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Purification and Molecular Characterization of Two-Antimicrobial Peptides Produced by *Lactobacillus plantarum* DU10

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ABSTRACT

The aim of this study was the partial characterization of plantaricin MZ, a two-component antimicrobial peptides produced by the putative probiotic bacteria *Lactobacillus plantarum* DU10, which was isolated from Algerian raw camel milk. This biomolecule was secreted in the supernatant of a *L. plantarum* DU10 culture and showed diverse spectrum of antimicrobial activities against several pathogenic bacteria. The activity as determined by the proteolytic action of trypsin, pepsin and proteinase K, plantaricin MZ was maintained even after a treatment at 121°C for 15°C and a pH range from 2-10. This putative probiotic strain was found to produce antimicrobial substances proteinaceous in nature. Molecular mass determination was estimated with tricine-sodium dodecyl sulphate-polyacrylamide gel electrophoresis, then confirmed by mass-assisted laser desorption ionization time of flight mass spectrometry. The producing strain and its antimicrobial peptides may find an application as a bio-preservative agent.

Key words: *Lactobacillus plantarum*, probiotic, bacteriocin, purification, antimicrobial peptide

INTRODUCTION

Algerian camel milk represents an important probiotic source, in which *Lactobacillus plantarum* was the major species of Lactic Acid Bacteria (LAB) isolated from this product (Zineddine *et al*., 2011; Marroki and Bousmaha-Marroki, 2014). This strain is able to produce antimicrobial peptides with a specific action such as bacteriocins or bacteriocin-like inhibitory substances (Xie *et al*., 2011).

These bio-molecules can inhibit even in low concentrations bacterial growth especially the closely related ones. Despite of their diversity and origins, they are all ribosomally synthesized

antimicrobial peptides. Bacteriocins produced by gram-positive bacteria can be classified into two main groups. Group 1 includes antibiotics which are post-traditionally modified bacteriocins, group 2 includes the unmodified bacteriocins and which can be divided into many subgroups due to their characteristics. For example, class IIa bacteriocins are called Listeria-active or pediocin-like. Class IIb are two-peptide bacteriocins, class IIc are circular and class IId are linear and non-pediocin bacteriocins. From these subgroups, class IIb bacteriocins depends on the action of both antimicrobial peptides.

Most of LAB bacteriocins are found as single peptides but some of them only were found as two-peptide systems. In the same operon, each system is encoded by adjacent Open Reading Frames (ORFs) situated in the same operon which are transcribed and synthesized in the same time. They are completely active usually because of the synergistic action of the two peptides, while each peptide alone has less or no activity. In all previous studies, the optimal combination ratio of the two peptides is about 1:1 (Chen *et al*., 2014).

This type of systems is found in lantibiotics which have not been categorized into a defined class and in modified two peptides bacteriocins belonging to class IIb.

The first two-peptide bacteriocin was described for the first time from *Lactococcus lactis* subsp. *lactis* LMG 2081 named as Lactococcin G. Its activity, structure and mode of action have been examined extensively (Nissen-Meyer *et al*., 1992).

Bacteriocins, especially those produced by probiotic bacteria are of particular interest because of their potential as biopreservative (Wang *et al*., 2008).

Nisin is a good model of safe biopreservative according to Food and Drug Administration and has hence beneficiated of many research works mainly probiotic ones.

Lactobacillus plantarum strains have gained particular interest these last two decades, most of their plantaricins were not only characterized but amino acid also sequenced.

Both class I and class II plantaricins have been previously identified from different *L. plantarum* strains (Atrih *et al*., 2001; Enan *et al*., 2002; Galvez *et al*., 2007; Gong *et al*., 2010; Hata *et al*., 2010; Hernandez *et al*., 2005; Holo *et al*., 2001; Tiwari and Srivastava, 2008; Zhang *et al*., 2013) and were found to show high antimicrobial activities against *L. monocytogenes* (Gong *et al*., 2010; Holo *et al*., 2001; Tiwari and Srivastava, 2008).

The probiotic potential of *L. plantarum* DU10 has already been established in our labs (Amina *et al*., 2014). Therefore, the present study was designed to partially characterize the two-antimicrobial peptides plantaricin MZ by molecular techniques after their purification to homogeneity.

MATERIALS AND METHODS

Sampling: Lactic acid bacteria were isolated from raw camel milk in Southern Algerian farms, from Ouargla (Afran village, 786 km far from the capital Algiers). Ten milk samples were collected in sterilized bottles and brought to applied microbiology laboratory at Oran 1 University (Algeria), then to the Department of Biotechnology at Pondicherry University (India) for further analysis.

Identification of the selected strain: The identification was determined by classical methods (Sharaf and Al Harbi, 2011), then finally by 16S rDNA sequencing using universal primers 16S1 (5‘AGA GTT TGA TCC TGG CTC AG 3‘) and 16S2 (5‘ACG GCT ACC TTG TTA CGA CTT 3‘) then sent to Microsynth Laboratories, Switzerland.

The obtained sequence was BLAST-analyzed using NCBI website and the results submitted to GenBank (Marroki *et al*., 2011; Kumar *et al*., 2010; Talpur *et al*., 2012; Amina *et al*., 2014).

Antimicrobial spectrum: The antimicrobial activity of Cell Free Supernatant (CFS) was determined by agar well diffusion method against several pathogenic bacteria. The MRS agar containing the indicator strain was poured on petri dishes and then 4 mm wells were punched into agar and filled with 80 µL of filter sterilized CFS of *Lactobacillus plantarum* using different dilutions and adjusted to pH 6.5.

Clear zones around each well confirmed the antimicrobial activity of the selected probiotic bacterium (Anas *et al*., 2012; Kumar and Arul, 2009).

Sensitivity to antibiotics: Twenty five milliliters of MRS agar was inoculated with 12 h old culture and mixed carefully, then poured on petri dish. After solidification, all antibiotic discs present in our lab were deposed on the medium and incubated at 37°C for 24 h. Clear zones of inhibition indicated the sensitivity of the probiotic strain towards selected antibiotics.

