JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

BIO-PROFILING OF A POTENTIAL ANTIMYCOBACTERIAL BACTERIOCIN PRODUCED BY BACILLUS SUBTILIS (MK733983) OF ETHNOMEDICINAL ORIGIN

Santhi Sudha S. [1], *Aranganathan V.* *[2]

Address(es): Aranganathan V
[1]Jain (Deemed to-be-University, Doctoral Research Scholar, Department of Biochemistry, 18, 3rd Block, 9th Main Rd, Jayanagar, Bengaluru, Karnataka 560011. Phone: +919035501749, 080 4322 6500.
[2]Jain (Deemed to-be-University, Faculty, Department of Biochemistry, 18, 3rd Block, 9th Main Rd, Jayanagar, Bengaluru, Karnataka 560011. Phone: +919035501749, 080 4322 6500.

*Corresponding author: v.aranganathan@jainuniversity.ac.in

https://doi.org/10.15414/jmbfs.3259

ARTICLE INFO

Received 12. 6. 2020
Revised 8. 3. 2021
Accepted 10. 3. 2021
Published 1. 8. 2021

Regular article

OPEN ACCESS

ABSTRACT

Antibiotic resistance is a global crisis and antimicrobial peptides or bacteriocins are the ascending alternatives. This study is reporting a potential bacteriocin from *B. subtilis* (MK733983) of ethnomedicinal origin showing significant activity with *Mycobacterium smegmatis* as an indicator organism on spot-on-lawn assay. Production optimization with suitable physical parameters such as modified lysogeny broth [1.5% peptone; 0.75% salt; 0.75% yeast], temperature [35 °C], pH [7], inoculum size [1%] and time course [24 h] has enhanced the activity up to 20%. This study also observed that supplements like minimal essential medium amino acids and B complex vitamins individually has further enhanced the bacteriocin activity by 13% (total 33%), biomass by 43%, total protein and activity by 17% and 22% respectively. However, addition of some common growth supplements like multivitamins with trace elements, $CaCl_2$ and $MgSO_4$ had shown inhibitory effect on bacteriocin activity. Molecular weight of the bacteriocin was estimated to be below 14kDa and bioassay guided TLC techniques showed distinctive antibacterial activity. The bacteriocin completely lost its activity with Trypsin, α-Chymotrypsin, Proteinase K but not with Amylase, and showed up to 40% activity with Papain. It showed heat stability up to 70°C with a wide antibacterial spectrum against standard strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli and Chromobacterium violaceum* with minimal inhibitory concentrations ranging between 0.325±0.02-0.75±0.02mg/mL, suggesting the bacteriocin may have wide application prospects.

Keywords: Bacteriocin; *Bacillus subtilis;* Ethnomedicinal origin; Bioassay guided TLC techniques; wide antibacterial spectrum

INTRODUCTION

Antimicrobial resistance (AMR) has become an escalating global burden with an increase in the microbial resistance endangering the efficacy of antibiotics across all socio-economic backgrounds, with other complicated risks such as drug-drug interactions, hospital acquired infections and others posing a serious challenge (**Ventola, 2017**). Although campaigning on minimization of antibiotic prescriptions, encouragement of antibiotic-free food industry, minimizing antibiotic use in meat industry and chemical preservatives, and novel diagnostics with generation of antimicrobial agents are in progress, the development of new antimicrobials has declined in recent years. One of the tangible reasons is due to its production cost and the complexity of the multiple resistance mechanisms developed by the AMR microbes and thus, development of potential antimicrobial agents has become very difficult and a critical priority in the present world (**Lopez *et al.*, 2017; WHO report, 2017**).
Antimicrobial peptides (AMPs) also known as host defensive proteins (HDPs) seem to be promising alternatives to traditional antibiotics, antiseptics, and other non-specific anti-bacterial substances due to their high potency, specific antibacterial spectrum, low risk of resistance development, limited effects on the normal flora and importantly, they can be chemically modified to more efficient forms (**Christopher *et al.*, 2012**). Some bacterial species produce biologically active proteins or peptides known as peptide antibiotics, also known as bacteriocins (**Zhang *et al.*, 2018**). Many of these ribosomally synthesized peptides or proteins are known to have narrow spectrum, inhibiting the bacteria that are taxonomically close, but a few others are known to be broad spectrum (**Silva *et al.*, 2018**). Even further ahead, bacteriocins were reported to have inhibited polymicrobial infections (**Ramya *et al.*, 2014**), capable of inhibiting biofilm formation (**Khalaf *et al.*, 2016**) and a few studies have shown to inhibit taxonomically unrelated bacteria and certain viruses (**Park *et al.*, 2002**). Although the productivity of bacteriocins by microbes like bacteria are time consuming with below par yield, these challenges can be compensated with an effective optimization and purification techniques. Furthermore, characterization of the biologically active compounds like bacteriocins is pivotal for comprehensive understanding of their scope in their future potential as therapeutic agents, preservatives, or other uses.
Present study was focused on optimizing *B. subtilis* (MK733983) strain capable of producing a potential antimycobacterial bacteriocin with *M. smegmatis* as an indicator microorganism (IMO). Purification by solid phase extraction of the bacteriocin, molecular weight determination, characterization with hydrolytic enzymes and organic solvents were delineated. The active compounds were also characterized by bioassay guided chromatographic (TLC) techniques for detecting antimicrobial activity under diverse occurrences (**Mahmoud *et al.*, 2018**). Antibacterial Inhibition spectrum of the bacteriocin was tested against some standard strains of gram positive and negative bacteria that fall under WHO critical and high priority pathogen list (**WHO report, 2017**).

