter culture for *kaddid* fermentation to improve and standardize this traditional meat product.

Introduction

Even today, traditional meat products are generally homemade as a mean to preserve meat to be consumed in times of scarcity. Among traditional products, the appreciation of fermented meats is probably related to their unique and specific sensory properties and alleged rootedness in a socio-cultural context. Meat products prepared in North African countries are usually dried or cooked because of the weather and are rarely smoked; they are produced from different meat (beef, lamb, goat and camel) depending on the geographic area. Dried *kaddid* has been traditionally used as

ingredient to prepare different winter dishes as stews and soups (Gagaoua and Boudechicha 2018). In Algeria, it is prepared by adding salt and spices to lamb meat cutting into thin strips, which are then hang on a string and exposed to the air until thoroughly dried and stored at room temperature; salt content in the final product ranges from 7 % to 12 % (Bennani *et al*. 1995; Benlacheheb *et al*. 2018).

Microbiological survey of dry-salted *kaddid* showed the presence of lactic acid bacteria (LAB) in addition to microorganisms related to the product hygiene (Bennani *et al*. 2000; Ben Belgacem *et al*. 2008, 2010; Essid *et al*. 2009; Benlacheheb *et al*. 2018). Physicochemical features of *kaddid* during ripening such as pH, moisture, salt content and water activity are main modulators of microbiological evolution. It is known that the presence of LAB induces desirable attributes to traditional fermented food products, enhancing their typical characteristics while conferring additional safety and health benefits. Distinctive features derived from LAB metabolic activities in salted meat will determine final quality of fermented products.

In view to design novel functional starter cultures, it would be necessary to exploit the autochthonous LAB with technological, functional and safety characteristics. A quick growth and high acidification rate in the presence of high salt content are the main criteria for strains selection by which a safe initial environment can be created and food pathogen and spoilage reduced (Fusco 2015; Fessard and Remize 2017). From a safety point of view, the production of antibacterial and antifungal compounds during fermentation are desired features as these metabolites inhibit pathogen and spoilage proliferation. The lack of antibiotic resistance, virulence factors and aminogenesis among other traits, are also required (Castellano *et al*. 2017). In addition, the United Nations Food and Agriculture Organization (FAO)/World Health Organization (WHO) stated the importance to conduct a minimum safety assessment including several specific metabolite productions, toxin production, and potential hemolysis, even for microbial populations classified as GRAS (FAO/WHO 2002). Moreover, changes in meat and fat are responsible for the flavour in fermented meat. Proteolysis and lipolysis by meat enzymes and LAB will impact on the development of typical sensory characteristics by peptides and amino acids generation (Fadda *et al*. 2010; Vignolo *et al*. 2019). Thus, the aim of this study was the identification of the LAB population from Algerian *kaddid* samples and traits related to technological and safety features of autochthonous isolated LAB were investigated.

Experimental

Samples collection and LAB isolation

Samples of homemade *kaddid* were collected from the Southwestern region of Algeria, namely Tindouf (one sample from camel meat) and Béchar (6 samples from lamp meat) provinces. Approximately 400 g of ready to consume *kaddid* pieces from each producer were placed in sterile plastic bags and transported refrigerated to the laboratory for analyses. Each sample (10 g) was suspended in 90 mL of sterile tryptone-salt diluents (tryptone 1 $g.L^{-1}$; NaCl 0.85 $g.L^{-1}$; Tween 80 1 $mL.L^{-1}$), homogenized for 3 min (Stomacher 400, Seward, Worthing, UK) and serially diluted. Dilutions were then plated in duplicate on MRS and M17 (Merck, Darmstadt-Germany) media, and microaerobically incubated at 30 °C during 72 h. An average of 20 – 25 colonies per *kaddid* sample were randomly picked from both media plates containing 100 – 300 colonies. Gram-positive and catalase-negative bacteria (presumptive LAB) were sub-cultured on the corresponding medium and stored in 25 % of glycerol at -80 °C for further use.

Physiological and biochemical characterization of LAB

Sixty-three presumptive LAB isolates were preliminarily identified according to Von Wright and Axelsson (2012) and the scheme described by Carr *et al.* (2002). Growth at 10 ºC and 45 ºC during 7 d and 24 h, respectively, at pH 4 to 9.6 and in the presence of 4 to 10 % NaCl were evaluated in MRS (bacilli) and M17 (cocci). Production of gas (CO_2) from glucose (Gibson and Abdelmalek 1945) and arginine hydrolysis (Møller 1955) were analyzed. For carbohydrate

fermentation MEVAG medium (Biokar Diagnostic, Allonne, France) supplemented with different sugars (Table 1) was used, LAB suspensions being inoculated by a central puncture and covered by a vaseline layer to promote anaerobiosis; after incubation (30 °C; 24 h) color changes due to the sugars utilization by bacteria were recorded.

