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Probiotic Properties and Physicochemical Potential of Lactic Acid Bacteria Isolated from Moroccan Table Olives

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HIGHLIGHTS

- *Lactococcus lactis* 9 and *Leuconostoc mesenteroides* 62 showed a good antibacterial and antioxidant activity.
- *Weissella paramedenteoides* 9 and *Enterococcus faecium* 168 were considered the most acidifying strains.
- *Lactiplantibacillus plantarum* 11 showed high antibacterial, antioxidant, and biosurfactant activity.

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Acronyms and abbreviations
CFU=Colony Forming Unit
DPPH=1,1-Diphenyl-2-Picrylhydrazyl
EI=Emulsification Index
LAB=Lactic Acid Bacteria
MALDI-TOF MS=Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometer
MRS=deMan Rogosa and Sharp

ABSTRACT

Background: Lactic Acid Bacteria are a group of Gram-positive bacteria which are widely used in the food industry as organic ferments called starter cultures. In this study, *Enterococcus faecium*, *Leuconostoc mesenteroides*, *Lactococcus lactis*, *Weissella paramesenteroides*, and *Lactiplantibacillus plantarum* isolated from Moroccan table olives were tested for their acquisition of probiotic and technological properties.

Methods: The 5 strains were previously isolated from table olives in 2017. Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometer (MALDI-TOF MS) and intergenic space sequencing were used for molecular identification. Following that, probiotic and physicochemical properties were evaluated, including growth at different pH levels (2, 3, and 10), temperatures (7, 45, and 50 °C), and sodium chloride (NaCl) concentrations (6.5 and 18% m/v). Antibacterial activity was tested out against Gram-positive and Gram-negatives bacteria.

Results: The 5 strains (*E. faecium* 168, *L. lactis* 9, *L. plantarum* 11, *L. mesenteroides* 62, and *W. paramesenteroides* 36) showed an ability to grow at low temperatures (7 °C). *L. lactis* 9 and *L. plantarum* 11 showed higher acid (pH 2) and salt (18% NaCl) tolerances. In addition, *L. lactis* 9 and *L. plantarum* 11 exhibited the highest level of free radical scavenging activity after 48 h of incubation, respectively). *L. plantarum* 11 and *E. faecium* 168 showed the highest antibacterial capacity. However, *E. faecium* 168 and *W. paramesenteroides* 36 demonstrated better and more rapid acid production capabilities.

Conclusion: *L. plantarum* 11, *E. faecium* 168, and *W. paramesenteroides* 36 were considered the best candidates as probiotic cultures for further *in vivo* studies and functional food product development.

Introduction

Lactic Acid Bacteria (LAB) are isolated from various food matrices. The most successful and competitive isolates are used as probiotics (Maldonado et al., 2018). They are defined as "live microorganisms that confer

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beneficial health effects on the host when consumed in adequate amounts" (FAO/WHO, 2001). They can inhibit the growth of pathogenic organisms through different mechanisms, such as adhesion to epithelial cells and modulation of the immune system through the secretion of antimicrobial compounds like bacteriocins (Wan et al., 2016).

Bacteriocins are small proteins synthesized by the ribosomes of LAB. They act on pathogenic and food spoilage bacteria and consider them fundamentally safe food additives after ingestion through the gastrointestinal system (Sari et al., 2018), which proves the capacity of LAB for the bio-conservation of food by their use as a starter culture in the process of controlled fermentation (Anagnostopoulos et al., 2020). Therefore, in recent years, the isolation and characterization of LAB have generated powerful research interests. They had isolated it from different foods and traditional fermented products (Argyri et al., 2020).

Probiotics are among the most promising alternatives to antibiotics. Their application is widely accepted today in the food industry as well as in aquaculture. The effects of probiotics are strain-specific. They can, for example, improve health performance by maintaining the intestinal microbial balance by inhibiting pathogens, strengthening the intestinal barrier, and modulating the immune system (Wan et al., 2016). Many strains have been described as probiotics. These are often LAB or yeasts introduced into the diet in the form of fermented milk products or food supplements. Probiotic lactic acid strains mainly belong to the genera *Lactobacillus*, *Bifidobacterium*, and *Enterococcus*, as well as the genus *Saccharomyces* for yeasts.