MATERIAL AND METHODS

Indicator microorganisms and culture conditions

Indicator microorganisms (IMO's) were taken in inoculum size of 10^6 CFU/mL cells measured at A_{600} for all the antimicrobial assays under prescribed suitable growth conditions. Standard bacterial strains of *M. smegmatis* (MC^2-155 wild type), *S. aureus* (MTCC 737), *P. aeruginosa* (MTCC 3541) and *C. violaceum* (MTCC 2656) were cultured using LB (Luria-Bertani) media, and *K. pneumoniae* (ATCC 700721), *E. coli* (ATCC 8739) were cultured using Muller-Hinton Media at 35°C for 24 h.

Bacteriocin Production Optimization

Standard LB media for the isolated *B. subtilis* (MK733983) was optimized by one-factor optimization method for maximum bacteriocin production using

carbon, nitrogen and salt concentrations, along with other biophysical parameters like pH (6.0-8.5 with interval of 0.5); Inoculum size (1-6%); temperature (20-50°C with interval of 5°C) and effect of time course on growth along with the bacteriocin production was studied by measuring the colony forming units (CFU/mL) at A_{600} up to 5 days. The bacteriocin preparation is described in detail by **Santhi & Aranganathan** (**2019**) and production optimization was determined step-by-step, advancing to next parameter with already optimal factors and their bacteriocin activity was determined by 30 µL of each of bacteriocin (1mg/mL) loaded into 6 mm wide wells made in LB agar plates pre-swabbed with 100 µL of *M. smegmatis* (10^6 CFU/mL) as an indicator and zone of inhibition (ZOI) in mm was taken as a measure of antimicrobial activity (**Tagg & McGiven, 1971**). The bacteriocin producer was further optimized with supplements like minimal essential medium (MEM) amino acids (1-2%), Multivitamins and B complex vitamins (0.5-2%), $CaCl_2$ and $MgSO_4$ (0.1-0.5g/mol). For testing the growth kinetic pattern of *B. subtilis* (MK733983), its culture could grow with the delineated optimal parameters, simultaneously, the bioactivity of its supernatant was assessed by optical density values measured spectrophotometrically at 600 nm. Their supernatants were collected at a regular time interval of 3 h, was used for preparing bacteriocin concentrate (1mg/mL). It was then checked for its antimicrobial activity through well diffusion assay with *M. smegmatis* as the indicator by taking each of 30µL per well (6 mm), this experiment was done twice in triplicates. All chemicals were purchased from SD Fine-Chem Ltd (India) and Himedia for all the experiments in the entire study.

Solid Phase Extraction

The bacteriocin obtained from optimized media was ultra-filtered with Polyether sulfone membrane syringe filter - pore size 0.22 mm (Merck) and then loaded to a C18 Sep-Pak cartridge (Flinn Scientific) that was thoroughly pre-washed with double distilled water, followed by the bacteriocin loading and was eluted with 100% methanol as an attempt for initial purification step. The eluted sample was further concentrated by drying in a hot air oven (45°C) and were dissolved in DMSO [Dimethyl sulfoxide] and was used for further study on antimicrobial assays, with *M. smegmatis* (10^6 CFU/mL) as an indicator.

