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PRELIMINARY PROBIOTIC POTENTIAL OF SELECTED *AEROCOCCUS SPP.*, *ENTEROCOCCUS SPP.*, AND *WEISELLA SP.* FROM ALGERIAN *GUEDID*

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ABSTRACT

Objective: The aim of the current study was to carry out probiotic criteria of ten candidates; 5 *Aerococcus spp.*, 4 *Enterococcus spp.*, and 1 *Weisella sp.* from Algerian culinary *Guedid* prepared traditionally to preserve and improve sensory and nutritional quality of this product.
Methods: Antimicrobial activity, acidity, growth Kinetics, quantification, heat and enzymes sensitivities were assayed against *Listeria ivanovii* CECT148, *Bacillus cereus* ATCC11778, *Staphylococcus aureus* ATCC25923 and *Clostridium perfringens* CECT486. Assessment of proteolysis, lipolysis, amylolysis, gelatinase, bile salts hydrolase, acetoin and exopolysaccharides production, acidity conditions, bile salts, gastric and intestinal resistances were determined. Survival lactic acid bacteria was then calculated using single plate-serial dilution spotting. Cholesterol assimilation, hemolysis and antibiotic resistance were also characterized. Statistical analysis was performed using originPro v9.5.
Results: The neutralized supernatants of *Aerococcus spp.* (Lbm19, 18, 3) and *Enterococcus sp.* (Lbm49, 46, 50) showed 9 mm inhibition zone, *Clostridium. perfringens* CECT486 was the most sensitive one. Lactic acid bacteria decreased by 1-2 log CFU/mL on gastrointestinal conditions and assimilated cholesterol by up to 89%. The antagonistic peak was obtained at the stationary phase where pH 3.5 was reached. The supernatant was sensitive to enzymes and heat. All candidates showed digesting ability for proteins but not for starch, lipids, gelatin, bile salts, also showed no hemolytic activity. All candidates were found to resist against two antibiotics and three isolates exhibited negative for exopolysaccharides and acetoin production.
Conclusion: *Enterococcus sp.*Lbm49, *Aerococcus sp.*Lbm19, *Aerococcus sp.*Lbm18, *Enterococcus sp.*Lbm46, and *Aerococcus sp.*Lbm3 showed the highest potential probiotic score. This study should be completed by a molecular characterization, *ex vivo* and *in vivo* tests.

Keywords: *Guedid*, lactic acid bacteria, screening, probiotic potential

INTRODUCTION

Lactic acid bacteria (LAB) are ubiquitous, unicellular gram positive, non-sporulating, catalase-negative, facultative anaerobic microorganisms. LAB are frequently found in food and traditional ones. As well, they live in a bacterial ecosystem such as the vagina and gastrointestinal tract (**Quinto *et al*., 2014**). They are heterotrophic that generate energy from carbon substrates fermentation to produce organic acids such as lactic acid as one of the main fermentation products **(Quinto *et al*., 2014; Carr *et al*., 2002)**. LAB are generally recognized as safe "GRAS" by the agency of the Food and Drug Administration (FDA) or qualified presumption of safety "QPS" by the European agency of the Food Safety Authority (EFSA). These friendly human microbes are essentially utilized as food, food supplements, food ingredients, medical food, recently, as drugs, for therapeutic purposes to enhance human health and treatment of some gastrointestinal microbial disorders. This is due to their ability to inhibit harmful bacteria **(Mokoena, 2017; Urdaci *et al*., 2004)**.This inhibition is related to the production of organic acids and various other metabolites; bacteriocins mainly **(Izuchukwu, 2017; Hor and Liong, 2014; Lairini *et al*., 2014; Guarner *et al*.,2011)**.
The International Scientific Association for Probiotics and Prebiotics (ISAPP) defined probiotics as "*a live organism, which provides a benefit to the host when provided in adequate quantities*" **(de Oliveira, 2018; Gasbarrini *et al*., 2016)**. Health benefits of probiotics include beneficial effects in prevention of diarrhea, inflammatory bowel disease, urogenital infections, lactose intolerance, lowering serum cholesterol level, colon cancer, regulation of microbial balance of intestinal microbiota, prevention of allergies, enhancement of the immune system, nutrient bioavailability, and calcium absorption **(Angmo *et al*., 2016 ; Tokatlı *et al*., 2015)**.

