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Inhibition of Methicillin Resistant *Staphylococcus aureus* by Bacteriocin Producing *Pseudomonas aeruginosa*

Thangarasu Arumugam[1] · Selvam Dhanam[1] · Neelamegam Rameshkumar[2] · Muthukalingan Krishnan[2] · Nagarajan Kayalvizhi[1]

Abstract

Bacteriocins are natural antimicrobial peptides with attractive possible applications in food preservation and health care. In the present study, bacteriocin producing bacterial strain *Pseudomonas aeruginosa* were isolated from soil which exhibited antagonistic activity against Methicillin Resistant *Staphylococcus aureus* (MRSA) bacteria. The bacteriocin producing strain TA6 was confirmed as *P. aeruginosa* by biochemical tests and 16S rRNA gene sequence analysis. Maximum bacteriocin activity (100 AU ml^{-1}) was observed at 37 °C with pH 6.0 in 24 h time duration. SDS–PAGE analysis of the extracellular protein of *P. aeruginosa* TA6 revealed a bacteriocin-like protein with a molecular mass of ~10 kDa. MRSA cells were treated with culture supernatant of *P. aeruginosa* TA6 and analyzed by FT-IR. The treated and untreated MRSA showed band variations at 671 and 3460 cm^{-1} corresponding to alkyl and amide group respectively. Mixed proportions of dead and live control populations were analyzed by flow cytometry to determine detection limits of the Dead/Live cells. The flow cytometry detection of defined proportions of dead (p2) and live (p1) cells at 3 h were p2 = 60.5%; p1 = 39.5% and 6 h p2 = 66.5%; p1 = 33.5% respectively. The scanning electron microscopy observation showed the main changes in the cell membrane structural integrity of *S. aureus* after exposure to the bacteriocin from *P. aeruginosa* TA6 at 12 h incubation. Together, the results suggested that bacteriocin from *P. aeruginosa* TA6 was effective against MRSA.

Keywords Bacteriocin · Methicillin-resistant *Staphylococcus aureus* · *Pseudomonas aeruginosa* TA6 · SDS–PAGE · SEM

Introduction

Bacteriocins are ribosomally synthesized antibacterial peptides that are active against narrow spectrum or broad spectrum microbiota. They are also involved in the host defense and cell signaling mechanisms with bacteriocidal mode of action (Tagg et al. 1976). Bacteriocin producing bacteria are immune to their own bacteriocins and that are mediated by specific immunity proteins (Cotter et al. 2005). They are produced by Gram-negative and Gram-positive bacteria (Heng et al. 2007), and some members of the Archaea (Riley and

Wertz 2002; Shand and Leyva 2008). Bacteriocin was first identified as a heat-labile colicin produced by *Escherichia coli* V that inhibited the growth of *E. coli* S (Gratia 1925).

Bacteriocins may offer an alternative to conventional antibiotics; they are small cationic or amphiphilic antimicrobial peptides that inhibit the growth of closely related species (Cotter et al. 2005). Bacteriocin family includes a diversity of proteins that are varied in size, microbial target, mode of action, release and immunity mechanisms respectively. Bacteriocins are classified depending upon their structures into three main groups (Cotter et al. 2005; de Jong et al. 2006). Once bound to lipid II, nisin also form pores in the cell membrane (Brotz et al. 1998). Nisin is active against various Gram-positive and Gram-negative bacteria, including MRSA, vancomycin-resistant *enterococci* (VRE), heterogeneous vancomycin-intermediate *Staphylococcus aureus* (hVISA), *Streptococcus pneumoniae* and *Clostridium difficile* (Bartoloni et al. 2004; Piper et al. 2011; Severina et al. 1998).

✉ Nagarajan Kayalvizhi
kayalvizhinagarajan@gmail.com;
kayal@periyaruniversity.ac.in

[1] Department of Zoology, School of Life Sciences, Periyar University, Salem, Tamil Nadu 636011, India

[2] Insect Molecular Biology Laboratory, Department of Environmental Biotechnology, Bharathidasan University, Tiruchirappalli, Tamil Nadu 620024, India

Bacteriocins are interesting candidates for the use in food and agricultural industries. For example, bacteriocin producer were used in animal health feed for pigs and other livestock and they protect animals from severe gastrointestinal infections (Kirkup 2006). The bacteriocin has been several attractive properties to build them suitable for food preservative. Bacteriocins are recognized as safe substances, they are not active and also nontoxic on eukaryotic cells. They are inactivated by digestive proteases, hence little influence on the gut microbiota, due to pH and heat-tolerant. They exhibit broad antimicrobial spectrum, against many food-borne pathogens and spoilage bacteria. They show a bactericidal mode of action, usually acting on the cytoplasmic membrane and therefore no cross resistance to antibiotics, and their genetic determinants are usually plasmid-encoded. Bacterial infection is one of the major complications in orthopaedic surgery and may lead to permanent damage of tissue or bone (Krishnan et al. 1993; Fialkov et al. 2001; Cosgrove and Carmeli 2003). Various organisms, including Grampositive and Gram-negative bacteria, fungi and yeasts have been implicated in causing skin infections (Church et al. 2006; Vindenes and Bjerknes 1995). *S. aureus* is a virulent pathogen mostly responsible for superficial and invasive skin and soft tissue infections (Baggett et al. 2003; Daum 2007; Fridkin et al. 2005).

Staphylococci, like MRSA strain, are the most frequent cause of biofilm associated infections, which are significant cause of morbidity and associated with indwelling medical devices (Otto 2008). *S. aureus* is a major human pathogen associated with a variety of moderate to severe infections that are present in the community as well as nosocomial settings. Methicillin resistant *S. aureus* (MRSA) strains, which are now resistant to most antibiotics, are most often found in medical institutions, but are becoming increasingly more associated with community-acquired infections (Okesola 2011). The ongoing emergence of multi-drug resistant pathogens has sparked an attention in seeking alternative therapeutic options (Mathur et al. 2017). The aim of this study is to isolate and characterize of bacteriocin-producing bacterium with anti-MRSA activity for potential wound healing application which would have positive impact in medicinal application. Furthermore, in depth investigation of the present study would have a positive impact in field of medicine in near future.