Bacteriocin activity was defined as the reciprocal of the highest dilution at which bacterial growth was inhibited and expressed as arbitrary units (AU) per milliliter. Activity was calculated as $a^b \times 100$, where a is the dilution factor and b is the final dilution factor that can produce minimum inhibition zone (2 mm) and the experiments were done in quadruplicates. The increase in yield optimization and purification was evaluated by Lowry's protein estimation **(Lowry *et al.*, 1951**), bacteriocin specific activity (**Lee *et al.*, 2018**) and bacteriocin activity by spot on lawn method.

Characterization of bacteriocin

Molecular weight determination

Molecular mass determination was done by tricine-Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) 5% stacking and 12% separating gels (**Schagger & Jagow, 1987**). Electrophoresis was run with mid-range marker [SRL protein marker, mid-range cat BCL – 038 (14.3-97.4)] and it was carried out at 50 V for first half an hour followed by 100 V. Since many bacteriocins are known to be sensitive to different stains, the gels were stained with widely used Coomassie brilliant blue (CBB) and Silver nitrate. One set of the gels were left in a fixative solution (50% methanol; 10% acetic acid) for 30min, followed by CBB (R)-250 staining overnight. Then gels were de-stained with 10% acetic acid and 20% methanol.

Another set of gels were stained by silver-stain by washing thoroughly in 50 mM (NH4)2HCO3 in 50% methanol and were then incubated in sensitizing solution (Na2S2O3.5H2O w/v 0.02%) for 2-3 min on gentle rotation (**Giulian *et al.*, 1983**). Later, washed thoroughly with chilled sterile water and were put in staining solution (0.2% w/v Silver nitrate in 0.076% formalin) and were kept under gentle rocking until the bands were visible. Once the bands were visible, the reaction was immediately stopped by adding terminating solution (12% acetic acid) and all the results were recorded. This experiment was done twice with duplicates.

Bio-Profiling

Thin Layer Chromatography-Direct Bioautography (TLC-DB)

Demetrio *et al.* (2016) TLC-DB protocol was followed with minor variation, ~5µL of bacteriocin (1mg/mL) was spotted manually on to a pre-conditioned silica gel plate (6×4 cm) (Fluka, silica gel 60 F-254) with organic solvents (butanol/methanol) for 30 min at a temperature range of 18 - 20°C. The plates were dried horizontally for a few minutes at room temperature before placing them in the development chamber and was run with a predetermined, suitable mobile phase of butanol-methanol-water in 3:1:1. Later, the plate was visualized at 395-570 nm, 450-495 nm and 380-450 nm in UV TLC viewer and the relative retardation factor (Rf) was calculated. For the Direct bioautography, when TLC plate was sprayed with 5-10mL of *M. smegmatis* cell suspension (10^6 CFU/mL) and were incubated at 37°C for 24 h and the antibacterial activity was identified as spots with clear zones of inhibition against the indicator organism. This experiment was done in triplicates.

Contact Bioautography

Khurram *et al.* (2009) protocol for contact bioautography was followed with a variation, where the TLC chromatogram of approximately (6×6 cm) was developed by spotting 100µL of the bacteriocin sample (1mg/mL) that was gently deposited at several spots on the silica gel plate under similar conditions mentioned in TLC-DB and was left for 30 min (at room temperature) for an evenly spread. Later, the TLC plate was placed face down, ensuring a proper contact on to the *M. smegmatis* (10^6 CFU/mL) inoculated agar layer in a petri dish for a period of 4-6 h to enable diffusion. Later the chromatogram was gently removed, and the agar plates were incubated at 37°C for 24 h and antibacterial activity was recorded. This experiment was done twice with duplicates.

Agar diffusion DB

The gels that were developed after CBB/ silver staining in tricine SDS PAGE were thoroughly washed with 0.1% Tween 80 at room temperature. Subsequently one set of SDS-free gel was aseptically placed in LB broth (0.7% agar, w/v) containing *M. smegmatis* (10^6 CFU/mL) as indicator and another set was placed under similar conditions with added trypsin (1mg/mL). The plates were incubated at 37°C for 24 h and were observed for the presence of inhibition zones (**Maricic & Dawid, 2014**; **Saikat *et al.*, 2014**). This experiment was done twice with duplicates.

