Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2018.093

RESEARCH ARTICLE

Characterization of Bacteriocin like Inhibitory Substances from *Enterococcus ratti* MF183967

Ayesha Riaz[1], Saleha Noureen[1], Muhammad Fiaz Qamar[2], Iram Liaqat[3], Muhammad Arshad[4] and Najma Arshad[1*]

[1]Department of Zoology, University of the Punjab, Quaid-i-Azam Campus, Lahore-54590, Pakistan
[2]University of Veterinary and Animal Sciences, Jhang, Pakistan; [3]Government College University, Lahore
[4]University of the Education, Lower Mall Campus, Lahore- Pakistan
*Corresponding author: najmaarshad@gmail.com; najmaarshad.zool@pu.edu.pu

ARTICLE HISTORY (18-223)

Received: June 24, 2018
Revised: September 06, 2018
Accepted: September 14, 2018
Published online: October 02, 2018

Key words:
Anti-MRSA peptide
Bacteriocin Like Inhibitory Substances
Cell free supernatant
Enterococcus ratti MF183967
Lactic acid bacteria

A B S T R A C T

This study was designed to characterize one Lactic acid bacteria (LAB), *Enterococcus ratti* showing admirable antimicrobial potential. Out of 54 LAB strains isolated from herbivores, cell free supernatant (CFS) of one strain *E. ratti* was active against wide range of pathogens including Methicillin resistant *Staphylococcus aureus* (MRSA), *Bacillus cereus, Clostridium perfringens, Listeria monocytogenes, Escherichia coli, Salmonella enteritidis, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Proteus mirabilis*. PCR amplification of enterocin genes indicated presence of *entL50 A* and *entP*. Time kill assay of foodborne pathogens in the presence of CFS further confirmed its bactericidal property. The antimicrobial component was stable at a wide range of pH (4-10) and temperature (4-100°C). The loss of activity with proteinase k and pepsin treatment indicated its proteinaceous nature. SDS-PAGE analysis confirmed production of short peptides of 20 and 30KDa Bioautography revealed 20kDa fraction was active against MRSA. Scanning Electron Micrograph further indicated that its bactericidal action involves damage to the cell wall. Our findings suggest safe nature of *E. ratti* MF183967 for consumer health.

To Cite This Article: Riaz A, Noureen S, Qamar MF, Liaqat I, Arshad M and Arshad N, 2019. Characterization of bacteriocin like inhibitory substances from *Enterococcus ratti* MF183967. Pak Vet J, 39(1): 1-6. http://dx.doi.org/10.29261/pakvetj/2018.093

INTRODUCTION

Foodborne and food spoilage bacteria are a major threat to the industry (Bitrus *et al*., 2016). Different methods including heating, refrigeration, salting, drying and synthetic chemicals are used to preserve food from spoilage bacteria and extend their shelf life (Gálvez *et al*., 2007). These methods of preservation have several drawbacks and limitations including change in nutritional quality and taste of food (Pereira and Vicente, 2010). Furthermore, the use of chemical preservatives has not been much appreciated due to their undesirable effects. Biopreservation is an alternative tool of increasing the shelf life of food items by using microorganisms and their metabolites (García *et al*., 2010).

Lactic acid bacteria (LAB) are potential candidate to be use for this purpose since these are known to produce antimicrobial metabolites such as lactic acid, acetic acid, ethanol, formic acid, fatty acids, hydrogen peroxide, diacetyl, exopolysaccharides, reuterin, reutericyclin, organic acids, bacteriocin and other enzymes (Cálix-lara *et al*., 2014). Some bacteriocins from LAB, such as nisin and pediocin, are currently used at commercial scale with variable success (Cheng *et al*., 2018).

Lactic acid bacteria (LAB) are cosmopolitan and possess strong broad spectrum antimicrobial activity, however their efficacy is strain and source specific. Extensive data is available on LAB from fermented food and dairy products, however, GIT is less explored site. GIT tract is a potential source of probiotic bacteria as they bacteria line the intestinal lumen and protect it from attachment of pathogenic bacteria, inhibit their growth and provide health benefits to the host (Yu *et al*., 2015; Carvalho *et al*., 2017). The current study was planned to characterize *Enterococcus ratti,* one of our field strain of LAB that displays excellent broad spectrum antimicrobial activity.

MATERIALS AND METHODS

Bacterial strains: The study was conducted on *Enterococcus ratti* MF183967 that was selected from a collection of 54 LAB isolates from herbivores on the basis of admirable broad spectrum antimicrobial activity during initial screening process. *Enterococcus ratti* MF183967 was identified on the basis of biochemical characteristics including gram staining, catalase, oxidase, spore formation and motility along with 16S rRNA gene sequencing. The strain was assigned accession number MF183967 from NCBI-BLAST. *Bacillus cereus,* MRSA (KY698020), *Clostridium perfringens, Listeria monocytogenes* ATCC 19115, *Escherichia coli* ATCC 8739, *Salmonella enteritidis, Pseudomonas aeruginosa, Proteus mirabilis* and *Klebsiella pneumoniae* were used as indicator strains.