Molecular identification

Genomic DNA was extracted from pure LAB cultures using InstaGene matrix (Bio-Rad Laboratories Inc., Hercules, USA). Molecular identification was performed as described by Montanari *et al*. (2015). The partial 16S rRNA gene sequence was amplified using the primers LpigF/LpigR (5'-TACGGGAGGCAGCAGTAG-3' and 5'-CATGGTGTGACGGGCGGT-3'; Eurofins Genomics Germany GmbH, Ebersberg, Germany). PCR amplifications were performed using Taq DNA polymerase kit from Thermo Fisher Scientific (Waltham, USA). Reaction mixtures consisted of buffer 5X, 2 mM $MgCl_2$, 50 mM each of the 4 deoxynucleoside triphosphates (dNTP), 1.25 U of Taq-polymerase, 0.5 mM of each primer, and 0.5 µL of appropriately diluted template DNA in a final volume of 50 µL. Amplification was performed on a Biometra T3000 thermal cycler (Analytik Jena GmbH, Jena, Germany) with initial denaturation at 94 °C for 5 min, then 34 cycles at 94 °C for 1 min, 55.5 °C for 2 min, and 72 °C for 2 min, followed by final extension at 72 °C for 10 min. PCR products were separated by electrophoresis on 1.5 % (w/v) agarose gel (Lonza, Italy) stained with ethidium bromide (0.5 $\mu g.mL^{-1}$). The 600 bp amplicons were eluted from an agarose gel, purified with the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) and sequenced at the BMR Genomics Srl. sequencing facility (Padova, Italy) using the same primers as for amplification. Sequence similarity searches were performed using BLAST network service (http://blast.ncbi.nlm.nih.gov/) and Ez-Taxon Server (http://147.47.212.35:8080/).

Antimicrobial activity

LAB (63 strains) inter-species inhibitory capacity was first investigated by using the disc method described by Tadesse *et al*. (2004) with modifications. Overnight LAB cultures (0.5 mL) used as indicators were mixed with 12 mL of melted and cooled MRS agar media, poured into plates, let solidify and dried. LAB cultures were centrifuged (7,000 × g; 15 min) and the obtained cell free supernatants were used to impregnate sterile filter paper discs that were deposited on the seeded agar. On the other hand, the antibacterial activity of identified *Weissella* isolates was evaluated by a semi-quantitative agar-spot-test as described by Fontana *et al*. (2015) against Gram positive and Gram-negative bacteria (Table 3). Overnight LAB cultures were centrifuged (7,000 × g; 15 min), and supernatants (5 µL) were spotted onto 10 mL of BHI agar (Britania, Argentina) plates (0.7 %) previously inoculated with 50 µL of each indicator strain. Plates were incubated at 30 °C for 48 h and the presence of a clear inhibition zone around the spots was considered as a positive antagonistic effect. Inhibitory activity was expressed as + (halos presence) or – (no halos) around the spot. Positive antibacterial activity LAB supernatants were neutralized (4N NaOH) and treated with catalase (1,000 $U.mL^{-1}$) (Sigma-Aldrich, St Louis, USA) to determine the chemical nature of the inhibitory substance. Antifungal activity against *Aspergillus flavus*, *Penicillium expansum*, and *Fusarium oxysporum albedinis* was investigated by a modified agar diffusion assay (Magnusson *et al*. 2003). Petri plates containing Potato Dextrose Agar (PDA) were inoculated with the fungus and incubated at 25 °C during 48 h. After visible formation of conidiospores, they were collected and adjusted to 10^5 spores/ml of sterile saline solution. LAB selected strains were streak-inoculated on MRS agar plates and after incubation (30 °C; 48 h), plates were overlaid with 10 mL of PDA soft agar (0.7 % agar) containing fungal spore suspensions ($10^4.mL^{-1}$) and incubated aerobically (30 °C; 48h). Plates were then examined for clear inhibition zones around the LAB streaks and scored as – (no growth inhibition), + (1 – 5 mm growth inhibition), ++ (5 – 10 mm growth inhibition) and +++ (> 10mm growth inhibition).

Biogenic amines and H_2S production

The ability to decarboxylate amino acids used as precursor was tested according to Bover-Cid and Holzapfel (1999). Briefly, the plates with the agar medium, supplemented with histidine, tyrosine, ornithine, and lysine 1 % (w/v) were spotted with the active *Weissella* (7 strains) and incubated anaerobically (30 °C; 2 – 5 d). Growth of decarboxylating strains was easily recognizable because of a purple halo in the yellow medium. Production of $H_2S$was investigated on TSI (Triple sugar iron) agar medium by a central puncture inoculation. After incubation (30 °C; 48 – 72 h), H_2S production was confirmed by the blackening of the medium and gas bubbles in the agar (Guiraud 2003).

