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SCREENING OF ANTILISTERIAL EFFICACY AND PARTIAL PURIFICATION OF CHROMOSOMALLY LOCATED BACTERIOCIN ISOLATED FROM *LACTO-BACILLUS PLANTARUM*

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ABSTRACT

To sustain life and promote good health, there is a need to provide sufficient amounts of safe and nutritious food as it is a basic human necessity. In the last few years, the risks associated with the food borne pathogens have greatly increased. The presence of *Listeria monocytogenes* in fresh and processed foods and listeriosis outbreaks has enforced the need of natural food preservatives. In this study, bacteriocin producing *Lactobacillus plantarum* KIBGE-IB45 was isolated from cheddar cheese. *L. plantarum* KIBGE-IB45 was identified on the basis of morphological, biochemical and 16S rDNA sequence analysis. The isolated strain was screened against several pathogenic strains for its bacteriocinogenecity. Results revealed that bacteriocin (BAC-IB45) possessed broad antibacterial potential against *Listeria monocytogenes* ATCC 7644 and other food borne pathogens. BAC-IB45 was partially purified using gradient precipitation method for further analysis. For the localization of bacteriocin coding gene, plasmid curing technique was performed which revealed that the bacteriocinogenic gene located on bacterial chromosome instead of plasmid. Broad antibacterial potential and antilisterial efficacy of BAC-IB45 suggests its plausible applications in food industry to overcome the unrestrained issues of chemical preservatives, food borne diseases, food quality, safety, shelf life and packaging.

Keywords: Bacteriocin, Cheddar cheese, Food borne pathogens, Gradient precipitation, *Listeria monocytogenes*, plasmid curing technique

INTRODUCTION

Food safety and stability is a matter of global concerned. The past few years have witnessed an increase in contamination of food by various food borne pathogens. Most of the pathogens responsible for > 90% of food related deaths are *Bacillus cereus*, *Listeria monocytogenes*, *Escherichia coli O157:H7*, *Staphylococcus aureus*, *Campylobacter* and *Salmonella* species (Scallan *et al.*, 2011; Van Cauteren *et al.*, 2017). *Listeria monocytogenes* is one of the most virulent food borne pathogen that causes a severe disease called listeriosis. The ability of the *L. monocytogenes* to survive and proliferate during storage of foodstuffs at refrigerated temperatures make it a focus of interest and an unrestrained issue in the industrialized world. Therefore, it is of great significance to develop some strategies to prevent the growth of *L. monocytogenes* for food safety and human health.

Lactic acid bacteria (LAB) are industrially important food grade bacteria that recognized for their nutritional, fermentative and health benefits (Sharma *et al*., 2006). LAB are ubiquitous in nature and are frequently isolated from nutrient rich habitats which include fermented products such as fruit, vegetables, milk, meat and dairy products. The applications of LAB and their metabolites in food and pharmaceutical industries have generated great interest towards their isolation and identification (Cleveland *et al.*, 2001; Kanmani *et al.*, 2013). LAB comprises of diverse genera including *Streptococcus, Pediococcus, Leuconostoc, Lactococcus, Weissella, Oenococcus, Sporolactobacillus, Teragenococcus, Vagococcus, Lactobacillus, Aerococcus, Carnobacterium* and *Enterococcus* (Jay *et al.*, 2008). Among these genera, species from *Lactobacillus* can produce a great variety of antimicrobial substances. These substances play a crucial role in bacterial interactions, among them bacteriocins are highly specific, natural and efficient antagonists (Yang *et al.*, 2014). Bacteriocins are ribosomally synthesized natural antimicrobial peptides produced by large number of bacteria. The genes responsible for the production and immunity of bacteriocin are generally clustered and located on plasmids, chromosome and/or transposons with minimum genetic machinery. Most of the LAB strains harbored plasmid-borne genetic determinants for production and immunity to bacteriocins (Klaenhammer, 1993). However, presence of bacteriocin producing gene on the

chromosome of some bacteria have also been reported (Banerjee *et al.,* 2013). The presence of bacteriocinogenic gene on the chromosome of any bacterium is an important character as it provides genome stability during curing experiments and /or in the absence of plasmid DNA.

The objective of current study was to isolate and screen *Lactobacillus* species from fermented food product capable of producing bacteriocin with broad antibacterial potential against food borne pathogens especially *Listeria monocytogenes.* Moreover, bacteriocin is partially purify and localization of its genetic determinant will be carried out using plasmid curing technique.