Detection of bacteriocin structural genes: Genomic DNA from *E. ratti* was extracted using Qiagen Kit and PCR amplification of the 8 most frequently reported enterocin structural genes was done using specific primers (Table 1). The amplified products were analyzed using gel electrophoresis on 1.5% agarose gel.

Safety assessment of strain: Gelatinase, Catalase, Dnase Coagulase and hemolytic activity tests were performed (Martín *et al*., 2006). The antibiotic resistance profile of *E. ratti* was determined following CLSI standards.

Preparation of cell free supernatant (CFS): *E. ratti* was grown in MRS broth at 150rpm, at 37°C for 48 hours and centrifuged at 10,000rpm for 15min at 4°C to obtain CFS. CFS was neutralized by adjusting its pH at 7±0.02 and sterilized by passing through 0.22µm membrane filter.

Characterization of Cell Free Supernatant (CFS)

a). Inhibitory activity of CFS: Inhibitory activity of CFS was evaluated against indicator pathogens using Agar Well Diffusion Assay (AWDA).

b). Growth Kinetics of Foodborne pathogens with CFS: Pair of glass flasks containing 50ml of nutrient broth was inoculated with 2% of each foodborne pathogen culture (adjusted at OD 1±0.02 $\simeq$ 10^9 CFU/ml) and incubated at 37°C. At exponential phase (3 hours), CFS was added (20% v/v) to one of each pair of flasks. Equal volume of preautoclaved CFS was added to the control flask. Aliquots were taken from each flask and growth was measured at 600nm.

c). Effect of different temperatures and pH on CFS: CFS was incubated at range of temperatures (4-121°C) for 15min and pH (2-11), later on activity was monitored by AWDA.

d). Effect of different enzymes and surfactant on antimicrobial potential of CFS: CFS was treated with different enzymes such as Protinase K, α- amylase, lipase and pepsin at a final concentration of 1mg/ml and incubated for 2 hours at 37°C. Later on, it was heated at 100°C to stop reaction. The effect of surfactant was analyzed by adding EDTA, SDS, Tween 80 and trintone X100 in CFS at a final concentration of 1% and activity was analyzed by AWDA.

Table 1: List of primers used in the study

Gene	F/R	Primer sequence	Product Size (bp)	References
Ent Q	F	GGA ATA AGA GTA GTG GAA TAC TGA AT AG C	653	(De Vuyst *et al*., 2003)
	R	AAA GAC TGC TCT TCC GAG CAG CC		
Ent A	F	GAG ATT TAT CTC CAT AAT CT	542	(Aymerich *et al*., 1996)
	R	GTA CCA CTC ATA GTG GAA		
Ent B	F	GAA AAT GAT CAC AGA ATG CCT A	159	(Toit *et al*., 2000)
	R	GTT GCA TTT AGA GTA TAC ATT TG		
Ent P	F	ATG AGA AAA AAA TTA TTT AGT TT	216	(Gutiérrez *et al*., 2005)
	R	TTA ATG TCC CAT ACC TGC CAA ACC		
Ent L50A	F	CCA TGG GAG CAA TCG CAA AA	135	(Batdorj *et al*., 2006)
	R	AAG CTT AAT GTT TTT TAA TCC ACT CAA T		
Ent L50B	F	ATG GGA GCA ATC GCA AAA TTA	252	(Cintas *et al*., 1998)
	R	TAG CCA TTT TTC AAT TTG ATC		
Ent 31	F	CCT ACG TAT TAC GGA AAT GGT	130	(De Vuyst *et al*., 2003)
	R	GCC ATG TTG TAC CCA ACC ATT		
Ent AS48	F	GAG GAG TAT CAT GGT TAA AGA	339	(De Vuyst *et al*., 2003)
	R	ATA TTG TTA AAT TAC CAA		

Table 2: The antibiotic resistance profile of *Enterococcus ratti*

Sr. #	Antibiotics	Abbre.*	Conc.$^{\beta}$ (µg)	$^{\epsilon}$ Susep.	ZI	CLSI Standards		
						S	I	R
1	Methicillin	MET	5	S	15	≥14	10-13	≤9
2	Penicillin G	P	10	R	0	≥15	-	≤14
3	Chloramphenicol	C	30	R	0	≥18	13-17	≤12
S4	Streptomycin	S	10	S	15	≥10	7-9	6
5	Linezolid	LNZ	10	R	0	≥23	19-22	≤20
6	Oxacillin	OX	01	R	0	≥20	-	-
7	Tetracycline	TE	30	R	12	≥19	15-18	≤14
8	Erythromycin	E	15	R	0	≥23	14-22	≤13
9	Vancomycin	VA	30	S	18	≥17	15-16	≤14
10	Sulbactam	SAM	20	R	0	≥15	12-14	≤13
11	Cefixime	CFM	5	R	9	≥19	16-18	≤15
12	Clindamycin	DA	2	R	12	≥21	15-20	≤14
13	Gentamicin	CN	10	S	14	≥10	7-9	6
14	Sulphamethoxazole	SXT	25	R	0	≥16	11-15	≤10
15	Furazolidone	FR	15	R	0	≥17	15-16	≤14
16	Clarithromycin	CLR	15	R	0	≥18	14-22	≤13