Antibiotic resistance

Antibiotics recommended for the European Food Safety Authority (EFSA 2012) to identify bacterial strains with potential acquired resistance were used. Ampicillin (AMP; 0.032 – 16 $\mu g.mL^{-1}$), vancomycin (VAN; 0.25 – 128 $\mu g.mL^{-1}$), chloramphenicol (CHL; 0.125 – 64 $\mu g.mL^{-1}$), gentamycin (GEN; 0.5 – 256 $\mu g.mL^{-1}$), streptomycin (STR; 0.5 – 256 $\mu g.mL^{-1}$), kanamycin (KAN; 2 – 1024 $\mu g.mL^{-1}$), tetracycline (TET; 0.125 – 64 $\mu g.mL^{-1}$), erythromycin (ERY; 0.016 – 8 $\mu g.mL^{-1}$) and clindamycin (CLI; 0.032 – 16 $\mu g.mL^{-1}$) were tested. The minimum inhibitory concentration (MIC) of antibiotics was determined by the broth micro-dilution method reported by the ISO 10932/IDF 233 standard. The strains were classified as susceptible or resistant according to the cut-off values proposed by EFSA (2012). A bacterial strain was defined as susceptible or resistant when it was inhibited or not, at a specific antimicrobial concentration equal or lower than the established cut-off value.

Hemolysin and gelatinase activity

Hemolysin activity was determined on Columbia Blood Agar (Oxoid) containing 5 % defibrinized horse blood after 48 h of incubation at 37 °C, both under aerobic and anaerobic conditions. The type of hemolysis (α, β or ɣ) was determined. *Staphylococcus aureus* ATCC25923 and *Escherichia coli* ATCC25922 were used as positive control for β- and α–hemolysis, respectively. Zones of clearing around colonies indicated β-hemolysin production. Gelatinase production was detected by inoculating LAB onto freshly prepared peptone yeast extract agar containing gelatin (30 $g.L^{-1}$; Difco). Plates were incubated overnight at 37 °C and cooled at room temperature for 2 h. The presence of turbid zone around the colonies was considered as positive result.

Growth and acidification

Selected *Weissella* strains were inoculated (1 %) into MRS broth, incubated at 10, 30, 37 and 44 °C for 48 h and growth at OD_{620} was measured. Similarly, the growth of each strain was evaluated in MRS with pH adjusted to pH 4.5, 5, 6, 7, 8 and 9.6 and supplemented with 4, 6.5 and 10 % NaCl. The OD_{620} was measured at 0, 2, 4, 6, 24 and 48 h. Acidification ability was evaluated as reported by Ammor *et al*. (2005), using Sausage-Broth (SB) medium. Eighty (80) ml of SB medium was inoculated with an overnight culture of each strain. The pH values and OD_{620} were recorded after 0, 3, 6, 24, 48, 72 and 96 h of incubation at 30 °C using a pH meter and a spectrophotometer.

Autolytic activity and thermoresistance

Each strain was suspended in PBS buffer (pH 7) at a DO_{620} : 0.2 and subjected to a freeze cycle (-20 °C for 24 h) and after thawing, strains were incubated at 30 °C for 24 h. Autolytic activity was determined by the decrease percentage in absorbance at D_{620} after time interval (Piraino *et al*. 2008) as %AA: (Ai-At) × 100/Ai, where AA: autolytic activity, Ai: initial Absorbance and At: Absorbance after 24 h of incubation. Autolysis was classified according to lactobacilli genus (Ayad *et al*. 2004), ranged from 70 – 96 % (good), 40 – 69 % (low) and 0 – 39 % (poor). Thermo-resistance was evaluated as reported by Badis *et al*. (2004); LAB strains inoculated in MRS broth were heated at 60.5 °C during 30 min, and then incubated at 30 °C for 24 to 48 h and the colonies in MRS agar were enumerated.

Proteolytic and lipolytic activities

Five microliters (5 µL) of each LAB strain suspended in PBS buffer (pH 7) were spot inoculated onto tryptone soy agar (TSA) supplemented with sterile skim milk (10 %) as described by Guiraud (2003). After incubation at 30 °C for 5 d, the caseinolytic activity (measured in mm) was determined by the presence of a clear area around the spot. For lipolytic activity, the technique reported by Mauriello *et al*. (2004) with minor modifications was used. One mL of LAB overnight cultures was inoculated into 10 mL of a broth containing tryptone (1 % (w/v)), yeast extract (0.5 % (w/v)), NaCl (3 % (w/v)), pH 7.0, supplemented with 4 % (w/v) lamb fat previously homogenized by vigorous shaking. After incubation at 30 °C for 7 d, free fatty acids were then determined. The lipids were extracted into 10 ml of petroleum ether by shaking for 1 min. The fatty acids of the upper phase were titrated with NaOH (0.1 M) in ethanol using 1 % phenolphthalein-ethanol solution as indicator. Results were expressed as % of oleic acid by a × N × 28.2/g, where a: mL NaOH used for titration, N: NaOH normality, 28.2: % of oleic acid equivalent weight and g: amount of lamb fat used.

Exopolysaccharides (EPS) production

Active cultures of LAB strains were spotted on MRS agar in which glucose was replaced by sucrose and incubated at 30 °C for 2 – 7 d. Ropiness was examined by the presence of a ropy condition after touching the colony with a loop.