MATERIALS AND METHODS

Sample collection and strain isolation

For the isolation of lactic acid bacteria (LAB), ten indigenous samples including decayed fruits (Mango and Peach), vegetable (Cabbage) and a dairy product (Raw milk, Yogurt, Cheddar cheese, Cheese slice, Meat, Chicken meat and skin) were collected from local market of Karachi, Pakistan. After collection, samples were processed and the serial dilutions were prepared from 10^{-1} to 10^{-6}. From each dilution, 0.1 mL was spread on MRS (DeMan Rogosa Sharpe) agar plate and incubated at 37 ºC for 24 h.

Screening of bacteriocin producing strain

All the selected isolates were screened for bacteriocin production using stab and overlay and cross and streak methods (Hanlin *et al.,* 1993). Stab and overlay method was used initially to screen multiple producer strains against *Listeria monocytogenes* ATCC 7644. Afterwards, the producer strains which showed maximum antibacterial activity against *L. monocytogenes* were selected and screened against several indicator strains using cross and streak method.

Taxonomic characterization of the bacterial strains

Phenotypic characterization

Bergey's manual of determinative bacteriology was used to identify the bacterial cultures (Holt *et al.,* 1994). For preliminary identification, the bacterial isolates were subjected to various biochemical tests including salt tolerance test, sugar fermentation, catalase, oxidase, nitrate reduction, glycerol test, starch and casein hydrolysis.

Genotypic characterization

16S rDNA sequence analysis was performed to further characterize and confirm the bacterial strain as stated by Ansari *et al.* (2012). Briefly, genomic DNA was extracted using DNA extraction kit (Promega, USA). For amplification, polymerase chain reaction (PCR) of 16S rDNA was performed using universal primer set as 16SF 5´-GAGTTTGATCCTGGCTCAG-3´ and 16SR 3´-AGAAAGGAGGTGATCCAGCC-5´. The amplified DNA was analyzed using agarose gel electrophoresis and purified through PCR purification kit (Promega, USA). The purified products were then sequenced and the sequence similarity was assessed using BLAST (GenBank, http://www.ncbi.nlm.nih.gov/blast/). Sequences were aligned and phylogenetic tree was constructed using neighbour joining method with Mega software (version 7.0.1).

Bacteriocin production

Bacteriocin production was carried out in MRS medium (10.0 g peptone, 8.0 g lab-lemco' powder, 4.0 g yeast extract, 20.0 g glucose, 1.0 mL Tween 80, 2.0 g di-potassium hydrogen phosphate, 5.0 g sodium acetate $3H_2O$, 2.0 g tri-ammonium citrate, 0.2 g magnesium sulphate.$7H_2O$ and 0.05 g manganese sulphate $4H_2O$/L of distilled water, pH 6.2 ± 0.2) (Oxoid). Inoculum (100 mL) was grown in the MRS medium at 35ºC for 24 hours and was transferred into 900 ml of MRS medium and incubated at 35 ºC for further 24 hours with an agitation of 135 rpm. Cells were harvested by centrifugation at 14000 *x g* for 15 minutes at 4 ºC. Cell free supernatant (CFS) was filtered through 0.22 µm syringe filters (Millipore, USA) and adjusted to pH 6.0 by sterilized 1N NaOH to rule out the effect of organic acids. To eradicate the inhibitory effect of hydrogen peroxide neutralized CFS was treated with 1 mg mL^{-1} of catalase (Sigma-Aldrich Corporation, USA) at 25 ºC for 30 minutes. Afterwards, the antibacterial activity of neutralized CFS was determined using agar well diffusion assay.

Determination of inhibitory spectrum

The antibacterial potential of bacteriocin was determined against various food borne pathogens including Methicillin resistant *Staphylococcus aureus* KC465400], *Bacillus cereus* ATCC 11778, *Pseudomonas aeruginosa*

and *Listeria monocytogenes* ATCC 7644 using agar well diffusion assay. Briefly, nutrient agar plates were spreaded with the log phase culture of indicator strains (10^5 CFU/mL). After 30 min, wells of 5 mm were prepared and 100 µL of partially purified BAC-IB45 was added and the plates were incubated at 37 ºC for 24 h. After incubation zones of inhibition were measured in millimeters (mm).