Table olives (*Olea europaea* L.) are considered a very important fermented food and a nutritionally complete food contain water, lipids, carbohydrates, proteins, fibers, phenols, vitamins, organic acids, and mineral elements. Due to their remarkable constitution of phytonutrients, antioxidants, and anti-inflammatories, as well as copper, iron, dietary fiber, and vitamin E, research shows that a regular diet of olives is linked to a reduced risk of cardiovascular disease and blood pressure. Antioxidants are important because of their antioxidant properties; they contribute to the organoleptic properties and determine the shelf life of foods. In olives, phenolic acids, phenolic alcohols, and flavonoids are the most abundant and have strong capacities to reduce the risk factors of cardiovascular diseases. Table olives are the most used as starter cultures because of their nutritional quality and their content of probiotic microorganisms. They can ensure better control of the fermentation process by inhibiting the growth of undesirable bacteria and enriching the finished product with probiotic microorganisms (Benítez-Cabello et al., 2019).

In this work, 5 isolates (*Enterococcus faecium* 168, *Lactiplantibacillus plantarum* 11, *Lactococcus lactis* 9, *Weissella paramesenteroides* 36, and *Leuconostoc mesenteroides* 62) were tested for their acquisition of technological and probiotic properties.

Materials and methods

Isolation of bacterial strains

Five LAB were isolated from table olives in Morocco, and results were published in a previous study (El Issaoui et al., 2017). Samples of green, black, red, pitted, and spicy olives were collected in 2017. The isolation was carried out on deMan Rogosa and Sharp (MRS; Biokar Diagnostics, Beauvais, France) agar, and dishes were incubated at 30 °C for 24-48 h.

Identification of strains

The isolates were identified by sequencing the 16S-23S rDNA intergenic space. Strains with an identification percentage less than or equal to 97% were identified by Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometer (MALDI-TOF MS) (Bruker Daltonic, Germany) (El Issaoui et al., 2020). For identification, the strain was sub-cultured twice on MRS agar and incubated at 37 °C before MALDI-TOF MS analysis. The pellet and the extract were prepared according to the method described by Wieme et al. (2012). On a stainless steel MALDI-TOF MS target plate, 1 µl of cell extract was spotted in duplicate. The extract was then overlaid with 1 µl of matrix solution consisting of 10 mg of α-cyano-4-hydroxycinnamic acid (*α*-CHCA) dissolved in 1 ml of acetonitrile:trifluoroacetic acid:Milli-Q water (50:2.5:47.5)% solvent. Before analysis, the MS was previously calibrated using the bacterial test standard (BrukerDaltonics, Germany). The sample was analyzed using the BrukerMicroflexTM LT/SH (flexControl version 3.4) device. For identification, the generated mass spectra were compared using the Bruker MBT Compass Explorer software (version 4.1) with the reference spectra present in the Bruker identification database (BDAL, DB-7,854 MSP) and in the internal reference database LM-UGent. Final identification scores were expressed using Logscore values. According to the manufacturer, Logscores <1.70 indicate "no organism identification possible," while Logscores of 1.70 to 2.299 and >2.3 provide acceptable identification at the genus and species levels, respectively.

Growth at different temperatures, pH, and salt levels

The growth of isolates at different temperatures was

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determined according to the method described by De Lourdes Pérez-Chabela et al. (2008) with some modifications. Each strain was cultured in 5 ml of 1% MRS broth and then incubated at temperatures of 7, 45, and 50 °C for 24 to 72 h. Under the same incubation conditions, the growth of strains was also tested in the MRS broth with pH adjusted to 2, 3, and 10 using hydrochloric acid (HCl) and a solution of sodium hydroxide (NaOH) at 0.1 N, respectively. Multiplication was revealed by the formation of a pellet. Similarly, the growth of strains was tested at different NaCl concentrations (6.5% and 18% (m/v)). Salt tolerance was revealed by the formation of a pellet in the medium.

Acidifying activity

The acidifying activity of the strains was tested according to the method adopted by Adesulu-Dahunsi et al. (2018) with minor modifications. Each isolate's culture was inoculated for 18 h in 2% MRS broth. The incubation was carried out for 3 days at 30 °C. Samples were taken at regular time intervals, and pH was measured using a pH meter (PHSJ-3F, Spain). The variation in pH (ΔpH) was calculated between the initial pH at time 0 and the pH at time t. The strain is considered fast, medium, or slow acidifying, when the ΔpH reaches a value of 0.4 U after 3, 3 to 5, and 5 h of incubation, respectively.