Results were compared to ACFMS (Antibiogram Committee of the French Microbiology Society) to confirm the obtained results. The dilution method was used to determine the Minimum Inhibitory Concentration (MIC) (Danielsen and Wind, 2003; Sahm and Washington, 1991; Vlkova *et al*., 2006).

Crude bacteriocin preparation and partial purification: The MRS Broth culture inoculated with 0.1% of DU10 inoculums grown for 16 h at 37°C. The cells were separated by centrifuging at 8,500 g at 4°C for 15 min, then the cell free supernatant neutralized to pH 6.5±0.1 with 1 N NaOH (Enan, 2006).

The antimicrobial activities against pathogenic bacterial strains were elucidated by agar well diffusion assay. Then the CFS was subjected to ultrafiltration using Amicon Ultra Centrifugal Filters, which enable the high concentration of proteins (Merck Millipore, Germany), as the desirable putative bacteriocins were suspected below this molecular weight.

Ammonium sulfate was gradually added to the procured filtrate to attain up to 90% saturation and kept for overnight at 4°C. Then the precipitated proteins collected by centrifugation at 10,000 g for 20 min at 4°C and dissolved in a minimal quantity of a 10 mM ammonium acetate buffer (pH 6.0) and dialyzed 16 h against the same buffer at 4°C with a 1 kDa pore size dialysis membrane (spectrum labs, USA).

Purification of the antimicrobial peptide: The dialyzed crude bacteriocin was partially purified by Akta Prime plus protein purification system (GE Healthcare, Uppsala, Sweden) which was connected with a size exclusion column (c) 10/20) and Sephadex G-25 (Sigma) as a matrix. Two milliliter of dialyzed sample was injected into the column which was equilibrated with 10 mM ammonium acetate buffer (pH 6.0) and containing 0.01 M sodium chloride at a flow rate of 0.5 mL min^{-1}. The eluent was collected as 1 mL size of fractions. Each fraction carried out for antibacterial activity and protein profiling by Tricine SDS-PAGE. The active fraction was lyophilized and the concentrated fraction further purified by reverse phase liquid chromatography RP-HPLC (Shimadzu, Japan).

Then, 25 µL of the concentrated bacteriocin which was collected from Akta prime plus column purification and able to inhibit pathogens was injected into an analytical C18 reverse-phase column (Luna 5 µm, 250×4.6 mm; Phenomenex, CA, USA). The elution was performed at a flow rate of 1 mL min^{-1} using a linear gradient from 90% solvent A [0.1% (w/v) TriFluoro-Acetic Acid (TFA) in 5% (v/v) acetonitrile in water] and 10% solvent B [0.1% TFA in 100% acetonitrile] to 42 and 58% of solvents A and B, respectively within 46 min. The peptide fractions were detected spectrophotometrically by measuring the absorbance at 220 nm and collected manually. The fractions were then lyophilized and dissolved in an ammonium acetate buffer (10 mM, pH 6.0) and kept for further analysis.

Effect of enzymes, pH and temperature on antibacterial activity: From the gel filtration chromatography an active fraction was obtained and adjusted to 1 mg protein mL^{-1} (pH 6.5) as partially purified antimicrobial peptides and followed by an antimicrobial activity test. Aliquots of these samples were co-incubated with several enzymes at 37°C for 2 h, pepsin (800 U mL^{-1}, 0.05 M citric acid buffer, pH 2.0, Sigma, USA), trypsin (250 U mL^{-1}, 0.05 M sodium phosphate buffer, pH 7.0, Sigma), proteinase K (38 U mL^{-1}, pH 7.5, 0.05 M sodium phosphate buffer, Amresco, USA), α-chymotrypsin (100 U mL^{-1}, 0.05 M sodium phosphate buffer, pH 7.5, Sigma) and catalase (2000 U mL^{-1}, 0.05 M sodium phosphate buffer, pH 7.0, Sigma). After incubation, all samples were adjusted to pH 6.5 with sterile 3 M NaOH or 3 M HCl and tested for antibacterial activity by using *Listeria monocytogenes* ATCC 15313 as an indicator strain. Partially purified antimicrobials in buffers without enzyme and buffers alone were used as controls.

The pH of samples was adjusted (1 mg protein mL^{-1}) to values varying from 2.0-10.0 with sterile 3 M NaOH or 3 M HCl to check its effect on the antimicrobial activity. After incubation at 37°C during 72 h, the samples were re-adjusted to pH 6.5 with sterile 3 M NaOH or 3 M HCl and tested again for antibacterial activities.

By heating the partially purified antimicrobials, the effect of temperature on antibacterial activity was tested (1 mg protein mL^{-1}) at 60°C/30 min, 80°C/30 min, 100°C/30 min 121°C/15 min, respectively, then the samples were cooled and residual antibacterial activities were tested by using *Listeria monocytogenes* ATCC 15313, as an indicator strain. In experiments of 121°C, heating and cooling time were about 15 min, respectively. All experiments were conducted in triplicate (Al-Otaibi, 2012).

Molecular mass determination: The molecular mass of the antimicrobial peptide was confirmed by Mass-Assisted Laser Desorption Ionization Time Of Flight Mass Spectrometry (MALDI-TOF/MS) using the Proteomic facility of the Molecular Biophysics Unit at the Indian Institute of Sciences, Bangalore, India (Kaur *et al*., 2013).

Tricine-sodium dodecyl sulfate polyacrylamide gel electrophoresis: The separated fractions obtained after an HPLC analysis, were used to determine the molecular size by Tricine SDS-PAGE (10%) which was run under reducing and non reducing conditions (Schagger and Jagow, 1987). The probe buffer wasn't neither boiled nor added of 2-mercaptoethanol. The SDS was removed by washing the gel 4 times for 10 min in distilled water and then the gel was placed in a petri dish containing a 2% agar medium overlaid with a precooled 0.7% agar medium inoculated with the

indicator organism at 37°C for 24 h. A low molecular mass protein marker with sizes ranging from 3.0-97.4 kDa (Bangalore Genei, Bangalore, India) was used. The gel was stained using the silver staining method (Morrissey, 1981).

