Plasmid encoded bacteriocin transformation studies in A*lcaligenes* and B*revundimonas* sp.

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Bacteriocins have attracted much attention due to their potential use as antibacterial agents for the treatment of infections, preservation of food and animal feed. A total of five bacterial strains (AZS, A2, P, U and LB) showing high bacteriocin activity against the target organism were screened for further studies. The stability of the crude extract of bacteriocin to high temperature 121°C, Proteinase K and Ultraviolet radiation indicated that these compounds could be a better option for utilization as a probiotics. The Sodium dodecylsulfate polyacrylamide gel electrophoresis analysis indicated that bacteriocin from both *Alcaligenes* (U) and *Brevundimonas* (A2) genera were of low molecular weight (10 to 25 kDa) whereas the molecular weight of *Alcaligenes* (U) strains was approximately 40 kDa**.** The bioactivities of the polyacralamide gel bands were also observed. The result of transformation studies revealed that bacteriocin producing gene was plasmid encoded in case of *Alcaligenes* (U) and *Brevundimonas*(A2).

Keywords: Bacteriocin; Plasmid; Transformation; Sodium dodecylsulfate polyacrylamide gel electrophoresis

1. Introduction

Microorganisms produce a large array of metabolic compound for their defense. These include classical antibiotics, metabolic by-products, lytic agents, numerous type of exotoxins and the antimicrobial peptide. Bacteriocins range from small peptides produced mainly by Gram-positive bacteria [1] to high-molecular weight complexes that resemble defective bacteriophages, such as the R- and F-type pyocins of *Pseudomonas aeruginosa* [2] Bacteriocins are ribosomally synthesized, generally cationic, have less than 100 amino acid residues [3] and contain a substantial portion (30% or more) of hydrophobic residues [4]. Many bacteriocins have a narrow host range, and is likely to be most effective against related bacteria with nutritive demands for the same scarce resources [5]. However, some bacteriocins exhibit a much broader spectrum of antimicrobial activity and may extend beyond the borders of bacteria to include protozoa, yeast, fungi and viruses. A few bacteriocins are cytotoxic, with activity against sperm and tumour cells [6]. One example of this second type is nisin [7] which is produced by certain strains of *Lactococcus lactis* subsp. *Lactis.* Other is pediocin, produced by *Pedicoccus pentosaceus.* Different mechanisms of action have been proposed for bacteriocins: alteration of enzymatic activity, inhibition of spore germination and inactivation of anionic carriers through the formation of selective and non-selective pores [8]. Both groups act on sensitive cells by targeting either the inner membrane by pore formation or an intracellular target using enzymatic activity such as DNAse or RNAse [9]. Genes encoding the biosynthesis of bacteriocins are organized in clusters located on either the chromosome, plasmids or on possibly both if located on a transposon [10]. Some studies of characterization of bacteriocins show that these molecules can be active under certain ranges of temperature and pH. Sensibility to proteolytic enzymes evidences the proteinaceous characteristic of bacteriocins [11]. No decrease in antibacterial activity was recorded after 90 min at 100ºC or 20 min at 121ºC [12]. The thermotolerance feature might be related to the molecular structure of the bacteriocin, usually composed by small peptides without tertiary structure. The antagonistic activity was detected through the well diffusion assay on agar plates [13]. It is possible that these bacteriocins contain only a minor proteinaceous component. Several bacteriocins contain a carbohydrate, lipid or phosphorous moiety. The presence of such a non-proteinaceous moiety can be verified by the sensitivity of these bacteriocins glycolytic (α-amylase), lipolytic (lipase) and phospholipolytic (phospholipase) enzymes. As a consequence of the rising incidence of resistance to most traditional antibiotics, numerous research programs have been implemented aiming to explore the potential role which naturally produced and genetically modified bacteriocins might have as replacements for traditional antibiotics. Other efforts focus on the use of antimicrobial toxins as food preservatives [14]. There is also an ever-increasing interest in the use of bacteria as biocontrol agents for the management of fungal and bacterial plant pathogens and, more recently, as the active agent in probiotic formulations. The present study was conducted to achieve the screening and characterization of the bacteriocins producing bacterial strains associated with indigenous environment, measuring the growth phase-related bacteriocin synthesis of the bacterial strains, confirming the bacteriocin-like character of their crude extract by preliminary detecting their antagonistic activity against indicator strain, checking the stability of crude extract of bacteriocin, estimating the molecular weight of bacteriocin, transforming the plasmid encoded bacteriocin gene in the reference *E.coli* JM107 strain.

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2. Material and methods

2.1 Bacterial Strains and Antagonistic activity

Samples from various natural sources like dry surface soil, soil from rhizosphere and pond water were collected from the indigenous areas of Punjab University, Lahore, Pakistan for the isolation and purification of the bacterial strains that exhibit the antimicrobial activity. The preliminary screening of bacterial strains exhibiting antimicrobial activity was done by the agar well diffusion technique [15] and agar spot test [16]. Those strains were then identified through morphological and biochemical analysis. Then the resistance frequencies of these selected strains against various antibiotics were checked. The stock concentrations of these antibiotics were prepared as described in Sambrook et al., 2001 [17]. L-agar plates with working concentrations of 50 μg/ml and 100 μg/ml for all antibiotics were prepared.

2.2 Time killing studies

Time-killing assays were used to assess the relative bacteriocin activity of the selected strains against the target organism. For the bacteriocin production experiment, 1 ml of 0.9 OD culture (at 600 nm) of each bacterial strain was inoculated in 100ml of both Tryptic soy broth (TSB) and Luria Bertani (LB) broth and incubated at 37°C at 100 rpm on shaker. TSB (ACUMEDIA) and LB broth (OXOID) without bacterial culture was used as control. Bacterial growth was monitored spectrophotometically at 600 nm (A600) after every 2 hrs intervals for 36 hrs. The bacterial cells after every 2 hrs were separated from culture medium by centrifugation at 14,000 rpm at 4°C for 10 min and supernatant of all selected strains from both medium was assayed against target organism by well diffusion method. Growth curves were prepared by plotting graph between time of incubation in hrs on X-axis and optical density on Y-axis.