Antibacterial activity

The antibacterial activity of isolates was tested using the good diffusion method (Khay et al., 2011). First, a dish with Mueller-Hinton Agar (MHA) medium (Biokar Diagnostics, Beauvais, France) was covered with a semi-solid Brain Heart Infusion (BHI; HiMedia Laboratories, Mumbai, India), inoculated with the indicator strain, and 4 wells of 6 mm were formed using the end of a sterile Pasteur pipette. Three wells were filled with 100 µl of Free Cell Supernatant (FCS). The fourth was filled with 100 µl of sterile distilled water as a negative control. The plates were pre-incubated at 4 °C for 4 h to allow uniform diffusion in the agar, and then they were incubated at 30 °C for 24 h. The antibacterial activity consisted of the inhibition zone around the wells. The antibacterial activity of the selected isolates was tested against 7 Gram-positive strains (*Listeria monocytogenes* CECT 4032, *Staphylococcus aureus* ATCC 25983, *S. aureus* ATCC 43300, *S. aureus* ATCC 3920, *S. epidermidis* ATCC 12228, *S. aureus* MBLA, *E. feacium* CECT 410) and 6 Gram-negative strains (*Escherichia coli* MBLA K12, *Klebsiella pneumoniae* ATCC 13883, *Proteus mirabilis* (Institute of Hygiene Rabat, Morocco), *Salmonella typhimurium* ATCC 14028, *E. coli* ATTC 87739, *E. coli* ATTC 25922). The determination of the bacteriocins genes (*entA, entB, entP, AS-48, ent1, 071 A and B, ent L50A and B, plnA, plnB, plnC, plnD, plnef, plnI, plnJ, plnK, plnG, plnN, plnnC8*, plnS*, plnW*, leucocinA, leucocin Ata33, leucocin Bta11a, leucocinK, mesenterocin 105B, mesenterocin Y105*) was carried out by Polymerase Chain Reaction (PCR) using the total genomic DNA of the lactic strains (El Issaoui et al., 2020).

Antioxidant activity

The free radical scavenging activity was evaluated using 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) to estimate the antioxidant activity of strains, according to method described by Son and Lewis (2002) with some modifications. In hemolysis tubes, 1 ml of a culture of 24, 48, or 72 h of incubation (10^8 Colony Forming Unit (CFU)/ ml) was centrifuged at 8,000 g for 20 min at 4 °C. The pellet was mixed with 2 ml of 95% ethanol. Then, a volume of 1 ml of a solution of DPPH (0.004%, m/v), in 100 ml of 95% ethanol was added to the ethanolic extract obtained. The tubes were incubated for 30 min in a dark room at room temperature. The absorbance of each sample was measured at 517 nm using a UV-visible spectrophotometer (Rayleigh UV-1,800, China). The antioxidant activity was expressed as a percentage of the DPPH activity according to the following formula:

DPPH inhibition activity (%)=(Ac–Ae)/Ac]×100

Ac is absorbance of DPPH; Ae is absorbance of sample.

Strain growth kinetics

The isolates were cultured in a mineral medium M (containing g/L: K_2HPO_4, 1; $MgSO_4$, 0.2; NH_4NO_3, 1; $CaCl_2$, 0.02; $FeCl_3$, 0.05; KH_2PO_4, 1; 1 L distilled water), added of crude oil. The sample was diluted from 10^{-1} to 10^{-7}, then 1 ml of each dilution was spread on the surface of the MRS agar. The viability of the strains was determined by counting the colonies during 4 days of incubation at 30 °C.

Determination of the Emulsification Index (EI)

The EI makes it possible to assess the ability of strains to emulsify a hydrophobic phase in a hydrophilic phase (the culture medium). Three ml of the fermentation culture was centrifuged at 10,000 g for 15 min. The supernatant was mixed with 3 ml of refined palm oil in a sterile tube. The solution was stirred vigorously for 3 min. The uninoculated broth was used as a control. Each tube was left to stand for 24 h at room temperature, and the emulsifying activity was measured by calculating EI (Bodour et al., 2004) according to the following relationship:

E24%=(he/ht)×100

he is the height of the emulsion, and ht is the total height of the mixture.

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Oil displacement test

To determine the surface activity, 1 ml of the bacterial culture was centrifuged at 8,000 g for 10 min. Twenty µl of crude oil was deposited on the surface of 20 ml of distilled water in a petri dish. Afterwards, 20 µl of the supernatant of the strain to be tested were placed in the center, and the diameter of the clear zone was determined. Negative control was maintained with MRS medium (Sari et al., 2014).