Materials and Methods

Isolation of Bacterial Strains

Soil sample was collected in the agricultural field, Kadambur village, Salem District, Tamilnadu, India (11°30′34.8″N 78°36′48.4″E). Bacterial strains were isolated from soil samples using the standard protocol. Soil samples were suspended and serially diluted in sterile saline solution (0.89% w v^{-1} NaCl). Tubes containing 0.1 ml of appropriately diluted solution were plated on nutrient agar (Himedia, Mumbai, India) plates and were incubated at 30 °C for 24 h. Morphologically distinct single colonies were subcultured on nutrient agar plates and screened for antibacterial activity.

Detection of Antibacterial Strains

The antibacterial activity of the isolates was determined by a deferred-antagonism plating assay (Tagg et al. 1976). Nutrient agar plates were streaked with test organisms and incubated at 30 °C for 24 h and then the plates were overlaid with soft agar (0.75% agar) containing 10^6 CFU ml^{-1} of the stationary-phase culture of indicator strains. The plates were incubated at 30 °C for 24–48 h and examined for the zone of inhibition.

Agar Well Diffusion Assay

The strain which was selected as potential bacteriocin producers were grown in Luria broth at 37 °C for 24 h. Cells were separated by centrifugation at 10,000 rpm for 10 min at room temperature. Around 6 mm diameter wells were made on pre-inoculated agar media and 100 µl of culture supernatant was added to each well (Toba et al. 1991). Inhibitory activity was performed against *S. aureus*. Inhibition zones around the wells were measured and recorded.

Genomic DNA Extraction of Bacteria

DNA was isolated by following standard methods with slight modifications (Sambrook et al. 1989). The DNA sample was separated according to their molecular weights under electrophoresis system. Finally the DNA band was visualized under gel documentation system (Lark, Germany). The DNA concentration was determined by measuring the absorbance ratio at 260/280 nm and the DNA suspension was stored in − 20 °C for further analysis.

PCR Amplification of 16S rDNA Gene

A reaction mixture containing approximately 50 ng of template DNA, 10× PCR buffer, a 20 pmol concentration of each PCR primer (27F AGAGTTTGATCMTGGCTCAG and 1492 R TACGGYTACCTTGTTACGACTT), a 2.5 mM concentration of dNTPs and 2.5 U of Taq DNA polymerase in a total volume of 50 µl were prepared. After 10 min denaturation at 94 °C, the reaction mixture was run through 35 cycles of denaturation for 20 s at 94 °C, annealing for 20 s at 58 °C, and extension for 1 min at 72 °C, followed by a final extension for 10 min at 72 °C. 10 µl of PCR product

was electrophoresed on 1% agarose gel to determine the size of the product.

Phylogenetic Analysis

The reference sequences required for comparison were obtained from the NCBI database (http://www.ncbi.nlm.nih.gov/Genbank). The aligned sequences were then manually checked for gaps in each row and saved in molecular evolutionary genetics analysis (MEGA) format using MEGA v.2.1 software. Pair-wise evolutionary distances were computed using the Kimura 2-parameter model. To obtain confidence values, the original dataset was resampled 1000 times using the bootstrap analysis method. The bootstrapped dataset was used directly for constructing the phylogenetic tree with the MEGA program or for calculating multiple distance matrixes. The multiple distance matrix obtained was then used to construct phylogenetic trees using the neighbor-joining method of Saitou and Nei (1987). All of these analyses were performed using MEGA v.2.1 (Kumar et al. 2004).

SDS–Polyacrylamide Gel Electrophoresis (SDS–PAGE)

The purity and molecular weight of the bacteriocin-like protein was confirmed on SDS–PAGE. The electrophoresis was carried out using a 15% w v^{-1} polyacrylamide gel using tris-tricine buffer system (Schagger and Jagow 1987). The gel was quick-stained with Coomassie brilliant blue (CBB) G-250 and then destained. Protein molecular weight markers (Bangalore Genei, India) in the range of 14.3–97.4 kDa were used as the standard. To localize the in situ bacteriocin activity, the gel was cut and separated before staining and the gel was fixed and washed as described by Bhunia et al. (1987). The washed gel was placed on a glass plate and overlaid with 0.7% agar containing 10^6 ml^{-1} of S. aureus. The overlaid gel was incubated overnight at 37 °C for 24 h and examined for zone of clearance.

Flow Cytometry

Samples were acquired on FACS Canto II flow cytometer equipped with 3-laser system (405, 488, 633 nm), eight color configuration and BD FACS Diva™ v6.1.3 software. The cytometer was checked daily by the Rainbow set up beads (BD Biosciences). The compensation matrix was performed by anti-mouse IgK/negative control (FBS) Compensation Particles Set (BD Biosciences) following manufacturer recommendations. BD CompBeads were stained as compensation controls for V450 anti-human CD11b and for FITC anti-human CD35, while pHrodo™ labeled bacteria were used as phycoerythrin (PE) fluorescence to calculate the compensation matrix. The compensation values were

calculated automatically by DiVa™ software. The BD High Throughput Sampler (HTS) system was used to run the plate samples. A total of 10,000 events were collected from each sample gated on live cells.

Forward scatter and Side scatter were acquired on a linear scale and fluorescence was acquired on a logarithmic scale. PE and fluorescein isothiocyanate (FITC) were excited using 488 nm laser and the emission of fluorescence was collected using 585/42 and 530/30 nm filters, respectively. V450 and LIVE/DEAD Fixable Aqua were excited by 405 laser and fluorescence emission was collected with 450/50 and 510/50 nm DF filters. After acquisition, all data were exported as Flow Cytometry Standard format 3.0 files (FCS files) and analyzed by FlowJo (Mac-Version 9.1; Treestar US, Ashland, OR).

FT-IR Analysis

In determining the possible functional groups by FT-IR analysis was performed using Perkin Elmer to detect the characteristic peaks and their functional groups. The vibration pattern that appears in the infrared spectra provides information about the chemical functional group of the sample. 100 AU ml^{-1} bacteriocin prepared and 10×0.6 OD indicator strain was mixed together and incubated at 37 °C for 3 h. A fraction of sample was encased directly in sample holder and spectra were scanned from 3460 to 671 cm^{-1}.