2.3 Extraction and purification of bacteriocins

Solvent extraction method of Westley *et al.,* 1979 [18] and Liu *et al.,* 1978 [19] was employed for partial purification of antibacterial compounds from these strains. The partial purification of the antibacterial compounds was done through ethyl acetate. These extracts were used for TLC and further analysis. In order to separate the atimicrobial peptide from other compound present in the partially purified extracts of all the bacterial strains, the extracts of bacteriocin of all the five strains were subjected to thin layer chromatography and after development, the chromatogram was exposed to UV radiation and iodine vapours. After running and observing the TLC band were cut and soaked in ethyl acetate for 2 hours and scratched the silica layer of the cut bands with the help of sterile cutter and then circular pieces of sterile filter paper were soaked in the scratched suspension in the petri plates. After that placed the filter paper disc on the lawn of indicator strain and the residual activity of the bacteriocin TLC band were observed by well diffusion method [20].

2.4 Analysis of abiotic factors on antimicrobial compound

Effects of enzymes, temperature, and UV light on bacteriocin activity were also investigated. 50 μl crude bacteriocins were dispensed into 1.5 ml microcentrifuge tubes containing 5μl proteinase K. The sensitivity to enzymes treatments was checked by agar well diffusion method. To test temperature sensitivity, 500 μl of crude bacteriocin was dispensed into 1.5 ml microcentrifuge tubes, autoclaved for 15 minutes at 121 ^{o}C. Heat treated crude bacteriocin was assayed for bacteriocin activity by agar well diffusion method. To test the effect of UV radiation, 1 ml of the crude bacteriocin was poured in sterile petriplates and exposed under UV light for 20 minutes. The residual activity of the treated preparation was checked by agar well diffusion method.

2.5 Extracellular and Intracellular bacteriocin extraction

The antimicrobial compounds were partially purified using ammonium sulfate precipitation method. TSB culture broth was incubated for 24 hrs at 37°C. After incubation, the culture broth was centrifuged at 400 rpm for 20 minutes. The cell free supernatant was transferred to a beaker containing equal volume of 60 % ammonium sulfate solution. The mixture was centrifuged at 14000 rpm for 20 minutes and the precipitated extracellular protein pellet was obtained, dissolved in nuclease-free water. 100 ml overnight TSB broth culture of intracellular bacteriocin producing strains was centrifuged at 4000 rpm for 20 minutes to obtain pellet. Proper amount of lysis buffer was added and mixed well. It was incubated at room temperature for 5 minutes, heat shocked (100ºC) in a pre-warmed water bath for 5minutes. The cells were sonicated for half an hr. The bacterial suspension was centrifuged at 14000 rpm for 20 minutes and supernatant was taken in a separate eppendorf and stored at 4ºC.

2.6 Protein profiling

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The molecular size of the crude extract was analyzed by Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) using 12 % acrylamide gel (Sambrook and Russell, 2001). Sealed the plates with tape to prevent the gel from leakage and fixed the assembled plates in gel apparatus. 10 ml of 12% resolving gel was prepared. After polymerization of resolving gel, 4ml of 5% stacking gel was prepared. The treatment is same for both extra and intracellular bacteriocin. 40 µl of supernatant/sample was taken and mixed with 20 µl of SDS gel loading buffer. Immediately a 5-7 minutes heat shock was provided, and then centrifuged for 2 minutes. Then the sample was run. Electrophoresis was done at constant voltage of 40 volts for 3 hrs; the gel was removed and allowed to stain in staining solution for 20 to 25 minutes. Placed the gel in fixing solution under shaking conditions for 15 minutes, then destained in destaining solution overnight. After observing the overnight destain gel for the appearance or presence of bacteriocin accordingly to the page ruler ™ prestained protein ladder SM 0671, the bacteriocin bands were cut and placed on the lawn of indicator strain for estimating the bacteriocin activity of the band.

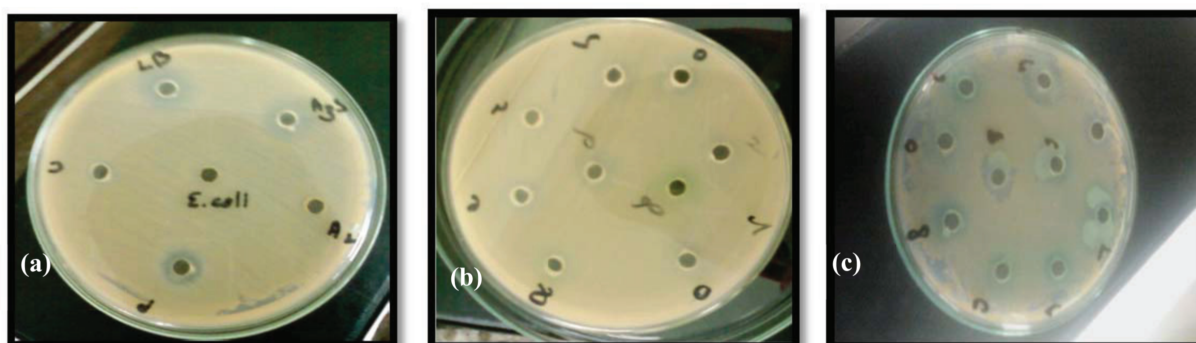
2.7 Genetic characterization and Transformation of bacteriocin-encoded plasmid in *E.coli* JM107

For molecular characterization all the five bacterial strains were sequenced through Macrogen (Korea). Genetic studies were conducted in two phases. Firstly, Plasmid isolation was done by two different method i.e. Kado and Liu, 1981 and alkaline lysis method [17]. Secondly, Transformation of Plasmid-encoded bacteriocin producing characters into the reference strain *E.coli* JM107 [21]. The transformation was done by using reference *E.coli* JM107 (genotype is ATCC 47014. F′ *tra*D36 *pro*A^+B^+ *lacI* ^{q} *E. coli* K-12 *sup*E D hskR17 F9 traD36 lacIR lacZ DM15 Amp^S and $Strep^R$) as a recipient strain and donor cells (AZS, LB, U, P and A2) genotype of which are Amp^R and $Strep^S$. The recipient cells were made competent using Calcium Chloride solution. The competency of cell was checked by taking 100 µl of competent cell in a sterile eppendrof and added 50 µl of plasmid DNA and placed on ice for 10 min. After that heat shocked cells for 2 minutes at 42 ^oC in water bath. Added 1 ml of LB medium at room temp and incubated for 1 hr at 37 ^oC. Plated several dilutions on LB/Ampicillin medium and incubated for 12 to 16 hours at 37 ^oC.