Partial purification of bacteriocin

Neutralized cell free supernatant was partially purified using gradient precipitation method. For this 20 to 80 % saturation of ammonium sulphate was used. The mixture was placed at 4 ºC for 18 hours for equilibration. Precipitates were collected by centrifugation at 35060 *x g* for 30 minutes at 4 ºC and dissolved in 50mM sodium acetate buffer of pH 6.0. After desalting through dialysis tubing (Cut off 2.0 kDa, Sigma), dialyzed sample was filter sterilized using 0.22 µm membrane filter (Millipore, USA) and the activity of partially purified bacteriocin was measured using agar well diffusion assay. This partially purified bacteriocin was designated as BAC-IB45 and stored at -20 ºC for further experiments (Ansari *et al*., 2018).

Localization of bacteriocin positive marker (Bac^+)

Plasmid curing

To determine the location of bacteriocin coding genes, a plasmid curing assay was performed. For this purpose, two different intercalating agents including ethyl methanesulfonate (EMS) and acridine orange (AO) were used. Bacteriocin producing strain was grown at 35 ºC for 24 h. The logarithmic phase culture was further transferred in several MRS broth tubes (2.0 mL) containing EMS and acridine orange separately in a concentration ranging from 0.025 to 1.0 mg mL^{-1} and incubated at 35 ºC for 16 h. Producer strain without any curing agent used as a control. After incubation, the tubes with the lowest concentration of the curing agents with growth of the producer strain were selected and further diluted from 10^{-1} to 10^{-4}. The isolated colonies were replicated and then analyzed for the bacteriocinogenesis using overlay method. The nutrient agar plates containing isolated colonies of producer strain were overlaid with 5 ml nutrient soft agar containing 0.1 mL of standardized inoculum of the indicator strain (10^5 CFU mL^{-1}). Plates were re-incubated at 35 ºC for further 24 h to observe a clear zone of inhibition around the producer strains treated for plasmid curing (Hanlin *et al*., 1993).

Plasmid extraction

For the confirmation of curing technique, the plasmid was isolated from both treated and untreated producer cells. Overnight culture was centrifuged at 35060 *x g* for 5 minutes. Cell pellet was suspended in 100 µL of suspension buffer (gL^{-1}: Tris-HCl, 3.8; EDTA, 3.7; glucose, 9.0) and mixed thoroughly. In this tube 150 µL of denaturation solution (gL^{-1}: NaOH, 8.0; SDS, 10.0) was than incorporated and mixed gently by inverting several time and kept at 30 ºC for 5 minutes. In the next step 200 µL of neutralization solution (potassium acetate, 5.0 M; glacial acetic acid, 11.5 mL) was added and again kept at 30 ºC for 10 minutes. After incubation, it was centrifuged at 35060 *x g* for 5 minutes and the supernatant was separated in a fresh tube. Absolute ethanol was added up to 2.5 volumes and centrifuged at 35060 *x g* for 10 minutes and the tube was kept at -20 ºC for 20 minutes. The supernatant was discarded and the pellet was washed with 200 µL of 70 % ethanol. The tube was spin for 1 minutes and the supernatant was again discarded whereas, the pellet containing the DNA was vacuum dried and stored at -20 ºC for further analysis. The extracted plasmids DNA were subjected to agarose gel electrophoresis and visualized in gel documentation system under UV trans illuminator (Ansari *et al*., 2015).

Statistical analysis

All the experiments were performed in triplicates and the data is presented as mean ± standard deviation of three observations.

RESULTS AND DISCUSSION

Isolation of bacteriocin producing strains

Lactic acid bacteria (LAB) constitutes a diverse group of organisms and gained extensive attention due to their GRAS status and antimicrobial properties particularly in the food industry. They are frequently isolated from fermented foods. In the current study, forty (40) LAB strains were isolated from different fermented products. Among all these, only eleven (11) strains showed bacteriocin production against tested indicator strains.

