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Genomic analysis of *Streptomyces amritsarensis* MTCC 11845 and characterization of novel lanthipeptide 2A

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Antibiotic resistance in pathogenic bacteria is on the rise, and regrettably, existing antibiotics are becoming less effective against these resilient pathogens. Genus *Streptomyces* members are potent producers of antibiotics and other bioactive secondary metabolites. In the current study, genome of *Streptomyces amritsarensis* (MTCC 11845) was analyzed to identify biosynthetic gene clusters responsible for encoding potent antimicrobial compounds based on its *in vitro* antimicrobial activity. Analysis with antiSMASH database showed presence of biosynthetic gene clusters encoding secondary metabolites. Average nucleotide identity (ANI) of the *S. amritsarensis* 2A whole genome sequence showed 99.04–83.31% identity, with type strains of all closely related species. Whole-genome analysis revealed the presence of multiple peptide synthetase, Non-ribosomal peptide synthetase (NRPS) and polyketide synthase (PKS) biosynthetic gene clusters producing various antimicrobials. The active antimicrobial compound produced by *S. amritsarensis* 2A, purified and characterized using chromatographic techniques. MALDI-TOF data revealed that the active novel peptide has molecular mass of 1341.7 Da, and exhibiting 37–60% similarity with other lanthipeptide sequences available in database. AntiSMASH data reveals that peptide has 14 amino acids and it belongs to the Class III lanthipeptides. Lanthipeptide 2A was stable at high temperatures and *p*H ranging from 4.0–10.0. It exhibits potent antimicrobial activity against various test strains, with MIC values ranging from 2.2 to 30.8 µg/mL. It is non-haemolytic even at 5× MIC concentration and effectively reduces bacterial load after 2 h of incubation. Lanthipeptide 2A, with its broad antimicrobial activity, stability, and non-haemolytic characteristics, emerges as a promising candidate for therapeutic applications.

Keywords: Lanthipeptide 2A, Antimicrobial, Therapeutic agent

Antibiotic resistance has reached an alarming stage, and to solve this problem we have to search for novel natural compounds. Nowadays innovative technologies are in demand for the discovery and development of novel antimicrobials active against drug-resistant pathogens. Bacteriocins, glycopeptides, lipopeptides, cyclic peptides are some emerging classes of novel antimicrobials[1]. They possess diverse mechanisms of action as compared to already existing antimicrobials in the market. Bacteriocins are evolving as a potent antimicrobial peptides produced by various microorganisms[2,3]. Bacteriocins can kill or inhibit closely related or non-related bacteria. Lantibiotics are ribosomally synthesized and post-translationally modified peptides (RiPPi) that belong to the class I bacteriocins[4]. They have gained attention of many pharmaceutical and biomedical researchers because they are highly potent and destroy target cells rapidly using multiple modes of action[5]. Nisin (class I lantipeptide) is largely used in food industry as an antimicrobial agent and food preservative[6].

Recent advances in genome mining and biosynthetic engineering have resulted in discovery and development of lanthipeptide as a new and potent class of antimicrobials[7,8]. Lanthipeptides display high diversity in their structures and possess linkages due to post-translational modification process. The first step in the modification process involves dehydrating Ser residues to 2, 3-didehydroalanine (Dha) and Thr residues to 2,3-didehydrobutyrine (Dhb) residues[9]. Lanthipeptide contains lanthionine, a bis-amino-bis acid with two alanine residues. A bis-amino-bis acid has two alanine residues linked through a thioether group connecting β-carbons. Lanthionine incorporates into a peptide chain through both the amino and acid groups this results in thioether crosslink[10]. Microorganisms synthesize lanthipepeptides, however

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genes encoding homologues of lanthipeptide biosynthetic enzymes are also reported from genomes of higher eukaryotes and some archaea[9].