Statistical analysis

Agar assays were performed by duplicate and growth curves by triplicate. In the case of antibiotic resistance, media values were compared with cut-off points. The media and SD were calculated for growth data, results (means OD ± SD) being evaluated by the application of ANOVA to define differences and statistical significances were determined by the Tukey test.

Results and Discussion

Physiological and biochemical characterization of LAB isolates

Sixty-three presumptive LAB isolates (42 cocci and 21 bacilli) were subjected to a preliminary characterization. Results showed that 10 % of the isolates produced gas from glucose (Table 1). Among homofermentative cocci/coccobacilli isolates, those tetrads-forming cocci (23.8 %) that grew at 10 °C and 40 – 45 °C, up to 10 % NaCl but not at pH > 8.0 were presumed as *Pediococcus.* However, chains-forming homofermentative cocci (47.7 %) that developed at the same temperatures, in a wider pH range (4.0 to 9.6) and up to 6.5 % NaCl were assigned to *Enterococcus* genus, differing from lactococci in that the latter are not able to grow at 40 – 45 °C. On the other hand, heterofermentative cocci/coccobacilli isolates (28.6 %) able to grow at 10 °C, up to 6.5 % NaCl and pH between 4.5 and 8.0 (arginine mostly positive and variable growth at pH > 6.0, between 40 – 45 °C and NaCl > 6.5 %) have been characterized as presumptive *Leuconostoc* or *Weissella.* Concerning bacilli (21 %) involving homo and heterofermentative isolates would be assigned to *Lactobacillus* or *Weissella* genus with variable arginine hydrolysis, ability to grow between 40 – 45 °C in a wide range of pH and resistant to NaCl and sugar fermentation capacity except for xylose. Sugars fermentation for cocci/coccobacilli isolates exhibited variable carbohydrates fermentation; heterofermentative coccobacilli fermented cellobiose, fructose, maltose, mannose, sucrose, and xylose but not melibiose, raffinose, sorbitol and trehalose. Based on phenotypic, morphologic and biochemical characterization, pH, temperature, salt tolerance and sugars fermentation, it can be preliminary suggested that cocci/coccobacilli isolates belong to *Pediococcus*, *Enterococcus*, *Leuconostoc*, *Weissella* and *Lactobacillus* genera. These results agree with the LAB from high salt-containing fish and meat products (Najjari *et al*. 2008; Ben Belgacem *et al*. 2010; Belfiore *et al*. 2013).

Molecular identification of isolates exhibiting antimicrobial activity

After the first inter-LAB species inhibition assay, selected isolates (19) were subjected to molecular identification.

Table 1. Physiological and biochemical characterization of LAB isolated from *kaddid.*

Physiological traits	**Cocci/Coccobacilli (42)**			**Bacilli (21)**
	Homofermentative		**Heterofermentative**	**Homo/Hetero**
Microscopy	**Cocci/ tetrads**	**Cocci/ chains**	**Coccobacilli single/pairs**	**Chains/ pairs**
CO_2 from glucose	–	–	+	+/–
Arginine hydrolysis	+(v)	+/–	–	+/–
Growth at 10 °C	+	+	+	+
45 °C	+	+	+/–	v
NaCl 4%	+	+	+	+
6.5%	+	+	+/–	+ (v)
10%	+	+/–	– (v)	+ (v)
pH 4.5	+	+	+	+
6.5	+	+	+ (v)	+
8.0	–	+	+/–	v
9.6	–	+	–	–
D-Glucose	+	+	+	+
D-Arabinose	+/–	+/–	+/–	+/–
D-Cellobiose	–	+/–	+	+
D-Fructose	+/–	+	+	+
D-Galactose	+/–	+	+/–	+
D-Lactose	+	+	+	+
D-Maltose	–	–	+	+
D-Mannose	–	+	+	+
D-Mannitol	+	–	+/–	+
D-Melibiose	–	+/–	–	+
D-Ribose	+	+/–	+	+
D-Raffinose	+/–	–	–	+/–
D-Sorbitol	+/–	+/–	–	+/–
D-Sucrose	+/–	+	+	+/–
D-Trehalose	–	+	–	+
D-Xylose	+/–	+/–	+	–

+ = Positive; – = negative; +/– = more or less positive; v = variable.