Scanning Electron Microscopy

To study the morphology of the cultures and to determine the mode of action of the bacteriocin produced by *P. aeruginosa* TA6 the SEM analysis was carried (McDougall et al. 1994). To know the effect of bacteriocin on cell morphology, the cell pellet of *S. aureus* grown in nutrient broth for 12 h at 37 °C was suspended in the bacteriocin preparation (100 AU ml^{-1}) and incubated for 1 h. The bacteriocin treated and untreated cells were processed for SEM. The cells were harvested by centrifugation at $6500 \times g$ for 15 min and fixed using 2.5% (v/v) aqueous glutaraldehyde for 2 h. These cells were dehydrated using a gradient of ethyl alcohol (10–100%) and final wash was done with absolute ethyl alcohol. The dried cells were gold plated and subjected to scanning electron microscopy (LEO 435-VP, England, UK).

Results

Screening of Bacterial Isolates for Bacteriocin Production

Eight bacterial isolates were obtained from soil samples and they were tested for the bacteriocin activity against indicator

bacteria MRSA. Among them, one bacterial isolate exhibited the antagonistic activity against *S. aureus*. The antagonistic bacterial strain designated as TA6 found to inhibit the growth of *S. aureus* (Fig. 1a).

Antibacterial Activity

The agar well diffusion assay was used to study the antibacterial activity of the TA6 strain of bacteriocin bacteria isolated from soil sample were shown to produce inhibition zones against *S. aureus*. The sensitivity of the indicator strains were estimated based on the diameter (mm) of the inhibition zones. The bacterial strain TA6 produced higher level of bacteriocin (100 AU ml^{-1}) (Fig. 1b).

Biochemical Test

The isolated strain TA6 was identified on the basis of its morphological, biochemical characterization. The microscopic analysis showed rod in shape, motile, non-spore former and can grow at 37 °C. Various biochemical and physiological tests (Table 1) showed that TA6 is catalase, urease, casein hydrolysis, nitrate broth, citrate utilization, gelatin liquefaction, oxidase and lipase positive; however

Table 1 Morphological characteristics and biochemical tests for isolated strain

Tests	Result
Media used for inoculums	NB medium
Colony character	Round, green
Colony on nutrient media	Green
Grams staining	Negative rods
Biochemical tests	
Indole tests	Negative
Methyl red tests	Negative
VP tests	Negative
Citrate utilization tests	Positive
Oxidase tests	Positive
Nitrate tests	Positive
Catalase tests	Positive
Gelatin	Positive
Casein hydrolysis	Positive
Urease	Positive
Lipase	Positive
Starch hydrolysis	Negative

starch hydrolysis, methyl red, voges-proskauer and indole negative. Based on the characterization and the TA6 stain was phenotypically identified as *Pseudomonas* sp.

Identification of Bacteriocin Producing Bacteria Gene Sequence

The ribosomal DNA gene sequences are relatively conserved among the bacteria. Therefore, 16S rDNA sequence was analyzed to confirm the species level identity of the bacterial isolate. The 16S rDNA region (1500 bp) was amplified from the genomic DNA of the bacterial isolate (Fig. 2). The sequences (1411 bp) were aligned and searched for sequence homology in Ribosomal Data base Project II (RDP). The results revealed that the sequence had high bit score of 99% homology with *P. aeruginosa*. The identity of selected bacterial isolate (TA6) was confirmed as *P. aeruginosa*. The 16S rRNA gene is universal in bacteria, and so relationships can be measured among all bacteria. The phylogenetic tree was constructed for the relative species of *Pseudomonas* by the neighbor joining method with 1000 bootstrap samplings. These results suggest that genetic variation appears within the species.

Optimization of Culture Condition for Bacteriocin Production

To determine the optimum pH and time interval for bacterial growth and bacteriocin production, *P. aeruginosa* TA6 was grown in nutrient broth with various initial pH
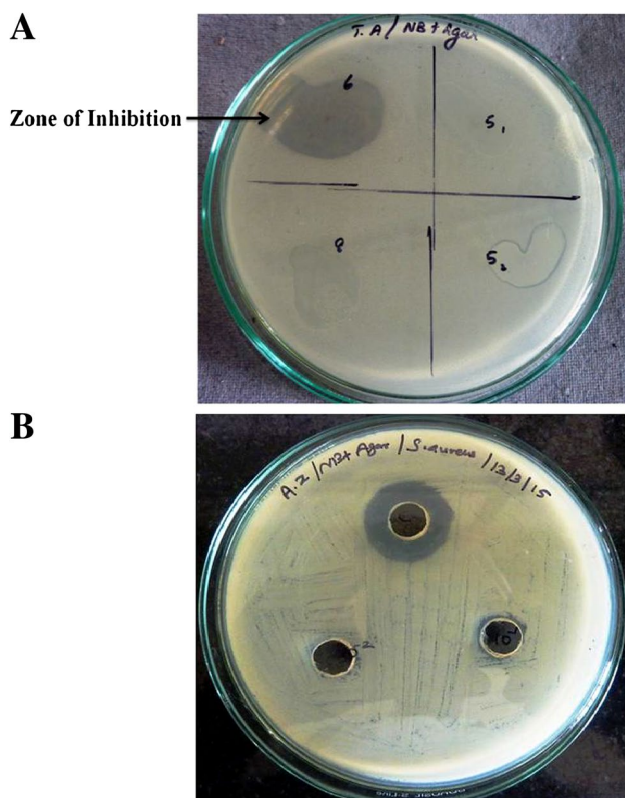

Fig. 1 a Screening of bacterial isolates for bacteriocin activity. **b** Antibacterial activity of bacteriocin produced by strain TA6 against *S. aureus*