*= Abbreviation ; $^{\beta}$= Concentration; $^{\epsilon}$= Susceptibility; The level of susceptibility to various antibiotics was recorded as resistant (R), intermediate (I) or sensitive (S) according to the Clinical and Laboratory Standards Institute (CLSI) standards (Wayne, 2009).

Partial purification of BLIS by Ammonium Sulphate Precipitation: CFS was subjected to 80% ammonium sulphate precipitation with constant stirring at 4°C. The resulting precipitate was centrifuged at 10,000 rpm for 15min at 4°C and pellet was resuspended in 1 ml of 20mM sodium phosphate buffer (pH 6). Dialysis membrane of 10KDa cut-off was used to desalt the suspension using sodium phosphate buffer with overnight constant stirring at 4°C. Resulting solution was designated as BLIS.

Microdilution assay: Inhibitory activity of BLIS was analyzed by microtiter plate assay and expressed as arbitrary units (AU/ml).

Molecular size determination: BLIS was analyzed for presence of protein by performing Tricine-SDS-PAGE. After electrophoresis, gel was divided in two parts, one part was stained with Coomassie Brilliant Blue R-250, and other part was fixed in 25% Isopropanol and 10% acetic acid for 1 hour. Then it was subjected to bioautography with 10^6 CFU of the MRSA and examined for zone of inhibition (Barboza-Corona *et al*., 2007).

Scanning Electron Microscope (SEM) analysis: The MRSA cells (2×10^7cells/ml) were incubated with 200µg/ml of BLIS for 4 hours at 37°C and examined under SEM (Pattanayaiying *et al*., 2014).

Statistical analysis: The experimental data was analyzed using one way ANOVA.

RESULTS

In current study, antimicrobial metabolite from one of our LAB isolate *E. ratti* was characterized due to its broad spectrum antagonistic activity against clinical and food spoilage bacteria. The strain was Gram positive cocci (0.8-2.2µm), negative for catalase, oxidase, spore formation and motility, facultative anaerobe and didn't show any hemolysis on sheep blood agar. It could tolerate the temperature of 45ºC, 6.2 pH and 2% NaCl concentration. The strain was confirmed by 16s RNA sequencing up to species level. Result of 16s RNA sequencing lead to conclusion that the strain was *Enterococcus ratti* (accession no: MF183967). DNA of *E. ratti* was tested for the presence of 8 frequently reported bacteriocin encoding genes. Under optimized PCR condition with all primers pair resulted in amplification of two fragments generating length of 216 and 135bp indicating the presence of *entP* and *ent*L50A genes in our strain (Fig. 1).

Safety assessment of strain showed negative results for catalase, gelatinase, Dnase, coagulase and oxidase. Further it was non-hemolytic indicating its safe nature. Antimicrobial profile of strain as verified by CLSI standards indicated that it was sensitive to Gentamycin, Methicillin, Streptomycin and Vancomycin and resistant to Penicillin G, Chloramphenicol, Linezolid, Oxacillin, Tetracycline, Erythromycin, Sulbactam, Cefixime, Clindamycin, Sulphamethoxazole, Furazolidone, Clarithromycin (Table 2).

Cell free supernatant of *E. ratti* was tested for its antimicrobial potential against clinical and foodborne pathogenic strains (*S. enteritidis, E. coli* ATCC 8739, *C. perfringens* and *L. monocytogenes* ATCC 19115). The target strains displayed variable zone of inhibition (ZI). CFS showed highest inhibition against MRSA and lowest activity against *P. aeruginosa* (Fig. 2). In general, Gram positive strains exhibited higher ZI as compared to Gram negative. Likewise, pronounced inhibition in growth curves of foodborne pathogens was observed. Overall, 2.5 fold reduction in *S. enteritidis*, 3 fold in *C. perfringens*, 2.4 fold reductions in *E. coli* and 5 fold in *L. monocytogenes*, thus confirming the inhibitory potential of CFS (Fig. 3). One interesting aspect was complete elimination of log phase of pathogenic strains following addition of (20% v/v) CFS of *E. ratti*.