In parallel with the physiological/biochemical characterization, amplification of partial 16S rRNA gene sequence allowed the identification of 15 isolates as *Weissella* (*W.) cibaria*/*confusa* with a similarity level of 99 to 100 %, one isolate as *W. paramesenteroides* (similarity 99.87 %), two isolates as *P. acidilactici* (similarity > 99 %) and one isolate as *E. hirae* (similarity 99.77 %), (Table 2). This result indicated that most of LABs were heterofermentative, while only three strains were homofermentative. Because *Weissella* species constituted the major population among identified LAB, they were used for further studies. High relatedness of *W. cibaria* and *W. confusa* observed in this study agrees with that described by Lynch *et al*. (2015). However, based on differential capacity to ferment carbohydrates, *W. cibaria* was described to ferment arabinose but not galactose and ribose, while these latter were utilized by *W. confusa* (Björkroth *et al*. 2002; Quattrini *et al*. 2019). Results from carbohydrates fermentation were used to complement molecular identification (Table 1). Thus, strains were distinguished as *W. cibaria* (BK1, BK2, BK3, BK9, BK10, BK13, BK18, BK19), *W. confusa* (BK4, BK5, BK6, BK7, BK11, BK12, BK16), *W. paramesenteroides* (BK8), *P. acidilactici* (BK14, BK17) and *E. hirae* (BK15) as shown in Table 2. Species of this genus have been isolated from a wide range of ecological niches including soil, plants, breast milk, oral cavity, urogenital and GIT tract of humans and animals as well as a huge variety of fermented foods (Abriouel

et al. 2015; Fusco *et al*. 2015; Maldonado *et al*. 2018). From a technological point of view, *Weissella* plays a key role in food fermentation based on vegetables and to a lesser extent in meat. However, the identification of *W. cibaria*, *W. confusa* and *W. paramesenteroides* from dry-salted Algerian kaddid agrees with those reported during dry-cured sausages fermentation (Fusco *et al*. 2015). Indeed, *W. cibaria* and *W. confusa* were previously retrieved from Portuguese fermented sausages, Thai pork sausages (*nham*), *morcilla* de Burgos and fermented fish (Santos *et al*. 2005; Srionnual *et al*. 2007; Albano *et al*. 2009; Kopermsub and Yunchalard 2010; Wongsuphachat *et al*. 2010). Likewise, *W. paramesenteroides* was identified from Italian fermented sausages (Urso *et al*. 2006; Papagianni and Papamichael 2011). Although *W. cibaria*has been first isolated from Thai fermented meat (Björkroth *et al*. 2002), together with *W. confusa* have been associated with a wide range of vegetable fermented products and their ability to use plant carbohydrates was reported (Fusco *et al*. 2015). Besides plant sugars utilization, *W. confusa* and *W. paramesenteroides* showed to use ribose, suggesting they also may grow in meat.

Table 2. Lactic acid bacteria (LAB) strains identified from dried salted Algerian *kaddid.*

LAB isolates	Isolation source	Closest relative	Identity [%]	Accession No.*
BK1	SK1	*Weissella cibaria*	99.41 %	MT158598.1
BK2	SK1	*Weissella cibaria*	99.87 %	MT012260.1
BK3	SK1	*Weissella cibaria*	99.87 %	MT012260.1
BK4	SK2	*Weissella confusa*	100 %	MK503640.1
BK5	SK2	*Weissella confusa*	100 %	MK503640.1
BK6	SK3	*Weissella confusa*	99.76 %	MK503640.1
BK7	SK3	*Weissella confusa*	100 %	MK503640.1
BK8	SK4	*Weissella paramesenteroides*	99.87 %	MN994365.1
BK9	SK4	*Weissella cibaria*	99.75 %	MT012260.1
BK10	SK5	*Weissella cibaria*	99.55 %	MT012260.1
BK11	SK5	*Weissella confusa*	99.50 %	MK503640.1
BK12	SK6	*Weissella confusa*	99.88 %	MK503640.1
BK13	SK6	*Weissella cibaria*	99.65 %	MT012260.1
BK14	SK6	*Pediococcus acidilactici*	99.74 %	CP050079.1
BK15	SK7	*Enterococcus hirae*	99.77 %	MT197246.1
BK16	SK7	*Weissella confusa*	99.05 %	MK503640.1
BK17	SK7	*Pediococcus acidilactici*	99.88 %	CP050079.1
BK18	SK7	*Weissella cibaria*	100 %	MT012260.1
BK19	SK7	*Weissella cibaria*	99.75 %	MT012260.1

SK – sample of *kaddid*. *Kaddid* Southwestern Algerian samples were from: SK1, SK2, SK3 (Béchar city), SK5 (BeniOunif), SK6 and SK7(Igli) from Béchar province, and SK4 from Tindouf province. *Sequence similarity searches were performed using BLAST networkservice (http://blast.ncbi.nlm.nih.gov/).