Fig. 2 **a** PCR amplification of 16S rDNA gene of bacteria TA6 using universal primer. M—marker; L1—PCR amplification of TA6. **b** Phylogenetic analysis of bacteria TA6 based on 16S rDNA sequences. The tree was constructed using the neighbourjoining method. To obtain confidence values, the original dataset was resampled 1000 time using the bootstrap analysis method
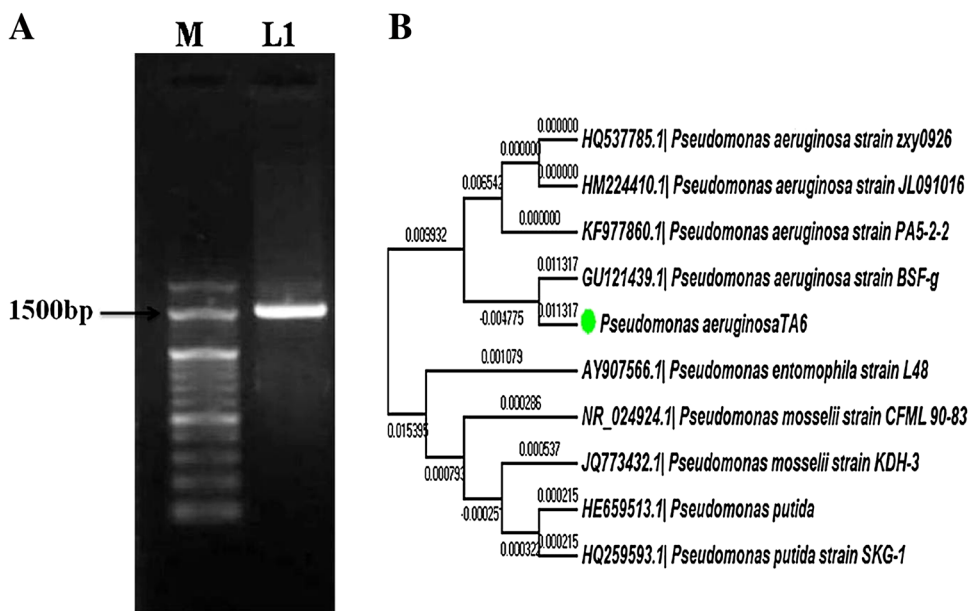
levels (5.0–9.0) at 37 °C for 36 h (Fig. 3a). The bacte-

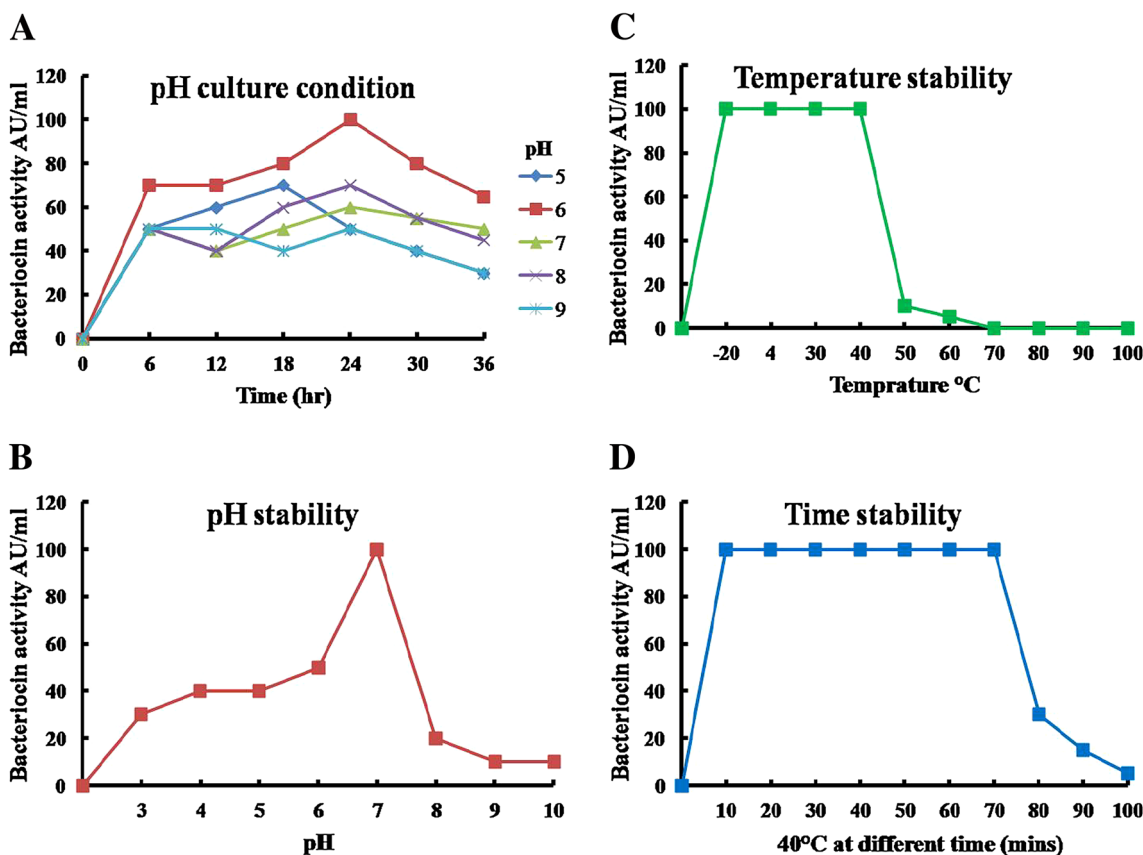in the medium that had an initial pH 6.0 at 24 h of

Fig. 3 **a** Effect of initial pH of the broth on bacteriocin production by *P. aeruginosa*. **b** Effect of pH stability on bacteriocin activity. **c** Effect of temperature stability on bacteriocin activity. **d** Effect of time interval of temperature stability on bacteriocin activity

riocin production was found maximum (100 AU ml^{-1})

incubation.

Effect of pH Stability on Bacteriocin Activity

To determine the stability of bacteriocin in different pH range (3.0–10.0). The sample was treated for 3 h incubation at room temperature. The maximum bacteriocin activity (100 AU ml^{-1}) was observed at pH 7.0 (Fig. 3b). Bacteriocin activity was lowered in other pH ranges (30 AU ml^{-1} at pH 3.0, 40 AU ml^{-1} at pH 4.0, 40 AU ml^{-1} at pH 5.0, 50 AU ml^{-1} at pH 6.0, 20 AU ml^{-1} at pH 8.0, 10 AU ml^{-1} at pH 9.0, 10 AU ml^{-1} at pH 10.0). Therefore, subsequent experiments were carried out at pH 7.0.

