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VIRULENCE FACTORS, ANTIBIOTIC RESISTANCE, AND ANTIMICROBIAL ACTIVITY OF *ENTEROCOCCUS* SPP. ISOLATED FROM DIFFERENT SOURCES IN ALGERIA

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ABSTRACT

This study aimed to isolate and identify different *Enterococcus* spp. from diverse sources including dromedary raw milk, rhizospheric soil and natural saline water samples and to determine their antimicrobial activities and safety aspects. From these sources, distinct strains of *E. faecium, E. durans, E. hirae,* and *E. gallinarum* species were detected. No virulence factors were observed for six *Enterococcus* strains while none of the tested Enterococci harboured the genes encoding cytolysin and aggregation substance. All tested strains showed a high sensitivity to many antibiotics, particularly vancomycin, and exhibited a remarkable antagonist activity toward *Salmonella typhimurium, Fusarium graminearum* and *Penicillium expansum*. PCR testing of enterocins revealed the co-presence of *ent*A and *ent*B among all tested Enterococci. No hemolytic reaction was also detected in Enterococci isolates, except for six strains. Based on these findings, potential candidates for use as biocontrol agents or probiotic starters were selected for further studies.

Keywords: Enterococci, virulence factors, antibiotic resistance, antimicrobial activity, enterocin

INTRODUCTION

Enterococci are commensal inhabitants of the animal gastrointestinal tracts that commonly isolated from different environmental habitats such as animal and human feces, soil, plants, and water **(Byappanahalli *et al.*, 2012)**. *Enterococcus* species often occur in various food sources, in particular those of animal origin, such as dairy products. Recently, they are of increasing interest due to their potential techno-functional roles such as their roles in the preparation of different types of cheeses and the development of their sensory characteristics **(Hanchi *et al.*, 2018; Schirru *et al.*, 2012)**. In Algeria, raw dromedary milk is an important part of the human diet traditionally consumed by nomadic peoples for its nutritional value and medicinal properties **(Hassaïne *et al.*, 2007; Merzouk *et al.*, 2013)**. These potential positive roles are due to the presence of different strains of lactic acids bacteria (LAB) including *Enterococcus* species in dromedary milk yet little information is available on the LAB microflora of dromedary milk.
Enterococci are also frequently found in different environmental sources due to their heat resistance and their ability to survive adverse environmental conditions **(Giard *et al.*, 2001; Giraffa, 2002)**. Many bacteria have been found in rhizospheres associated with both plants and soil, and these bacteria have coevolved with phytopathogenic bacteria and fungi **(Fhoula *et al.*, 2013; Garbeva *et al.*, 2004)**. Nevertheless, reports on Enterococci isolation from rhizospheric soil and their safety aspects remain scarce. In addition, *Enterococcus* species can be isolated from different aquatic environments and coastal marine habitats **(Erdem *et al.*, 2007; Ferguson *et al.*, 2005; Moore *et al.*, 2008)**, due to their capacity to tolerate high salt concentrations. However, little information is available on the isolation of *Enterococcus* species from saline water in different natural caves of salt mountains in which salt levels are generally higher than in sea and rivers.
In fact, Enterococci are widely distributed in different environmental habitats and their diversity depends on several environmental factors such as the geographical area and potential sources **(Byappanahalli *et al.*, 2012; Moore *et al.*, 2008)**. To the best of our knowledge, the present study is the first research in Algeria that aims to provide new relevant information about the characterization of *Enterococcus* species isolated from dromedary raw milk, rhizospheric soil and natural saline water samples then to evaluate their antimicrobial activities and safety aspects in order to select potential candidates to be used as biocontrol agents or probiotic starters.