RESULTS AND DISCUSSION

Identification of *Lb. plantarum* from raw camel milk: Among 50 lactic acid bacteria isolated from Algerian raw camel milk, only one was selected in this study for its remarkable characteristics. The Isolate was identified as *Lactobacillus plantarum* DU10 by morphological, biochemical and phylogenetic 16S rDNA sequence method as described previously by Amina *et al*. (2014). The obtained sequence was identified and deposited in Genbank under the Accession Number KF724943. The phylogenic tree is shown in Fig. 1.

Inhibitory spectrum of DU10: CSF of *L. plantarum* DU10 gave clear zones around the indicator pathogenic strains. The largest diameter of inhibition which is 25 mm, was obtained with the CFS of this probiotic strain (Yateem *et al*., 2008) on *L. monocytogenes* and *L. innocua*. Table 1 indicates that *L. plantarum* is able to inhibit the growth of both gram-positive and gram-negative bacteria.

Table 1: Antimicrobial activity of DU10 towards pathogenic bacteria

Pathogenic strains	Sensitivity
Listeria monocytogenes ATCC 15313	+++
Listeria innocua ATCC 33090	+++
Staphylococcus aureus ATCC 6538	+++
Pseudomonas aeruginosa ATCC 27853	+++
Escherichia coli ATCC 8739	++
Klebsiella pneumoniae ATCC 700603	+
Salmonella enterica ATCC 14028	++
Salmonella typhi ATCC 700720	++
Vibrio fischeri ATCC 700601	+
Vibrio parahaemolyticus ATCC 17802	+

+: >5 mm, 5< ++: <10 mm and +++: <10 mm

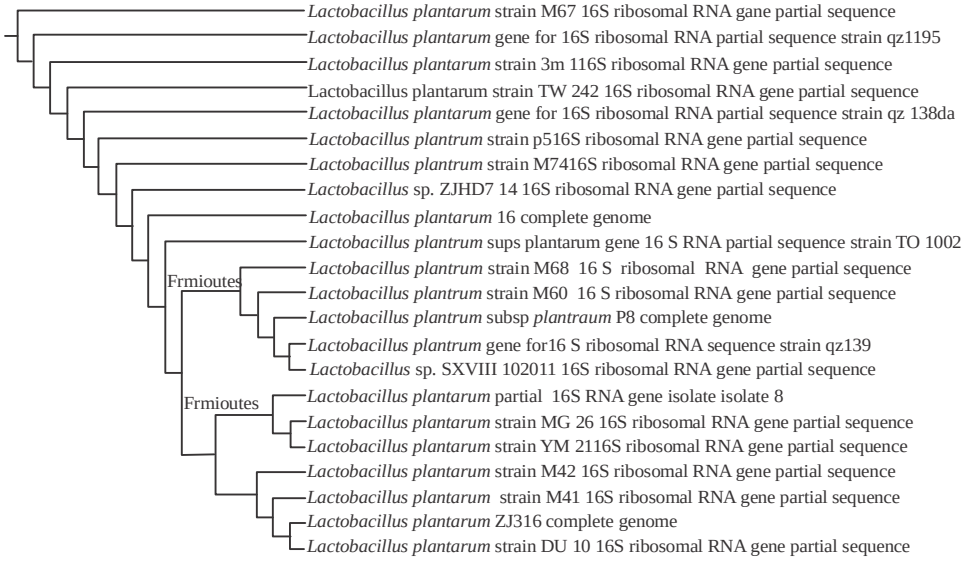

Fig. 1: Blast tree view of *L. plantarum* DU10 using blast pairwise alignments

Adeniyi *et al*. (2006) have used the CFS of *Lactobacillus* strains to prove their growth inhibition of all organisms implicated in urinary tract infection.

Lactobacillus plantarum (Yateem *et al*., 2008) has been isolated from un-pasteurized camel milk which was allowed to ferment spontaneously for one week, was able to inhibit the growth of the Gram-negative tested bacteria such as *Salmonella* spp. and *E. coli* strains. Dialyzed sample (semi-crude preparation) of *L. plantarum* DU10 has shown a broad spectrum antibacterial activity towards several gram-positive and gram-negative pathogens. Similarly, plantaricin 423 reported by Van Reenen *et al*. (1998) and plantaricin LP84 reported by Suma *et al*. (1998) showed inhibitory activity towards several pathogens such as *S. aureus* and *B. subtilis*.

Plantaricin 35d produced by a *L. plantarum* strain showed a broad spectrum of antimicrobial activity including *S. aureus* and *L. monocytogenes* according to Messi *et al*. (2001). Lash *et al*. (2005) described a bacteriocin produced by *L. plantarum* (ATCC 8014), which inhibited *S. aureus*, *E. coli*, *L. innocua* and *P. aeruginosa*. Plantaricin 35d produced by a *L. plantarum* strain showed a large broad spectrum of antimicrobial activity including *S. aureus* and *L. monocytogenes* (Messi *et al*., 2001). Valenzuela *et al*. (2008) described an antimicrobial peptide that inhibited pathogenic bacteria like *B. cereus*, *E. coli* and *S. enteric*a. Gong and Xie have reported plantaricin MG produced by *L. plantarum* KLDSI 0391 that inhibited many pathogens such as *L. monocytogenes*, *S. aureus*, *S. thyphimurium* and *E. coli* (Gong *et al*., 2010; Xie *et al*., 2011).