To ensure that the probiotic candidate strains exert their beneficial effect on human health, several required characteristics main currently examined, through *in vitro* tests as recommended by FAO/WHO guidelines **(Morelli and Capurso, 2012; FAO/WHO, 2002)**. Taxonomic identification must be achieved using reliable phenotypic and molecular techniques **(Reid, 2005)**. The origin of the strain must be human origin, preferably from the same site to obtain maximum efficiency and effectiveness. Those probiotic strains must be assessed by clinical trials on animals and on humans to avoid side effect. In addition, they must display an antimicrobial activity and resist to the gastrointestinal environment **(Zhang *et al*., 2018; Ashraf and Smith, 2016)**. A high percentage to adhere to the intestinal cell epithelium or mucosa is an essential property. It may eventually stimulate the immune system through mucin production and prevent the implantation of pathogens. Antibiotic susceptibility profile is the most important parameter for the LAB candidates, to exclude transferability of antibiotic resistance to commensal or pathogen bacteria present inside the gut **(Zhang *et al*., 2018; Sanders, 2011; Foligne *et al*., 2013)**.
We carried out this study aiming to screen, through a series of tests, functional, technological, and safety proprieties of ten (10) autochthonous LAB. Those LAB were previously isolated from an ethnic Algerian salted and dried meat (*Guedid*), characterized at the genus level by phenotypic tests and selected for possessing an important antibacterial activity.

MATERIAL AND METHODS

Laboratory bacterial strains and culture media

The four gram-positive potentially pathogenic bacteria used in the current study as target strains were: *Listeria ivanovii* CECT148, *Bacillus cereus* ATCC11778, *Staphylococcus aureus* ATCC25923, and *Clostridium perfringens* CECT486.

Working target strains were activated in TSB broth and/or agar (Trypticase Soy broth; Condalab) for 24 hours at 37°C. The ten autochthonous LAB used in the current study as test cultures: *Aerococcus spp.* (Lbm3, 10, 17, 18 and 19), *Enterococcus spp.* (Lbm46, 47, 49 and 50) and *Weisella sp.* Lbm22, were obtained from *Guedid* prepared from lamb meat by salting and sun-drying **(Gagaoua, and Boudechicha, 2018; Boudechicha *et al.*, 2017)**. Samples from homemade preparation were collected in sterile bags. LAB were isolated on elective growth MRS agar (De Man, Rogosa, &Sharpe; Condalab) and phenotypically identified **(Axelsson 2004; Doyle et *al.*, 2006)**. Working LAB cultures were activated in MRS broth and/or agar for 24 to 72 hours at 30°C under anaerobic atmosphere. Pathogens and LAB were regularly checked out by catalase reaction test and gram staining with microscopic observation according to standard procedures **(Goldman and Green, 2015)**. Their stock cultures were maintained in their respective media; as refrigerate stock in agar slants at 4°C and as frozen stock in 20% (v/v) glycerol-supplemented broth at -20°C **(Spencer, 2001)**.

Antagonistic activity against pathogenic bacteria

Antibacterial activity was screened using two methods: agar spot test in dual culture and agar well diffusion assay. The first assay was employed for the direct detection of antibacterial activity as described by **Fleming *et al.* (1975)** and **Schillinger and Lücke (1989)**. An overnight LAB cultures (30°C on MRS agar) were spotted on the surface of solidified MRS base agar (1.5% agar) seeded with 200 µl (approximately 10^8 CFU/mL) of the target pathogenic bacteria standardized at 0.5 McFarland from overnight cultures (37°C in TSB broth). The plates were incubated under anaerobic atmosphere at 30°C for 24 hours. The second assay was used for the indirect detection to determine the cell-free supernatants (CFS) antibacterial activity as described by **Schillinger and Lücke (1989)**. Culture supernatants (6000 rpm for 15 minutes at 4°C) from overnight cultures (MRS broth inoculated with 0.2% of LAB fresh cultures and incubated at 30°C for 48 hours) were treated at 80°C to eliminate vegetative forms for 10 min and then refrigerated at 4°C. Ten (10) mL of solidified TSA (Trypticase Soy agar; Condalab) base agar (1.5% agar) were overlaid with 10 mL TSA soft agar (0.75% agar) inoculated with 200 µl (approximately 10^8 CFU/mL) of the target pathogenic bacteria standardized at 0.5 McFarland from overnight cultures (37°C in TSB broth). Wells (7 mm in diameter) were bored at equal distance on the solidified TSA agar and filled with 40 µl of culture supernatants. The central well, as control, received 40 µl of sterile MRS broth. The plates were stored at room temperature (20°C for 2 hours) until the culture supernatant was absorbed. LAB were tested in duplicate and incubated under anaerobic atmosphere at 30°C for 24 hours.
Anaerobic conditions were ensured to bypass catalase inhibition as reported by **Vermeiren *et al.*, 2004** and a buffered TSA agar, at pH 7.0 and 0.2 M, was utilized to avoid acid inhibition. The buffered TSA agar was prepared by dissolving 19.5 parts of K_2HPO_4 at 0.1 M with 30.5 parts KH_2PO_4 at 0.1 M in a total of 100 parts of distilled water. The presence of inhibition zone (inhibition halo), surrounding the test culture spot or the culture supernatant higher than 0.5 mm, was considered as positive antagonistic activity **(Fleming *et al.*, 1975)**. Both assays were performed in duplicate.