Antimicrobial activity

In a first attempt to distinguish those isolates exhibiting inhibitory activity, from 63 LAB isolates preliminarily assigned to different LAB genera, 19 of them showed inter-species antagonistic activity (Result not shown) suggesting the presence of antibacterial metabolite/s in the supernatant. Indeed, when neutralized supernatants and catalase addition were evaluated, the inhibitory activity was suppressed indicating that organic acids or some compound of protein nature would be responsible for the antibacterial effect. The production of inhibitory metabolites active against food pathogens could be an important improvement for starter cultures and might be of interest in controlling meat fermentation, which naturally contain competing food-borne pathogens. Therefore, as a dominant population, sixteen LABs assigned to *Weissella* genus were investigated for their antibacterial and antifungal activity against a range of pathogens and contaminants (Table 3).
Results showed *Weissella* isolates BK2, BK3 and BK19 as the highest inhibitory strains against the assayed pathogens. Bacteriocin production by *Weissella* species was widely reported, especially

with activity against other LAB species (Fusco *et al*. 2015). The exceptional inhibitory ability against *E. coli*, *S. typhimurium* and *Pseudomonas* was in coincidence with that reported for *Weissella* species (Woraprayote *et al*. 2015; Fessard and Remize 2017). In addition, supernatants of the examined presumptive *Weissella* exhibited a high inhibitory activity against *L. monocytogenes* Scott A, GM1 and GM2 and *L. innocua* ATCC51742 and DSM20649 as well as *S. aureus* ATCC 29213 indicator strains, with inhibition zones between 8 to 20 mm, whereas *Enterococcus* and *Pediococcus* were not inhibited. The ability of *Weissella* isolates to prevent *Listeria* and *Staphylococcus* growth agree with those reported for *W. paramesenteroides*, *W. hellenica*and *W. viridescens* from pickles, sea foods and meat fermented products (Papagianni and Papamichael 2011; Masuda *et* al. 2012; Leong *et al*. 2013; Chen *et al*. 2014; Castilho *et al*. 2019). Moreover, the antifungal activity of 10 out of 16 *Weissella* isolates against fungal indicators (Table 3) coincide with that reported for *W. cibaria*, *W. confusa* and *W. paramesenteroides* against other phytopathogenic or food fungal strains (Trias *et al*. 2008; Valerio *et al*. 2009; Ndagano *et al*. 2011; Bianchini *et al*. 2015; Quattrini *et al*. 2019). The production of lactic and acetic acids by heterofermentative *Weissella* may account for their great inhibitory activity, in agreement to fungal inhibitory compounds production reported by Gerez *et al*. (2013). Due to their antimicrobial activity, *Weissella* have been found to act as foodbiopreservatives and probiotics in humans and animals (Abriouel *et al*. 2015; Fusco *et al*. 2015). The production of antimicrobial compounds is desired, thus the proliferation of pathogens or spoilage microorganisms can be controlled during fermentation. Based on these antimicrobial features, *W. cibaria* BK2, BK3 and BK19, *W. confusa* BK4, BK6 and BK11, and *W. paramesenteroides* BK8 were selected to investigate their major safety and technological properties.

Safety evaluation

Although many LAB species have been recognized as GRAS organisms by FDA (1999) or have attained the QPS status by EFSA (2004), no *Weissella* species were included. Studies on antibiotic resistance profile of this genus are limited, and MIC cut-off has not still defined by EFSA. When *Weissella* strains resistance/sensitivity to clinical antibiotics was investigated (Table 4), a multiresistance pattern was found, this being in correlation with that described by Abriouel *et al*. (2015). Similar to other LAB, *W. cibaria*, *W. confusa* and *W. paramesenteroides* exhibited intrinsic resistance to VAN (Ouoba *et al*. 2008). However, additional high resistance to KAN, GEN and TET was exhibited for all *Weissella* strains, strains BK3 and BK19 were also resistant to STR and CHL, this being in coincidence with those reported for strains isolated from Chinese dry fermented meat product (Wang *et al*. 2018) and fermented salted squid (Le and Yang 2018). Similar resistance to GEN and KAN of *W. cibaria* strain from goat milk was described (Elavarsi *et al*. 2014), and STR resistance of *W. cibaria* and *W. confusa* from fruit/juices was also reported (Xu *et al*. 2018). However, the investigated strains were sensitive to ERY, which disagree with that found for *W. cibaria*of vegetable origin (Xu *et al*. 2018; Dentice Maidana *et al*. 2019). Antibiotic resistance patterns found here are related to the controversial nature of *Weissella* genus reported by Abriouel *et al*. (2015), and the lack of use as commercial starter so far (Fessard and Remize 2017). Despite the resistance to aminoglycosides KAN and GEN, *W. cibaria* BK2 was the more sensitive among assayed strains. Within the framework of food safety, *Weissella* strains were also investigated for their hemolytic and gelatinase activity as well as biogenic amines production. Results showed neither gelatinase nor β-hemolytic activity was exhibited by *Weissella* strains, only γ-hemolysis being observed. Similarly, biogenic amines were not produced by the analyzed strains (data not shown). Due to their controversial status, determination of these safety traits for *Weissella* strains help in carefully selecting strains lacking pathogenic potential.