Susceptibility to antibiotics

The phenotypic and genotypic susceptibilities of the 5 isolates to antibiotics were tested in a previous study, according to the method described by Muñoz et al. (2014). The antibiotics (amoxicillin, ampicillin, ciprofloxacin, clindamycin, gentamicin, kanamycin, streptomycin, erythromycin, teicoplanin, vancomycin, chloramphenicol, tetracycline, trimethoprim (Sigma, Spain)) and the primers (*tetW, tetQ, tetS, tetO, ermA, ermB, ermC, ermF, ereA, ereB, bla, dfrA, dfrD, aac (6 ')-Ie-aph (2')-Ia, aph (2')-Ib, aph (2')-Ic, aph (2')-Id, aph(3 ')-IIIa, ant (4')-Ia*) used to detect the presence of resistance genes have been previously published (El Issaoui et al., 2020).

Gelatinase test

In 5 ml of solidified gelatin, a suspect colony was inoculated by central pricking. After incubation at 37 °C for 24 h, liquefaction of the medium was obtained if the bacterium was gelatinase positive, if the gelatin had not been hydrolyzed, the bacterium was therefore said to be gelatinase negative.

Statistical analysis

All analyses were carried out in triplicate, and the data was performed using Unidirectional Analysis of Variance (ANOVA) using statistical software (IBM SPSS software version 16.0) in order to assess the differences between the means of the treatments. The results were considered to be statistically different at $p<0.05$.

Results

Isolation and identification

The 5 strains were isolated from table olives marketed in Morocco. The results of identification by 16S-23S rDNA intergenic space sequencing were confirmed by MALDI-TOF MS identification for the lactic isolates analyzed, except for the BL9 strain, whose identification by 16S-23S rDNA intergenic space sequencing revealed a *W. paramesenteroides* species with a percentage identity less than 97%. However, the result obtained during identification by MALDI-TOF MS showed a profile of *L. lactis* with a Logscore of 2.480.

Growth at different temperatures, pH, and salt levels

L. plantarum 11 and *W. paramesenteroides* 36 exhibited a similar profile when cultured under defined conditions of temperature, pH, and salt concentration. Both strains showed the ability to grow at a temperature of 7 to 50 °C as well as in an acidic environment (pH 3). Except for growth at a temperature above 45 °C, the same profile was detected in *L. lactis* 9. Both *L. mesenteroides* 62 and *E. faecium* 168 showed the ability to grow at a temperature above 45 °C. However, they were unable to grow under acidic and highly saline conditions (pH 2 and 18% NaCl (m/v); Table 1).

Acidifying activity

Strains were classified into 2 groups according to their rate of acidification in the medium. *L. plantarum* 11, *L. lactis* 9, and *E. faecium* 168 showed moderate ability to lower pH and were classified moderate to low acidifying strains, whose ΔpH recorded during the first 6 h were between 0.1 and 0.7. *L. mesenteroides* 62 and *W. paramesenteroides* 36 were considered the slowest, with ΔpH of 0.02 and 0.07, respectively. However, acid production by these isolates increased later (after 12 h) which leads to a significant decrease ($p<0.05$) in pH from the accumulated acids.

Indeed, we obtained a high ΔpH after 72 h of incubation. *E. faecium* 168 was marked as the most acidifying strain with a ΔpH of 2.8, followed by *W. paramesenteroides* 36 (ΔpH 2.56).

Antibacterial activity

All 5 strains showed antagonistic activity against at least 6 indicator strains. The greatest antibacterial activity was detected against *L. monocytogenes* CECT 4032 and *Staphylococcus* spp., as shown by the results of *L. mesenteroides* 62 and *W. paramesenteroides* 36. The greatest zones of inhibition against *L. monocytogenes* CECT 4032 were obtained by *L. plantarum* 11 and *L. lactis* 9 (>20 mm). Gram-negative indicator strains were the least sensitive, with recorded inhibition diameters generally less than 10 mm. The 5 species showed the presence of several genes coding for bacteriocin production. *L. plantarum* 11 was the most dominant, with loci coding for one enterocin and 8 plantaricins.

Antioxidant activity

The results of radical reduction capacity, measured for the 5 cell fractions after 24, 48, and 72 h of incubation,

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are presented in Table 2. The inhibition activity was between 48.88% and 77% during the 72 h of incubation. The strongest antioxidant activity was recorded by *L. mesenteroides* 62, *L. lactis* 9, and *L. plantarum* 11, with values significantly greater than 75% (p=0.021). The production of antioxidant molecules was maximal during the first 2 days of incubation. It was maximal after 24 h of incubation, as is the case for *W. paramesenteroides* 36 and *L. mesenteroides* 62, and after 48 h for *L. plantarum* 11 and *L. lactis* 9, which recorded the highest antioxidant capacities with 76.66% and 77%, respectively, as a percentage of DPPH inhibition.