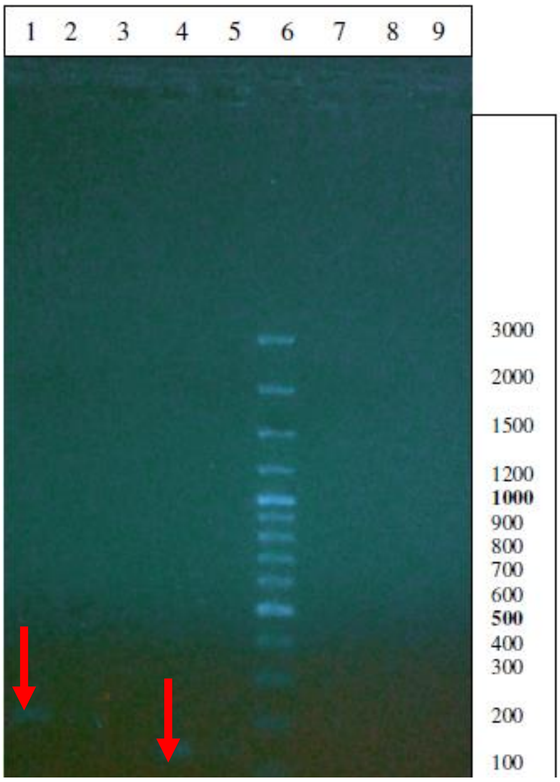

Fig. 1: Detection of bacteriocin genes by PCR amplification in *Enterococcus ratti* MF183967. (Lane 1: *entP*, 2: *entQ*, 3: *entA*, 4: *ent* L50A 5, *entB*, 6: Marker 1000bp 7: *ent L50B* 8: *ent31*, 9: *entAS48*). Out of eight genes, *entp* (216bp) and *ent L50A* (135bp) were amplified.

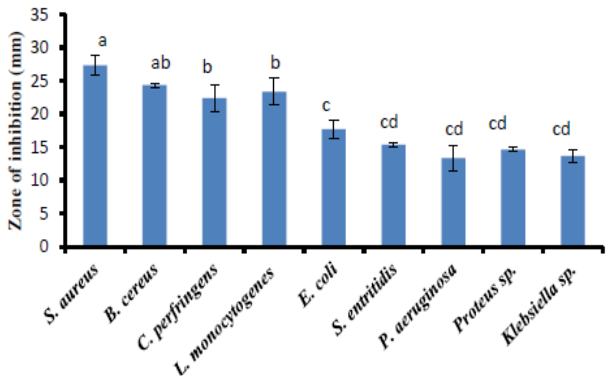

Fig. 2: Comparison of zone of inhibition against indicator pathogens by CFS of *Enterococcus ratti* MF183967. Comparison was made using One way ANOVA followed by DMRT. Bars having no common letters are significantly different from each other at P≤0.05.