Technological characterization

In view to select *Weissella* strain/s to be used for *kaddid* fermetnation, several technological traits

were evaluated. The strains tested showed a good adaptation towards cultural stresses, such as temperature, NaCl concentration and pH (Table 4). Acidification showed ΔpH values between 2.10 and 2.37 units after four days, showing a decrease to 4.03 – 4.27 after 6 h of incubation at 30 °C, reaching final values (96 h) in the range of 3.80 – 4.10 (data not shown). The pH reduction is in correlation with the acid production by LAB strains, *W. paramesenteroides* BK8 being the most acidogenic. The average acidification rate of assayed strains resulted in 0.55 units/day, which was higher than that of traditional *kaddid* (Benlacheheb *et al*. 2018). As expected, optimal temperature for *Weissella* strains was 30 °C reaching OD_{620} between 1.96 and 2.11 at 48 h with an average growth rate of 0.47/h. When NaCl concentration increased from 4 to 10 % a decrease in *Weissella* growth from OD_{620} of 1.68 to 0.06 was produced, *W. paramesenteroides* BK8 being the most resistant to 10 % of NaCl (Table 4). Osmotic adaptation of strains correlated with their growth under the high salt concentration of *kaddid*. To prevent spoilage/pathogens proliferation, quick growth/acidification capacity is an important criterion for the selection of LAB starter. In addition, the evaluation of enzymatic activities that could play a role in the flavor development, such as the release of intracellular enzymes by cell lysis (autolysis) showed values in the range of 4.23 – 8.08 %, while proteolytic activity was only exhibited by *W. confusa* BK11.Autolysis of *Weissella* strains at 24 h here obtained showed lower values compared to that reported for *W. confusa* strain isolated from Indian fermented foods (Sharma *et al*. 2018). All *Weissella* strains showed to be thermoresistant when heated at 60.5 ºC during 30 min while no lipolytic activity was detected (data not shown). Similarly, a lack of lipolytic activity was also reported for *Lactobacillus plantarum* isolated from Tunisian *kaddid* (Essid *et al*. 2009), but were able to hydrolyze casein, contrarily to the results for *W. cibaria* strains in this study. *W. cibaria* has been reported to have an extensive peptidolytic activity (Lynch *et al*. 2015); perhaps the use of casein as a protein source did not allow evidencing this activity.

Furthermore, the lack of H_2S and EPS production favor their use as starter culture in meat fermentation; even when the ability to produce EPS is a common trait for *W. cibaria* and *W. confusa* (Fusco *et al*. 2015; Lynch *et al*. 2015; Quattrini *et al*. 2019), formation of these compounds in meat products would lead to an indication of sensory spoilage. Therefore, based on antimicrobial activity, antibiotic resistance patterns, growth, and acidification, NaCl tolerance and moderate protein hydrolysis as well as the lack of virulence factors and adverse sensory traits, the strains *W. cibaria* BK2, *W. confusa* BK6 and BK11 as well as *W. paramesenteroides* BK8 may be selected as candidates to be used in the fermentation of Algerian *kaddid*.

Conclusion

Microbiological examination of Southwestern Algeria dried and salted *kaddid* samples, was performed. Antimicrobial activity and other safety traits of LAB isolates were used to select those inhibitory against food pathogens and contaminants, with low antibiotic resistance, unable to produce virulence factors and not aminogenic. Molecular identification showed *Weissella* species as dominant population and in a lesser extent *P. acidilactici* and *E. hirae*. In view to use these strains as autochthonous starter and functional culture, *Weissella* strains showing technological and safety traits allowed selecting *W. cibaria* BK2, *W. confusa* BK6 and BK11 as well as *W. paramesenteroides* BK8 as valuable candidates which may contribute not only to improve overall quality but also preserve typicality, which benefits for both producers and consumers.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Table 3. Antimicrobial activity of *Weissella* strains isolated from Algerian *kaddid.*