Identification and screening of producer strains

The LAB isolates were initially identified on the basis of their morphological and colonial characteristics (Table 1). Biochemical characterization was also performed. All bacterial strains ferment sugars with the production of acid only. The isolates showed negative results for catalase, oxidase, gelatin, casein hydrolysis, nitrate reduction, starch hydrolysis, methyl red tests which is represented in (Table 2). All the isolated strains from the fermented foods fall in the classification of LAB as Gram's positive, oxidase and catalase negative (Ishola and Adebay Tayo, 2012). Preliminary characterization confirmed the genus *Lactobacillus.* The identified isolates were screened for bacteriocin production. Among eleven producer strains, FI-5 and FI-10 exhibited maximum bacteriocin production against *L. monocytogenes* ATCC 7644 (Fig. **1**). Therefore, these strains were selected for further genotypic analysis. After 16S rDNA sequence analysis FI-5 was confirmed as *Lactobacillus curvatus* KIBGE-IB44 [MG814033] whereas, FI-10 was confirmed as *Lactobacillus plantarum* KIBGE-IB45 [MG814034]. The phylogenetic tree was constructed and it showed that *L. plantarum* KIBGE-IB45 and *L. curvatus* KIBGE-IB44 fits into a distinct clade and share maximum identity with other *Lactobacillus* species (Fig. **2**).

Table 1. Taxonomic characterization of the lactic acid bacterial species isolated from fermented foods.

		Taxonomic Characteristics		
Bacterial strain ID	**Sources**	**Colonial characteristics**	**Cell morphology**	**Microscopic characteristics**
FI-1	Cheese Slice	white, irregular margin	Gram positive	coccobacilli, chains
FI-2	Cabbage	white, pinpointed, smooth margin	Gram positive	cocci, chains
FI-3	Raw milk	white, medium, concave, mucoid smooth margin	Gram positive	cocci, chains
FI-4	Cheddar Cheese	grey, concave, mucoid, smooth margin	Gram positive	short rods, scattered
FI-5	Yogurt	white, concave, mucoid, smooth margin	Gram positive	coccobacilli, scattered
FI-6	Meat	white, small, concave, mucoid, smooth margin	Gram positive	rods, chains
FI-7	Peach	yellow, small, concave, mucoid smooth margin	Gram positive	coccobacilli, chains
FI-8	Chicken meat	grey white, concave, mucoid, smooth margin	Gram positive	cocci, scattered
FI-9	Raw milk	yellow, large, concave, mucoid smooth margin	Gram positive	short rods, scattered
FI-10	Cheddar Cheese	white, medium, smooth margin, mucoid	Gram positive	short rods, scattered
FI-11	Chicken skin	transparent, small, concave, smooth margin	Gram positive	coccobacilli, chains

Antibacterial potential of producer strains

After confirmation, antibacterial spectrum of the producer strains was determined against various food borne pathogens including Methicillin resistant *Staphylococcus aureus* [KC465400], *Bacillus cereus* ATCC 11778, *Pseudomonas aeruginosa* and *Listeria monocytogenes* ATCC 7644. Results revealed that bacteriocin produced by *L. plantarum* KIBGE-IB45 was effective against various food borne pathogens as compared to *Lactobacillus curvatus* KIBGE-IB44 (Fig. **3**). Therefore, in the current study *L. plantarum* KIBGE-IB45 was selected for the production of bacteriocin and *L. monocytogenes* ATCC 7644 as an indicator strain as the aim of study was the screening of antilisterial bacteriocin producing *Lactobacillus* species. *L. monocytogenes* ATCC 7644 is the most common food borne pathogen. This formidable food borne pathogen causes listeriosis. It is a life-threatening disease which results in high mortality rate. However, there is a need to establish some natural strategies to eradicate and overcome the deterioration caused by *L. monocytogenes* (Li *et al.,* 2016).

Production and partial purification of BAC-IB45

L. plantarum KIBGE-IB45 was grown in MRS medium for the production of bacteriocin. MRS is an enriched medium containing different complex nitrogen and carbon sources. Maximum bacteriocin production was achieved in MRS broth. Yang *et al*. (2018) also reported MRS broth for the production of bacteriocin from lactic acid bacteria. It is important to use an appropriate medium for the production of bacteriocin as production strongly dependent on medium composition. After production BAC-IB45 was partially purified using ammonium sulphate gradient precipitation method. Results revealed that maximum bacteriocin activity was achieved when the CFS was saturated with 80 % ammonium sulphate as bacteriocin are low molecular weight proteins therefore maximum bacteriocin was precipitated out at 80 % saturation of ammonium sulphate. Banerjee *et al*. (2013) also reported the precipitation of bacteriocin at 80 % saturation of ammonium sulphate. After precipitation, dialysis was performed and the desalted precipitates were further analyzed for antibacterial activity.