3. Results

3.1 Screening of bacteriocinogenic strains and bioassay

A total of 10 bacterial strains were isolated from various sources and screened for antibacterial activity against target organism. Out of 10 isolates, 5 showed greater antibacterial activity against target organism. These five isolates were selected and then used for further studies. Gram staining revealed that 3 out of five (P, A2 and U) were gram negative rods and 2 out of 5 (AZS and LB) were gram positive bacilli. Spore staining revealed that both gram positive bacilli were spore-former whereas all the gram negative rod were non-spore former. Motility test indicated that the two gram negative rod (P and U) were motile while rest of the three (A2, AZS and LB) were non-motile. The genectic characterization result showed that strain P belong to *Pseudomonas aeruginosa* , U (*Alcaligenes*), LB (*Bacillus cereus*), AZS (*Bacillus cereus*) and A2 (*Brevundimonas*) . The sensitivity and resistance pattern of all the five strains showed that all the strains were resistant to pencillin G and Ampicillin at both 50 µg/ml and 100 µg/ml concentrations while all the five isolate showed sensitivity against Streptomycin, Chloramphenicol and Tetracycline except A2 which showed resistance to 50µg/ml concentration of Tetracycline. The screening of the antibacterial activity of all the five isolates against target organism i.e. gram positive cocci by well diffusion method revealed that three isolates P, AZS and LB showed the best activity against the target strain after 18 to 24 hrs incubation at 37 ^oC while the isolates U and A2 showed best activity after 24 to 48 hrs incubation at 37 ^oC. The maximum zone of inhibition (i.e. 300nm) was found in case *Pseudomonas aeruginosa* and U (*Alcaligenes*) (figure 1a).



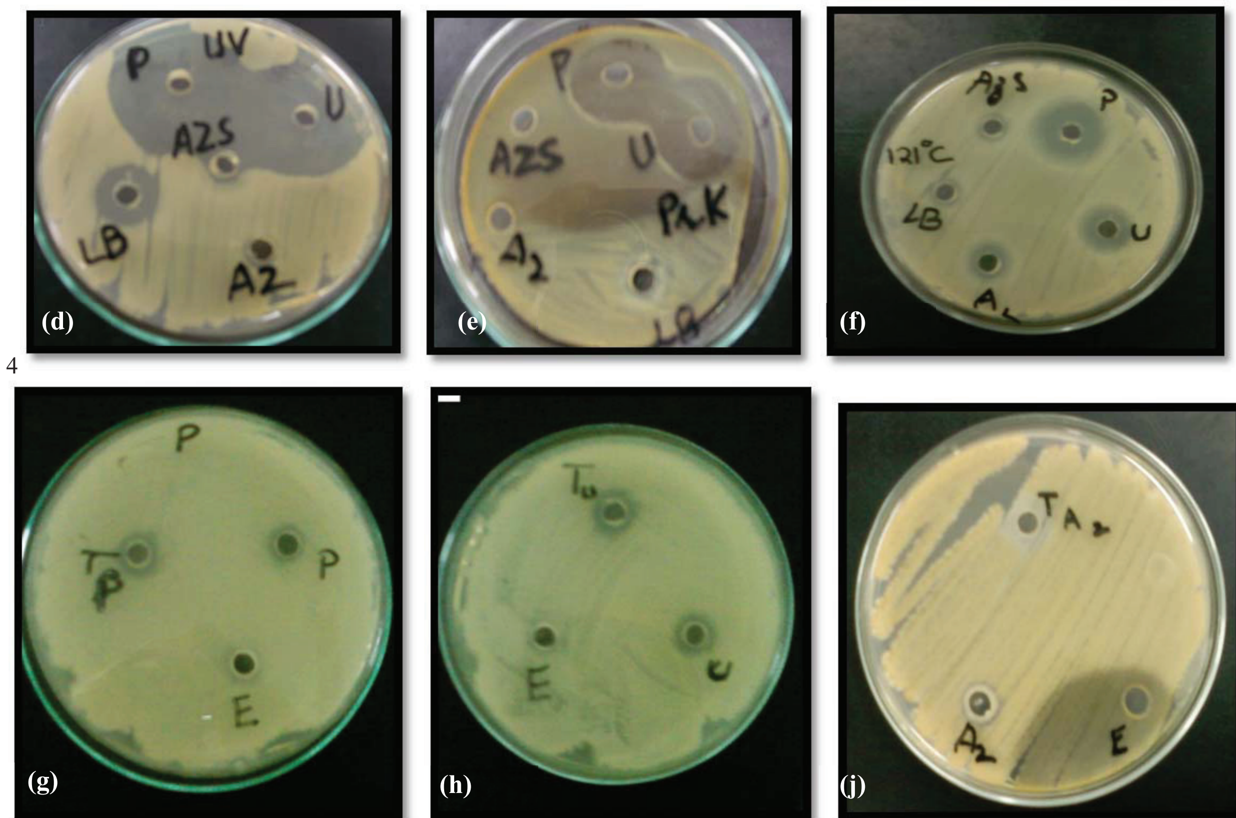


Fig. 1 Screening of the antibacterial activity of all the five bacterial strain i.e. P(Pseudomonas aeruginosa) , U (Alcaligenes), LB (Bacillus cereus), AZS (Bacillus cereus) and A2 (Brevundimonas) (a) Screening of bacteriocin acivity against target bacterium by well diffusion method; (b) Time profiling of bacteriocin activity for strain P against test organism in case of LB medium; (c) Time profiling of bacteriocin activity for strain P against test organism in case of TSB medium ; (d) Antagonistic activity of crude extract of bacteriocin by well diffusion method ; (e) Estimation of effect of UV light on the crude extract by well diffusion method; (f) Estimation of effect proteinase K on the crude extract by well diffusion method; (g) Estimation of effect of heat on the crude extract by well diffusion method;(h) Result of bacteriocin activity of transformant (TP) by well diffusion method; (i) Result of bacteriocin activity of transformant (TU) by well diffusion method; (j) Result of bacteriocin activity of transformant (TA2) by well diffusion method.