MATERIAL AND METHODS

Sampling, isolation and genotypic characterization of Enterococci

In this study, three different sources were used for the isolation of enterococci. Briefly, samples of dromedary raw milk were obtained from four different farms in the Laghouat region of Algeria. Samples were aseptically collected in sterile flasks after elimination of the first jets of milk, transferred to the laboratory in a cool box and kept under refrigeration at 4 °C until analysis. Enterococci were isolated by spreading 0.1 mL of the selected dilutions of samples on Enterococcus Selective Agar (ESA) (SIGMA ALDRICH Co., St. Louis, MO, USA) and typical maroon or dark red colonies were isolated after 48 hours of incubation at 37 °C. Regarding the other samples, rhizospheric soil was obtained from five different agricultural enterprises in the Jijel region of Algeria. Soil samples were taken from the rhizosphere area of olive plants with sterile spoons (15–20 cm depth) and saved into sterile nylon bags. For the natural saline water, samples were obtained from three different natural caves of a salt mountain located in Tadjrouna, Laghouat region of Algeria. Saline water samples were collected in sterile flasks from natural sources and kept in a cool box during the transportation to the laboratory. The enrichment method was then used for enterococci isolation from both rhizospheric soil and saline water samples following the procedure described by **Zamudio-Maya *et al.* (2008)** with some modifications. The samples were inoculated into Brain Heart Infusion (BHI) broth and incubated at 37 °C for 48 hours. Then, 0.1 mL from diluted cultures was spread onto ESA plates and typical colonies were isolated after incubation. The purity of all isolates from milk, soil, and water samples was checked by further spreading on ESA, followed by microscopic examination, Gram staining, catalase and growth tests: esculin hydrolysis, growth at pH 9.6 and in the presence of 6.5% NaCl, as well as growth at 10 °C and 45 °C. Phenotypic identification was also carried out using the miniaturized API 20 Strep tests (BIOMÉRIEUX, Marcy l'Etoile, France).
RAPD-PCR analysis was used for the genotypic discrimination of all isolates and conducted with primer M13 as described elsewhere **(Dertli *et al.*, 2016)**. The ~1.5 kb 16S rRNA gene of each selected strain was amplified with primers AMP_F and AMP_R, as described by **Baker *et al.* (2003),** and PCR analysis was performed under the conditions described previously by **Dertli *et al.* (2016)**. Sequence analysis results were examined with the NCBI database using the BLAST program. The phylogenetic tree was created using neighbour-joining bootstrap method (1000 replicates) and MEGA7 was used for all phylogenetic

analyses, as described by **Tamura *et al.* (2011)**. The 16S rRNA sequences of the isolates were submitted to the Genbank under the accession numbers of MT880256-MT880272.

Screening of Enterococci for virulence determinants and hemolytic activity

The screening of Enterococci for different virulence determinants was carried out by PCR using specific conditions and primers **(Eaton and Gasson, 2001)**. Eight virulence factors were investigated: *cob, agg, efaAfs, efaAfm, cylA, cylB, esp,* and *gelE*. As described above, genomic DNA was isolated from Enterococci strains and used as the DNA template in PCR reactions. PCR and the melting temperature of each primer set were performed according to **Eaton and Gasson (2001)**.
For the detection of hemolysin production, overnight cultures of *Enterococcus* strains were spread onto Blood Agar Base (MERCK, Kenilworth, NJ, USA) plates supplemented with 5% (v/v) of sheep blood as described by **Yoon *et al.* (2008)**. No hemolytic reaction around colonies, a partial hydrolysis or the presence of a clear zone of hydrolysis was interpreted as γ hemolysis, α hemolysis and β hemolysis, respectively, after incubation at 37 °C for 24–48 h.

Antibiotic sensitivity assay

The antibiotic resistance of the Enterococci strains was tested against nine antibiotics using the agar disc diffusion test. The following antibiotics were tested: streptomycin (S, 10 µg), tetracycline (TE, 30 µg), kanamycin (K, 30 µg), ampicillin (AMP, 10 µg), erythromycin (E, 15 µg), penicillin (P, 10 µg), chloramphenicol (C, 30 µg), oxytetracycline (OT, 30 µg), and vancomycin (VA, 30 µg) (OXOID, Basingstoke, UK). For each strain, 1% inoculum from overnight culture was added to BHI agar at 45-50 °C and poured into plates to obtain a final concentration of 10^6–10^7 CFU/mL, and then antibiotic discs were dispensed onto the inoculated agar surface. Inhibition zone diameters around the discs were measured after overnight incubation at 37 °C and expressed as millimeter (mm) following the recommendations of **NCCLS (2004)**.