Table 2. Biochemical characterization of lactic acid bacterial isolates.

Biochemical Tests	**LAB Isolates**		**Sugars**	**Sugar Fermentation**	
	FI-5	**FI-10**		**FI-5**	**FI-10**
Catalase	-	-	Glucose	+	+
Oxidase	-	-	Maltose	+	+
Glycerol	+	+	Mannitol	+	+
Methyl Red	-	-	Lactose	+	+
Voges Proskauer	+	+	Fructose	+	+
Casein	-	-	Sucrose	+	+
Starch	-	-	Arabinose	+	+
Salt tolerance (10%)	-	+	Xylose	+	+
Nitrate	-	-	Sorbitol	+	+

+ = Positive, - = Negative

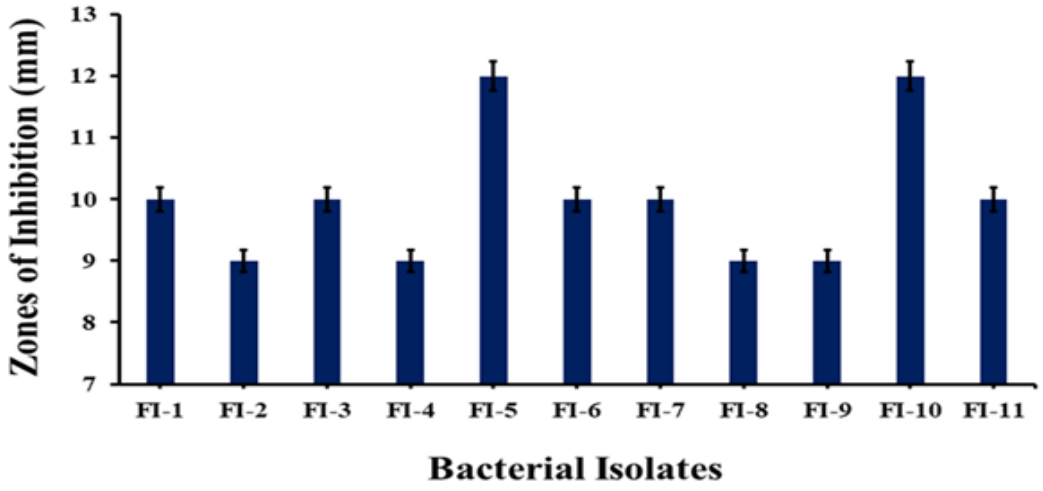

Fig. 1**.** Bacteriocin production by different lactic acid bacteria against *Listeria monocytogenes* ATCC 7644.

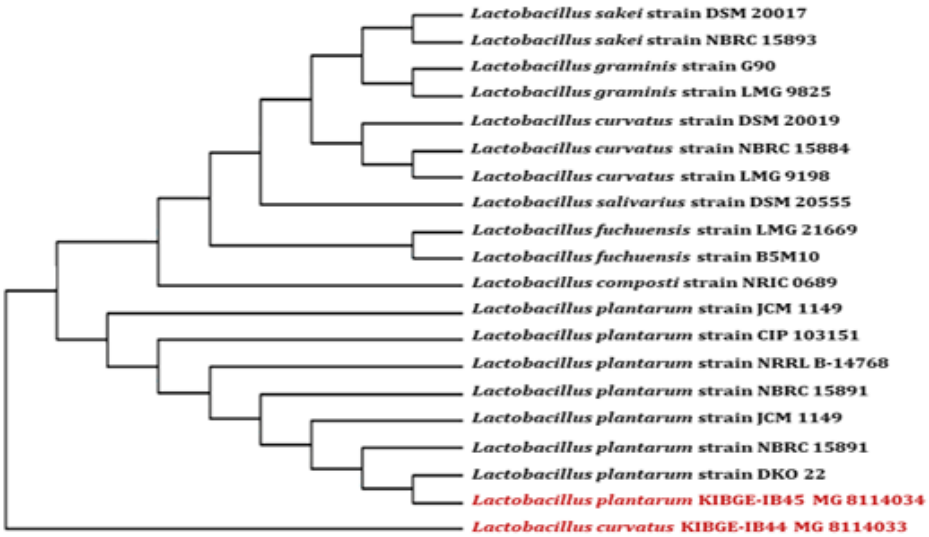

Fig. 2. Phylogenetic relations of *Lactobacillus plantarum* KIBGE-IB45 (FI-10) and *Lactobacillus curvatus* KIBGE-IB44 (FI-5) with other related species of lactic acid bacteria.