3.2 Induction time assay

The induction time assay of the selected bacterial strain using two different broth media i.e. TSB and LB were performed. The purpose of doing that assay was to determine the time of intiation of bacteriocin production of the selected strains while simultaneously monitoring the growth of that source strains at 600 nm. The graphs (figure 2 & 3i) and the time killing activity against target strain showed that in case of TSB medium, the growth and zone of inhibition (mm) increased exponentially with increasing time of incubation while in case of LB medium, the growth started decreasing after 28 hr incubation. The result from both these diagram (figure 1b & c) indicated that TSB was an ideal media for bacteriocin production.

3.3 Antimicrobial activity of the partially purified compounds extracted

The antibacterial activities of the partially purified extract of bioactive compounds were assayed by well diffusion method (figure 1d). During extraction it was noted that both P *Pseudomonas aeruginosa* and U (*Alcaligenes*), were extracellular bacteriocin producer while rest of the three i.e. LB (*Bacillus cereus*), AZS (*Bacillus cereus*) and A2 (*Brevundimonas*) were produced bacteriocin intracellularly.

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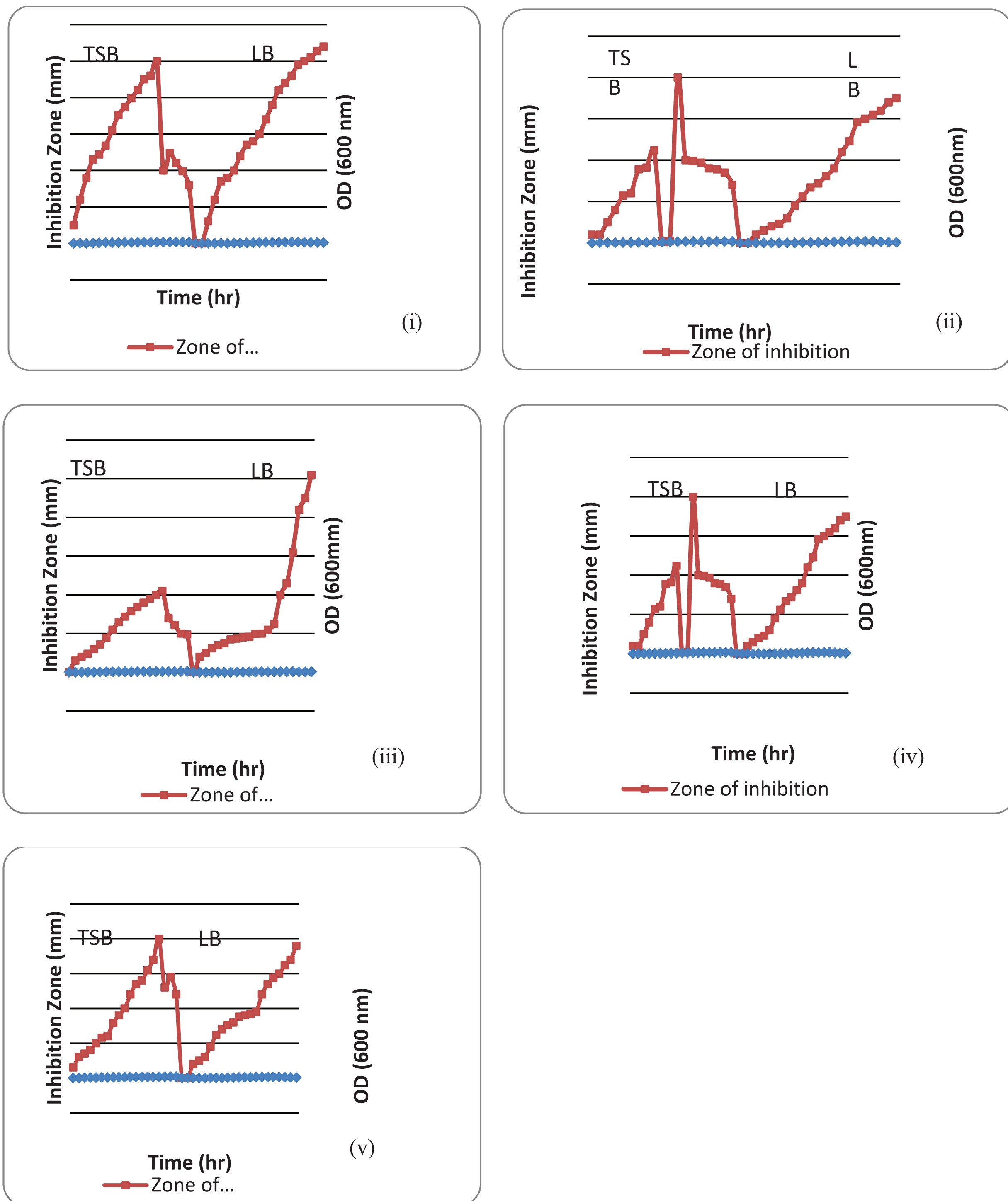

Fig. 2 Growth kinetics of (i) P (*Pseudomonas aeruginosa*) (ii) A2 (*Brevundimonas*) (iii) U (*Alcaligenes)* (iv) AZS (*Bacillus cereus*) (v) LB (*Bacillus cereus*) with two different media (i.e. TSB and LB).

3.4 Effect of biotic and abiotic factors on activity of antimicrobial compounds

The crude extract of all the five bacterial strains were exposed to UV radiation for 20 min and the residual activity was checked against target organism by agar well diffusion method. It was then revealed that UV radiation enhanced the antibacterial activity of these strains against the target organism (figure 1e) which indicated that the antimicrobial substances was not a pure protein but instead it was a conjugated protein. The crude extract of all the five bacterial strains were treated with 5µl of proteinase K. The residual activity was then checked against target organism by agar well diffusion method. It was then revealed that treatment with proteinase K would totally inhibit the antibacterial activity of intracellular crude extract of three bacterial strains i.e. AZS, A2 and LB whereas treatment with proteinase

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K would have little effect on the antibacterial activity of extracellular crude extract of bacterial strains i.e. U and P (figure 1f). The crude extract of all the five bacterial strains were exposed to 121°C for 15 min and the residual activity was then checked against target organism by agar well diffusion method. It was then noted that heat treatment would have no effect on the antibacterial activity of isolates P and U against the target organism whereas in case of AZS, A2 and LB. Heat treatment had reduced the antibacterial activity against the target organism(figure 1g).