Biosurfactant activity

The growth of isolates in the mineral medium was reduced compared to their growth in MRS medium (Figure 1). For *E. faecium* 168, a significant reduction (p<0.018) in microbial biomass (3 log CFU/ml) was obtained.

In the case of *L. mesenteroides* 62, *L. lactis* 9, and *W. paramesenteroides* 36 isolates, the growth was reduced by more than 1.5 log CFU/ml. *L. plantarum* 11 showed the highest adaptability to medium M, whose growth was approximately 6.98 log CFU/ml recorded in medium M compared to 7.22 log CFU/ml recorded on MRS. *L. lactis* 9, *L. plantarum* 11, and *E. faecium* 168 showed a significant (p<0.05) capacity to emulsify the crude oil contained in the culture medium, of which the EI obtained for the 4 strains corresponds to 53.33%, followed by *W. paramesenteroides* 36 with an EI of 50%. Maximal EI values were obtained after 24 h of incubation for *L. plantarum* 11 and after 72 h for *L. lactis* 9.

According to the results, maximal EI values were generally proportional to the microbial concentration. Except for *E. faecium* 168, the strains *L. lactis* 9, *L. plantarum* 11, *W. paramesenteroides* 36, and *L. mesenteroides* 62 showed maximal values of E (24%) after an incubation time proportional to their maximal growth. The production of biosurfactants was at its peak during the exponential phase (Figure 1), but EI values dropped dramatically during the decline phase of growth.

The results obtained by the oil displacement test showed corroboration with those obtained by the emulsion test (E24%), whose largest displacement diameters were recorded by *L. plantarum* 11 as sown in Table 3.

Susceptibility to antibiotics

The results of phenotypic and genotypic susceptibility were previously published. The Minimum Inhibitory Concentrations (MIC) obtained was compared with the microbiological guidelines defined by Clinical and Laboratory Standards Institute (CLSI). For the 5 species studied, high resistance was detected against erythromycin, vancomycin, tetracycline, gentamycin, and trimethoprim. *L. plantarum* 11, *L. mesenteroides* 62, *L. lactis* 9, and *W. paramesenteroides* 36 showed a sensitive profile against ampicillin, clindamycin, and teicoplanin. However, no antibiotic resistance genes were detected in any of the strains except *L. plantarum* 11.

Gelatinase test

Gelatinase production was absent in *L. plantarum* 11, *E. faecium* 168, *L. lactis* 9, and *W. paramesenteroides* 36. On the other hand, *L. mesenteroides* 62 showed liquefaction of the culture medium.

Table 1: Growth of strains under different temperature, pH, and salt conditions

		***Enterococcus faecium* 168**	***Leuconostoc Mesenteroides* 62**	***Lactococcus Lactic* 9**	***Weissella paramesenteroides* 36**	***Lactiplantibacillus Plantarum* 11**
NaCl (%)	6.5%	+	+/-	+	+	+
	18%	-	-	+	+	+
T (°C)	7	+	+	+	+/-	+
	45	+	+	-	+	+
	50	+/-	+	+/-	-	+/-
pH	2	+/-	-	+/-	+	-/+
	3	+	+	+	+/-	+
	10	+/-	+/-	-	+/-	-

+: Growth; +/-: Low growth; -: Absence

Table 2: Antioxidant activity in cell pellet (%) recorded for the isolates tested

	Incubation time (h)		
	24	48	72
Enterococcus faecium 168	62.19	61.12	51.97
Leuconostoc mesenteroides 62	75.61	55.48	64.11
Lactococcus lactis 9	51.51	77	48.88
L.actiplantibacillus plantarum 11	50.33	76.66	51.75
Weissella paramesenteroides 36	70.30	59.37	65.38
Ascorbic acid	99.99	99.98	99.99

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Table 3: Oil displacement diameters obtained by the 5 strains

Strain	Displacement diameter of oil (mm)
Enterococcus faecium 168	20.3±0.09
Leuconostoc mesenteroides 62	17.4±0.73
Lactococcus lactis 9	19.3±0.75
Lactiplantibacillus plantarum 11	21.1±0.26
Weissella paramesenteroides 36	17.0±0.20