Xie *et al*. (2011) described an active bacteriocin against strains of *Lactobacillus*, *Listeria*, *Sreptococcus* and *Pediococcus*. Plantaricin LD1 produced by food isolate of *Lactobacillus plantarum* LD1 inhibited not only related strains but also other gram-positive and gram-negative bacteria such as *S. aureus*, *P. aeruginosa*, *S. typhi* as described by Gupta and Tiwari (2014). Plantaricin Y produced by *L. plantarum* 510 reported by Chen *et al*. (2014) showed strong inhibitory activity against *L. monocytogenes* BCRC 14845.

Sensitivity to antibiotics: The probiotic bacterium was sensitive to most selected antibiotics except for polymyxin, kanamycin, sulfafurazole, cefpodoxim and vancomycin. Various reports showed that *Lactobacillus plantarum* DU10 was naturally resistant to some antibiotics, such as penicillin G, ampicillin, vancomycin, chloramphenicol or ciprofloxacin. These results are not in concordance with ours, except for vancomycin (Coppola *et al*., 2005; Halami *et al*., 2000) (Table 2).

This antibiotic belongs to glycopeptide antibiotics and inhibits the peptidoglycan synthesis which is important structural component of cell wall bacterial. Consequently, lactic acid bacteria are particularly vulnerable to vancomycin treatment (Reynolds, 1989).

Purification of microbial peptides: To purify plantaricin MZ, the CFS was ultrafiltered through a 10 kDa membrane, precipitated with ammonium sulfate, dialyzed and subjected to Sephadex G-25 gel chromatography (Fig. 2). The resulting active fraction was lyophilized and dissolved in a minimum volume of solvent A for an HPLC analysis.

Upon injection of the Sephadex G-25-purified sample into the reverse-phase HPLC, two distinct peaks were observed, corresponding to a retention time of 16.90 and 23.38 min, respectively

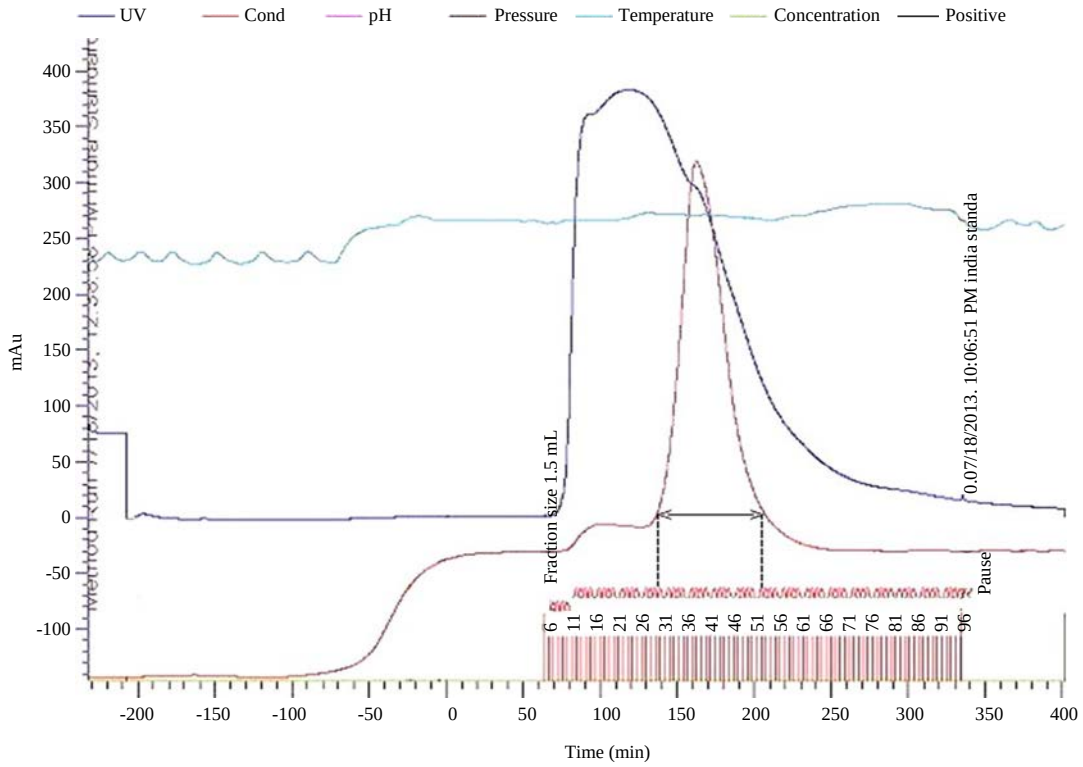

Fig. 2: Sephadex G-25 chromatogram for the whole bacteriocin sample. The bacteriocin sample was purified by Sephadex G-25 gel chromatography and eluted at a flow rate of 0.5 mL min^{-1}

Table 2: Antibiotic sensitivity of *Lactobacillus plantarum* DU10

Pathogenic strains	Sensitivity	Disc concentration (µg/disc)
Chloramphenicol	S	30
Amoxycillin	S	10
Vancomycin	R	30
Gentamycin	S	10
Oxytetracycline	S	30
Erythromycin	S	10
Amikacin	S	30
Penicillin	S	10
Cefpodoxim	R	10
Neomycin	S	30
Methicillin	S	30
Novobiocin	S	30
Kanamycin	R	30
Rifampicin	S	30
Tetracycline	S	5
Ampicillin	S	10
Polymyxin	R	300
Sulfafurazole	R	300
Ciprofloxacin	S	5

S: Sensitive and R: Resistant

(Fig. 3). These peaks were also shown to be active against *Listeria monocytogenes* ATCC 15313 and *Staphylococcus aureus* ATCC 6538 and used for the molecular mass determination.