Detection of antimicrobial activity and enterocin coding genes

To determine the antimicrobial activity of Enterococci against pathogenic bacteria and fungi, the agar well diffusion assay was used. Briefly, the supernatants of isolates were obtained from overnight cultures by centrifugation for 5 min at 14,000 rpm, followed by filtration through a sterile syringe filter (0.22 µm). The filtered supernatants were treated with catalase (MERCK) for 30 min at 25 °C after adjusting the pH to 6.0 with NaOH. In this study, four bacterial strains were evaluated: *Escherichia coli* BC 1402, *Salmonella typhimurium* RSSK 95091, *Staphylococcus aureus* BC 7231, and *Yersinia enterocolitica* ATCC 27729. The indicator strains were obtained from Pamukkale University, Food Engineering Department Culture Collection (PUFECC, WDCM 1019). All pathogens were cultured aerobically in Tryptic Soy Broth (TSB) medium at 37 °C for 24 h. Wells (5 mm diameter) were cut into TSB agar plates containing 10^6 cells per mL of the target pathogen strains and then 50 µL supernatants were added to the wells. After overnight incubation at 37 °C, the inhibition zones were scored and expressed as follows: ++, strong inhibition with detectable clear zones around the wells; +, weak inhibition around the wells; –, no inhibition zone.
For the antifungal assay, 4 filamentous fungal strains were tested: *Fusarium graminearum* MUCL 53452, *Aspergillus parasiticus* CBS 100926, *Aspergillus flavus* NRRL 3251, and *Penicillium expansum* MUCL 29192. For each fungal strain, inoculum was obtained from 7–14 days cultures grown on Potato Dextrose Agar (PDA) at 25 °C, and then 50 µL supernatants were added to the wells cut into PDA plates containing 0.4–5×10^4 fungal spores per mL. After aerobic incubation at 30 °C for 48 h, the inhibition zones around the wells were examined and graded as described above (**Magnusson and Schnurer, 2001**).
The PCR detection of the genes encoding enterocins (A and B) was performed using the conditions described by **Fontana *et al.* (2015)** with the specific enterocin PCR primers **(Du Toit *et al.*, 2000)**. Amplicon sizes of 126 and 162 bp were checked on agarose gels in order to confirm the presence of *ent*A and *ent*B, respectively.

RESULTS

Identification and genotypic characterization of isolates

Among 100 strains randomly selected from different samples, 33 isolates were identified as presumptive Enterococci based on the criteria mentioned above. Further, the isolates were confirmed as members of *Enterococcus* based on phenotypic characterization using biochemical identification tests provided by the API 20 Strep System. Following these processes and genotypic discrimination, identification of the selected isolates revealed the presence of 8 *Enterococcus faecium,* 4 *Enterococcus hirae,* 3 *Enterococcus durans,* and 2 *Enterococcus gallinarum* strains from different sources among these isolates as can be seen in table 1.

Phylogenetic tree in Figure 1 shows the relationship between Enterococci strains based on the MEGA7 alignments of the 16S rRNA genes using the neighbor-joining method. The cluster alignments analysis of the Enterococci showed that *E. faecium, E. hirae* and *E. durans* were close but formed different subgroups and importantly *E. faecium* strains DM 19, KM 1 and KM 2 were separated from the other tested *E. faecium* strains suggesting potential differences in terms of ancestral genetics. Compared with the other strains, *E. gallinarum* strains also formed a different subgroup (Figure 1). Our results revealed the diversity of *Enterococcus* in different environments and these strains were further tested for both antimicrobial activity and safety properties.

Table 1 Selected *Enterococcus* strains for 16S rRNA gene sequencing and their natural sources

Codes	16S rRNA Sequencing	Sources
DM 29	*Enterococcus gallinarum*	Dromedary raw milk
DM 26	*Enterococcus gallinarum*	
DM 20	*Enterococcus durans*	
DM 19	*Enterococcus faecium*	
DM 4	*Enterococcus durans*	
DM 34	*Enterococcus durans*	
KM 8	*Enterococcus hirae*	Natural saline water
KM 6	*Enterococcus hirae*	
KM 2	*Enterococcus faecium*	
KM 1	*Enterococcus faecium*	
KM 14	*Enterococcus faecium*	
KM 12	*Enterococcus hirae*	
KM 11	*Enterococcus hirae*	
RS 31	*Enterococcus faecium*	Rhizospheric soil
RS 29	*Enterococcus faecium*	
RS 4	*Enterococcus faecium*	
RS 25	*Enterococcus faecium*	

Figure 1 Phylogenetic tree based on the MEGA7 alignments of the 16S rRNA genes showing the relationship between Enterococci strains