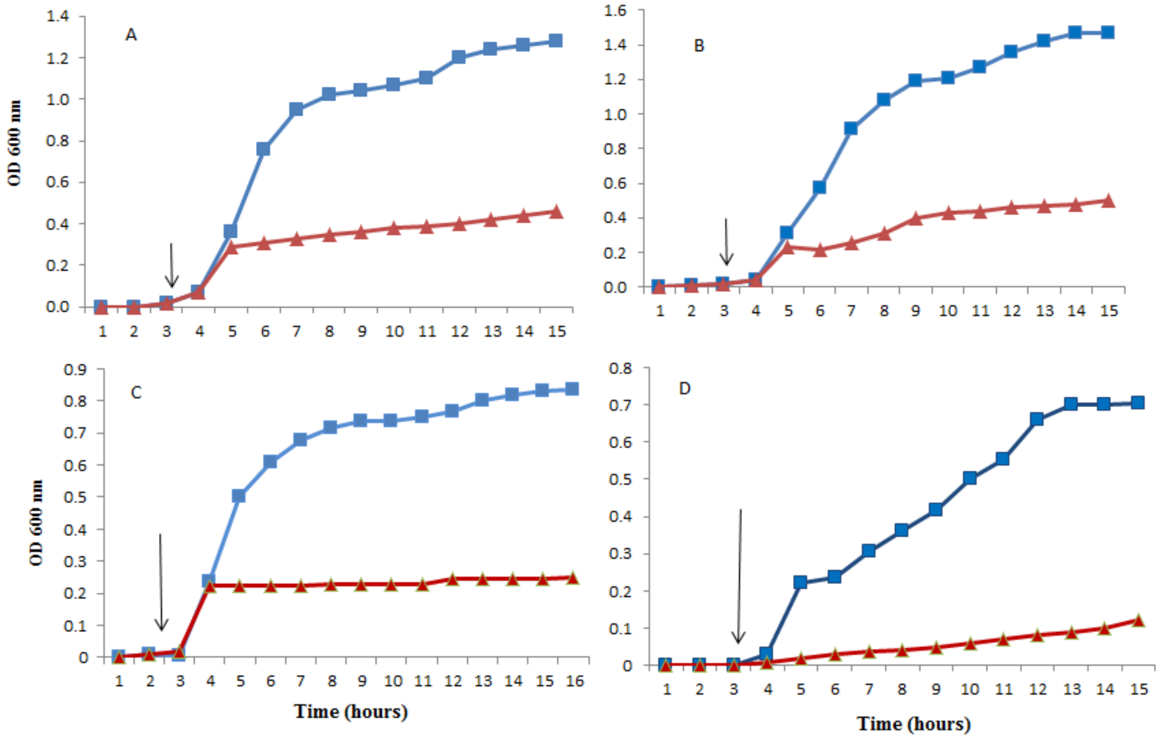

Fig. 3: Growth inhibition of foodborne pathogens in the presence of 20% (v/v) CFS. A: *Salmonella enteritidis* B: *Clostridium perfringens* C: *Escherichia coli* and D: *Listeria monocytogenes* in the absence of CFS ( ) and presence of CFS ( ). 1ml of fresh culture of each target strain was adjusted to OD 1±0.02 corresponding to 10^9 CFU/ml used in the study.

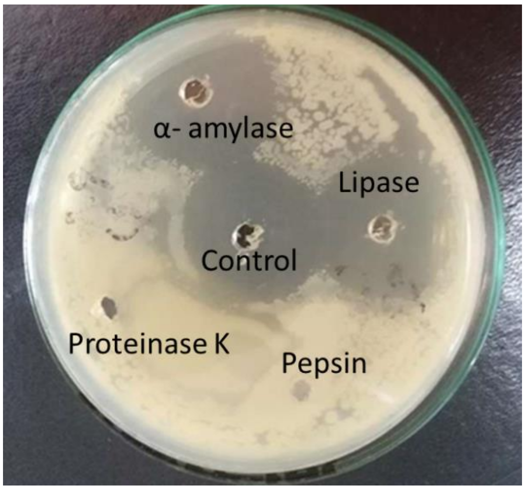

Fig. 4: Effect of different enzymes on the stability of BLIS.

Experiments on chemical nature of CFS indicated the proteinaceous nature of antimicrobial metabolite since its inhibitory activity vanished when it was treated with protinase K and pepsin but remained stable after treatment with α-amylase and lipase (Fig. 4). Stability of antimicrobial metabolite in CFS was determined at different pH and temperatures. Our results showed that antagonistic activity of CFS remains stable at pH (2-10) and temperatures (4-100°C). Among surfactants, SDS and EDTA showed no effect on activity of CFS in our study.

Keeping in mind chemical nature of active metabolite on CFS, it was subjected to ammonium sulphate precipitation. The protein fraction was tested against four strains using microdilution method. The highest reciprocal units of MIC was noticed against MRSA (10240 AU/ml) followed by *E. coli* (3200 AU/ml), *L. monocytogenes* ATCC 19115 (2560 AU/ml), *S. enteritidis (*1280 AU/ml), and *Clostridium perfringens* (640 AU/ml). Owing to inhibitory effect of the protein fraction of *E. ratti* towards above mentioned pathogens, it was named as bacteriocin like inhibitory substance (BLIS). Just like CFS, BLIS was also more active against Gram positive compared to Gram negative bacteria.

In order to identify the active fraction in the BLIS, it was subjected to SDS-PAGE electrophoresis followed by overlay assay. Two proteins of molecular weight of 30 and 20 KDa were observed on the gel. The fraction of 20KDa inhibited the growth of MRSA (Fig. 5). SEM analysis was performed to check the mode of action of BLIS. It was observed that BLIS (200µg/ml) cause roughening and disintegration of cell wall of target cell (Fig. 6).

DISCUSSION

Foodborne pathogens contaminate food and cause detrimental health issues which may lead to mortality. Among food borne pathogens, *L. monocytogenes*, *E. coli*, *C. perfringens* and *S. aureus* are some of the most important pathogens which are responsible for gastroenteritous, abortion, urinary tract infection, meningitis, fatal septicemia and food poisoning in human (Sánchez-Maldonado *et al.*, 2018). Food preservation industry has been practicing different physical, chemical and biological strategies for supplementation of standard preserved food. However, emergence of resistance in pathogens and adverse effect of chemical preservatives has directed researchers to search safer and novel antimicrobials from natural resources. LAB may offer a relief in this scenario. They are known for their probiotic nature and are recognized for production of diverse type of antimicrobial peptides / bacteriocins which may be