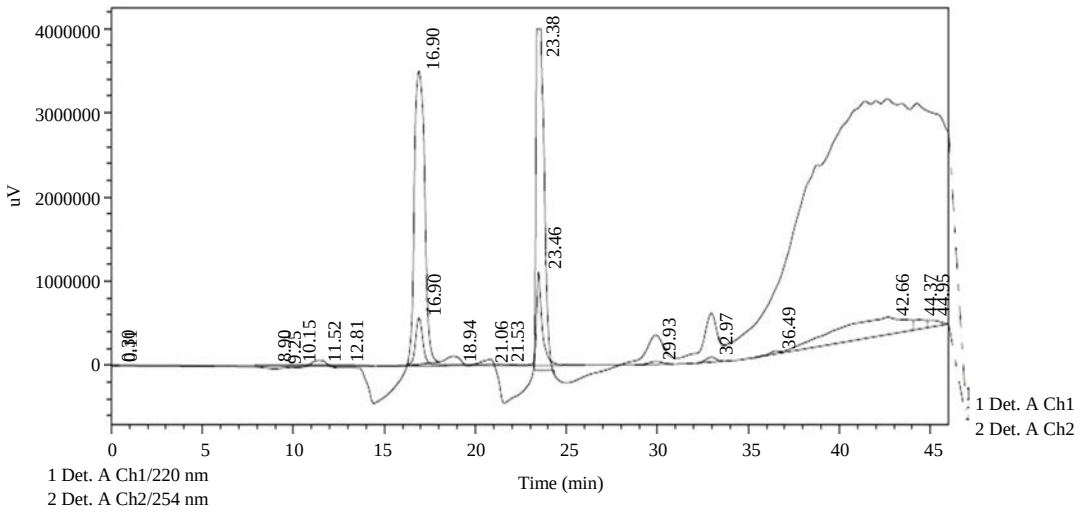

Fig. 3: High Performance Liquid Chromatography chromatogram of plantaricin M and Z. The lyophilized partially purified samples were resuspended in a minimal quantity of solvent A. A 25 µL sample was eluted using a linear gradient from 90% solvent A [0.1% (w/v) trifluoroacetic acid (TFA) in 5% (v/v) acetonitrile in water] and 10% solvent B (0.1% TFA in 100% acetonitrile) to 42 and 58% of solvents A and B, respectively within 46 min. Two distinct peaks of purified plantaricin M and Z were obtained at 16.90 and 23.38 min, respectively

Table 3: Antimicrobial spectrum expressed as residual bacteriocin activity of mixed fractions (1:1 molar ratio)

Residual bacteriocin activity (%)		
Temperature and time	Surfactant	pH
60°C, 30 min (100)	Catalse (100)	2.0 (100)
80°C, 30 min (100)	Proteinase-K (0)	6.0 (100)
100°C, 30 min (100)	A-Chymotrypsin (0)	8.0 (100)
121°C, 15 min (100)	Trypsin (0)	10.0 (100)
	Pepsin (0)	
	Lipase (100)	

Negative results were found when both fractions were tested separately, this means that the two-peptide bacteriocins acts synergistically

Effect of enzyme and heat treatment on antimicrobial activity: Plantaricin MZ was found to be heat resistant to 121°C for 15 min. At temperatures of 60, 70 and 80°C for 30 min, 100% activity was retained and stable. This may constitute an advantage in view of its potential use as a food additive in processes like pasteurization and drying (Bhattacharya and Das, 2010). It was sensitive to pepsin, trypsin, chymotrypsin and proteinase-K but insensitive to catalase confirming that inhibition was due to proteinaceous molecule (bacteriocin). Its activity was not reduced by lipase indicating that there was no structural modification by lipid moiety (Table 3). In previous studies, most plantaricins treated with trypsin were inactivated (Gong *et al*., 2010; Gonzalez *et al*., 1994; Jimenez-Diaz *et al*., 1993; Tiwari and Srivastava, 2008; Van Reenen *et al*., 1998; Zhang *et al*., 2013). Treatment of plantaricin Y with trypsin provided a similar result using *W. paramesenteroides* BRCR 14006 as the indicator strain but inhibitory activity was observed

when *L. monocytogenes* BCRC 14845 was used as the indicator strain. In order to determine whether trypsin-treated plantaricin MZ has inhibitory activity against other tested bacteria, activity against all the indicator strains listed in Table 1 was tested. To clarify this, more detailed analyses are necessary in future studies.

Negative results were found when both fractions were tested separately, this means that the two-peptide bacteriocins acts synergistically.

Molecular mass determination: Plantaricin M and Z were purified to homogeneity and MALDI-TOF/MS confirmed the purity of samples and determined the exact molecular mass of peptides to be 6.7956 and 7.1922 kDa, respectively as shown in Fig. 4 and 5.

These bacteriocins could temporary been classified as a class II bacteriocin based on their molecular mass [9]. Previous reports on molecular weight of bacteriocins isolated from *L. plantarum* strains did not exactly corresponded to Plantaricin M and Z.

Tricine-sodium dodecyl sulfate polyacrylamide gel electrophoresis: A clear zone in native PAGE was observed (Fig. 6) which was corresponding to purified antimicrobial peptides Plantaricin M and Z with a molecular weight around 6 and 7 kDa, respectively.