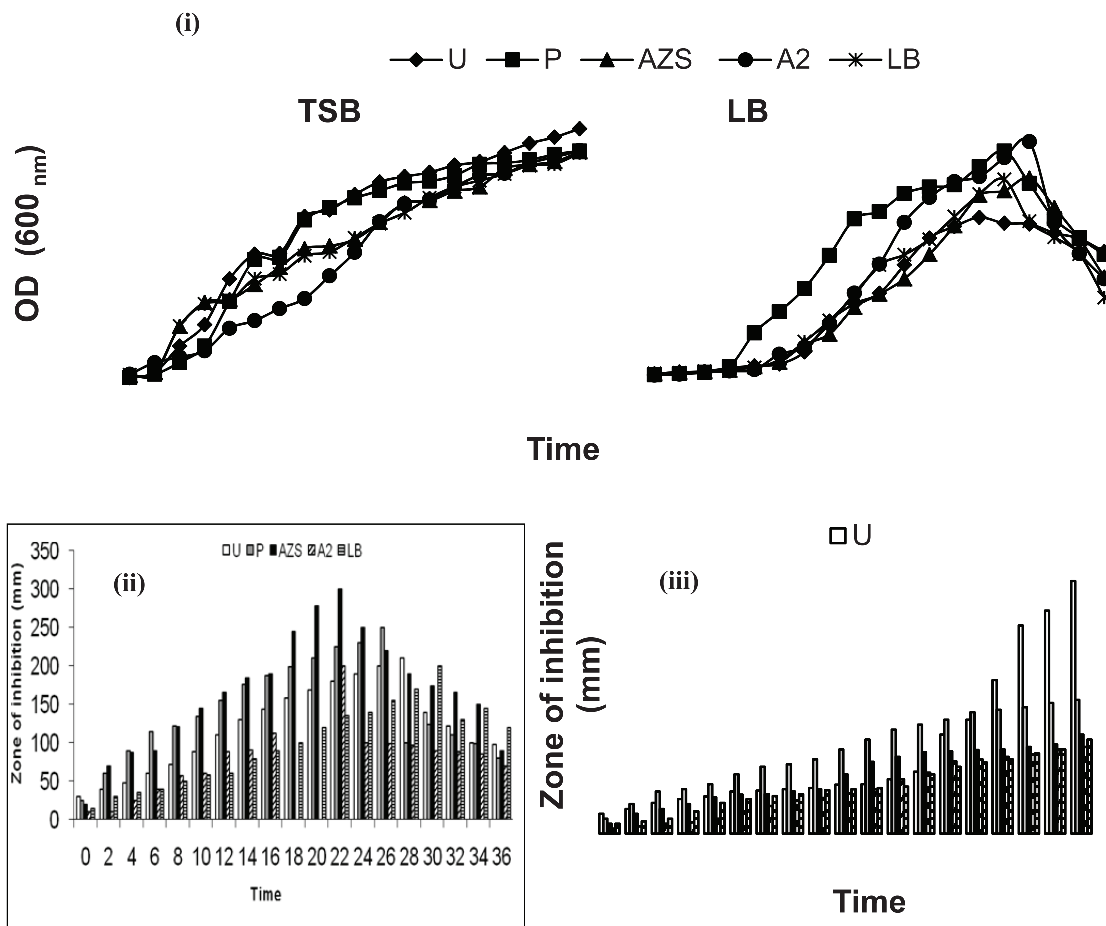

Fig. 3 (i) Comparative analysis of growth kinetics with two different broth media i.e. TSB and LB. (ii) Time killing studies and zone of inhibition with LB broth. (iii) Time killing studies and zone of inhibition with TSB broth.

3.5 Plasmid Isolation and Transformation

Plasmid DNA of all the five isolates were isolated from two different method i.e. MiniPrep alkaline lysis method and second one was Kado and Liu method. Figures 6, illustrated that most of strains contained plasmid of shorter length and due to that reason the band were appeared down to wells. The isolated plasmid of these strains were then used for transformation studies. As it was known that bacteriocin production was plasmid encoded so that why the isolated plasmid were then introduced in competent *E.coli* strain that lack the bacterocin through transformation. The double selection marker to check that whether the transformation was successful or not was 100 µg/ml Ampicillin supplemented with 100 µg/ml streptomycin. The result indicated that transformation was successful only in strains P, U and A2 (figure 1h, i & j) but not sufficiently successful in case of rest of the two strain i.e LB (*Bacillus cereus*) and AZS (*Bacillus cereus*).

3.6 Thin Layer Chromatography and SDS-PAGE

The different combination of organic solvents were used but the best resolution was with methanol: chloroform (5:5). The compounds were separated on the basis of their increased solubility in an organic solvent (ethyl acetate in that case). Various color band or spots were visualized under UV light. Brown spot were developed on exposure to iodine vapors. The activity of TLC bands of bacteriocin were checked by filter paper disk diffusion method and the result indicated that zone of inhibition were appeared around the disk but the antagonistic activity of all the five extracts were reduced [34] . The molecular weights of both intracellular and extracellular bacteriocin were determined by using SDS-Page analysis. The result of SDS-Page along with page ruler ™ prestained protein ladder SM 0671 revealed that the

molecular weight of both P and U extracellular bacteriocin were approximately 40 kDa according to reference protein ladder band while the molecular weight of AZS, A2 and LB were estimated to be in the range of 10 to 25 kDa. After the estimation of molecular weight, the respective bands were cut and were observed for bacteriocin activity against indicator organism (Fig. 5).