Virulence determinants and hemolysin production

Screening of the presence of *Enterococcus* virulence factors is considered as one of the main concerns about their safety for use as biocontrol agents or probiotic starters. As shown in table 2, six strains were negative for the tested virulent factors. However, *E. faecium* DM 19 harboured multiple virulence determinants in which five virulence genes encoding *cob, efaAfs, efaAfm, esp,* and *gelE* were presented. Two virulence genes encoding *efaAfm* and *cob* were also detected in the strain *E. faecium* RS 29, whereas the other tested strains possessed only one virulence factor (*efaAfm*). In general, all tested Enterococci strains were negative for the tested genes encoding cytolysin (*cylA* and *cylB*) and aggregation substance (*agg*) which is a good characteristic for their potential biotechnological applications.
Another important role in enterococcal virulence is the hemolysin production by enterococci which is considered as a health risk factor. A partial hydrolysis (α

hemolysis) of sheep blood was observed for six *E. faecium* while no hemolytic reaction (γ hemolysis) was detected in the other tested strains (Tab 2).

Table 2 Occurrence of virulence traits and hemolytic activity among tested *Enterococcus* strains

Strains	*Agg*	*cob*	*cylA*	*cylB*	*efaAfs*	*efaAfm*	*esp*	*gelE*	α hemolyse	γ hemolyse
Enterococcus gallinarum DM 29	–	–	–	–	–	–	–	–	–	+
Enterococcus gallinarum DM 26	–	–	–	–	–	–	–	–	–	+
Enterococcus durans DM 20	–	–	–	–	–	+	–	–	–	+
Enterococcus faecium DM 19	–	+	–	–	+	+	+	+	+	–
Enterococcus durans DM 4	–	–	–	–	–	+	–	–	–	+
Enterococcus durans DM 34	–	–	–	–	–	+	–	–	–	+
Enterococcus hirae KM 8	–	–	–	–	–	–	–	–	–	+
Enterococcus hirae KM 6	–	–	–	–	–	–	–	–	–	+
Enterococcus faecium KM 2	–	–	–	–	–	+	–	–	–	+
Enterococcus faecium KM 1	–	–	–	–	–	+	–	–	–	+
Enterococcus faecium KM 14	–	–	–	–	–	+	–	–	+	–
Enterococcus hirae KM 12	–	–	–	–	–	–	–	–	–	+
Enterococcus hirae KM 11	–	–	–	–	–	–	–	–	–	+
Enterococcus faecium RS 31	–	–	–	–	–	+	–	–	+	–
Enterococcus faecium RS 29	–	+	–	–	–	+	–	–	+	–
Enterococcus faecium RS 4	–	–	–	–	–	+	–	–	+	–
Enterococcus faecium RS 25	–	–	–	–	–	+	–	–	+	–

–: not detected, +: detected

Antibiotic resistance

The results of antibiotic resistance of Enterococci strains are reported in table 3. With the exceptions of *E. faecium* RS 29 and RS 31, our isolates were resistant to streptomycin and kanamycin, but sensitive to other tested antibiotics. By contrast, both *E. faecium* RS 29 and RS 31 were found to be resistant to ampicillin and penicillin, but susceptible to other antibiotics (Tab 3). In general, no vancomycin resistance was recorded among tested Enterococci strains, while seven isolates were intermediate-resistant to erythromycin.

Table 3 Antibiotic sensitivity profiles of *Enterococcus* isolates

Strains	S*	K	TE	AMP	E	C	P	OT	VA
Enterococcus gallinarum DM 29	R	R	S	S	S	S	S	S	S
Enterococcus gallinarum DM 26	R	R	S	S	S	S	S	S	S
Enterococcus durans DM 20	R	R	S	S	I	S	S	S	S
Enterococcus faecium DM 19	R	R	S	S	S	S	S	S	S
Enterococcus durans DM 4	R	R	S	S	I	S	S	S	S
Enterococcus durans DM 34	R	R	S	S	S	S	S	S	S
Enterococcus hirae KM 8	R	R	S	S	S	S	S	S	S
Enterococcus hirae KM 6	R	R	S	S	S	S	S	S	S
Enterococcus faecium KM 2	R	R	S	S	I	S	S	S	S
Enterococcus faecium KM 1	R	R	S	S	I	S	S	S	S
Enterococcus faecium KM 14	R	R	S	S	I	S	S	S	S
Enterococcus hirae KM 12	R	R	S	S	S	S	S	S	S
Enterococcus hirae KM 11	R	R	S	S	S	S	S	S	S
Enterococcus faecium RS 31	S	S	S	R	S	S	R	S	S
Enterococcus faecium RS 29	S	S	S	R	S	S	R	S	S
Enterococcus faecium RS 4	R	R	S	S	I	S	S	S	S
Enterococcus faecium RS 25	R	R	S	S	I	S	S	S	S
Resistant (R)	<13**	≤13	≤14	≤16	≤13	≤17	≤14	≤14	≤14
Intermediate-resistant (I)	–	14–17	15–18	–	14–22	18–20	–	15–18	15–16
Sensitive (S)	≥15	≥18	≥19	≥17	≥23	≥21	≥15	≥19	≥17