Effect of enzymes and heat on bacteriocin

Effect of hydrolytic enzymes was evaluated by taking each of 1:5 ratio of bacteriocin to Trypsin (pH-8.0; T-8918; Sigma), Proteinase K (pH-7.5; P-2308; Sigma), α- Chymotrypsin (pH-7.8; RM801; Himedia), Papain (pH-7.0; GRM058; Himedia) and Salivary amylase (pH-7.0; (Human Saliva in 1:9 of 0.85% saline solution) and the enzyme treated samples were incubated at 37°C for 24 h. The bacteriocin untreated with enzyme was used as positive control and 30µL of DMSO alone is taken as blank. Thermal stability was examined by incubating bacteriocin for 10 min under moist heat treatment ranging from 40°C to 121°C by 10°C rise and samples unexposed to heat were taken as control. Residual activity of all the samples (each of 30µL) tested for antimicrobial activity determined by agar well diffusion method with *M. smegmatis* (10^6 CFU/mL) as indicator. The assay was performed twice in triplicates.

Inhibitory spectrum and Minimal Inhibitory Concentrations (MIC)

Inhibitory spectrum assays were done by well diffusion assays, 30µL and 50µL of bacteriocin (1mg/mL), was placed in the wells of the media overlaid with 100µL of gram positive and negative indicator microorganisms (10^6 CFU/mL) respectively, they were incubated at 37°C for 24 h and the antimicrobial activity was evaluated along with determination of the MIC values as described by **Santhi & Aranganathan (2019)**. All the assays were done in triplicates for a minimum of two independent experiments.

Statistical Analysis

All experimental results are expressed as means ± standard error of the mean or standard deviation (SD) and the significant differences were examined by regression analysis by using [Microsoft 365 MSO (16.0.12827.20200) 32-bit] and One-way analysis of variance (ANOVA) by Tukey test (Post-hoc analysis) with SPSS 27.0. p-value < 0.05 was considered statistically significant. Relative Percentage and residual activity were calculated in comparison to the Positive control values of the experiment performed.

RESULTS AND DISCUSSION

Optimization and purification results

Optimization of standard media and biophysical parameters had approximately increased the bacteriocin activity by 20% comparing to that of the crude. The growth kinetic pattern of *B. subtilis* (MK733983) suggested that the bacteriocin activity reached its maximum at the end of the exponential phase (figure 1). Further additional supplements like MEM amino acids or B complex vitamins had approximate enhancement of biomass by 43%, total protein by 17% and antimicrobial activity by 33%. Some of the common supplements like CaCl2, MgSO4 and multivitamins with trace elements had shown decreased bacteriocin activity (Tab 1). On elution with C18 SEP-PACK, specific activity of the bacteriocin increased as noted in Tab 2 and antimicrobial activity increased approximately by 33.3% with a ZOI of 20.17±0.07 mm.

Table 1 Optimization Results

Optimization Factors	Range tested	Optimal Range	ZOI (mm)
Standard Media	Standard constituents	-	15
Peptone conc (%)	0.5-2 (0.25 interval)	1.5	16.06±0.02
Low NaCl conc (g/mol)	0.25-1.75 (0.25 interval)	0.75	16.15±0.03
High NaCl conc (g/mol)	3,4 & 5	3	8.16±0.04
Yeast %	0.25-1 (0.25 interval)	0.75	16.17±0.04
Time course (Days)	1 – 5 (24h interval)	24h	16.06±0.03
pH	6 – 8.5 (0.5 interval)	7	16.2±0.02
Inoculum size (%)	1 – 6 (1% interval)	1	18.08±0.02
Temperature °C	20-50 (5°C interval)	35	18.4±0.3
Physical parameters Optimization	Physical optimal parameters	-	18.4±0.3
B complex vitamins (%)	0.5 – 3.5 (0.5 interval)	2.5	20.12±0.02
MEM amino acids (%)	1 - 2 (0.25 interval)	1	20.16±0.08
$CaCl_2$ g/mol	0.1-0.5 (0.1interval)	0.2	18.17±0.05
$MgSO_4$ g/mol	0.1-0.5 (0.1interval)	0.1	15.22±0.02
Multivitamins with trace elements (%)	1 – 2 (0.25 interval)	1%	10.1±0.2
Complete Optimization	With supplements		20.16±0.08

Legend: IMO-*M. smegmatis*; Optimal range represents the bacteriocin conc that was reported to exhibit highest antagonism towards IMO; ZOI – Zone of Inhibition in diameter on petri plates in (mm) and values are means ± standard deviation of triplicates ($p < 0.05$).