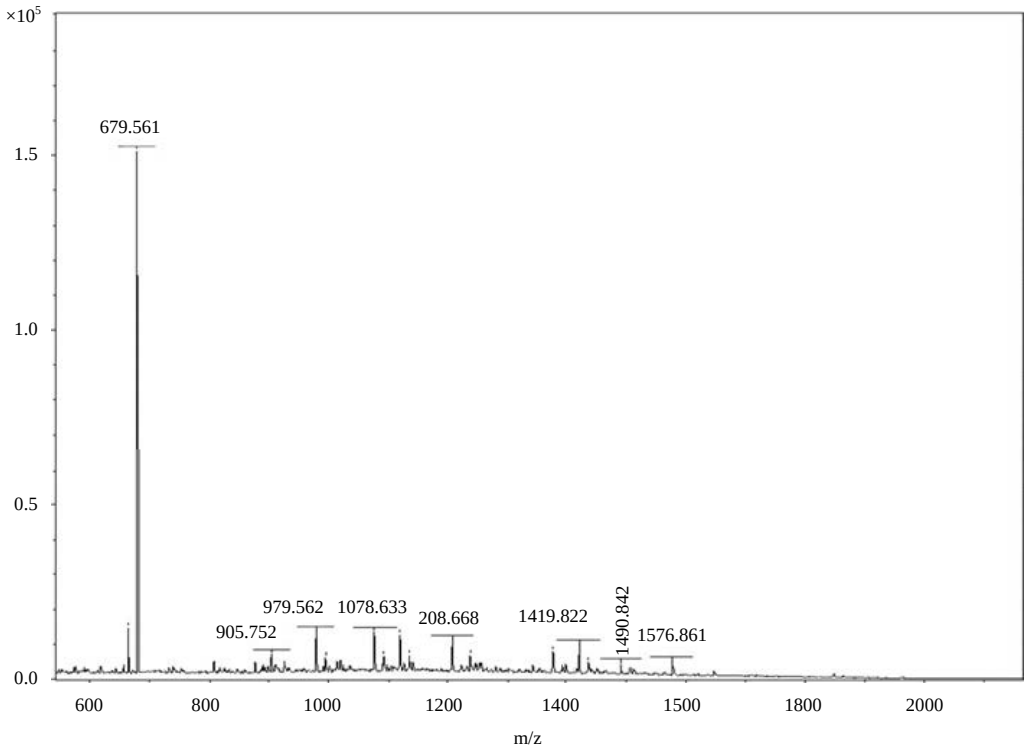

Fig. 4: Mass-assisted laser desorption ionization time of flight mass spectrum of plantaricin M. The single active peak fraction of HPLC was subjected to mass spectrometry. The mass-assisted laser desorption ionization time of flight analysis showed a distinct peak around 670 m z^{-1}, indicating that the molecular mass of the bacteriocin protein was 6.7956 kDa

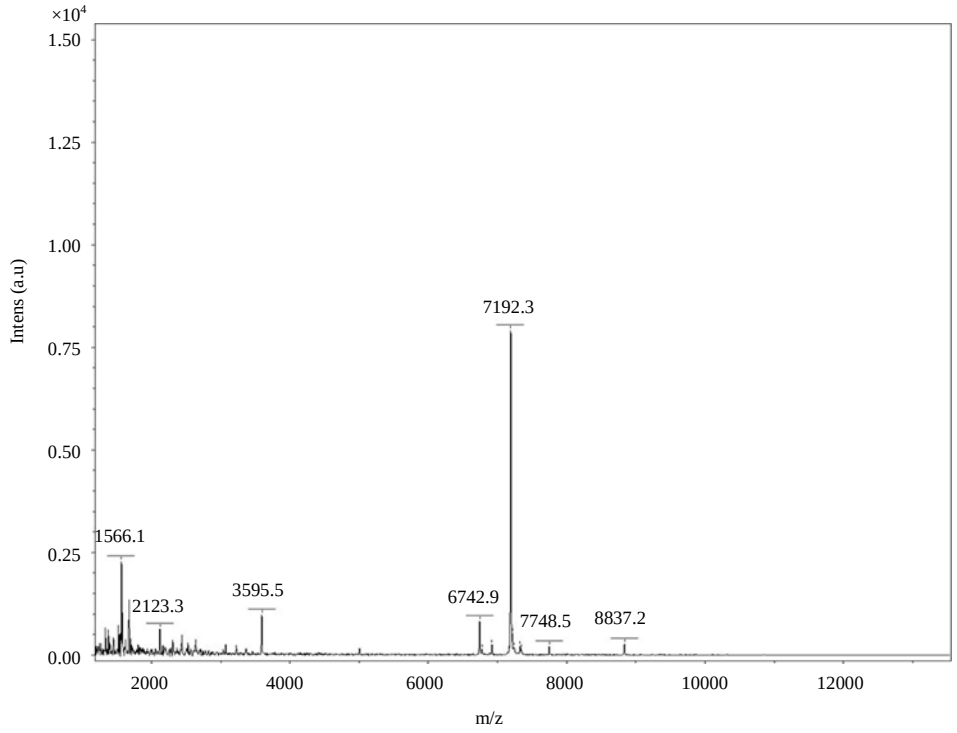

Fig. 5: Mass-assisted laser desorption ionization time of flight mass spectrum of plantaricin Z. The single active peak fraction of HPLC was subjected to mass spectrometry. The Mass-assisted laser desorption ionization time of flight analysis showed a distinct peak around 7250 m z^{-1}, indicating that the molecular mass of the bacteriocin protein was 7.1922 kDa

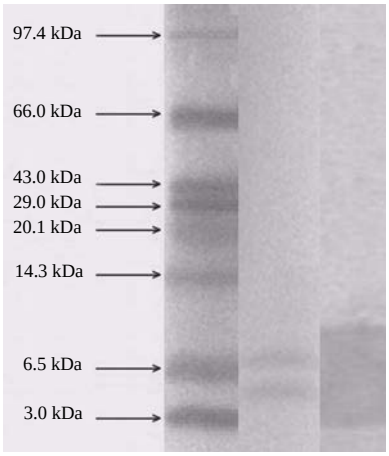

Fig. 6: Tricine SDS-Page of DU10 antimicrobial peptides, Lane 1: Molecular weight markers, Lane 2: Position of peptide bands and Lane 3: Gel overlaid with the pathogenic strain

These results have validated further the molecular mass of the antimicrobial peptides as 6.7956 and 7.1922 kDa. Data obtained indicate that plantaricin M and Z have a different molecular mass and characteristics to other known bacteriocins from *L. plantarum*.

CONCLUSION

In vitro screening of Lactobacilli from raw camel milk constitutes a valuable strategy for the large-level preliminary selection of putatively safe LAB expected for use as probiotic cultures.