Legend: * S – Streptomycin, K – Kanamycin, TE – Tetracycline, AMP – Ampicillin, E – Erythromycin, C – Chloramphenicol, P – Penicillin, OT – Oxytetracycline, VA – Vancomycin
** Inhibition zone diameters expressed as millimeter (mm)

Determination of antimicrobial activity and enterocin coding genes

Table 4 summarizes the antimicrobial activity of Enterococci strains toward pathogenic bacteria and fungi. *E. faecium* RS 29 and RS 31 were the only two strains which showed no inhibition against the tested pathogenic bacteria. With the exception of *E. faecium* RS 4, no activity was observed against *E. coli.* Additionally, only two strains, *E. durans* DM 34 and *E. faecium* RS 4 indicated moderate inhibitory activities against *Y. enterolitica.* However, the tested Enterococci strains exhibited considerable levels of inhibitory activity against *S. typhimurium.* Apart from 6 strains, the isolates also showed antagonistic effects toward *S. aureus* (Tab 4). For the antifungal activity, all Enterococci strains exerted strong inhibitory effects toward *F. graminearum* and *P. expansum.* The isolates also displayed inhibition of the growth at a lower extent against *A. parasiticus,* except for five strains. Similarly, 8 out of 17 Enterococci strains did not show any antifungal activity against *A. flavus* (Tab 4).

In this study, the characterized strains (17 isolates) were also subjected to PCR detection of the genes encoding enterocins (*ent*A and *ent*B) using specific primers. The results revealed that all isolates harboured both *entA* and *ent*B genes within their genome (data not shown).

Table 4 Antimicrobial activity of Enterococci strains toward selected pathogenic bacteria and fungi

Strains	*S. typhimurium* RSSK 95091	*E-coli* BC 1402	*S. aureus* BC 7231	*Y. enterolitica* ATCC 27729	*F. graminearum* MUCL 53452	*A. parasiticus* CBS 100926	*A. flavus* NRRL 3251	*P. expansum* MUCL 29192
Enterococcus gallinarum DM 29	++	–	+	–	+	+	+	++
Enterococcus gallinarum DM 26	++	–	++	–	++	–	+	++
Enterococcus durans DM 20	+	–	+	–	++	+	–	++
Enterococcus faecium DM 19	+	–	++	–	++	–	+	++
Enterococcus durans DM 4	+	–	–	–	++	–	+	++
Enterococcus durans DM 34	+	–	+	+	++	+	–	++
Enterococcus hirae KM 8	+	–	+	–	+	+	–	++
Enterococcus hirae KM 6	++	–	+	–	+	+	+	++
Enterococcus faecium KM 2	+	–	+	–	++	+	+	++
Enterococcus faecium KM 1	+	–	–	–	++	+	+	++
Enterococcus faecium KM 14	+	–	–	–	++	+	–	+
Enterococcus hirae KM 12	++	–	+	–	++	+	+	++
Enterococcus hirae KM 11	+	–	+	–	+	+	+	+
Enterococcus faecium RS 31	–	–	–	–	+	+	–	++
Enterococcus faecium RS 29	–	–	–	–	++	–	–	++
Enterococcus faecium RS 4	+	+	–	+	++	+	–	++
Enterococcus faecium RS 25	++	–	++	–	++	–	–	++

++: Strong inhibition with detectable clear zones around the wells, +: weak inhibition around the wells, –: no inhibition zone