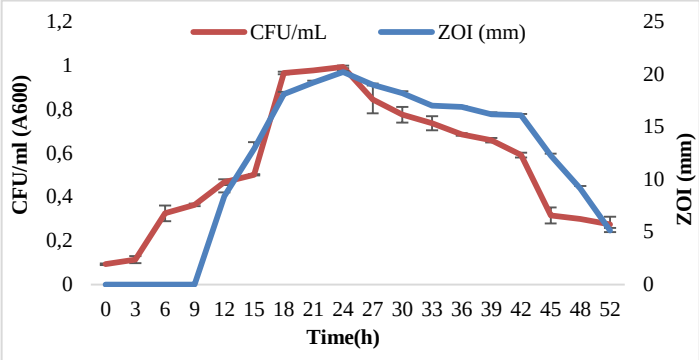

Figure 1 Growth of *B. subtilis* (MK733983) on incubation (h) and bacteriocin production. Cells growth at A_{600} and its bacteriocin production measured on the scale of its inhibition (mm) against imo, *M. smegmatis.* Values represented in the graph are means ± standard deviation of triplicates ($p < 0.05$).

Characterization by tricine SDS-PAGE and Bio-profiling

The bacteriocin showed migration as a single band with an estimated molecular weight to be below 14.3 kDa on assessment with tricine SDS- PAGE. CBB staining showed better result comparing to silver staining (figure 2, Lane 1&2). Figure 3 shows the TLC Chromatogram with a separation at Rf value of 0.85±0.005 which fluoresce when viewed under 450-495nm & 380-450nm and correspondingly a spot of clearing as an indicative of antibacterial activity against *M. smegmatis* was observed in its bioautogram. In contact bioautogram, the chromatogram taken showed a significant antibacterial activity inhibiting the growth of *M. smegmatis*, substantiating the ability of its antimicrobial entities to diffuse from complex substrates on contact and sustain its inhibitory action (figure 4). On agar diffusion DB of the gel band from PAGE, growth inhibition of the indicator (*M. smegmatis*) was observable in place of gel band and the gel band that was incubated in media treated with trypsin, showed growth of indicator organism, highlighting the proteolytic nature of the bacteriocin (Lane 3).

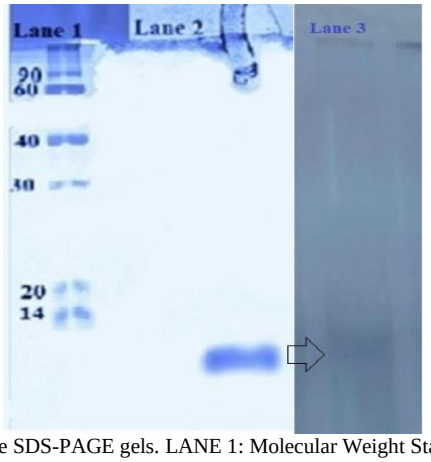

Figure 2 Tricine SDS-PAGE gels. LANE 1: Molecular Weight Standard; Lane 2: Migration of the bacteriocin; Lane 3: Trypsin digestion of the bacteriocin Gel Band.

Figure 3 TLC Chromatograms and Bioautogram. Chromatograms recorded with 3 Filters Of TLC Viewers & TLC Bioautogram shows microbial clearing observed at Rf = 0.85±0.005 ($P < 0.05$) with IMO, *M. Smegmatis.*

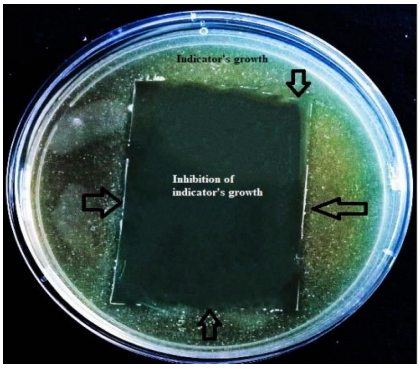

Figure 4 Contact Bioautogram. Bioautogram (6×6 Cm) showing the inhibition of IMO growth (*M. Smegmatis)*.