Effect of Temperature Stability on Bacteriocin Activity

The effect of various temperatures ranging from − 20 to 100 °C the bacteriocin activity by *P. aeruginosa* TA6 was evaluated (Fig. 3c). The maximum bacteriocin activity (100 AU ml^{-1}) at − 20, 4, 30 and 40 °C in 10 min. Bacteriocin activity was reduced when temperature increases 50 °C (10 AU ml^{-1}) and 60 °C (5 AU ml^{-1}), no activity was found at 70, 80, 90 and 100 °C. Further this bacteriocin activity was carried out at different time ranging from 10 to 100 min. Maximum bacteriocin stability found to be at 70 min (Fig. 3d), where as the activity reduced 30 AU ml^{-1} at 80 °C, 15 AU ml^{-1} at 90 °C, 5 AU ml^{-1} at 100 °C.

Effect of Chemicals and Enzymes on the Bacteriocin

Chloroform, iso-propanol, Tween 80, TCA, Triton X100, Tween 20 and SDS had no effect on the antibacterial activity of bacteriocin. However, the bacteriocin lost the activity

Table 2 Effect of chemicals and enzymes on bacteriocin

Chemicals (concentration)	Residual activity (%) Bacteriocin
Chloroform (50% v v^{-1})	100
Ethanol (50% v v^{-1})	100
Isopropanol (50% v v^{-1})	100
Methanol (50% v v^{-1})	100
Butanol (50% v v^{-1})	0
Acetone (50% v v^{-1})	0
EDTA (10 mM)	0
TCA (0.6 mM)	100
Triton X100 (10% v v^{-1})	100
Tween 80 (10% v v^{-1})	100
SDS (10% w v^{-1})	100
Trypsin 1 mg ml^{-1}	100
Proteinase K 1 mg ml^{-1}	100
Pronase 1 mg ml^{-1}	100

on treatment with acetone, EDTA and *n*-butanol (Table 2). The bacteriocin was also resistant to proteinase K, pronase E and trypsin treatment.

Bactericidal Activity

The mode of action of the peptides was examined to find out if they have bacteriocidal or bacteriostatic action on the indicator strains (Fig. 4). Upon the treatment with this bacteriocin 50 AU ml^{-1}, the cell density of *S. aureus* decreased rapidly and cell lysis eventually occurred at 100 AU ml^{-1} concentrations, the bacteriocin rapidly decreased the viability of *S. aureus* and hence action of these bacteriocins is considered to be bactericidal.

SDS–PAGE and Zymogram Analysis

To estimate the molecular weight of the bacteriocin, the protein in the active fraction was resolved on 15% tricine SDS–PAGE. Based on the electrophoretic mobility, the molecular weight of the single protein band that exhibited antimicrobial activity was estimated to be 10 kDa (Fig. 5).

Flow Cytometry

Flow cytometry has potential as a powerful tool for the investigation of cell membrane damage and repair mechanisms. However, suspensions of cells previously attached to solid surfaces have been analyzed by flow cytometry (Williams et al. 1999). Bacteriocin-treated cells were stained with propidium iodide (PI) subsequently analyzed in the flow cytometry. In this case, cellular damage facilitates the entry of PI into the cells. This suggests some kind of cooperation. Two possible mechanisms could account for this: (1) both molecules cooperate to form either
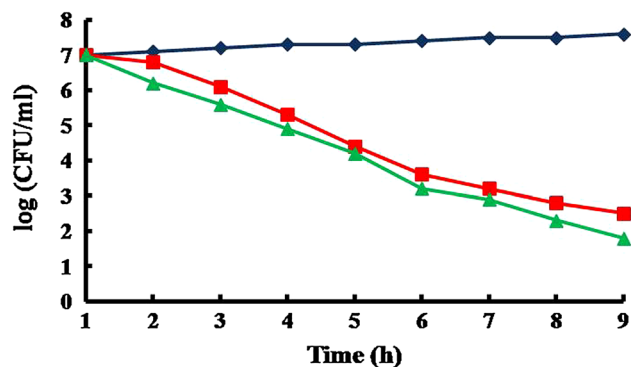

Fig. 4 Bactericidal activity of bacteriocin on *S. aureus*. Filled diamond—control; filled square—50 AU ml^{-1} concentration of bacteriocin; filled triangle—100 AU ml^{-1} concentration of bacteriocin
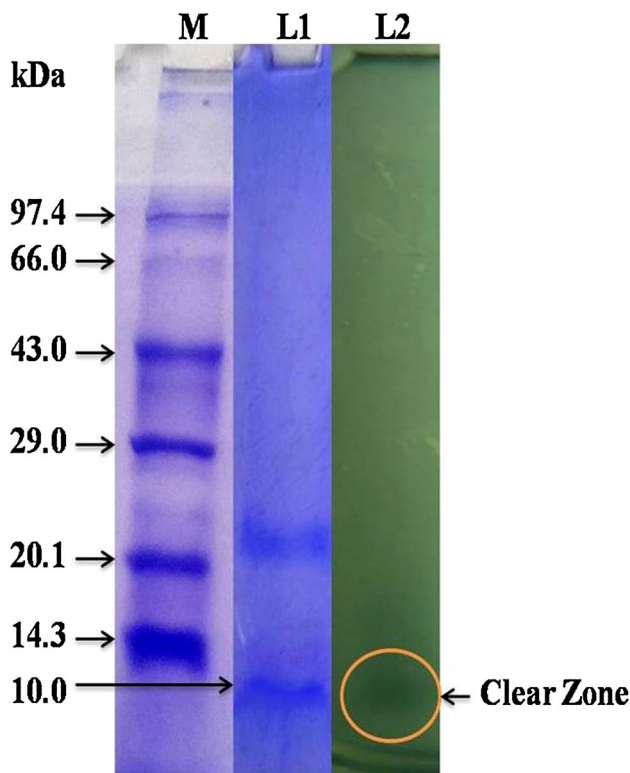
Fig. 5 Tricine SDS–PAGE of the bacteriocin of *P. aeruginosa* TA6. Lane-M: molecular weight protein markers, lane-1: coomassie blue stain gel; lane-2: the protein with *S. aureus* to determine the bacteriocin activity (inhibition of bacterial growth)

wider or more effective channels, and (2) the accumulation of injuries produced by each bacteriocin separately enhances the effects on the integrity of bacterial membranes. Increasing time duration of bacteriocin treatment (3, 6 h) produced increasing dead cells of to *S. aureus*. (A) Control 43.9% dead cells; (B) 3 h treatment 60.5% dead cells; (C) 6 h treatment 66.5% dead cells. The bacteriocin activity dead cell was increased in different time duration compared to control (Fig. 6).