This study shows that filter sterilized CFS from *L. plantarum* DU10 exhibited antimicrobial activity against both gram-positive and gram-negative bacteria. The purified two-component antimicrobial peptides plantaricin MZ were reported for the first time in this research work.

Some additional studies should be done to know the power of adhesion, mode of action and the stability of the isolate to manufacturing processes.

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REFERENCES

Adeniyi, B.A., F.A. Ayeni and S.T. Ogunbanwo, 2006. Antagonistic activities of lactic acid bacteria isolated from Nigerian fermented dairy food against organisms implicated in urinary tract infection. Biotechnology, 5: 183-188.

Al-Otaibi, M.M., 2012. Isolation of lactic acid bacteria from some traditional Saudi food. Am. J. Food Technol., 7: 690-699.

Amina, Z., S. Noureddine, A. Venkatesan, V. Perumal and B. Hichem *et al*., 2014. Characterization and potential probiotic attributes of *Lactobacillus plantarum* DU10 isolated from Algerian raw camel milk. Biotechnology, 13: 282-288.

Anas, M., B.A. Zinedine, H.A. Rizk, H.J. Eddine and K. Mebrouk, 2012. Screening of autochthonous *Lactobacillus* species from Algerian raw goats' milk for the production of bacteriocin-like compounds against *Staphylococcus aureus*. Afr. J. Biochem., 11: 4595-4607.

Atrih, A., N. Rekhif, A.J.G. Moir, A. Lebrihi and G. Lefebvre, 2001. Mode of action, purification and amino acid sequence of plantaricin C19, an anti-Listeria bacteriocin produced by *Lactobacillus plantarum* C19. Int. J. Food Microbiol., 68: 93-104.

Bhattacharya, S. and A. Das, 2010. Study of physical and cultural parameters on the bacteriocins produced by lactic acid bacteria isolated from traditional Indian fermented foods. Am. J. Food Technol., 5: 111-120.

Chen, Y.S., Y.C. Wang, Y.S. Chow, F. Yanagida, C.C. Liao and C.M. Chiu, 2014. Purification and characterization of plantaricin Y, a novel bacteriocin produced by *Lactobacillus plantarum* 510. Arch. Microbiol., 196: 193-199.

Coppola, R., M. Succi, P. Tremonte, A. Reale, G. Salzano and E. Sorrentino, 2005. Antibiotic susceptibility of *Lactobacillus rhamnosus* strains isolated from Parmigiano Reggiano cheese. Lait, 85: 193-204.

Danielsen, M. and A. Wind, 2003. Susceptibility of *Lactobacillus* spp. to antimicrobial agents. Int. J. Food Microbiol., 82: 1-11.

Enan, G., 2006. Behaviour of *Listeria monocytogenes* LMG 10470 in poultry meat and its control by the bacteriocin plantaricin UG 1. Int. J. Poult. Sci., 5: 355-359.

Enan, G., S. Alalyan, H.A. Abdel-Salam and J. Debevere, 2002. Inhibition of listeria monocytogenes LMG10470 by plantaricin UG1 *in vitro* and in beef meat. Nahrung, 46: 411-414.

Galvez, A., H. Abriouel, R.L. Lopez and N.B. Omar, 2007. Bacteriocin-based strategies for food biopreservation. Int. J. Food Microbiol., 120: 51-70.

Gong, H.S., X.C. Meng and H. Wang, 2010. Plantaricin MG active against Gram-negative bacteria produced by *Lactobacillus plantarum* KLDS1.0391 isolated from Jiaoke, a traditional fermented cream from China. Food Control, 21: 89-96.

Gonzalez, B., P. Arca, B. Mayo and J.E. Suarez, 1994. Detection, purification and partial characterization of plantaricin C, a bacteriocin produced by a *Lactobacillus plantarum* strain of dairy origin. Applied Environ. Microbiol., 60: 2158-2163.

Gupta, A. and S.K. Tiwari, 2014. Plantaricin LD1: A bacteriocin produced by food isolate of *Lactobacillus plantarum* LD1. Applied Biochem. Biotechnol., 172: 3354-3362.

Halami, P.M., A. Chandrashekar and K. Nand, 2000. *Lactobacillus farciminis* MD, a newer strain with potential for bacteriocin and antibiotic assay. Lett. Applied Microbiol., 30: 197-202.

Hata, T., R. Tanaka and S. Ohmomo, 2010. Isolation and characterization of plantaricin ASM1: A new bacteriocin produced by *Lactobacillus plantarum* A-1. Int. J. Food Microbiol., 137: 94-99.

Hernandez, D., E. Cardell and V. Zarate, 2005. Antimicrobial activity of lactic acid bacteria isolated from Tenerife cheese: Initial characterization of plantaricin TF711, a bacteriocin-like substance produced by *Lactobacillus plantarum* TF711. J. Applied Microbiol., 99: 77-84.

Holo, H., Z. Jeknic, M. Daeschel, S. Stevanovic and I.F. Nes, 2001. Plantaricin W from *Lactobacillus plantarum* belongs to a new family of two-peptide lantibiotics. Microbiology, 147: 643-651.

Jimenez-Diaz, R., R.M. Rios-Sanchez, M. Desmazeaud, J.L. Ruiz-Barba and J.C. Piard, 1993. Plantaricins S and T, two new bacteriocins produced by *Lactobacillus plantarum* LPCO10 isolated from a green olive fermentation. Applied Environ. Microbiol., 59: 1416-1424.

Kaur, B., P. Balgir, B. Mittu, A. Chauhan and B. Kumar, 2013. Purification and physicochemical characterization of anti-*Gardnerella vaginalis* bacteriocin HV6b produced by *Lactobacillus fermentum* isolate from human vaginal ecosystem. Am. J. Biochem. Mol. Biol., 3: 91-100.

Kumar, R.S. and V. Arul, 2009. Purification and characterization of phocaecin PI80: An anti-listerial bacteriocin produced by *Streptococcus phocae* PI80 Isolated from the gut of *Peneaus indicus* (Indian white shrimp). J. Microbiol. Biotechnol., 19: 1393-1400.