Effect of enzyme and heat

Fig 5 shows the results of proteolytic enzymes effect on the bacteriocin, it showed a complete loss of activity with the Trypsin, α-Chymotrypsin and Proteinase K. On the other hand, bacteriocin treated with salivary amylase did not show any loss of activity and treatment with Papain showed a residual activity of 8.1±0.07 mm ($p < 0.05$). compared to the positive control (20.1±0.09 mm). Bacteriocin showed a consistent thermostability up to 70°C and retained ~59% activity at 100°C (Tab 3).

Table 2 Purification of Bacteriocin

Purification Steps	Volume Taken (ml)	Total Protein (mg)	Total Activity (Au)	Specific Activity*	Recovery %	Purification-Fold
CFS	100	12800	20500	1.6	100	1%
Optimization	100	15000	25000	1.66	121.90	1.03%
C18 SEP-PACK	80	900	8500	9.44	41	5.88%

Legend: * Specific activity is the ratio of total activity to that of total protein.

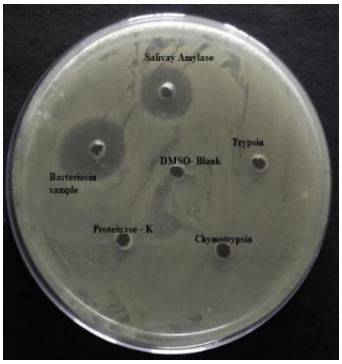

Figure 5 Effect of enzymes. IMO - *M. smegmatis*; ZOI of positive control (bacteriocin sample) - 20.1±0.09 mm, similarly with amylase treatment; No activity observed in blank/negative control (DMSO) or bacteriocin samples treated with Trypsin, Proteinase K, and α - Chymotrypsin.

Table 3 Thermostability of Bacteriocin

S.NO.	**Temperature** (°C)	**ZOI** (mm)	**Relative activity** (%)
1	Room Temperature	20.17±0.07	100
2	40	20.15±0.05	99.9
3	50	20.14±0.15	99.85
4	60	20.05±0.05	99.4
5	70	20±0.05	99.15
6	80	17.7±0.25	87.75
7	90	14.8±0.6	73.3
8	100	12±0.17	59.4
9	121	No inhibition	-

Legend: IMO-*M. smegmatis*; The bacteriocin is exposed to mentioned temperatures for 10 min; ZOI - Zone of inhibition by bacteriocin after heat exposure against IMO. values are means ± standard deviation of triplicates ($p < 0.05$).

Antibacterial spectrum and their MIC's

The bacteriocin showed inhibitory action with both gram-positive and negative bacteria and their MIC values shown in Tab 4, revealed that the gram-negative bacteria needed more concentration of bacteriocin compared to that of gram-positive bacteria, ranging 0.325±0.02-0.75±0.02mg/mL ($p < 0.05$).

Table 4 Antibacterial spectrum with MIC values

S.NO.	**Indicator Microorganism**	**Gram's character**	**ZOI** (mm)	**MIC** (mg/mL)
1.	*Staphylococcus aureus*	Positive	18.3±0.1	0.325±0.02
2	*Mycobacterium smegmatis*	Positive	20.17±0.02	0.35±0.004
3	*Pseudomonas aeruginosa*	Negative	19.5±0.39	0.6±0.02
4	*Klebsiella pneumoniae*	Negative	16.03±0.5	0.75±0.02
5	*Escherichia coli*	Negative	16.9±0.28	0.7±0.02
6	*Chromobacterium violaceum*	Negative	16.58±0.23	0.75±0.04

Legend: The bacteriocin taken per well for gram positive and negative IMOs is 30µL and 50µL (1mg/mL); MIC-minimal inhibitory concentrations. values are means ± standard deviation of triplicates ($p < 0.05$).

B. subtilis is a gram positive, aerobic and endospore forming rod shaped cells and are ubiquitous in nature. In the present investigation, a potential strain of *B. subtilis* (MK733983) isolated from ethnomedicinal plant origin was optimized for its bacteriocin production. The factors like time course, temperature and B complex vitamins showed a significant role in enhancing bacteriocin production. Rationale for escalatory and inhibitory effects of nutrient supplements on the bacteriocin producer must be further investigated. Growth kinetic study of *B. subtilis* shows that the potential entities of the bacteriocin was produced maximum between 18-24 h.