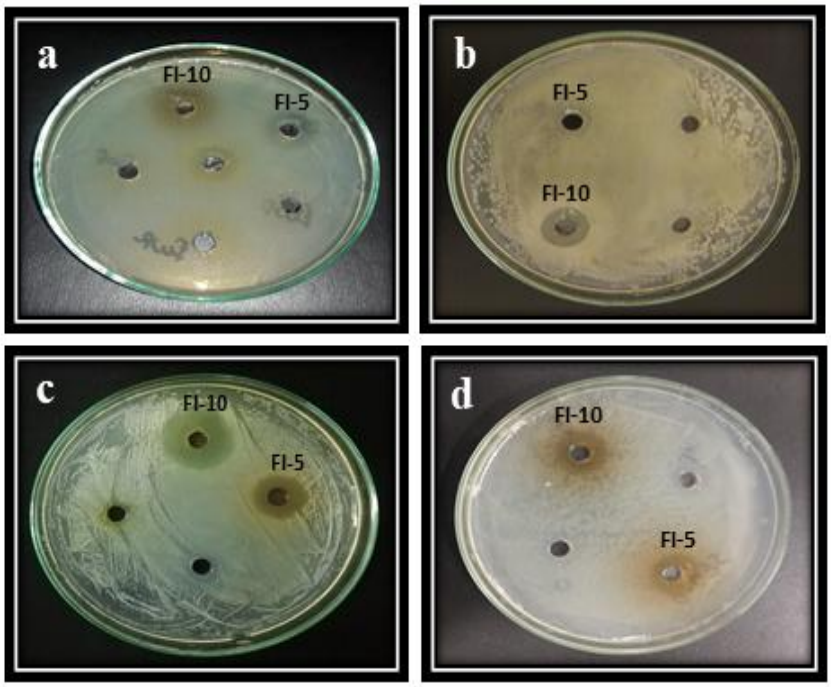

Fig. 3**.** Antibacterial spectrum of *Lactobacillus plantarum* (FI-10) and *Lactobacillus curvatus* (FI-5) against different indicator strains: a) Methicillin resistant *Staphylococcus aureus* [KC465400], b) *Bacillus cereus* ATCC 11778, c) *Pseudomonas aeruginosa*, d) *Listeria monocytogenes* ATCC 7644.

Localization of bacteriocin positive marker (Bac$^+$)

Bacteriocinogenic genes responsible for the production of bacteriocin by *L. plantarum* KIBGE IB-45 was localized using plasmid curing technique. It was observed that only the treatment with acridine orange (0.25 mg mL^{-1}) resulted in curing of the plasmid from the producer strain while, ethyl methanesulfonate inhibited the growth of producer strain even at low concentration (0.025 mg ml^{-1}). Initially, 424 colonies were obtained after curing which showed that approximately 53.0 % of bacterial colonies were cured. Agarose gel electrophoresis further confirmed that uncured colonies contained plasmid as compared to the cured colonies which have lost their plasmid after the treatment with acridine orange (Fig. **4**). All of the cured and uncured colonies showed the zone of inhibition against the indicator strain which clearly indicated that the gene responsible for the bacteriocinogenecity in KIBGE-IB45

was located on the chromosome because after removal of plasmid from *L. plantarum*, still its property to produce bacteriocin was stable. Similarly, Diep *et al.*, (1994) reported that the structural gene of plantaricin A was located on the chromosome. Moreover, there are also some bacteriocins reported such as pneumocin, bacteriocin 28b (Mindich, 1966; Guasch *et al.*, 1995) and other bacteriocins produced by *Lactobacillus brevis* NM 24 and *Lactobacillus fermentum* NM 332 have their genes located on chromosomes (Mojgani *et al.*, 2009). On the other hand, plasmid borne bacteriocins were also reported by some other bacteria such as *Lactobacillus curvatus* CWBI-B28 and *Bacillus subtilis* KIBGE-IB17 (Ghalfi *et al.*, 2009; Ansari *et al.*, 2015).