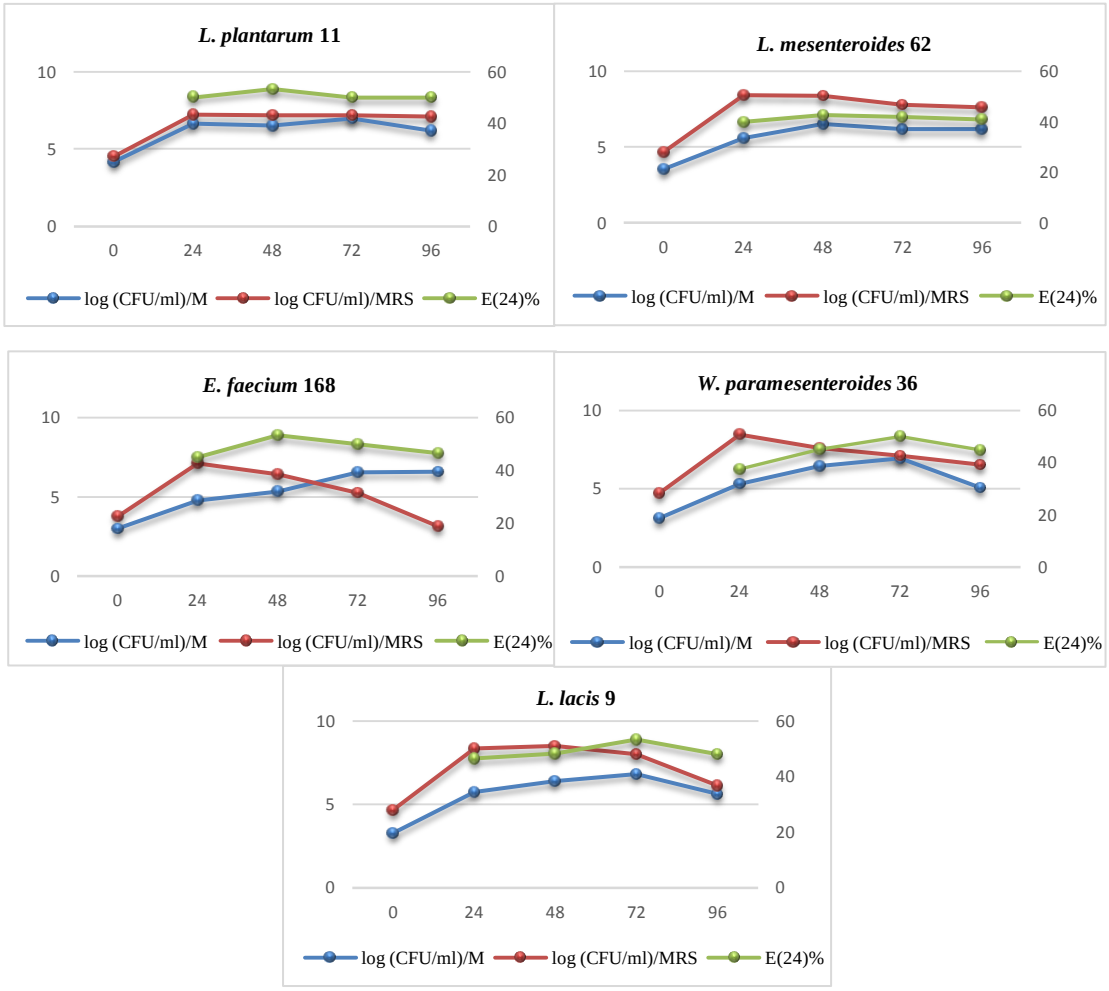

Figure 1: Graphical presentation of Emulsion Index (EI) and the growth kinetics of isolates in the mineral (log (Colony Forming Unit (CFU)/mL/M)) and the deMan Rogosa and Sharp (MRS) medium (log (CFU/mL/MRS)) as a function of fermentation time. (*E. faecium*: *Enterococcus faecium*; *L. mesenteroides: Leuconostoc mesenteroides*; *L. lactis: Lactococcus lactis*; *W. paramesenteroides: Weissella paramesenteroides*; and *L. plantarum: Lactiplantibacillus plantarum*)

Discussion

In this study, 5 lactic acid strains were isolated from table olives in Morocco. Results showed minor differences between phenotypic and genotypic identification, which could be attributed to the experimental conditions used. In a study by Gad et al. (2014), they observed some false positive results after comparison between phenotypic characterization and molecular identification, and they attributed this difference to the experimental conditions

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used in isolation and identification. Similar findings have also been reported in a comparative study by Strejcek et al. (2018). They found that only 35% of the bacterial isolates identified by MALDI-TOF MS coincided with those identified by 16S rRNA gene sequencing analysis. They attribute this discrepancy to the insufficient coverage of bacterial species in the databases. In general, the MALDI-TOF MS method is considered 100% reliable for molecular identification of microorganisms when compared to 16S-23S intergenic space sequencing (Singhal et al., 2015).