Acidity, growth kinetics and production of bacteriocin-like substances

Determination of acidity, growth kinetics and production of bacteriocin-like substances were carried out during the same experiment. From overnight cultures of LAB, MRS broth was inoculated with 0.2% and incubated at 30°C with shaking (100 rpm/min), Samples were collected after 0, 2, 6, 12, 18, 24, and 48 hours. Acidity was measured by pH-meter, optical density by spectrophotometer at 620 nm, and bacteriocin-like substances production was investigated by agar well diffusion assay on buffered TSA agar as noted above. The experiment was performed in duplicate **(Musikasang *et al.*, 2012; Musikasang *et al.*, 2009)**.

Heat and enzymes sensitivities of bacteriocin-like substances

Samples of CFS obtained by centrifugation (6000 rpm for 15 minutes at 4°C) from an overnight test cultures in MRS broth inoculated with 0.2% of fresh LAB incubated at 30°C for 48 hours. Samples were treated at 100°C for 10, 30, and 60 min and at 120°C for 15 min, then were cooled at 4°C. Other samples were exposed to the action of two proteolytic enzymes (pepsin and α chymotrypsin at a final concentration of 1 mg/mL) (Sigma-Aldrich). Resistance or sensitivity of CFS containing bacteriocin-like substances after these treatments were determined by the fact of the presence or absence of antibacterial activity by agar well diffusion assay applied as noted above. All tests were performed in duplicate **(Hanchi *et al.*, 2014; Monteagudo-Mera *et al.*, 2011)**.

Exopolysaccharides and acetoin production

Capacity to produce exopolysaccharides (EPS) was determined on modified MRS agar with 10% (w/v) sucrose (Sigma-Aldrich). Overnight LAB cultures (MRS agar at 30°C for 24 hours) were streaked on the surface of the agar. After incubation at 30°C for 24 to 48 hours, was considered as positive result streaks with viscous colonies **(Angmo *et al.*, 2016)**. For the capacity to produce acetoin, 1 mL from the overnight LAB cultures (MRS broth at 30°C for 24 hours) was inoculated in Clark Lubs broth (Condalab). After incubation at 30°C for 24 hours, 0.5 mL of both VP1 and VP2 reagents were added. Ten (10) minutes of rest at room temperature, acetoin production is indicated by pink ring and/or the diffusion of the pink color on the surface of the tube. Each experiment was performed in triplicate.

Assessment of proteolytic, lipolytic, amylolytic, and bile salts hydrolase

Assessment of proteolytic, lipolytic, amylolytic, and bile salts hydrolase activities was performed on modified MRS agar containing skimmed milk (1%, 2%, and 10% w/v), tween 80 (at 1% and 3% v/v), soluble starch (2% w/v), and bile salts (0.5% w/v with 0.037% of $CaCl_2$ w/v) (for all reagents Sigma-Aldrich), respectively. Overnight LAB cultures (MRS agar at 30°C for 24 hours) were spotted on the surface of media. After incubation at 30°C for 24 to 48 hours, a positive activity was indicated by higher than 1 mm clear zone around the spot. The starch agar plate must be flooded with iodine solution and examined 15-30 min later. The zone diameters were then measured. Experiment was performed in duplicate **(Ruiz Rodríguez *et al.*, 2016; Musikasang *et al.*, 2012; Monteagudo-Mera *et al.*, 2011; Musikasang *et al.*, 2009)**.

Surviving in acidity conditions, bile salts, simulated gastric, and intestinal juice

The capacity of the LAB to survive in acidity conditions was tested in phosphate-buffering saline (PBS) adjusted to pH 2 and 3 (0.9% NaCl, 0.9% K_2HPO_4, 0.15% KH_2PO_4). The capacity to survive in gastric and intestinal conditions was also tested in a saline solution (0.85% NaCl) supplemented with 3 mg/mL of pepsin adjusted to pH 3 and with 3 mg/mL of bile salts adjusted to pH 8 (for all reagents Sigma-Aldrich), respectively. These broths were inoculated by aliquots from overnight LAB cultures (MRS broth at 30°C for 24 hours) to attend 10^8 CFU/mL. These preparations were examined before and after 4 hours of incubation at 37°C and survival LAB were then calculated using single plate-serial dilution spotting (SP-SDS) on MRS agar in duplicate. Percentages of survival LAB were calculated as follow: % survival = (log CFU of viable cells survived / log CFU of initial viable cells inoculated) x 100 **(Ruiz Rodríguez *et al.*, 2016; Serrano-Nino *et al.*, 2016; Tokatlı *et al.*, 2015; Oh and Jung, 2015 ; Thomas *et al.*, 2015)**.