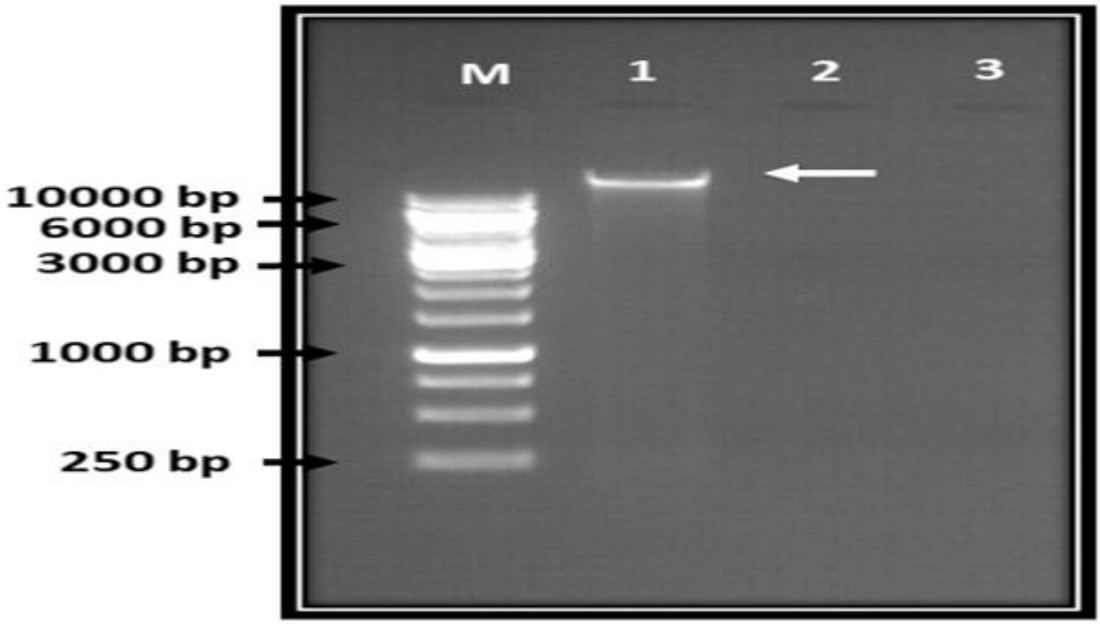

Fig. 4. Agarose gel electrophoresis showing curing of plasmid. Lane M: DNA ladder (1.0 kb), Lane 1: Parental cells of the *lactobacillus plantarum* KIBGE-IB45, Lane 2 and 3: Cured cells.

Conclusions

Lactic acid bacteria and their bacteriocins have emerged as a great alternatives to chemical preservatives and traditional antibiotics in the fields of food science and technology. In the present study, BAC-IB45 produced by *Lactobacillus plantarum* KIBGE-IB45 was isolated from cheddar cheese and exhibited antilisterial potential. The gene responsible for BAC-IB45 was localized on chromosome. The broad inhibitory spectrum of BAC-IB45 against several food borne pathogens especially *L. monocytogenes* have enforced its application to resolve the uncontrolled issues of food industries.

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REFERENCES

Ansari, A., S. Pervez, U. Javed, M.I. Abro, M.A. Nawaz, S.A.U. Qader and A. Aman (2018). Characterization and interplay of bacteriocin and exopolysaccharide mediated silver nanoparticles as an antibacterial agent. *International Journal of Biological Macromolecules*, 115: 643-650.

Ansari, A., A. Aman, N.N. Siddiqui, S. Iqbal and S.A. Ul Qader (2012). Bacteriocin (BAC-IB17): Screening, isolation and production from *Bacillus subtilis* KIBGE IB-17. *Pakistan Journal of Pharmaceutical Sciences*, 25: 195-201.

Ansari, A., N.N. Siddiqui, M. Ghani, A. Aman and S.A. Ul Qader (2015). Plasmid borne BAC-IB17: Localization of a potential antibacterial positive marker (Bac+) encoded broad inhibitory spectrum bacteriocin. *Pakistan Journal of Pharmaceutical Science*, 28: 1331-5.