Following the selection process, the 5 strains presented interesting probiotic and technological properties. Indeed, the difference in the acidifying capacity of lactic strains has been linked to their ability to degrade carbon and nitrogen compounds in the medium and to assimilate the nutrients essential for growth (Fguiri et al., 2016). Strains with rapid acidifying activity are widely used as starter cultures in food fermentation processes; however, those considered less rapid can be used as auxiliary crops depending on their other important properties. *Streptococcus thermophilus* SJRP107 defined as starter culture for Mozzarella cheese production, showed fast acidification (0.4 U in 2-4 h), and high production of organic acids (Silva et al., 2020).

L. lactis 9, *L. mesenteroides* 62, *L. plantarum* 11, and *W. paramesenteroides* 36 showed a slower acidifying capacity during the first h of fermentation. However, after 24 h of incubation, high rates of acidification were observed. The same observations were noted by Perin et al. (2017), who demonstrated that high levels of acidification produced by enterococci (isolated from raw goat milk in Italy) were obtained only after 24 h of incubation. Also, various previous studies have reported that most *Enterococcus* and *Lactococcus* strains generally produce little acid at the start of fermentation. Hassaîne et al. (2007) reported that *Enterococcus durans*, isolated from camel milk, was unable to reduce pH to fewer than 5.0 after 24 h of fermentation. Furthermore, Malek et al. (2012) determined that *Enterococcus* is poor acidifying bacteria after analyzing 35 strains of *E. faecium* isolated from Egyptian cheeses.

L. lactis 9, *L. mesenteroides* 62, and also *W. paramesenteroides* 36 showed the highest acidifying capacities, with values equal to or greater than 2 units being obtained after 72 h. On the other hand, the acidification developed by *L. plantarum* 11 was significantly lower, especially compared to *L. mesenteroides* 62 and *W. paramesenteroides* 36, which was in agreement with the study carried out by Georgieva et al. (2009). They reported the low acidifying ability of probiotic *L. plantarum* isolated from Bulgarian cheeses. Contrary to our observations, another study presented that 2 strains of *L. plantarum*, isolated from Nigerian traditional fermented cereal gruel, were characterized by a high acidification capacity throughout the incubation period compared to strains belonging to the genus *Weissella* (Adesulu-Dahunsi et al., 2018). Regarding *E. faecium*, it presented the highest levels of acidification. Similar to our results, some studies were able to select, from a fermented cereal mixture, two lactic strains possessing a significant acidifying property, belonging to the genera *Enterococcus* and *Leuconostoc*, with a rapid and high acidifying activity, respectively, for the two genera (Deza et al., 2018). Another study showed that the acidifying activity of several species of *Enterococcus* isolated from raw milk and traditional dairy products were quite low (Mohammed et al., 2009).

Antibacterial activity is a property characterizing several LAB and an alternative to reducing microbial contamination (Arqués et al., 2015). LAB with high antimicrobial activities can be used as bio-preservatives (Delcarlo et al., 2019). In this study, high antibacterial activity was recorded against Gram-positive bacteria (*L. monocytogenes* CECT 4032 and *S. aureus* MBLA). However, *E. coli* 87739 was the least inhibited. The same results were obtained by Stanojević-Nikolić et al. (2016). They showed that the strongest inhibitory effect of the lactic acid produced was against Gram-positive bacteria. Lactic acid or other organic acids synthesized by LAB can penetrate the cell membrane, affecting its function, acidifying the cytoplasm, and inhibiting acid-sensitive enzymes.

The greatest zones of inhibition were obtained by *L. plantarum* 11 and *L. lactis* 9. Similar results were obtained by Adeyemo et al. (2018) and Qian et al. (2020), who demonstrated that *L. plantarum* showed the highest zones of inhibition in the presence of Gram-positive and Gram-negative bacteria from cereal, legumes, and yogurt. On the one hand, Akbar et al. (2019) reported a potential antimicrobial effect of *L. lactis* subsp. *lactis* isolated from fermented milk products against various pathogens tested, and the highest antibacterial activity was detected against *L. monocytogenes* and *S. aureus*. In terms of *E. faecium*, Kivanç et al. (2016) demonstrated that the antibacterial activity of *E. faecium* was strain-dependent, with all strains tested being able to inhibit the indicator strain *L. monocytogenes*; however, only a few were able to prevent the growth of certain target bacteria such as *S. aureus* and *Bacillus subtilis*.