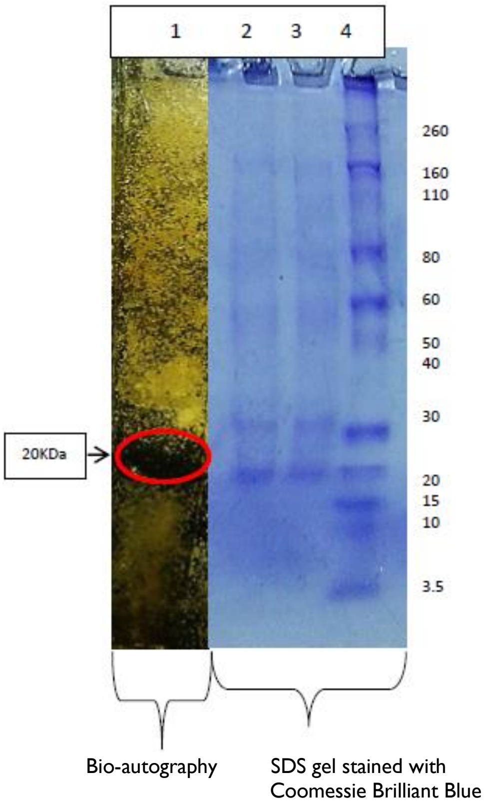

Fig. 5: Inhibitory activity of BLIS on SDS PAGE gel. Gel was divided in two halves. One half was overlaid with *S. aureus* containing 0.2% molten agar. In the lane I peptide band of 20KDa showed zone of inhibition against *Staphylococcus aureus*. Lane 2 &3 show protein band on SDS gel stained with Coomassie Brilliant Blue dye Lane 4 showed standard protein marker for comparison.

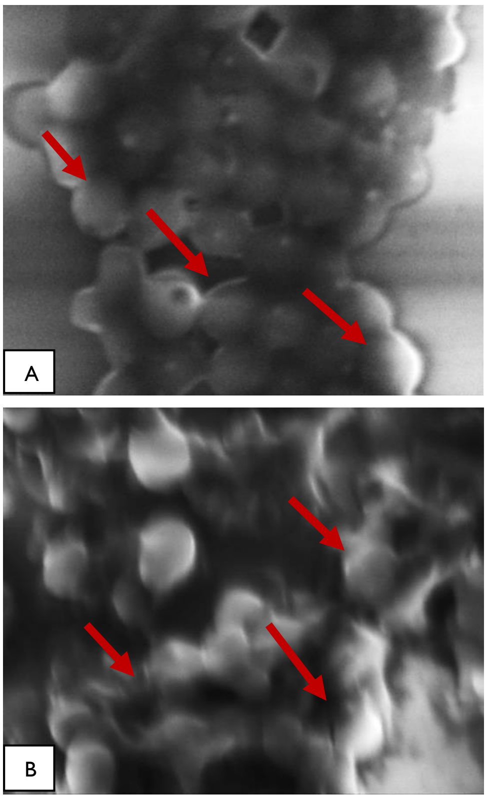

Fig. 6: Bactericidal effect of BLIS on *Staphylococcus aureus*. Scanning electron micrographs of A. Control: Red arrows represent the intact cell surface of *S. aureus,* B: Treated: arrows represent surface roughening and the cell debris accumulation of *S. aureus* cells.

utilized in food industry. While several studies have focused on purification and characterization of bacteriocin like inhibitory substance (BLIS) from other enterococci *viz*., *E. faceium* and *E. fecalis* (Jaouani *et al*., 2014) but to the best of our knowledge, BLIS from *E. ratti* has not been studied so far.

Generally, LAB show the narrow spectrum of antagonistic activity and inhibit only closely related species. Furthermore, their bacteriocin production, spectrum and amounts are also strain specific. However, the activity of CFS from *E. ratti* MF183967 isolated and identified in this study was apparently superior as it could reduce the growth of both gram positive and negative antibiotic resistant bacteria of clinical and food spoilage origin. The type of bacteriocin could be presumed by identifying specific structural genes. The investigation of bacteriocin structural genes revealed presence of two genes *entP* and *ent L50A*. These genes code for class II of enteriocin which are reported to have greater antilisterial activity (Franz *et al*., 2011). These results are similar to other studies who reported other bacteriocin producing genes in LAB from many resources (Jia *et al*., 2017; Borrero *et al*., 2018).

Safety assessment of probiotic strains is considered mandatory for their application in pharmaceutical and food industry. In this study, absence of hemolytic behavior and sensitivity to Methicillin, Vancomycin and Streptomycin was noticed in *E. ratti* suggesting inoffensive nature of our isolate. However, the strain was found to possess antibiotic resistance against other common antibiotics which make its use controversial. Therefore, we decided to characterize the active component for its application in food industry.

CFS is routinely used to primarily screen the antimicrobial capacity of LAB by well diffusion method. Moreover, CFS may contain many molecules, besides those secreted by bacteria i.e., medium components and/or intracellular compounds which may be accidentally released during CFS preparation (Al Kassaa *et al*., 2014). The secretion of antimicrobial metabolites of *E. ratti* was confirmed when CFS was used against clinical and food borne pathogens in agar well diffusion assay and zones of inhibition were noticed against target strains. The greater ZI was recorded against Gram positive compared to gram negative, which might be due to absence of lipopolysaccharide membrane in gram positive strains (Chen *et al*., 2014). Growth kinetic assay of pathogens in the presence of neutralized CFS resulted in complete elimination of log phase of pathogens culminating the complete inhibitory nature of active component in CFS.