Genus *Streptomyces* is a potential producer of a variety of antibacterial, antifungal and antiparasitic drug[11]. *Streptomyces* spp. are reported to produces lanthipeptide belonging to various classes: Cinnamycin (Class II), Roseocin (Class II), Labyrinthopeptin, NAI-112 (class III), SapB (class III) Sfl (class IV) and Lexapeptide (Class V)[7,12,13]. SapB is synthesized by *Streptomyces coelicolor* A3(2), acts as a biological surfactant by reducing the surface tension of substrate mycelium[14]. The class III lantipeptide displays other various activities such as antiviral (Labyrinthopeptin), anti-allodynic/antinociceptive (Labyrinthopeptin and NAI-112), and antimicrobial activity (SapT and NAI112)[7,15,16]. However, *Streptomyces* still remains a rich natural source of antimicrobials and keeping this in view, we analysed the genome of *S. amritsarensis* 2A (MTCC 11845, JCM 19660), exhibiting broad-spectrum antimicrobial activity[17]. The present study aimed to identify the potent biosynthesis gene clusters of *S. amritsarensis* 2A producing antimicrobial compounds. Genomic studies revealed that *S. amritsarensis* 2A produces various antimicrobial compounds including lanthipeptide 2A which exhibited 37–60% similarity with other lanthipeptide sequences available in database. Further, lanthipeptide 2A produced by *S. amritsarensis* 2A was purified and characterized as novel peptide belonging to Class III lanthipeptide.

Materials and Methods

Genomic analysis

Recent technological advances have sparked a resurgence in the discovery of antimicrobial peptides from various bacterial species. *S. amritsarensis* 2A was isolated and reported to produce potent broad-spectrum antimicrobial compound[17]. Therefore, whole genome analysis of *S. amritsarensis* was carried out to study secondary metabolite biosynthesis gene clusters. Whole genome sequences of strain *S. amritsarensis* 2A (NZ_MQUR01000208.1) and its closely related strains *Streptomyces* sp. strain fd1-xmd (CP019798.1), *S. flavotricini* strain NGL1 (NZ_JAINUL010000045.1), *S. katrae* strain NRRL ISP-5550 (NZ_JZWV01001871.1), *S. toxytricini* strain JCM 4421 (NZ_BMTY01000053.1) and *S. globosus* strain LZH-48 (NZ_CP030862.1) [17], were downloaded from NCBI Genome database in FASTA format. Secondary metabolite biosynthetic gene clusters of *S. amritsarensis* 2A and its closely related strains were predicted using antiSMASH 6.0[18]. The average nucleotide identity (ANI) of *S. amritsarensis* 2A was calculated using an ANI calculator to compare it with other closely related members[19]. AntiSMASH software was used to predict secondary metabolite biosynthesis gene clusters and genome annotation was done using Rapid Annotation Subsystem Technology (RAST) 144 server[20].

Purification and characterization of peptide

For purification of active peptide from *S. amritsarensis* 2A, microorganism was grown in Starch Casein Nitrate (SCN) broth (Himedia, India) at 28°C for four days. After incubation, culture broth was centrifuged at 10,000 rpm for 30 min at 4°C. The antimicrobial activity of cell-free fermented broth was checked and it was mixed with preactivated XAD-4 resin (5%). The crude antimicrobial compound was eluted with methanol. Antimicrobial peptide was purified using Toyopearl resin HW-40 (Tosoh Bioscience, Germany) and reverse phase-HPLC (1260 Infinity Agilent Technologies, United States). The mobile phase consisted of HPLC grade water and acetonitrile, with 0.1% and 0.12% trifluoroacetic acid as mobile phases A and B, respectively. A gradient of mobile phases A and B was applied over a duration of 55 min (0%–60% for 45 min, 60%–80% for 45–50 min, 80%–100% for 50–55 min)[21]. The peaks were monitored and collected using a UV detector set at 220 nm. The active peak (RT 19.8min) was collected, concentrated and residual mass was dissolved in water, and purity was confirmed by reinjecting it onto HPLC. The resulting HPLC-purified peptide was used for all subsequent studies. These fractions were tested for antimicrobial activity the active peaks were pooled through multiple runs, and solvent was evaporated. Peptides were suspended in water and used for characterization studies.

The molecular weight of the purified peptide was determined using Matrix-Assisted Laser Desorption Ionization (MALDI). A mixture containing peptide (0.5 mg/mL) and α-cyano-4 hydroxycinnamic acid (CHCA) matrix (10 mg/mL) in 1:1 ratio was prepared[22]. Two microliters of this mixture were spotted onto a well of a MALDI 96-well stainless-steel sample plate. The sample was allowed to air dry. The analysis was performed using an Applied Biosystem Sciex 5800 MALDI-TOF instrument equipped with a unique OptiBeam on-axis laser. The instrument operated in positive ion reflectron mode to obtain mass spectra.