Fourier Transmission-Infra Red Analysis (FT-IR)

The FT-IR spectral analysis, the extracellular metabolite extract of *P. aeruginosa* TA6 strain revealed that the spectral range of obtained functional group ranged was between 300 and 4000 cm^{-1} from the results, it was observed that the peak signal recorded for 671.70, 1637.27, 2083.07 and 3460.46 cm^{-1}. The peak observed at 671.70, was due to alkyl group (C–Br). The sharp peak at 1637.27 cm^{-1} was due to the presence of *o*-amino- or *o*-hydroxyarylketones. The vibration stretch recorded at 2083.07 cm^{-1} represents the possible presence of thiocyanate (–N=C=S). Finally a

broad band at 3460.46 cm^{-1} showed the presence of amide group (–CONH–) (Fig. 7a).

Scanning Electron Microscopy

Scanning Electron Microscopy has been widely used in microbiology to study the surface structure of biomaterials, to measure cell attachment and changes in morphology of bacteria. The SEM-generated photomicrograph of pathogen *S. aureus* after treatment with bacteriocin from *P. aeruginosa* TA6, which are known to affect cell death by pore formation in cell membrane. This indicated that the bacteriocin of *P. aeruginosa* TA6 exhibit drastic degradation of cytoplasmic constituents as seen in SEM of *S. aureus* treated with bacteriocin, wherein complete cell lysis was observed (Fig. 7b).

Genbank Submission

A newly isolated bacteriocin producing bacterial isolate, *P. aeruginosa* TA6 (GenBank accession number-KX548262) was employed in this study.

Discussion

The number of methicillin resistant *S. aureus* (MRSA) infection has been increasing and becoming a serious problem in public health worldwide. Novel antimicrobial agents are immediately needed to combat this drug resistant problem. Recently, a variety of bacteriocins have involved thought for their potential use as food preservative while less research has been conducted on the therapeutic applications as antimicrobial agent. The use of bacteriocin as an alternative agent to overcome the problem is promising (Papagianni 2003). Some of the bottlenecks which are currently hindering the development of bacteriocins as viable therapeutic options has been addressed to predict clinical outcomes of bacteriocin-antimicrobial combinations (Mathur et al. 2017). Hence in this study, bacteriocin producing bacteria were isolates and characterized. The colony morphology of Gram negative isolated bacteria strain TA6 showing high activity against *S. aureus* were identified as *P. aeruginosa* with 99% identity based on 16S rDNA gene sequence analysis. It is well documented with *P. aeruginosa* is produced bacteriocin was previously reported (Naz et al. 2015). The bacteriocin from *P. aeruginosa* was stable upto 60 °C on temperature treatment and also active at pH 7. However, our result was contradicted to earlier report on thermostability exhibited by R-type pyocin lost activity at a temperature above 60 °C (Rubiee et al. 1988). Regarding the influence of pH on the biological activity, pyocin SA189 remained stable within the pH range 2 to 11. Retention of bioactivity of the pyocins
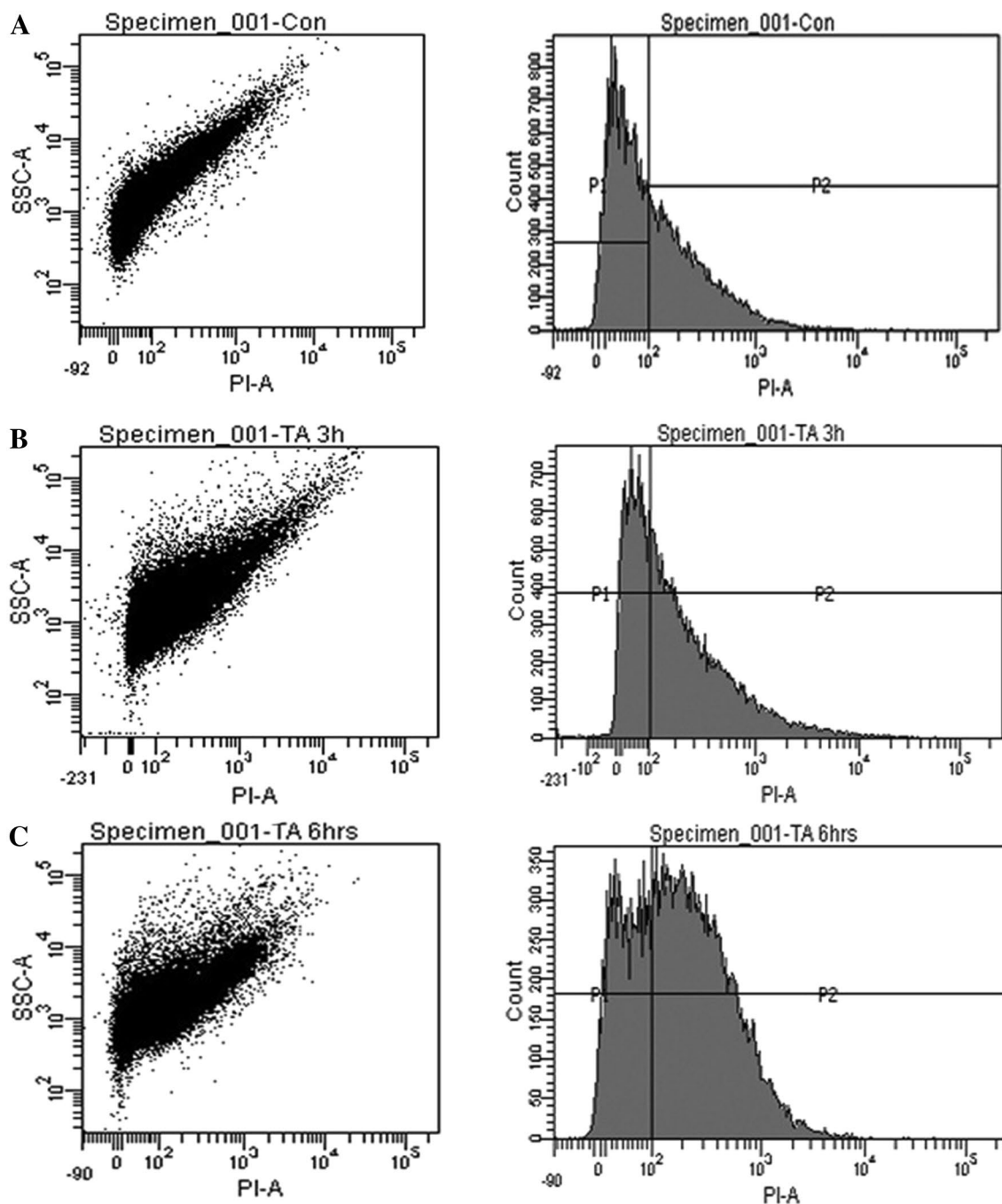
Fig. 6 Flow cytometry analysis of effect of bacteriocin on membrane integrity of *S. aureus*. **a** Control; **b** 3 h treatment; **c** 6 h treatment