Next objective was to find out the nature of the active component, for which, CFS was subjected to different physical and chemical treatments *viz*; temperature, pH, enzymes and surfactants. Treatment with enzymes indicated proteinaceous nature of the active component. Further experiments including monitoring effect of pH and temperature on CSF as well as exposure to surfactant indicated its stable nature. Our data is in agreement with (Hadji-Sfaxi *et al*., 2011) who reported that Bacteriocins are thermostable peptides which resist 121°C and remain active at a wide range of pH. Interestingly, CFS from *E. ratti*, remained active up to 100ºC, but degraded at 121°C.

Industries may have different approach towards application of LAB or their bacteriocins, therefore, an effort was made to purify and identify the active fraction in CFS produced by *E. ratti*. SDS-PAGE analysis of precipitated proteinaceous component revealed two bands having molecular weight of 20 and 30KDa, of which only 20KDa fraction was confirmed to possess active antimicrobial activity. Previously, Goh and Philip (2015) reported the production of a bacteriocin, BaC1 of 10KDa form *E. faecium* C1 through autobiography on SDS-PAGE gel. Different mass of active protein fraction was observed in this study. It might be an indication of different types of peptides in CFS of *E. ratti*.

The final objective was to find the mode of action of BLIS using MRSA as model organism. Bacteriocins can kill target strains by either acting on their cell wall, cell membrane or by interfering with metabolic processes (Alvarez-Sieiro *et al*., 2016). Keeping this in mind, bacterial cell wall integrity was measured as an indicator of cell lysis. SEM analysis of BLIS treated target strain revealed that its antagonistic effect involves lysis of cell wall. Laterosporulin, a bacteriocin from *Brevibacillus* sp. has also been reported to act on cell wall of the target strain (Singh *et al*., 2012).

Previous studies suggest that several bacteriocin have been associated with *Enterococcus* genus (Gao *et al*., 2016; Ogaki *et al*., 2016). However, studies of BLIS production from *E. ratti* remain insufficient. To our knowledge this is the first study of BLIS from *E. ratti* which showed inhibitory activity against pathogenic and food spoilage bacteria. The compounds secreted by *E. ratti* could be used to control food spoilage bacteria. However, N-terminal amino acid sequencing of the active peptide is recommended.

Authors contribution: AR and NA designed the study plan, carried out all experiments and wrote the manuscript. SN helped in experiments and acquisition of data and its analysis. NA supervised the experiments and helped in data analysis and manuscript writing. MFQ, IL and MA helped in interpretation of analyzed data and revising manuscript critically for important intellectual contents. All authors read and approved the manuscript in final version before submitting.

Acknowledgements: We acknowledge Higher Education Commission (HEC) Pakistan for providing financial support vide letter # 112-25549-2BMI-570(50022668) to complete research work of MS Ayesha Riaz. Facilities for experimental work was provided by Department of Zoology, University of the Punjab Lahore, Pakistan.

REFERENCES

Al Kassaa I, Hamze M, Hober D, *et al*., 2014. Identification of vaginal lactobacilli with potential probiotic properties isolated from women in North Lebanon. Microb Ecol 67:722-34.

Alvarez-Sieiro P, Montalbán-López M, Mu D, *et al*., 2016. Bacteriocins of lactic acid bacteria: extending the family. Appl Microbiol Biotechnol 100:2939-51.

Aymerich T, Holo H, Håvarstein LS, *et al*., 1996. Biochemical and genetic characterization of enterocin A from Enterococcus faecium, a new antilisterial bacteriocin in the pediocin family of bacteriocins. Appl Environ Microbiol 62:1676-82.

Barboza-Corona JE, Vázquez-Acosta H, Bideshi DK, *et al*., 2007. Bacteriocin-like inhibitor substances produced by Mexican strains of *Bacillus thuringiensis*. Arch Microbiol 187:117.

Batdorj B, Dalgalarrondo M, Choiset Y, *et al*., 2006. Purification and characterization of two bacteriocins produced by lactic acid bacteria isolated from Mongolian airag. J Appl Microbiol 101:837-48.

Bitrus AA, Zakaria Z, Bejo SK *et al*., 2016. Persistence of antibacterial resistance and virulence gene profile of methicillin resistant *Staphylococcus aureus* (MRSA) isolated from humans and animals. Pak Vet J 36:77-82.

Borrero J, Kelly E, O'Connor PM, *et al*., 2018. Plantaricyclin A, a novel circular bacteriocin produced by *Lactobacillus plantarum* NI326: purification, characterization, and heterologous production. J Appl Environ Microbiol 84:01801-7.

Cálix-lara TF, Rajendran M, Talcott ST, *et al*., 2014. Inhibition of *Escherichia coli* O157: H7 and *Salmonella enterica* on spinach and identification of antimicrobial substances produced by a commercial Lactic Acid Bacteria food safety intervention. Food Microbiol 38:192-200.