DISCUSSION

Enterococcus species are found in various sources of raw foods of animal origin, such as raw milk from goat, ewe and cow **(Yerlikaya and Akbulut, 2020; Hanchi *et al.,* 2018; Foulquié Moreno *et al.,* 2006)**. In the present work, six Enterococci strains were isolated from dromedary raw milk and identified as *E. durans* (3 isolates)*, E. gallinarum* (2 isolates)*,* and *E. faecium* (1 isolate), which clustered in different subgroups. Only few reports have shown the predominance of Enterococci in dromedary raw milk and suggested that they are good candidates for potential dairy applications such as fermented camel milk products **(Hassaïne *et al.,* 2007; Hassaïne *et al.,* 2008; Khay *et al.,* 2011)**. Enterococci are also found frequently in soils, water, and plants as a result mainly of fecal contamination **(Giraffa, 2002)**, but other studies have suggested that these bacteria were possibly derived from nonfecal sources (**Klibi *et al.,* 2012**). Our results showed the predominance of *E. faecium* species in rhizospheric soil samples while saline water samples revealed the presence of *E. hirae* and *E. faecium* species. Similarly, **Klibi *et al.* (2012)** reported the prevalence of *E. faecium* species (97%) isolated from olive rhizospheres in Tunisia. In addition, *Enterococcus* species such as *E. durans*, *E. hirae,* and *E. gallinarum* were commonly found in variety of habitats especially soil, water, and vegetables **(Abriouel *et al.,* 2008)**. *E. faecium, E. faecalis, E. hirae, E. casseliflavus* and *E. mundtii* are also the predominant species isolated from intertidal and marine sediments and ocean water **(Ferguson *et al.,* 2005; Moore *et al.,* 2008)**. This predominance of Enterococci could be related to their broad spectrum of natural tolerance to adverse environmental conditions and high salt concentrations **(Giraffa, 2002; Hardwood *et al.,* 2000)**.

Several studies have extensively focused on the incidence of virulence determinants in Enterococci species reporting that a remarkable low occurrence of virulence genetic determinants was detected in *Enterococcus* isolates from foods or used as starters **(Eaton and Gasson, 2001)**, but also in those from water and soil **(Abriouel *et al.,* 2008)**. In the present study, six *Enterococcus* strains isolated from dromedary raw milk and natural saline water samples were free of the tested virulence factors while none of our Enterococci isolates harboured cytolysin (*cylA* and *cylB*) and aggregation substance (*agg*) genes. **Eaton and Gasson (2001)** reported that all tested *E. faecium* and *E. durans* strains were clear of the cytolysin and aggregation substance genes compared with *E. faecalis* isolates. In agreement with our findings, previous reports also concluded that Enterococci strains not carrying virulence factors, especially cytolysin genes, may be considered as a good characteristic for their food applications **(Ahmadova *et al.,* 2013; De Vuyst *et al.,* 2003)**. However, five virulence genes encoding *efaAfs, efaAfm, esp, cob,* and *gelE* were harboured in *E. faecium* DM 19 while the other tested strains carried one to two virulence genes. It is noteworthy that *efaAfm* was highly detected among our isolates, especially rhizospheric Enterococci. Similarly, high incidence of virulence determinants *efaA* (*efaAfs* and/or *efaAfm*) among food and water isolates of *Enterococcus* was previously recorded by **Eaton and Gasson (2001)** and **Abriouel *et al.* (2008)**, nevertheless, very little information on the incidence of virulence factors among rhizospheric Enterococci has been published **(Klibi *et al.,* 2012)**. In addition, a much lower presence of *esp*, *cob,* and *gelE* genes or even absence in Enterococci isolates from dromedary raw milk, saline water and rhizospheric soil samples is in accordance with the previous reports concerning food, water and soil isolates (**Abriouel *et al.,* 2008; Eaton and Gasson, 2001; Klibi *et al.,* 2012**).

The present findings also indicate that none of the tested *Enterococcus* strains showed β hemolysis. However, a partial hydrolysis (α hemolysis) was observed for six *E. faecium* strains while no hemolytic reaction (γ hemolysis) was detected in the other tested strains. The absence of hemolytic activity within Enterococci species should be regarded as a significant selection criterion for their safe use taking into account vancomycin sensitivity and absence of cytolysin gene (**De Vuyst *et al.,* 2003**).