Banerjee, S.P., K.C. Dora and S. Chowdhury (2013). Detection, partial purification and characterization of bacteriocin produced by Lactobacillus brevis FPTLB3 isolated from freshwater fish: Bacteriocin from Lb. brevis FPTLB3. *Journal of Food Science and Technology*, 50: 17-25.

Cleveland, J., T.J. Montville, I.F. Nes and M.L. Chikindas (2001). Bacteriocins: Safe, natural antimicrobials for food preservation. *International Journal of Food Microbiology*, 71: 1-20.

Diep, D.B., L.S. Havarstein, J. Nissen-Meyer and I.F. Nes (1994). The gene encoding plantaricin A, a bacteriocin from Lactobacillus plantarum C11, is located on the same transcription unit as an agr-like regulatory system. *Applied Environmental Microbiology*, 60: 160-6.

Ghalfi, H., N. Benkerroum, M. Ongena and J. Van Beeumen (2009). Purification and characterization of three novel plasmid-born bacteriocins (curvalicins 28) produced by *Lactobacillus curvatus* CWBI-B28. *Antonie van Leeuwenhoek*, 96: 361.

Guasch, J., J. Enfedaque, S. Ferrer, D. Gargallo and M. Regue (1995). Bacteriocin 28b, a chromosomally encoded bacteriocin produced by most Serratia marcesens biotypes. *Research Microbiology,* 146: 477-483.

Hanlin, M.B., N. Kalchayanad, P. Ray and B. Ray (1993). Bacteriocins of lactic acid bacteria in combination have greater antibacterial activity. *Journal of Food Protection*, 56: 252-255.

Holt, J.G., N.R. Krieg, P.H. Sneath, J.T. Staley and S.T. William (1994). Bergey's manual determinative bacteriology. In: Williams, Wilkins A. editors. Baltimore: Wavley Company. 787.

Ishola, R. and B. Adebayo-Tayo (2012). Screening of Lactic Acid Bacteria Isolated from Fermented Food for Bio-molecules Production. *AU Journal of Technology,* 15: 205-217.

Jay, J.M., M.J. Loessner and D.A. Golden (2008). *Modern food microbiology* 7th edition. Newyork, Springer

Kanmani, P., R. Satish Kumar and N. Yuvaraj (2013). Probiotics and Its Functionally Valuable Products-A Review. Crit. Rev. *Food Science and Nutrition,* 53: 641-58.

Klaenhammer, T.R., (1993). Genetics of bacteriocins produced by lactic acid bacteria. *FEMS Microbiology Reviews*, 12: 39-86.

Li, S., G. Chen, M. Wu, J. Zhang and S. Wu (2016). Restraining of reactive oxygen species promotes invasion of Listeria monocytogenes into glia cells. *FEMS Microbiology Letters*, 363

Mindich, L., (1966). Bacteriocins of Diplococcus pneumoniae. I. Antagonistic relationships and genetic transformations. *Journal of Bacteriology,* 92: 1090-1098.

Mojgani, N., G. Sabiri, M.P. Ashtiani and M.A.K. Torshizi (2009). Characterization of bacteriocins produced by *Lactobacillus brevis* NM 24 and *Lactobacillus fermentum* NM 332 isolated from green olives in Iran. *International Journal of Microbiology,* 6: 1-9.

Scallan, E., R.M. Hoekstra and F.J. Angulo (2011). Foodborne illness acquired in the United States-Major pathogens. *Emerging Infectious Disease*, 17: 7–15.

Sharma, N., G. Kapoor and B. Neopaney (2006). Characterization of a new bacteriocin produced from a novel isolated strain of Bacillus lentus NG121. *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology,* 89: 337-43.

Van Cauteren, D., Y. Le Strat and C. Sommen (2017). Estimated annual numbers of foodborne pathogen–Associated illnesses, hospitalizations, and deaths, France, 2008–2013. *Emerging Infectious Disease*, 23: 1486-1492.

Yang, E., L. Fan, J. Yan, Y. Jiang, C. Doucette, S. Fillmore and B. Walker (2018). Influence of culture media, pH and temperature on growth and bacteriocin production of bacteriocinogenic lactic acid bacteria. *AMB Express,* 8: 10-23.

Yang, S.C., C.H. Lin, C.T. Sung and J.Y. Fang (2014). Antibacterial activities of bacteriocins: Application in foods and pharmaceuticals. *Frontiers in Microbiology*, 5: 241.

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