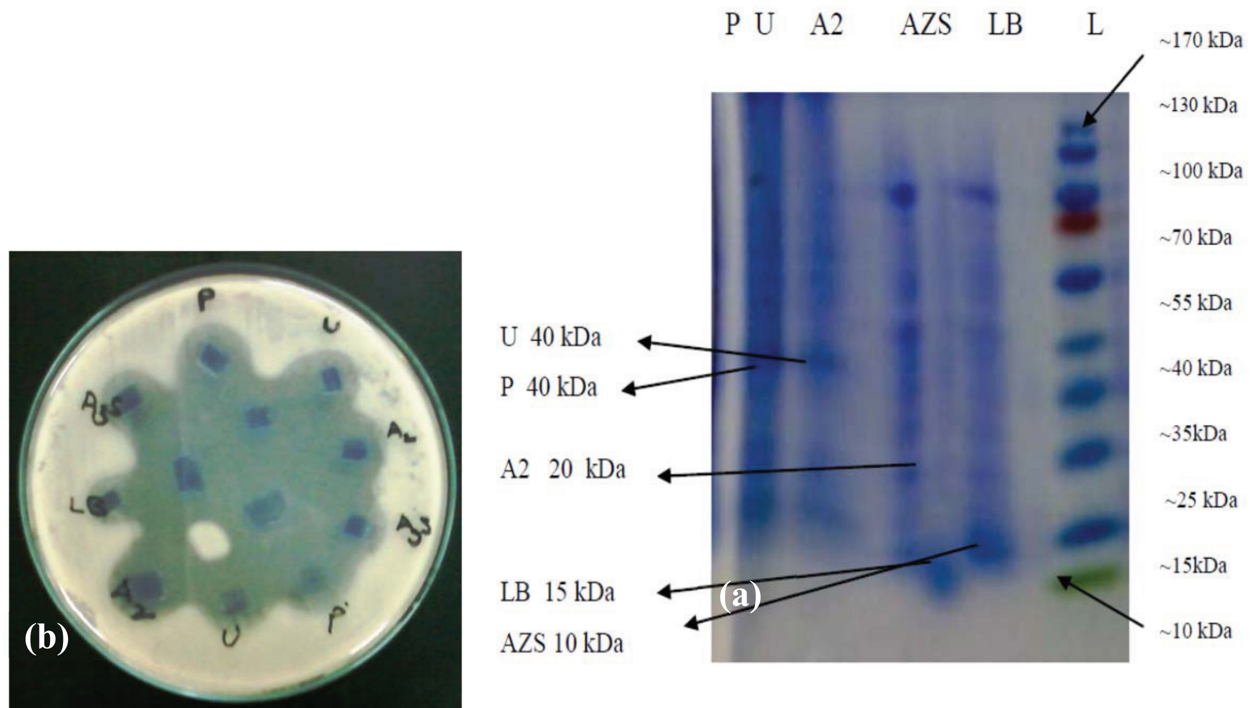

Fig. 4 (a) SDS –PAGE analysis of the bateriocin extract of P, U, AZS, A2 & LB (b) Antagnostic activity of the SDS –PAGE bands against indicator organism.

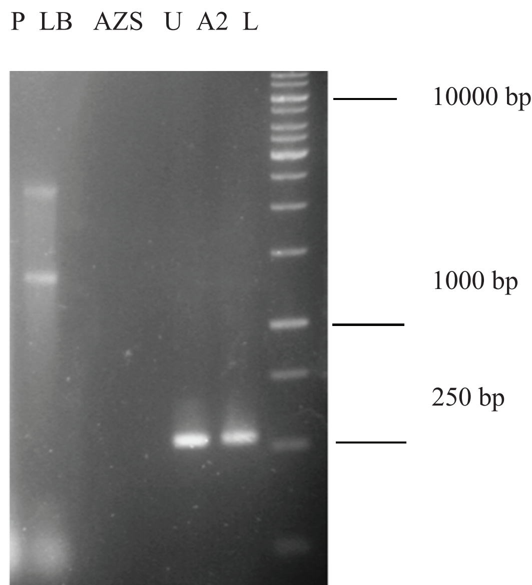

Fig. 6 Agarose gel electrophoresis of Plasmid isolation by Akaline lysis method, P (*Pseudomonas aeruginosa*) (ii) A2 (*Brevundimonas*) (iii) U (*Alcaligenes)* (iv) AZS (*Bacillus cereus*) (v) LB (*Bacillus cereus*), L (1Kb DNA ladder).

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4. Discussion

People have used antibiotics to treat illnesses caused by pathogens for over fifty years. However, the excessive and inappropriate use of these antibiotics in clinical treatment of humans and animals has increased pathogen resistance to these compounds, turning them into less effective agents. There has also been an increase in the generation of multi-drug resistant pathogens, primarily bacteria and fungi that resist the effects of most currently available antibiotics [22]. This clearly shows the need to develop new biomedical treatments with different action mechanisms from those of conventional antibiotics [23]. For this purpose, total 10 bacterial strains were isolated from the indigenous environment of Punjab University, Lahore. These strains were then screened for antibacterial activity against three different target organism. Out of 10 bacterial isolates, only five isolates showed antibacterial activity against one of the target organism which was gram positive cocci. These five isolates and target organism (gram positive cocci) were then selected for further studies. Although these five bacterial isolates were sequenced through MACROGEN (Korea) and the sequences result indicated that the strain U was *Alcaligenes,* P (*Pseudomonas aeruginosa*), A2 (*Brevundimonas)*, LB and AZS (*Bacillus cereus*)*.* Members of the genus *Bacillus* carry tremendous importance because of their antimicrobial activity since they produce a variety of peptide antibiotics representing several different basic chemical structures. The production of bacteriocins or bacteriocin-like substances has been described for *B. coagulans, B. brevis, B. lichniformis, B. cereus, B. subtilis, B. amyloliquefaciens* and other *Bacillus* species [24]. Colicin V from *E. coli* and pyocin from *Pseudomonas aeruginosa*, are the two best studied peptides in the Gram negative bacteria group. Gram negative bacilli of the genus *Pseudomonas* are common inhabitants of soil, fresh water, and marine environments. *Pseudomonas aeruginosa* receives more attention since it is also an opportunist pathogen, causing human diseases [25].