The DPPH free radical scavenging ability of the cell fractions of *L. plantarum* 11, *L. mesenteroides* 62, and *L. lactis* 9 exhibited the highest antioxidant activities. *Lactobacillus* genus is most widely described as possessing strong antioxidant activity. Ding et al. (2017) reported that *L. delbrueckii* subsp. *bulgaricus* F17 isolated from spontaneously fermented yak milk exhibited the greatest probiotic potential due to high antioxidant activity. Addi-

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tionally, *L. curvatus* SR6 and *L. paracasei* SR10-1, isolated from traditionally fermented sour meat products in China, showed strong DPPH and hydroxyl radical (OH) scavenging activities (Zhang et al., 2017). Uugantsetseg and Batjargal (2014) showed that a strain of *L. plantarum*, isolated from airag, recorded the highest antioxidant activity during a period of 72 h of fermentation, unlike *L. lactis*, which presented a lower percentage of inhibition. In addition, in recent research by Lee and Kim (2019), *L. mesenteroides* showed potent antioxidant activity with a DPPH free radical scavenging capacity of 82%. In another study by Virtanen et al. (2007), all LAB showed 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity, however, *Lactobacillus* and *Leuconostoc* genera showed higher antioxidant activity than *Lactococcus*.

According to the results, 3 different lactic species, *L. lactis*, *L. plantarum*, and *E. faecium*, showed a significant capacity to produce biosurfactants, with an EI of 53.33%. These results were confirmed by oil displacement tests, of which the 3 previous species showed the largest displacement diameters. Comparing our results with those obtained by Kaur et al. (2015), ours are more important. They discovered that the displacement diameters of 15 *Lactobacillus* species ranged between 0.2 and 1.5 cm, whereas in the current study, the displacement diameter of *L. plantarum* was greater than 2 cm.

The production of biosurfactants by the tested strains was closely related to the culture. It started during the exponential phase and continued until the end of the stationary phase of growth (48 to 72 h of incubation). Djerbaoui (2011) showed that the production of biosurfactants by a strain of *Pseudomonas aeruginosa* was associated with an increase in biomass. Similarly, in a recent study by Vallejo et al. (2021), the highest level of biosurfactant production by 3 *Lactobacillus* isolates from lactoserum was in the exponential phase.

The carbon source is also a very important factor influencing the production of biosufactants. By comparing the growth of strains in a mineral medium supplemented with oil with their growth in a nutrient medium (MRS), it can be seen that strains showed significant growth in the MRS medium compared to the medium based on crude oil (Hentati, 2018).

The need to assess the safety aspects of strains is recommended by the Food and Agriculture Organization and World Health Organization (FAO/WHO, 2001). Any probiotic strain intended for human consumption should not carry antibiotic resistance genes, due to the associated risk of horizontal gene transfer to opportunistic pathogens. Probiotic strains intended for human consumption must not carry antibiotic resistance genes. Based on the characteristics studied, the 5 strains tested can be considered safe for use as beneficial cultures in food industry. Except for *L. mesenteroides* 62, no strain showed the presence of gelatinase. *L. mesenteroides* 62 can be considered virulent due to the risk of causing disruption of the mucoid lining and, therefore, the formation of infection pathways. Sieladie et al. (2011) isolate, from raw cow milk, 15 lactobacilli that are gelatinase-negative and may be considered safe following these activities. In addition, the antibiotic resistance was limited to the certain antibiotics (tetracycline, erythromycin, gentamycin, and vancomycin), except for *L. lactis*, which showed very high multidrug resistance (El Issaoui et al., 2020).

Conclusion

Based on the probiotic and technological characteristics, as well as the safety profile identified in this study, *E. faecium* 168 showed significant antibacterial, antioxidant, and rapid acidifying activity. Although *Enterococcus* species are often found in fermented foods, they are not used directly as starter cultures, in part because they are still used as fecal contamination indicators and also because some strains can transfer antibiotic resistance genes. *W. paramesenteroides* 36 showed moderate antibacterial activity and strong acidification activity with a medium rate of acidification. The production of antioxidant and biosurfactant molecules by this strain was significant. *L. lactis* 9 and *L. mesenteroides* 62 showed strong acidifying activity and high antibiotic resistance, as well as considerable antibacterial and biosurfactant activity. In the case of *L. plantarum* 11, the better performances were recorded, with a significant inhibitory activity due to the production of antibacterial molecules (bacteriocins), a remarkable antioxidant and biosurfactant activity, and a sensitive profile against antibiotics.

Author contributions

K.E.I. and E.O.K. designed the study; K.E.I. and A.W. conducted the experimental work; K.E.I., E.O.K., J.A., N.S.S. analyzed the data; and K.E.I. wrote the manuscript. All the authors read and approved the manuscript.

Conflicts of interest

The authors declare that they have no competing interests.

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