Cholesterol assimilation

Cholesterol assimilation was assayed in MRS Broth supplemented with 50 μg/mL of cholesterol (Quimicaclinica) and inoculated with 1% of overnight LAB cultures (MRS broth at 30°C for 24 hours) standardized at 10^8 CFU/mL. After incubation at 30°C for 24 hours, CFS (6000 rpm for 15 min at 4°C) and inoculated control (sterile MRS broth) were assayed for their cholesterol content by spectrophotometry at 505 nm. The difference in cholesterol content between the control and the CFS was considered as a percentage of assimilated cholesterol **(Dubey and Jeevaratnam, 2015; Tokatlı *et al.*, 2015)**.

Hemolysis, gelatinase and antibiotic resistance

For safety considerations, hemolysis was searched on MH agar (Muller Hinton; Condalab) supplemented with 5% of human fresh blood. Overnight LAB cultures (MRS agar at 30°C for 24 hours) were streaked on the surface of the medium. After incubation at 30°C for 24 to 48 hours, hemolytic activity was confirmed by the presence of a clear zone around bacterial streak. Were considered as non-hemolytic (green zones; α-hemolysis or non-staining; γ-hemolysis) and hemolytic (yellow zones; β-hemolysis) **(Angmo *et al.*, 2016; Leite *et al.*, 2015; Oh and Jung, 2015; Rather *et al.*, 2015; Monteagudo-Mera *et al.*, 2011)**. Gelatinase was searched on modified MRS agar containing gelatin (3% w/v). Overnight LAB cultures (MRS agar at 30°C for 24 hours) were streaked on the surface of MRS gelatin agar. After incubation at 30°C for 24 to 48 hours, the surface of the medium was sprayed by a saturated solution of ammonium sulphate $(NH_4)\ 2SO_4$ (Sigma-Aldrich) to make detectable halos of lysis **(Monteagudo-Mera *et al.*, 2011)**. The experiment was performed in triplicate.
Antibiotic resistance of the ten LAB to 11 antibiotics from different classes: penicillin (10 µg.disk^{-1}), ampicillin (10 µg.disk^{-1}), amoxicillin (30 µg.disk^{-1}) ceftiofur (30 µg.disk^{-1}), oxytetracyclin (30 µg.disk^{-1}), tylosin (10 µg.disk^{-1}), sulfadiazin (10 µg.disk^{-1}), florfinicol (10 µg.disk^{-1}), vancomycin (30 µg.disk^{-1}), erythromycin (15 µg.disk^{-1}), and gentamicin (1 µg.disk^{-1}) (for all antibiotics Oxoid, Australia), was characterized using Kirby-Bauer disc diffusion test. MRS agar was inoculated by swabbing overnight LAB cultures (MRS broth at 30°C for 24 hours) standardized at 10^8 CFU/mL. Discs were placed onto the surfaces of MRS agar. After incubation at 30°Cfor 24 hours, clear zones (zones of inhibition) around the discs including the disc diameter were measured and expressed in millimeters. Susceptibility was calculated as follow: number of resistances / number of antibiotics tested **(Angmo *et al.*, 2016; Ruiz Rodríguez**

***et al*., 2016; Dubey and Jeevaratnam, 2015; Oh and Jung, 2015; Vera-Pingitore *et al*., 2016; Musikasang *et al*., 2012; Kohajdová, 2006)**.

Statistical analysis

Statistical analysis was performed using originPro v9.5. Probiotic and technological potential was calculated as (observed score / maximum score) x 100. Indication of score was: (0) lytic / no producer or (1) no lytic / producer for technological properties, and (0) sensitivity or (1) resistance for functional properties.