Present study and others [26] have provided some initial insights as production of bacteriocins in Gram-positive and Gram-negative bacteria was generally associated with the shift from log phase to stationary phase. For example, nisin production begins during mid-log phase and increases to a maximum as the cells enter stationary phase. The regulation of expression is not cell cycle dependent, per se, but rather culture density dependent [27]. Time killing studies with both TSB and LB broth media indicated that TSB was an ideal medium for the estimation of bacteriocin production with respect to growth kinetics (Figure 3i). Kinetics of bacteriocin production showed that its synthesis or secretion of bacteriocin started in the late exponential phase of growth and maximum activity of bacteriocin in case of both the broth media (i.e. TSB and LB) was observed at the early stationary phase of growth. Afterward, the bacteriocin activity slowly decreased (Figure 3ii & 3iii). Similar findings were reported by Saleem *et al.,* (2009) [28] who reported the production of (bacillocin Bb and pyocin) bacteriocins started during early exponential growth phase and continued till late stationary phase. Adequate purification of bacteriocins is necessary for their characterization. Different strategies such as solvent extraction method (by ethyl acetate) and ammonium sulphate precipitation method were employed to partially purified the bateriocin. The partially purified extracts of bacteriocin were further purified by TLC. Thin layer chromatography (TLC) was frequently used in the 1980's to characterize compounds with respect to their polarity. In TLC, the best separation of chromatograms were obtained by developing solvent chloroform: methanol (5:5). Various coloured spots were seen when exposed to UV radiation, confirming that the compound was unsaturated in nature and may be aromatic with extended conjugation. When exposed to iodine vapours, brown spot were detected. Iodine has a strong affinity for both unsaturated and aromatic compounds. The iodine vapours dissolve to form weak complexes with lipophilic organic compound, which show up as dark brown colour spots. The appearence of brown spots to iodine, explained that the compound (bacteriocin) is lipophilc organic compound and may be aromatic in nature. The bioactivities of TLC spots on Silica card against the target organism were checked by filter paper disk diffusion method [20], it was observed that the diameter of the zone of inhibition of spot scratched from TLC card were smaller than the original extract, so it can be suggested that the one of the major concerns when assessing the bioactivity of a crude extract is the purity of the extract and their synergetic affinity to both developing solvent and Silica.

The present study was also provide deep insight to establish an idea that whether or not rhizospheric strains of *Bacillus* (AZS &LB) and *Pseudomonas* (P) produce bacteriocins like compounds and, if so, investigate their stability to UV light, temperature and proteinase K. For this purpose, the partially purified crude extracts of bacteriocin were exposed UV radiation for 20 min and after that activity were observed by well diffusion method. It was observed that activity of the crude extract of bacteriocin was stable and the UV light did not affect the activity of bacteriocin produced by the *Pseudomonas* and *Alcaligenes* (P & U), *Brevundimonas*(A2) *Bacillus* (AZS & LB), confirming the protein status of bacteriocins. A similar result was reported by Ogunbanw *et al.,* in 2003 [29].

The bioactivity of *Bacillus* and *Brevundimonas* strains (AZS, LB & A2) was completely lost after treatment with proteinase K thereby suggesting their proteinaceous nature [28] while the bioactivity of both *Pseudomonas and Alcaligenes* (P & U) were reduced to a lesser extent which may indicated the presence of unusal amino acid in the bacteriocin structure or cyclic N-and/ or C-terminally blocked peptides render this compound resistance to proteases [30]. Heat treatment of the partially purified extracts of bacteriocin showed that heating at 121°C (at 15 psi for 15 minutes) that their bioactivity of all these extracts were markedly decreased but not completely diminished which may indicated the presence of carbohydrate moiety along with protein. This was due to the lengthy time of exposure to heat and pressure. Accordingly, Joshi *et al.,* (2006) [31] described that the activity of the bacteriocin produced by *Lactobacillus* CA44 was completely lost at 121°C for 15 min. A similar result was reported for thuricin 7 produced by

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B. thuringiensis BMG1.7 [32]. The interesting feature of heat stability of these bacteriocin at 100°C for 15 min, supports the fact that it might constitute an advantage in view of its potential use as a food additive, in processes like pasteurization, drying, refrigeration and freezing.

In general, bacteriocin production has been known to be induced by coding of plasmid DNA in both Gram positive and negative bacteria [33]. This study has provided a novel experimental evidence that plasmid-encoded bacteriocin gene can be transformed in any bacterial strain that lack the bacteriocin activity. So to carried out this novel approach, the plasmid from all the five bacterial strains were isolated and were then transformed in reference *E.coli* JM107 (genotype of which is known) as a recipient strain. To check that whether the transformation was successful or not, the cross marker was also done. The result indicated that transformation was successful in case of *Pseudomonas* and *Alcaligenes* (P & U) and only in one strain (A2) *Brevundimonas* but the resulting bacteriocin activity of transformants was not as much pronounced as the donor bacterial strains. Quadric *et al.,* in 1995 had also done the transformed the *E.coli* to overexpressed the production of Carnobacteriocin B2, a 48-amino acid antimicrobial peptide containing a YGNGV motif that is produced by the lactic acid bacterium *Carnobacterium piscicola* LV17B. The molecular weight of the bacteriocin were analyzed by SDS-PAGE analysis. It was found that the molecular weight of *Brevundimonas* and *Bacillus cereus* (A2, AZS & LB) were approximately in the range of 10 to 25 kDa whereas the molecular weight of both *Pseudomonas* and *Alcaligenes* (P & U) strains was approximately 40 kDa . The bioactivities of the SDS-PAGE bands were checked against the target organism. The clear zones around the bands indicated that our selection of bacteriocin bands with reference to page ruler ^{TM} prestained protein ladder SM 0671 were correct.

It was concluded that this study and others [34] have provided some initial insights as to the potential use of bacteriocin-like producer strains in the biocontrol of bacterial disease. The lack of effective control measures to manage this disease justifies a further investigation of this strategy, perhaps involving a more thorough screen for these types of compounds.