Generally aromatic, polycyclic compounds (Polycyclic aromatic Hydrocarbons - PaHs) with non-polar nature have higher Rf values and fluoresce when viewed under longer wavelengths, the bacteriocin in this study shows higher Rf value and fluoresce when viewed under 450-495nm & 380-450nm and not under 495-570nm, in this preliminary assessment and needs other confirmatory evaluation. A single band on tricine SDS-PAGE of the bacteriocin indicates that the sample deposited is a compound with minimal impurities. The presence of single band at this initial step of purification do not imply that the bacteriocin is pure, because of the possibility of presence of other sensitive molecules which are undetectable by the chosen method of experimentation.

Bio-profiling using Bio-autography is an association of planar chromatographic analyses with biological detection methods and known as effect-directed analyses (EDA) or Bioassay guided chromatography techniques and they are simple tools for characterizing antimicrobial properties of analyzed substances in minute quantities (**Grzelak *et al.*, 2013**). On the other hand, they are fast, accurate, inexpensive, and reliable methods to detect antimicrobial agents in a mixture of compounds, the bacteriocin demonstrated its ability to sustain activity in complex substrates in smaller quantities through TLC-DB. It also showed in-situ activity and its enzyme inhibition by trypsin affirming the proteinaceous nature by agar diffusion DB and its ability to diffuse by contact DB.

The bacteriocin treated with hydrolytic enzymes further reaffirmed its proteinaceous nature assuring its position as a GRAS (generally accepted as safe) protein along with its thermostability, as an added advantage (**Hammami *et al.*, 2009**) for its prospects as a bio-preservative. The improvement in thermostability of the bacteriocin on optimization and partial purification might be due to the increase of thermostable antimicrobial entities with conducive growth parameters. Bacteriocins from various microbes have generally exhibited a narrow inhibitory spectrum toward related producer strains (**Klaenhammer, 1993**). However, bacteriocins such as Thuricin 17 (**Gray *et al.*, 2006**), Cerein 8A (**Bizani *et al.*, 2005**), Gas 101 (**Garima *et al.* 2018**) have exhibited broad spectral inhibition. In agreement with the same, the bacteriocin in this study also showed antagonism towards certain critical and high prioritized gram positive and negative standard strains exhibiting its potential. *C. violaceum* is an emerging pathogen, widely studied for its quorum sensing mechanisms associated with the biofilm formation in many bacterial species (**Batista & Silva, 2017**). The bacteriocin shows inhibition towards this emerging pathogen substantiating its probable capability to disrupt quorum sensing mechanisms, which must be further investigated. The plausible cause for more bacteriocin concentration to inhibit gram negative bacterial strains may be due to their unapt taxonomical characteristics.

Many bacteriocins from lactic acid bacteria and *Bacillus* species are proven competent natural preservatives that can inhibit the spoilage and spread of pathogenic bacteria in food (**Gautam & Sharma, 2009**). They are emerging as potential surrogates and synergists to the chemical food preservatives with increase in demand for the natural bio-preservatives (**Wayah & Philip, 2018; Sarika *et al.*, 2019)** and are proficient to be considered as therapeutic agents **(Christopher *et al.*, 2012**). There are very few reports proving that the bacteriocins are antiviral (**Naruse *et al.*, 1990**) and antimycobacterial (**Sosunov *et al.*, 2007**) in nature. As per our knowledge, the results of the present study report the potential ethnomedicinal plant originated bacteriocin that has not only an effective antimycobacterial activity but antibacterial towards certain primal bacterial standard strains.

CONCLUSION

Currently multisectoral approach with divergent strategies are being developed globally to treat deleterious infections. Bacteriocins with their distinct features of selective toxicity towards wide microbial spectrum without developing resistance, are promising emerging alternatives. Their capacity to inhibit developed biofilms along with strong synergy with several antibiotics compared to conventional antibiotics are drawing attention as potential drug candidates for food and pharmaceutical industry. The significant characteristic features of the bacteriocin reported in this study has justified its ability to combat broad bacterial species and can be used as a precursor for the development of promising anti-mycobacterial / anti-bacterial drugs and as a bio-preservative. Further, characterization and mode of action, might enhance its efficacy and open new avenues for its wider application prospects.

Funding: Not Applicable

Acknowledgements: The authors express their gratitude to Dr.Anusha (Department of Biochemistry, Jain (Deemed to-be) University, for assiatance with Tricine SDS PAGE.

Author contributions: The authors have contributed to the theme, design, practical work, data analysis and in writing the manuscript.

Conflict of interest disclosure: The authors declare that they have no conflict of interest.

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