RESULTS AND DISCUSSION

Antimicrobial activity assessment

Potential bacteria candidates require minimum criteria in order to display probiotic status and exert their beneficial effect on human health. Antimicrobial activity represents the essential criteria due to inhibition of growth and attachment of pathogens in the intestine (**FAO/WHO, 2002**). In this study, we pointed out that the ten tested LAB inhibit pathogenic bacteria as follow: in the spot MRS agar test with 2 to 3 mm of diameter, two *Aerococcus spp.* (Lbm18 and 19), *Enterococcus sp.* Lbm46 and *Weisella sp.* Lbm22 could eliminate *L. ivanovii* CECT148, *B. cereus* ATCC11778, *St. aureus* ATCC25923 and *C. perfringens* CECT486. Concerning *Enterococcus spp.* (Lbm47, 49 and 50), they eliminated *B. cereus* ATCC11778, *St. aureus* ATCC25923 and *C. perfringens* CECT486. *Aerococcus sp.* Lbm17, eliminated *L. ivanovii* CECT148 and *C. perfringens* CECT486. The last two isolates *Aerococcus spp.* (Lbm3 and 10) eliminated only *C. perfringens* CECT486. However, in the agar well diffusion assay, two *Aerococcus spp.* (Lbm3 and 18) and *Enterococcus sp.* Lbm50 eliminated all target pathogens with a diameter of 9 mm (well included). *Weisella sp.* Lbm22 and *Enterococcus sp.* Lbm49 eliminated *B. cereus* ATCC11778, *St. aureus* ATCC25923 and *C. perfringens* CECT486. *Aerococcus sp.* Lbm19 exhibited inhibition against *L. ivanovii* CECT148, *St. aureus* ATCC 25923, and *C. perfringens* CECT486. *C. perfringens* CECT486 was the most sensitive pathogenic bacteria followed by *B. cereus* ATCC11778 and *St. aureus* ATCC2592 (Figure 1). LAB cultures were found to inhibit the growth of gram-positive pathogenic bacteria with variable inhibitory extents, as thus, in the study of **Dhewa *et al*. (2010)**10 to 12 mm of Lactobacilli cultures inhibition were found for gram-positive pathogens as *E. faecalis*, *St. aureus* and *B. cereus.* In addition, **Angmo *et al*. (2016**) found inhibition zones of about 5 mm against *B. cereus*, *S. aureus*, and *S. dysenteriae*. The pic of bacteriocinogenic activity (9 mm) versus *L. ivanovii* CECT148, *B. cereus* ATCC11778, *St. aureus* ATCC25923 and *C. perfringens* CECT486 was obtained at the stationary phase where pH 3.50 was reached (16 to 18 hours of culture). Regarding acidity, pH ranging from 3.03 (*Aerococcus sp.* Lbm 19) to 3.95 (*Enterococcus spp.* Lbm49) was reached and demonstrated by all LAB isolates (Figure 1). This could underline that our LAB candidates are considered as appropriate acidifiers by an accumulation of acids, manly lactic during lactic fermentations. Our results agree with those obtained by **Hanchi *et al*. (2014)** and **Benreguieg *et al*. (2013)** who reported at pH 2-3 a pic of antimicrobial activity at a stationary phase between 12 and 18 hours. The results are displayed on Figure 2. Furthermore, the CFS of *Aerococcus spp.* (Lbm3, 17) and *Enterococcus spp.* (Lbm47, 49) isolates were heat-sensitive. However, the CFS of the rest of LAB were heat-resistant (100°C for 10, 30, and 60 min). Furthermore, only CFS of *Aerococcus spp.* (Lbm3, 17) and *Enterococcus spp.* (Lbm47, 49) isolates were sensitive to enzymes (pepsin and α chymotrypsin). This sensitivity can predict the proteinaceous character of inhibitor factor, bacteriocin-like substances **(Musikasang *et al*., 2012)**. Bacteriocin resistance for α-chymotrypsin was reported (**Monteagudo-Mera *et al*., 2011)**.

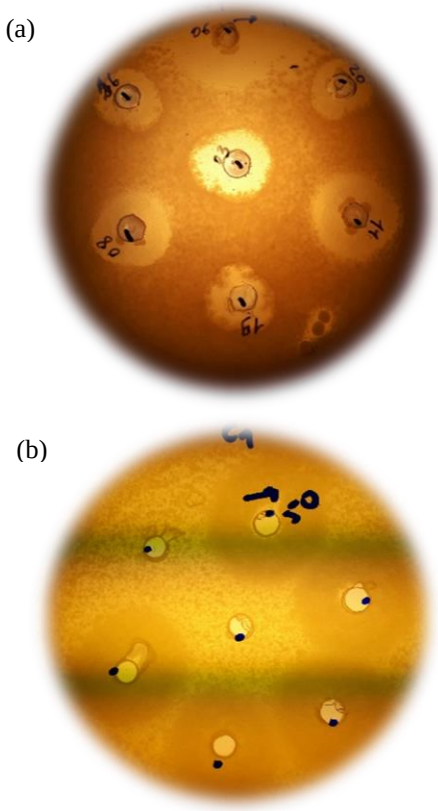
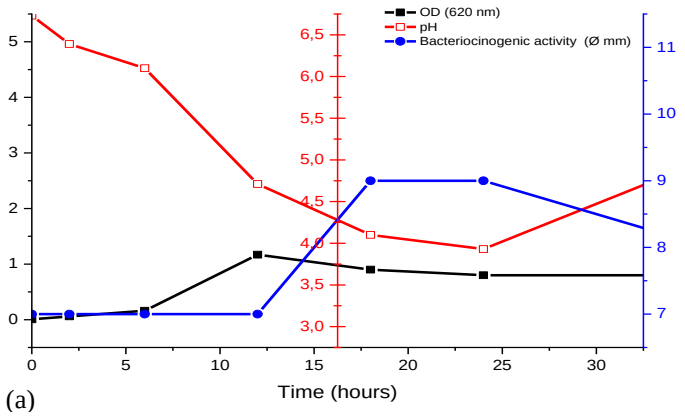
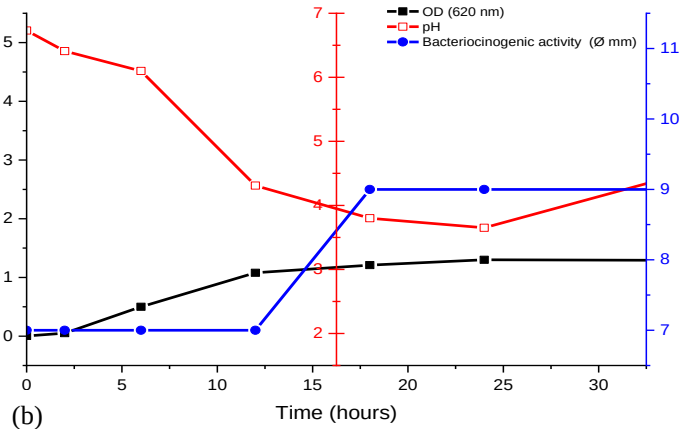