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References

[1] Héchard Y, Sahl H-G. Mode of action of modified and unmodified bacteriocins from Gram-positive bacteria. Biochimie. 2002; 84:545-557.
[2] Michel-Briand Y, Baysse C. The pyocins of Pseudomonas aeruginosa. Biochimie. 2002; 84:499-510.
[3] Jenssen H, Hamill P, Hancock RE. Peptide antimicrobial agents. Clinical microbiology reviews. 2006; 19:491-511.
[4] Dijkshoorn L, Brouwer CP, Bogaards SJ, Nemec A, Van Den Broek PJ, Nibbering PH. The synthetic N-terminal peptide of human lactoferrin, hLF (1-11), is highly effective against experimental infection caused by multidrug-resistant Acinetobacter baumannii. Antimicrobial agents and chemotherapy. 2004; 48:4919-4921.
[5] Deegan LH, Cotter PD, Hill C, Ross P. Bacteriocins: biological tools for bio-preservation and shelf-life extension. International dairy journal. 2006; 16:1058-1071.
[6] Reddy K, Aranha C, Gupta S, Yedery R. Evaluation of antimicrobial peptide nisin as a safe vaginal contraceptive agent in rabbits: in vitro and in vivo studies. Reproduction. 2004; 128:117-126.
[7] Cleveland J, Montville TJ, Nes IF, Chikindas ML. Bacteriocins: safe, natural antimicrobials for food preservation. International journal of food microbiology. 2001; 71:1-20.
[8] Martinez RC, de Martinis EC. Effect of Leuconostoc mesenteroides 11 bacteriocin in the multiplication control of Listeria monocytogenes 4b. Food Science and Technology (Campinas). 2006; 26:52-55.
[9] Cascales E, Buchanan SK, Duché D, Kleanthous C, Lloubes R, Postle K, Riley M, Slatin S, Cavard D. Colicin biology. Microbiology and Molecular Biology Reviews. 2007; 71:158-229.
[10] McAuliffe O, Ross RP, Hill C. Lantibiotics: structure, biosynthesis and mode of action. FEMS microbiology reviews. 2001; 25:285-308.
[11] de Martinis EC, Santarosa PR, Freitas FZ. Preliminary characterization of bacteriocins produced by six lactic acid bacteria strains isolated from vacuum-packaged meat products. Food Science and Technology (Campinas). 2003; 23:195-199.
[12] Todorov S, Dicks L. Lactobacillus plantarum isolated from molasses produces bacteriocins active against Gram-negative bacteria. Enzyme and Microbial Technology. 2005; 36:318-326.
[13] Toro C. Use of probiotic lactic acid bacteria in food shrimp Litopenaeus vannamei as inhibitors of pathogens and immune system stimulants. Brazil: Federal University of Parana, Curitiba; 2005.
[14] Gillor O, Kirkup BC, Riley MA. Colicins and microcins: the next generation antimicrobials. Advances in applied microbiology. 2004; 54:129-146.
[15] Jagadeeswari S, Vidya P, Kumar DM, Balakumaran M. Isolation and characterization of bacteriocin producing Lactobacillus sp. from traditional fermented foods. Electronic Journal of Environmental, Agricultural and Food Chemistry. 2010; 9:575-581.
[16] Schillinger U, Lücke FK. Antibacterial activity of Lactobacillus sake isolated from meat. Applied and Environmental Microbiology. 1989; 55:1901-1906.
[17] Sambrook J, Fritsch EF, Maniatis T. Molecular cloning. Cold spring harbor laboratory press New York; 2001
[18] Westley JW, Liu CM, Evans RH, Blount JF. Conglobatin, a novel macrolide dilactone from Streptomyces conglobatus ATCC 31005. The Journal of antibiotics. 1979; 32:874-877.
[19] LiuU WC, Slusarchyk DS, Astle G, Trejo WH, Brown WE, Meyers E. Ionomycin, a new polyether antibiotic. The Journal of antibiotics. 1978; 31:815-819.

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[20] Uzair B, Ahmeda N, Mohammadb FV, Ahmadb VU, Edwards D. Screening of marine bacteria of Pakistan coast for drug discovery potential. Proc. Pakistan Acad. Sci. 2009; 46:137-144.
[21] Ausubel F BR, Kingston R, Moore D, Seidman J, Smith J, Struhl K Short protocol in Molecular Biology. 5th ed. John Wiley and Sons Inc; 1994
[22] Angulo FJ, Heuer OE, Hammerum AM, Collignon P, Wegener HC. Human health hazard from antimicrobial-resistant enterococci in animals and food. Clinical Infectious Diseases. 2006; 43:911-916.
[23] Parisien A, Allain B, Zhang J, Mandeville R, Lan C. Novel alternatives to antibiotics: bacteriophages, bacterial cell wall hydrolases, and antimicrobial peptides. Journal of applied microbiology. 2008; 104:1-13.
[24] Lisboa MP, Bonatto D, Bizani D, Henriques JA, Brandelli A. Characterization of a bacteriocin-like substance produced by Bacillus amyloliquefaciens isolated from the Brazilian Atlantic forest. International Microbiology. 2010; 9:111-118.
[25] Waite RD, Curtis MA. Pseudomonas aeruginosa PAO1 pyocin production affects population dynamics within mixed-culture biofilms. Journal of bacteriology. 2009; 191:1349-1354.
[26] Ghanbari M, Rezaei M, Soltani M, Shah-Hosseini G. Production of bacteriocin by a novel Bacillus sp. strain RF 140, an intestinal bacterium of Caspian Frisian Roach (Rutilus frisii kutum). Iranian Journal of Veterinary Research. 2009; 10:267-272.
[27] Dufour A, Hindré T, Haras D, Le Pennec J-P. The biology of lantibiotics from the lacticin 481 group is coming of age. FEMS microbiology reviews. 2007; 31:134-167.
[28] Saleem F, Ahmad S, Yaqoob Z, Rasool SA. Comparative study of two bacteriocins produced by representative indigenous soil bacteria. Pak J Pharm Sci. 2009; 22:252-258.
[29] Ogunbanwo S, Sanni A, Onilude A. Characterization of bacteriocin produced by Lactobacillus plantarum F1 and Lactobacillus brevis OG1. African Journal of Biotechnology. 2003; 2:219-227.
[30] Khalil R, Djadouni F, Elbahloul Y, Omar S. The influence of cultural and physical conditions on the antimicrobial activity of bacteriocin produced by a newly isolated Bacillus megaterium 22 strain. African Journal of Food Science. 2009; 3:011-022.
[31] Joshi VK, Sharma S, Rana NS. Production, purification, stability and efficacy of bacteriocin from isolates of natural lactic acid fermentation of vegetables. Food technology and Biotechnology. 2006; 44:435-439.
[32] Cherif A, Ouzari H, Daffonchio D, Cherif H, Ben Slama K, Hassen A, Jaoua S, Boudabous A. Thuricin 7: a novel bacteriocin produced by Bacillus thuringiensis BMG1. 7, a new strain isolated from soil. Letters in Applied Microbiology. 2001; 32:243-247.
[33] Maqueda M, Gálvez A, Bueno MM, Sanchez-Barrena MJ, Gonzalez C, Albert A, Rico M, Valdivia E. Peptide AS-48: prototype of a new class of cyclic bacteriocins. Current Protein and Peptide Science. 2004; 5:399-416.
[34] Sarika A, Lipton A, Aishwarya M. Bacteriocin production by a new isolate of Lactobacillus rhamnosus GP1 under different culture conditions. Adv J Food Sci Technol. 2010; 2:291-297.

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