The frequent Enterococci resistance to commonly used antibiotics and the risk of transmitting antibiotic resistant genes to other opportunistic or pathogenic bacteria are particular concerns for their safe use in foods or as biocontrol agents **(Giraffa, 2002)**. In the present study, antibiotic susceptibility results showed that our isolates were resistant to streptomycin and kanamycin, except for two strains. Resistance to cephalosporins and aminoglycosides among certain Enterococci species has been reviewed by **Morrison *et al.* (1997)** and **Tendolkar *et al.* (2003)**. Moreover, several authors have reported on the resistance of Enterococci to kanamycin and streptomycin **(Ahmadova *et al.,* 2013; Hosseini *et al.,* 2009; Mathur and Singh, 2005)**. In this study, only two *E. faecium* stains isolated from rhizospheric soil samples were found to be resistant to ampicillin and penicillin. A similar observation was reported by **Abriouel *et al.* (2008)** on the resistance to ampicillin and penicillin within *Enterococcus* species especially *E. faecium* and *E. faecalis* isolates from soil and water. Nevertheless, few studies have also reported on the antibiotic resistance of *Enterococcus* from rhizospheric soil (**Klibi *et al.,* 2012; Fhoula *et al.,* 2013**). Generally, our results showed a high sensitivity of all isolates to the tested antibiotics, especially vancomycin which is considered as a major health concern worldwide.

Enterococci produce powerful enterocins which are of great interest because of their large spectra of activity against pathogenic bacteria and fungi. In this study, only two isolates did not inhibit the tested pathogenic bacteria while the others exhibited varying levels of antibacterial activity. In addition, some isolates showed considerable inhibitory effects against *S. typhimurium* and *S. aureus.* Several authors concluded that any activity of enterocins against Gram-negative bacteria is very rare due to the lipopolysaccharide layer in their outer membranes **(Ahmadova *et al.,* 2013; Schirru *et al.,* 2012; Belgacem *et al.,* 2010; Gong *et al.,* 2010)**. However, our isolates exhibited remarkable antagonist activity toward *S. typhimurium* which could be of great interest. Previous reports also proved that bacteriocin-producing Enterococci displayed a strong inhibitory effect against a broad range of Gram-positive bacteria including *Staphylococcus* and *Listeria* **(Yerlikaya and Akbulut, 2020; Fhoula *et al.*, 2013; Rivas *et al.*, 2012; Schirru *et al.,* 2012)**, which are in agreement with our findings. Moreover, all the tested Enterococci strains exerted strong inhibitory effects toward *F. graminearum* and *P. expansum.* Similar to our results, *E. faecium* PC4.1 exhibited strong inhibition growth at the same extent for *Fusarium* ssp. and *Cladosporium* **(Hadji-Sfaxi *et al.,* 2011)**, while **Fhoula *et al.* (2013)** confirmed the antifungal efficacy of *Enterococcus* strains against most of the tested postharvest fungi (*A. niger, P. expansum,* and *B. cinerea*) and highlighted their potential use as biocontrol agents. Many *Enterococcus* species produce a large number and diverse classes of useful antimicrobial peptides or enterocins **(Foulquié Moreno *et al.,* 2006)**. Our results revealed the co-presence of *ent*A and *ent*B among all the isolates which has already been reported by other studies **(Özden Tuncer *et al.*, 2013; Rivas *et al.*, 2012)**. **Vendera *et al.* (2019) and De Vuyst *et al.* (2003)** also demonstrated the synergistic effects of enterocin A and enterocin B, and the combined use of both enterocins could be more better in exhibiting a large spectrum of activity against a wide range of pathogenic microorganisms.

CONCLUSIONS

In conclusion, the obtained results showed the diversity of *Enterococcus* strains isolated from different sources in Algeria which may depend on different geographic regions and environmental habitats from which they were isolated. Among the tested *Enterococcus* isolates, six strains (*E. gallinarum* DM 29, *E.*

gallinarum DM 26, *E. hirae* KM 6, *E. hirae* KM 8, *E. hirae* KM 11, and *E. hirae* KM 12) were selected for further investigations based on their safety aspects. These included the lack of tested virulence factors and hemolytic activity, the high sensitivity to vancomycin and the exhibition of remarkable antagonist effect against tested pathogenic bacteria and fungi. This indicated their potential as candidates for biocontrol agents or probiotic starters. However, more research is needed to study the chemical characterization of the molecular structure of active compounds produced by these strains.

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