Figure 1 Antagonistic activity against *St. aureus* ATCC25923 of candidates LAB in the spot MRS agar (a) and in the agar well diffusion assay (b)





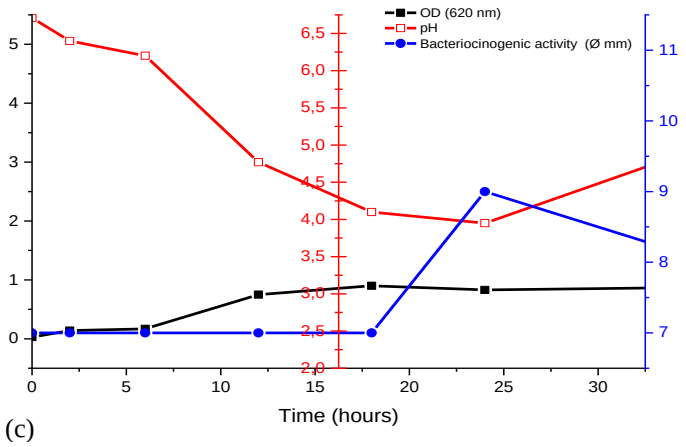


Figure 2 Acidity (□), growth kinetics, optical density (OD) at 620 nm (■) and production of bacteriocin like substances anti-*St. aureus* ATCC 25923 (●) by (a) *Enterococcus sp.* Lbm46, (b) *Enterococcus sp.* Lbm49, and (c) *Aerococcus spp.* Lbm10.

Technological properties assessment

Approaches for improving probiotic status include but not limited to the selection of technological advantageous properties. All LAB tested exhibited protein digesting on modified MRS with skimmed milk at 2% and 10% with 17 to 22 mm for *Aerococcus spp.* (Lbm3, 10, 17, 18), *Enterococcus sp.* Lbm50 and *Weisella sp.* Lbm22, with 10 to 15 mm for *Aerococcus sp.* Lbm19 *Enterococcus spp.* (Lbm46, 47, 49). In comparison, **Essid *et al.* (2009)** found that *L. plantarum* have a proteolytic activity up to 5 mm on 10% skimmed milk. However, digesting capacity on modified MRS was not exhibited for protein at 1%, starch at 3%, and lipids at 1% and 3% of tween 80. Numerous probiotic studies with various strains showed negative results for amylolytic and lipolytic activities **(Mechai *et al.*, 2014; Musikasang *et al.*, 2012; Musikasang *et al.*, 2012; Musikasang *et al.*, 2009 ; Essid *et al.*, 2009)**. In fact, meat substrate, muscle proteins and fat, should be more suitable to assess lipolytic and proteolytic activities than commonly used substrates such as powdered milk, gelatin, and Tween 80 **(Essid *et al.*, 2007; Mauriello *et al.*, 2004).** EPS production was demonstrated only by *Aerococcus sp.* Lbm10 and *Enterococcus sp.* Lbm50, while, acetoin production was demonstrated by *Aerococcus spp.* (Lbm3, 18) and *Enterococcus spp.* (Lbm46, 47, 49 and 50), excepting three isolates *Aerococcus spp.* (Lbm17, 19) and *Weisella sp.* Lbm22 which appeared neither EPS nor acetoin producers. **Quinto *et al.* (2014)** highlights the benefits of EPS in the resistance to acid.