at different pH values has been reported earlier (Sano and Kageyama 1981; Padilla et al. 2002; Saleem et al. 2009). The bacteriocins were resolved by SDS–PAGE, the peptide bands visualized after Commassie Blue staining (Fig. 5). SDS–PAGE gels were also revealed with bioindicator *S. aureus* and an inhibition zone 10 kDa was detected. This molecular mass is similar to that found bacteriocin like substances (Meyer et al. 2009). The present results show that increasing time duration of bacteriocin treatment (3, 6 h) produced increasing dead cells of *S. aureus*. (A) Control 43.9% dead cells; (B) 3 h treatment 60.5% dead cells; (C) 6 h treatment 66.5% dead cells. The bacteriocin activity dead cell was increased in different time duration compared to control (Fig. 6). Within the area of bacteriocin research, flow cytometry has potential as a powerful tool for the investigation of cell membrane damage and repair mechanisms.
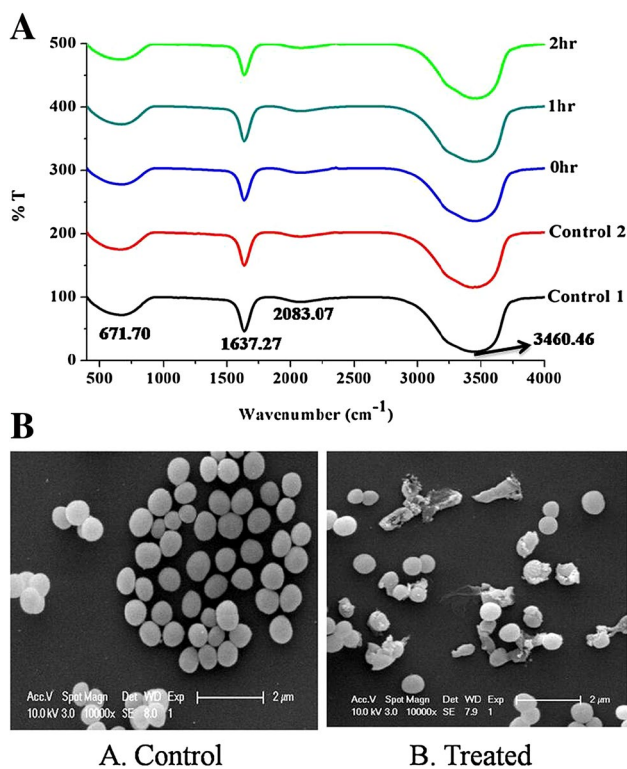
Fig. 7 a Fourier Transmission-Infra Red Analysis (FT-IR) of intra cellular metabolites. **b** SEM analysis of inhibition of *S. aureus* by bacteriocin

aureus test has optimum activity at pH 7 with obstacles measuring 100 AU ml^{-1}, bacteriocin *P. aeruginosa* TA6 active at neutral pH. Bacteriocin *P. aeruginosa* TA6 has a molecular weight of 10 kDa were included in the group of class III bacteriocins, generally large (> 10 kDa), and are not heat resistant. The stable nature of the bacteriocin to high temperature and resistant to various chemicals it also exhibited antimicrobial activity against MRSA make this bacteriocin as potent attractive antimicrobial agent.

Compliance with Ethical Standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

However, suspensions of cells previously attached to solid surfaces have been analyzed by flow cytometry (Williams et al. 1999). The FT-IR spectrum of *P. aeruginosa* TA6 bacteriocin treated *S. aureus*. The strain was revealed that functional range at 300 and 4000 cm^{-3}. In bacteriocin treated cells shift in absorbance in low frequency at 671.70, 1637.27, 2083.07 and 3460.46 cm^{-1}. FT-IR spectroscopy has been applied as a reliable method to study the putative mode of action of cell lytic bacteriocins from *P. aeruginosa* on *S. aureus* (Motta and Brandelli 2008), (Fig. 7a), the main changes observed under SEM analysis were structural disorganization of *S. aureus* the cellular membrane 3 and 6 h after exposure to the bacteriocin of *P. aeruginosa* TA6.

The advancement of science and technology has led to an increase in bacteriocin research. As a consequence, a number of novel LAB bacteriocins with unique and promising properties have been discovered. In this study, the microbe have been isolated from soil sample, the isolated microbe showed bacteriocin production and their effects against *S. aureus*. Bacteriocin producing microbe strain TA6 was identified as *P. aeruginosa* TA6 which is an ideal candidate for higher yielding and enhancement of bacteriocin production and further use. The selected isolated strain producing bacteriocin have been further characterized. Bacteriocin *P. aeruginosa* TA6 bacteria *S.*

References

Baggett HC, Hennessy TW, Leman R, Hamlin C, Bruden D, Reasonover A (2003) An outbreak of community-onset methicillin-resistant *Staphylococcus aureus* skin infections in southwestern Alaska. Infect Control Hosp Epidemiol 24:397–402

Bartoloni A, Bartalesi F, Mantella A, Dell'Amico E, Roselli M, Strohmeyer M (2004) High prevalence of acquired antimicrobial resistance unrelated to heavy antimicrobial consumption. J Infect Dis 189:1291–1294