Carvalho RD, Do Carmo FL, De Oliveira Junior A, *et al*., 2017. Use of wild type or recombinant lactic acid bacteria as an alternative treatment for gastrointestinal inflammatory diseases: a focus on inflammatory bowel diseases and mucositis. Front Microbiol 8:800-3.

Chen YS, Wang YC, Chow YS, *et al*., 2014. Purification and characterization of plantaricin Y, a novel bacteriocin produced by *Lactobacillus plantarum* 510. Arch Microbiol 196:193-9.

Cheng Q, Shi X, Liu Y, *et al*., 2018. Production of nisin and lactic acid from corn stover through simultaneous saccharification and fermentation. Biotechnol Biotechnol Equip 32:420-6.

Cintas LM, Casaus P, Holo H, *et al*.,1998. Enterocins L50A and L50B, two novel bacteriocins from *Enterococcus faecium* L50, are related to staphylococcal hemolysins. J Bacteriol 180:1988-94.

De Vuyst L, Moreno MF and Revets H, 2003. Screening for enterocins and detection of hemolysin and vancomycin resistance in *Enterococci* of different origins. Int J Food Microbiol 84:299-318.

Franz CM, Huch M, Abriouel H, *et al*., 2011. *Enterococci* as probiotics and their implications in food safety. Int J Food Microbiol 151:125-140.

Gálvez A, Abriouel H, López RL *et al*., 2007. Bacteriocin-based strategies for food biopreservation. Int J Food Microbiol 120:51-70.

Gao Y, Li B, Li D *et al*., 2016. Purification and characteristics of a novel bacteriocin produced by *Enterococcus faecalis* L11 isolated from Chinese traditional fermented cucumber. Biotechnol Lett 38:871-6.

García P, Rodríguez L, Rodríguez A *et al*., 2010. Food biopreservation: promising strategies using bacteriocins, bacteriophages and endolysins. Trends Food Sci Technol 21:373-82.

Goh H and Philip K, 2015. Isolation and mode of action of bacteriocin BacC1 produced by nonpathogenic *Enterococcus faecium* C1. J Dairy Sci 98:5080-90.

Gutiérrez J, Criado R, Citti R, *et al*., 2005. Cloning, production and functional expression of enterocin P, a sec-dependent bacteriocin produced by Enterococcus faecium P13, in *Escherichia coli*. Int J Food Microbiol 103:239-50.

Hadji-Sfaxi I, El-Ghaish S, Ahmadova A, *et al.,* 2011. Antimicrobial activity and safety of use of *Enterococcus faecium* PC4. 1 isolated from Mongol yogurt. Food Control 22:2020-7.

Jaouani I, Abbassi M, Alessandria V, *et al*., 2014. High inhibition of Paenibacillus larvae and *Listeria monocytogenes* by *Enterococcus* isolated from different sources in Tunisia and identification of their bacteriocin genes. Lett Appl Microbiol 59:17-25.

Jia FF, Pang XH, Zhu DQ, *et al*., 2017. Role of the luxS gene in bacteriocin biosynthesis by *Lactobacillus plantarum* KLDS1. 0391: A proteomic analysis. Scientific reports 7:13871.

Martín M, Gutiérrez J, Criado R, *et al*., 2006. Genes encoding bacteriocins and their expression and potential virulence factors of enterococci isolated from wood pigeons (*Columba palumbus*). J Food Protect 69:520-31.

Ogaki MB, Rocha KR, Terra MR, *et al*., 2016. Screening of the Enterocin-encoding genes and antimicrobial activity in *Enterococcus* species. J Microbiol Biotechnol 26:1026-34.

Pattanayaiying R, Aran H and Cutter CN, 2014. Effect of lauric arginate, nisin Z, and a combination against several food-related bacteria. Int J Food Microbiol 188:135-46.

Pereira R and Vicente A, 2010. Environmental impact of novel thermal and non-thermal technologies in food processing. Food Res Int 43:1936-43.

Sánchez-Maldonado AF, Lee A and Farber JM 2018. Methods for the control of foodborne pathogens in low-moisture foods. Annu Rev Food Sci Technol 9:177-208.

Singh PK, Sharma V, Patil PB *et al*., 2012. Identification, purification and characterization of laterosporulin, a novel bacteriocin produced by *Brevibacillus sp.* strain GI-9. PloS one 7:e31498.

Toit MD, Franz C, Dicks L *et al*., 2000. Preliminary characterization of bacteriocins produced by *Enterococcus faecium* and *Enterococcus faecalis* isolated from pig faeces. Appl Microbiol 88:482-94.

Wayne P, 2009. Clinical and Laboratory Standards Institute (CLSI) performance standards for antimicrobial disk diffusion susceptibility tests 19th ed. approved standard. CLSI document M100-S19 29.

Yu H, Yu J, Zhu Y, *et al*., 2015. Comparison of improved effect of antibacterial and antiviral activity of four probiotic *Lactobacillus* expressing porcine lactoferrin in mice. Pak Vet J 35:123-7.