Functional properties assessment

The selection criteria for potential probiotic candidates include also abilities to survive gut transit **(Giraffa *et al.*, 2010)**, as well as their abilities to resist gastric acidity and bile salts in the small intestine **(Lähteinen *et al.*, 2010)**. The pH of the stomach ranges from 2.5 to 3.5 and bile salts concentration reaches to 0.3%, reason why they are considered as standard to assess gastric and intestinal tolerance **(Ashraf and Smith, 2016; Tokatlı *et al.*, 2015; Leite *et al.*, 2015; Dubey and Jeevaratnam, 2015)**. For the capacity to survive gut transit, the ten LAB were found to resist pH 2 less than pH 3 with percentages ranging from 82.83% to 92.56% for *Aerococcus spp.* and from 93.16% to 97.09% for *Enterococcus spp.* and with 76.58% for *Weisella sp.* Lbm22 (Table 1). The decrease in cell viability was approximately 1-2 log CFU/mL (in the two pH values tested). In comparison, different probiotic LAB displayed survival ability at pH 2.5 up to 85% **(Tokatlı *et al.*, 2015)** and reductions in counts at least 2 logarithmic units compared with controls **(Leite *et al.*, 2015)**. For others, the maximum 4.8 log CFU/mL decline in viability at pH 2 (**Angmo *et al.*, 2016**) and 3.3 log at pH 3 **(Dhewa *et al.*, 2010)**.

Table 1 Acidity resistance and cholesterol assimilation

		In PBS adjusted top H 2			In PBS adjusted top H 3		In MRS broth supplemented with 50 µg/mL of cholesterol
		t0	t1	%	t1	%	%*
Aerococcus spp.	Lbm03	8.42 ±0.39	7.76 ± 0.32	92.17	8.27 ± 0.10	98.18	97.91 ± 1.67
	Lbm10	9.03 ± 0.32	8.36± 0.22	92.56	8.79 ± 0.35	97.33	91.66 ± 0.70
	Lbm17	9.16 ± 0.23	8.42 ± 0.35	91.85	8.53 ± 0.40	93.12	93.74 ± 1.30
	Lbm18	8.76 ± 0.21	7.26 ± 0.07	82.83	8.00 ± 0.02	91.33	95.83 ± 2.21
	Lbm19	8.42 ± 0.35	7.26 ± 0.73	86.22	7.95 ± 0.60	94.52	91.66 ± 0.87
Enterococcus spp.	Lbm46	8.48 ±0.21	8.23 ± 0.35	97.09	8.42 ± 0.30	99.27	89.57 ± 0.98
	Lbm47	8.73 ± 0.12	8.42 ± 0.46	96.37	8.65 ±0.27	99.09	93.74 ± 1.13
	Lbm49	8.56 ± 0.19	8.15 ± 0.74	95.21	8.40 ± 0.24	98.15	95.83 ± 1.55
	Lbm50	8.81 ± 0.35	8.20 ± 0.05	93.16	8.60 ± 0.31	97.68	89.57 ± 1.43
Weisella sp.	Lbm22	8.85 ± 0.19	6.78 ± 0.83	76.58	7.85 ± 0.37	88.63	93.74 ± 1.10

Legend: t0 : initial time, t4 : after 4h of incubation, % :percentage of survival LAB (log CFU), %* : percentage of assimilated cholesterol
Values represented as mean ± standard deviation

Table 2 Capacity to survive gut transit

		Gastric in saline solution suspending with 3 mg/ml of pepsin adjusted to pH 3			Intestinal in saline solution suspending with 3 mg/ml of bile salts adjusted to pH 8		
		t0	t1	%	t0	t1	%
Aerococcus spp.	Lbm03	10.44 ± 0.46	10.32 ± 0.50	98.80	10.74 ± 0.95	10.66 ± 0.54	99.28
	Lbm10	10.55 ± 0.54	10.17 ± 0.25	96.35	10.70 ± 0.52	10.26 ± 0.79	95.93
	Lbm17	10.55 ± 1.09	10.22 ± 0.51	96.88	10.53 ± 0.30	10.45 ± 0.39	99.24
	Lbm18	10.34 ± 1.07	10.45 ± 0.43	101.01	11.27 ± 0.37	10.38 ± 0.64	92.15
	Lbm19	10.65 ± 0.66	10.64 ± 0.60	99.88	10.65 ± 0.69	10.59 ± 0.43	99.48
Enterococcus spp.	Lbm46	10.52 ± 0.39	9.98 ± 0.51	94.86	10.64 ± 0.32	10.41 ± 0.56	97.84
	Lbm47	10.66 ± 0.15	10.16 ± 0.40	95.30	10.63 ± 0.58	10.41 ± 0.03	97.91
	Lbm49	10.69 ±1.27	10.04 ± 0.46	93.91	11.18 ± 0.58	10.33 ± 0.04	92.43
	Lbm50	10.45 ± 1.07	10.13 ± 0.05	96.97	10.95 ± 0.98	10.44 ±0.04	95.34
Weisella sp.	Lbm22	10.60 ± 0.01	10.42 ± 0.65	98.36	10.95 ± 0.90	10.26 ± 0.04	93.62

Legend: t0 : initial time, t4 : after 4h of incubation, % :percentage of survival LAB (log CFU)
Values represented as mean ± standard deviation