Bhunia AK, Johnson MC, Ray B (1987) Direct detection of an antimicrobial peptide of *Pediococcus acidilactici* in sodium dodecyl sulphate-polyacrylamide gel electrophoresis. J Ind Microbiol 2:319–322

Brotz H, Josten M, Wiedemann I, Schneider U, Gotz F, Bierbaum G (1998) Role of lipid-bound peptidoglycan precursors in the formation of pores by nisin, epidermin and other lantibiotics. Mol Microbiol 30:317–327

Church D, Elsayed S, Reid O, Winston B, Lindsay R (2006) Burn wound infections. Clin Microbiol Rev 19:403–434

Cosgrove SE, Carmeli Y (2003) The impact of antimicrobial resistance on health and economic outcomes. Clin Infect Dis 36:1433–1437

Cotter PD, Hill C, Ross RP (2005) Bacteriocins: developing innate immunity for food. Nature Rev Microbiol 3:777–788

Daum RS (2007) Skin and soft-tissue infections caused by methicillin-resistant *Staphylococcus aureus*. N Engl J Med 357:380–390

de Jong A, van Hijum SAFT., Bijlsma JJE, Kok J, Kuipers OP (2006) BAGEL: a web-based bacteriocin genome mining tool. Nucleic Acids Res 34:273–279

Fialkov JA, Holy C, Forrest CR, Phillip JH, Antonyshyn OM (2001) Postoperative infections in craniofacial reconstructive procedures. J Craniofac Surg 12:362–368

Fridkin SK, Hageman JC, Morrison M, Sanza LT, Como Sabetti K, Jernigan JA (2005) Methicillin-resistant *Staphylococcus aureus* disease in three communities. N Engl J Med 352:1436–1444

Gratia A (1925) Sur un remarquable exemple d'antagonisme entre deux souches de coilbacille. Comp Rend Soc Biol 93:1040–1041

Heng NCK, Wescombe PA, Burton JP, Jack RW, Tagg JR (2007) The diversity of bacteriocins in Gram-positive bacteria. In: Riley MA, Chavan M (eds) Bacteriocins: ecology and evolution. Springer, Berlin, pp 45–92

Kirkup BC (2006) Bacteriocins as oral and gastrointestinal antibiotics: theoretical considerations, applied research, and practical applications. Curr Med Chem 13:335–3350

Krishnan V, Johnson JV, Helfrick JF (1993) Management of maxillofacial infections: a review of 50 cases. J Oral Maxillofac Surg 51:868–873

Kumar S, Tamura K, Nei M (2004) Mega3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform 5:150–163

Mathur H, Field D, Rea MC, Cotter PD, Hill C, Ross RP (2017) Bacteriocin-antimicrobial synergy: a medical and food perspective. Front Microbiol 8:1205

McDougall PP, Shane S, Oviatt BM (1994) Explaining the formation of international new ventures: the limits of theories from international business research. J Bus Ventur 9(6):469–487

Meyer J, Rogne P, Opegard C, Haugen H, Kristiansen P (2009) Structure-function relationships of the non-lanthionine-containing peptide (class II) bacteriocins produced by gram-positive bacteria. Curr Pharm Biotechnol 10:19–37

Motta AS, Brandelli A (2008) Evaluation of environmental conditions for production of bacteriocin-like substance by *Bacillus* sp. strain P34. World J Micro Biotech 24:641–646

Naz SA, Jabeen N, Sohail M, Rasool SA (2015) Biophysicochemical characterization of Pyocin SA 189 Produced by *Pseudomonas aeroginosa* SA189. Braz J Micro 46 (4):1147–1154

Okesola AO (2011) Community-acquired methicillin-resistant *Staphylococcus aureus* - a review of literature. Afr J Med Sci 40:97–107

Otto M (2008) *Staphylococcal* biofilms. Curr Top Microbiol Immunol 322:207–228

Padilla C, Lobos O, Brevis P (2002) Effect of the bacteriocin PsVP- 10 produced by *Pseudomonas* sp. On sensitive bacterial strains. De Microbiologia 44:19–23

Papagianni M (2003) Ribosomally synthesized peptides with antimicrobial properties: biosynthesis, structure, function, and applications. Biotech Adv 21:465–499

Piper C, Hill C, Cotter PD, Ross RP (2011) Bioengineering of a nisin A-producing *Lactococcus lactis* to create isogenic strains producing the natural variants nisin F, Q, and Z. Microb Biotech 4:375–382

Riley MA, Wertz JE (2002) Bacteriocins: evolution, ecology, and application. Annu Rev Microbiol 56:117–137

Rubiee R, Mudhaffar S, Hassan F (1988) Purification and characterization of pyocins from *Pseudomonas aeroginosa*. Folia Microbiol 30:25–29

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Saleem F, Ahmed S, Yaqoob Z (2009) Comparative study of two bacteriocins produced by representative indigenous soil bacteria. Pak J Pharma Sci 22:252–258

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Nova York

Sano Y, Kageyama M (1981) Purification and properties of an S-type pyocin, Pyocin AP41. J Bacteriol 146:733–739

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

Severina E, Severin A, Tomasz A (1998) Antibacterial efficacy of nisin against multidrug-resistant Gram-positive pathogens. J Antimicrob Chemother 41:341–347

Shand RF, Leyva KJ (2008) Archaeal antimicrobials: an undiscovered country. In: Blum P (ed) Archaea: new models for prokaryotic biology. Caister Academic, Norfolk, pp 233–242

Tagg JR, Dajani AS, Wannamaker LW (1976) Bacteriocins of Gram-positive bacteria. Bacteriol Rev 40:722–756

Toba T, Yoshioka E, Itoh T (1991) Potential of Lactobacillus gasseri isolated from infant faeces to produce bacteriocin. Lett Appl Microbio 12:228–231

Vindenes H, Bjerknes R (1995) Microbial colonization of large wounds. Burns 21:575–579

Williams I, Paul F, Lloyd D, Jepras R, Critchley I, Newman M (1999) Flow cytometry and other techniques show that *Staphylococcus aureus* undergoes significant physiological changes in the early stages of surface-attached culture. Microbiology 145:1325–1333