Indicator microorganism	Isolated bacteria	Antibacterial and antifungal activity of bacterial CFS															
		BK1	BK2	BK3	BK4	BK5	BK6	BK7	BK8	BK9	BK10	BK11	BK12	BK13	BK16	BK18	BK19
	Samples source	SK1	SK1	SK1	SK2	SK2	SK3	SK3	SK4	SK4	SK5	SK5	SK6	SK6	SK7	SK7	SK7
Gram (-)Bacteria*																	
Escherichia (E.) coli	Algerian meat	-	10.5	2.5	-	-	-	-	-	-	-	-	-	-	-	-	6
E. coli	ATCC25922	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Salmonella (S.) bongori	Algerian meat	-	2.5	5.5	7.5	-	-	-	6.5	-	-	-	-	-	-	-	3
S. typhimurium	ATCC2572	-	11.5	5.5	7.5	-	7.5	-	6.5	-	-	8	-	7	4.5	-	12
Klebsiella pneumoniae	Algerian kaddid (SK_4)	-	10.5	4.5	-	-	2.5	-	-	-	-	-	-	-	-	-	10
Citrobacter farmeri	Algerian kaddid (SK_1)	-	5.5	-	-	-	-	-	-	-	-	-	-	-	-	-	4
Pseudomonas (P). frederiksbergensis	Algerian kaddid (SK_2)	-	11.5	4.5	-	-	-	-	-	-	-	6	-	-	-	7	14
P. aeruginosa	ATCC27853	-	9.5	8.5	-	-	-	-	-	-	-	-	-	-	-	4	5
Acenitobacter baumanii	ATCC 19606	-	5	-	-	-	-	-	-	-	-	4	-	-	-	-	6
Gram (+) Bacteria*																	
Listeria (L.) monocytogenes Scott A		8	9	11	14	8	9	11	15	10	9	12	19	18	15	16	16
L. monocytogenes	ATCC13932	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
*L. monocytogenes*GM1	Italian chicken meat	9	15	14	15	15	13	10	12	13	14	9	8	12	15	12	14
L. monocytogenes GM2	Italian chicken meat	6	7	11	14	13	10	13	14	14	14	12	8	10	7	13	15
L. monocytogenes	ATCC 15313	-	7.5	-	-	-	-	-	-	-	-	-	-	-	-	-	10
L. innocua	ATCC51742	10	16	13	11	12	13	10	13	13	12	13	13	13	10	15	15
L.innocua	DSM20649	-	13	8	12	-	16	15	15	9	16	20	13	15	11	14	19
Bacillus cereus	ATCC 10876	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Enterococcus (En.) faecalis	ATCC 49452	-	9	-	-	-	-	-	-	-	-	-	-	-	-	-	7
En. faecalis	ATCC 29212	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
En. hirae BK15	Algerian kaddid (SK_5)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Staphylococcus (S.) aureus	ATCC 25923	-	10	-	-	-	7	-	-	-	-	-	-	-	-	-	-
S. aureus	ATCC 29213	9	18	16	16	15	15	13	13	15	14	14	14	14	13	13	14
Staphylococcus sp.	Algerian kaddid (SK_3)	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pediococcus acidilactici BK14	Algerian kaddid (SK_6)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Molds**																	
Penicillium expansum	Algerian wheat	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Aspergillus flavus	Algerian wheat	+	+++	++	-	-	-	-	-	-	-	-	-	+++	+	+	++
Fusarium oxysporum albedinis	Algerian dates	+	+++	-	-	-	+++	-	+++	-	-	+++	-	-	++	++	+++

CFS – cell free supernatant. *Kaddid* Southwestern Algerian samples SK1, SK2, SK3 (Béchar city), SK5 (BeniOunif), SK6 and SK7 (Igli) from Béchar province, and SK4 from Tindouf province. *****Antibacterial activity is expressed by inhibition zone diameter (mm); ** Antifungal activity expressed as: – no growth inhibition), + (1 – 5 mm growth inhibition), ++ (5 – 10 mm growth inhibition) and +++ (> 10 mm growth inhibition).

Table 4. Safety and technological characterization of *Weissella* strains from Algerian *kaddid.*

Weissella strains	Antibiotic resistance	ΔpH*	Growth (OD_{620}) at 48 h						Autolytic activity [%]
			Temperature [°C]			NaCl [%]			
			10	30	44	4	6.5	10	
W. cibaria BK2	VAN/KAN/GEN	2.10 ± 0.02	0.13 ± 0.01	2.10 ± 0.40	1.10 ± 0.3	1.63 ± 0.12	1.23 ± 0.14	0.04 ± 0.00	6.03 ± 0.05
BK3	VAN/KAN/CHL/STR	2.10 ± 0.01	0.15 ± 0.02	2.10 ± 0.08	1.87 ± 0.33	1.63 ± 0.32	1.23 ± 0.00	0.02 ± 0.01	6.06 ± 0.17
BK19	VAN/KAN/GEN/TET/CHL	2.10 ± 0.00	0.15 ± 0.02	2.08 ± 0.11	1.85 ± 0.26	1.63 ± 0.17	1.23 ± 0.11	0.02 ± 0.0	8.08 ± 0.2
W. confusa BK4	VAN/KAN/GEN/TET	2.10 ± 0.03	0.15 ± 0.00	1.96 ± 0.23	1.87 ± 0.70	1.57 ± 0.09	1.23 ± 0.03	0.06 ± 0.01	4.23 ± 0.37
BK6	VAN/KAN/GEN	2.10 ± 0.11	0.19 ± 0.03	2.11 ± 0.07	1.85 ± 0.29	1.63 ± 0.44	1.23 ± 0.10	0.05 ± 0.00	4.23 ± 0.10
BK11	VAN/KAN/GEN	2.15 ± 0.07	0.13 ± 0.01	2.10 ± 0.17	1.68 ± 0.03	1.70 ± 0.05	1.35 ± 0.12	0.04 ± 0.01	5.75 ± 0.44
W. paramesenteroides BK8	VAN/KAN/GEN	2.37 ± 0.03	0.07 ± 0.00	2.10 ± 0.04	1.68 ± 0.15	1.99 ± 0.12	1.40 ± 0.14	0.18 ± 0.01	5.33 ± 0.21

*ΔpH was determined in SB as pH (96 h) – pH (0 h).
VAN – vancomycin; CHL – chloramphenicol; GEN – gentamycin; STR – streptomycin; KAN – kanamycin; TET – tetracycline.

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