Kumar, R.S., D.R. Varman, P. Kanmani, N. Yuvaraj, K.A. Paari, V. Pattukumar and V. Arul, 2010. Isolation, characterization and identification of a potential probiont from South Indian fermented foods (*Kallappam*, *Koozh* and *Mor Kuzhambu*) and its use as biopreservative. Prob. Antimicrob. Prot., 2: 145-151.

Lash, B.W., T.H. Mysliwiec and H. Gourama, 2005. Detection and partial characterization of a broad-range bacteriocin produced by *Lactobacillus plantarum* (ATCC 8014). Food Microbiol., 22: 199-204.

Marroki, A. and L. Bousmaha-Marroki, 2014. *Lactobacilli* isolated from Algerian goat's milk as adjunct culture in dairy products. Braz. Arch. Biol. Technol., 57: 410-420.

Marroki, A., M. Zuniga, M. Kihal and G. Perez-Martinez, 2011. Characterization of *Lactobacillus* from Algerian goat's milk based on phenotypic, 16S rDNA sequencing and their technological properties. Braz. J. Microbiol., 42: 158-171.

Messi, P., M. Bondi, C. Sabia, R. Battini and G. Manicardi, 2001. Detection and preliminary characterization of a bacteriocin (plantaricin 35d) produced by a *Lactobacillus plantarum* strain. Int. J. Food Microbiol., 64: 193-198.

Morrissey, J.H., 1981. Silver stain for proteins in polyacrylamide gels: A modified procedure with enhanced uniform sensitivity. Anal. Biochem., 117: 307-310.

Nissen-Meyer, J., L.S. Holo, Havarstein, K. Sletten and I.F. Nes, 1992. A novel *Lactococcal* bacteriocin whose activity depends on the complementary action of two peptides. J. Bacteriol., 174: 5686-5692.

Reynolds, P.E., 1989. Structure, biochemistry and mechanism of action of glycopeptide antibiotics. Eur. J. Clin. Microbiol. Infect. Dis., 8: 943-950.

Sahm, D.F. and J.A. Washington, 1991. Antibacterial Susceptibility Tests: Dilution Methods. In: Manual of Clinical Microbiology, Balows, A. (Ed.). 5th Edn., American Society for Microbiology, Washington, DC., USA., ISBN-13: 9781555810290, pp: 1105-1116.

Schagger, H. and G. von Jagow, 1987. Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. Anal. Biochem., 166: 368-379.

Sharaf, E.F. and R.M. Al Harbi, 2011. Isolation, Identification and antimicrobial activity of some local isolates of lactic acid bacteria. Res. J. Microbiol., 6: 826-838.

Suma, K., M.C. Misra and M.C. Varadaraj, 1998. Plantaricin LP84, a broad spectrum heat-stable bacteriocin of *Lactobacillus plantarum* NCIM 2084 produced in a simple glucose broth medium. Int. J. Food Microbiol., 40: 17-25.

Talpur, A.D., A.J. Memon, M.I. Khan, M. Ikhwanuddin, M.M.D. Daniel and A.B. Abol-Munafi, 2012. Isolation and screening of lactic acid bacteria from the gut of blue swimming crab, *P. pelagicus*, an *in vitro* inhibition assay and small scale *in vivo* model for validation of isolates as probiotics. J. Fish. Aquat. Sci., 7: 1-28.

Tiwari, S.K. and S. Srivastava, 2008. Purification and characterization of plantaricin LR14: A novel bacteriocin produced by *Lactobacillus plantarum* LR/14. Applied Microbiol. Biotechnol., 79: 759-767.

Valenzuela, A.S., G.D. Ruiz, N. Ben Omar, H. Abriouel and R.L. Lopez *et al*., 2008. Inhibition of food poisoning and pathogenic bacteria by *Lactobacillus plantarum* strain 2.9 isolated from ben saalga, both in a culture medium and in food. Food Control, 19: 842-848.

Van Reenen, C.A., L.M. Dicks and M.L. Chikindas, 1998. Isolation, purification and partial characterization of plantaricin 423, a bacteriocin produced by *Lactobacillus plantarum*. J. Applied Microbiol., 84: 1131-1137.

Vlkova, E., I. Trojanova and V. Rada, 2006. Distribution of *Bifidobacteria* in the gastrointestinal tract of calves. Folia Microbiologica, 51: 325-328.

Wang, Y., Z. Ahmed, W. Feng, C. Li and S. Song, 2008. Physicochemical properties of exopolysaccharide produced by *Lactobacillus kefiranofaciens* ZW3 isolated from Tibet kefir. Int. J. Biol. Macromol., 43: 283-288.

Xie, Y., H. An, Y. Hao, Q. Qin, Y. Huang, Y. Luo and L. Zhang, 2011. Characterization of an anti-*Listeria* bacteriocin produced by *Lactobacillus plantarum* LB-B1 isolated from koumiss, a traditionally fermented dairy product from China. Food Control, 22: 1027-1031.

Yateem, A., M.T. Balba, T. Al-Surrayai, B. Al-Mutairi and R. Al-Daher, 2008. Isolation of lactic acid bacteria with probiotic potential from camel milk. Int. J. Dairy Sci., 3: 194-199.

Zhang, H., L. Liu, Y. Hao, S. Zhong, H. Liu, T. Han and Y. Xie, 2013. Isolation and partial characterization of a bacteriocin produced by *Lactobacillus plantarum* BM-1 isolated from a traditionally fermented Chinese meat product. Microbiol. Immunol., 57: 746-755.

Zineddine, B.A., M. Anas, H.A. Rizk, H.J. Eddine and K. Mebrouk, 2011. Identification and characterization of functional and technological *Lactobacillus plantarum* strains isolated from raw goat and camel milk collected in Algeria. J. Pure Applied Microbiol., 5: 553-566.