Furthermore, LAB tested were able to survive after passing through the gastric, for 4 hours, by 96.35% to 99% for *Aerococcus spp.* and from 93.91% to 96.97% for *Enterococcus spp.* and with 98.36 % for *Weisella sp.* Lbm22. As for intestinal environments, survival variability ranged from 92.15% to 99.48% for *Aerococcus*

spp. and from 92.43% to 97.91% for *Enterococcus spp.* and was 93.62 % for *Weisella sp.* Lbm22 (Table 2). They were most tolerant to gastrointestinal stress factors with similar behavior and decreased by approximately 1-2 log CFU/mL in cell viability. This finding is in accordance with previous studies, that found minor reduction illustrating the high resistance to gastrointestinal passing **(Hanchi *et al.*, 2014)** and those reporting a decrease of approximately 3 log CFU/ml in simulated gut juices. As it is recommended that 6 log at 7 log CFU/mL (10^6 at 10^7 CFU) constitutes a minimum number of each viable probiotic strain at the end of their shelf life for the exhibition of health benefit **(Ashraf and Smith, 2016; Angmo *et al.*, 2016; Quinto *et al.*, 2014)**.

Additionally, the ten LAB demonstrated no bile salts hydrolase at 0.5%. Previous studies reported negative and positive results using different concentrations, where negative ones are frequently observed in weak concentrations (0.05, 0.10 and 0.30%). Concerning cholesterol reduction, hypocholesterolemic is a desired character. Tested LAB reduced its level by 89-97% (Table 1). Different rates of cholesterol assimilation levels were cited using different strains and initial concentrations of cholesterol **(Dubey and Jeevaratnam, 2015; Tokatlı *et al.*, 2015)**.

Safety assessment

Moreover, safety criteria for potential probiotic candidates require determination of the antibiotic resistance profile, hemolytic and gelatinase activities. The hemolytic activity of LAB candidates showed α-hemolytic for *Aerococcus spp.* (Lbm10, 17, 18, 19) and *Enterococcus spp.* (Lbm46, 47, 49) and γ-hemolytic for *Aerococcus sp.* Lbm3, *Enterococcus sp.* Lbm50 and *Weisella sp.* Lbm22. LAB producing α- or γ-hemolysis were considered non-hemolytic **(Rather *et al.*, 2015; Maragkoudakis *et al.*, 2009)**. All LAB showed no gelatinase activity. In most cases, probiotic bacteria candidates demonstrated no presence of this virulence factors (**Angmo *et al.*, 2016**; **Leite *et al.*, 2015**). Such no presence of this virulence factors indicates the safety in the use of such LAB as probiotic cultures **(Monteagudo-Mera *et al.*, 2011; FAO/WHO, 2002)**.

All LAB were found to resist to vancomycin and sulfadiazin, whereas *Enterococcus spp.* resisted to oxytetracyclin and ceftiofur, except *Enterococcus sp.* Lbm50 resisted to only ceftiofur. Inversely, they were all sensitive for ampicillin, amoxicillin, tylosin, florfinicol, erythromycin and gentamicin. Consequently, resistance coefficient was ranging from 0.28 to 0.10 and sensitivity coefficient was ranging 0.72 to 0.90. Resistance for tetracycline, erythromycin, chloramphenicol, streptomycin and vancomycin is clearly reviewed **(Quinto *et al.*, 2014)**. These results are consistent with many previous tests reporting a vancomycin resistance (**Angmo *et al.*, 2016; Leite *et al.*, 2015; Dubey and Jeevaratnam, 2015**), and were weakly antibiotic resistant to response at the requirement to avoid transfer genes inside the gut microbiota **(Mathur & Singh, 2005)** and thus were safe to use as potential probiotics **(Vera-Pingitore *et al.*, 2016)**.

Probiotic and technological potential

*Enterococcus sp.*Lbm49, *Aerococcus sp.* Lbm19, *Aerococcus sp.* Lbm18, *Enterococcus sp.* Lbm46, *Aerococcus sp.* Lbm3 and *Enterococcus sp.* Lbm50 isolates showed the highest potential probiotic score (up to 55%).

CONCLUSION

The objective of the current work was to study the potential probiotic of 5 *Aerococcus spp.*, 4 *Enterococcus spp.*, and 1 *Weisella sp.* isolated from an Algerian traditional dried and salted meat (*Guedid*). The obtained results showed that the ten assessed LAB cumulate probiotic potential scores between (0-1) especially, *Enterococcus sp.* Lbm49, *Aerococcus sp.* Lbm19, *Aerococcus sp.* Lbm18, *Enterococcus sp.* Lbm46, *Aerococcus sp.* Lbm3 and *Enterococcus sp.* Lbm50 that displayed the highest potential probiotic score (up to 55%). In addition, *Aerococcus sp.* Lbm18, *Enterococcus sp.* Lbm46, and *Enterococcus sp.* Lbm49, revealed the best technological properties. Finally, those promising candidates should be better characterized throughout a molecular assessment; and to be tested thorough *ex vivo* and *in vivo* to confirming their human or veterinary utilization as probiotics.

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