Minimum inhibitory concentration (MIC) and stability profile

MIC values were determined by using microtiterplate dilution assay[23]. Test strains used in study includes: *Bacillus subtilis* (MTCC 619), *Staphylococcus aureus* (MTCC 96), *Escherichia coli* (MTCC 1885), *Klebsiella pneumoniae* sub sp. *pneumoniae* (MTCC 109), *Pseudomonas aeruginosa* (MTCC 1688) and *Candida albicans* (MTCC 3017). All test strains were grown upto Mid-log-phase (5×10^5 CFU/mL) and inoculated with different concentrations of the peptides (1–32 μg/mL) using 96-well flat-bottomed polystyrene plate. After incubation absorbance was recorded at 600 nm using ELISA microplate reader (Thermo Fisher Scientific, USA). The lowest concentration that inhibited 90% growth of test strain was considered as MIC. Ampicillin and fluconazole were used as controls antibiotics. The 96-well plates were incubated for 24–48 h at 37°C.

For pH stability, peptide **(**5mg/mL) was dissolved in buffers ranging from *p*H 3.0–11.0. Citrate phosphate buffer of *p*H 3.0–8.0 (0.1 M citric acid solution adjusted to the desired *p*H with a 0.2 M di-sodium hydrogen phosphate solution) and carbonate bicarbonate buffer of the *p*H 10.0–11.0 (0.2 M anhydrous sodium carbonate solution adjusted to the desired *p*H with 0.2 M sodium bicarbonate solution) was prepared. The peptide solutions were incubated at 4°C for one hour. After incubation, the solutions were neutralized to *p*H 7.0, and the residual activity was evaluated by comparing it with the inhibition zone of the untreated sample.

To determine temperature susceptibility, peptide was incubated at different temperatures ranging from 50 to 100°C for 1 h and 121°C for 15 min, and then residual antimicrobial activity was determined after cooling the samples to room temperature (25°C). Similarly, proteolytic enzymes such as trypsin and proteinase K (Sigma-Aldrich, USA) of 1mg/mL concentration was used to ensure their effect on the activity of antimicrobial peptide. The enzyme solutions (1mg/mL) were prepared in 50 mM phosphate buffer (*p*H 7.0). All reactions were performed at 37°C for 6 h followed by deactivation of the enzyme by heating the solution at 80°C for 5 min before performing the activity assay. Residual activity (%) was calculated by comparing with the untreated control.

Toxicity testing and time-kill assay

For hemolysis assay rabbit blood was centrifuged and washed with phosphate-buffered saline (PBS). The cells count adjusted to 2×10^8 cells/mL. Cells treated with peptide (50–200μg) and incubated in CO_2 incubator for 24 h at 37°C[23.] The cell free supernatant separated by centrifugation (1000 × *g* for 5 min), 100μL supernatant was transferred to a 96-well flat-bottomed polystyrene plate and absorbance was recorded at 540 nm using ELISA microplate reader. PBS and 0.1% Triton X-100 used as negative and positive controls for haemolytic activity, respectively.

Purified peptide time-kill assay against *Staphylococcus aureus* (MTCC 96) was performed. Actively growing bacterial cultures with 0.2 OD (at 600 nm) were centrifuged and washed three times with PBS[24].The resulting pellet was suspended in PBS and simultaneously treated with 2× and 5× minimum inhibitory concentration (MIC) values of the purified peptide. Negative controls consisted of bacterial cultures without antimicrobial peptide (AMP) treatment. Samples were withdrawn at various time points (ranging from 0 to 8 h), and bacterial cells were centrifuged, serially diluted, and plated on nutrient agar. For each treatment, negative controls underwent the same procedure and plated on nutrient agar as well. After overnight incubation at 37°C, CFUs recorded at different time intervals following treatment.

Statistical analysis

Statistical significance among hemolysis groups (treated and positive control) and killing kinetics groups (treated and untreated) were calculated using student’s *t*-test. A *P*-value of < 0.05 was considered significant ($^{*} P < 0.05$, $^{**}P < 0.005$, and $^{***}P < 0.0005$). All the experiments were perform in triplicates and repeated thrice.

Results

Genome sequence analysis

S. amritsarensis 2A genome size is 7.81 Mb with 208 contigs (with PEGs). The G +C content is 72.6 mol%. The RAST Server was used to analyse the genome sequence. The analysis revealed the presence of 7,473 coding sequences (CDS), which included various gene clusters associated with secondary metabolism, stress response, cofactors, vitamins, and pigments (Fig. 1). The ANI analysis of *S. amritsarensis* 2A against its closely related strains *Streptomyces* sp. strain fd1-xmd (CP019798.1),

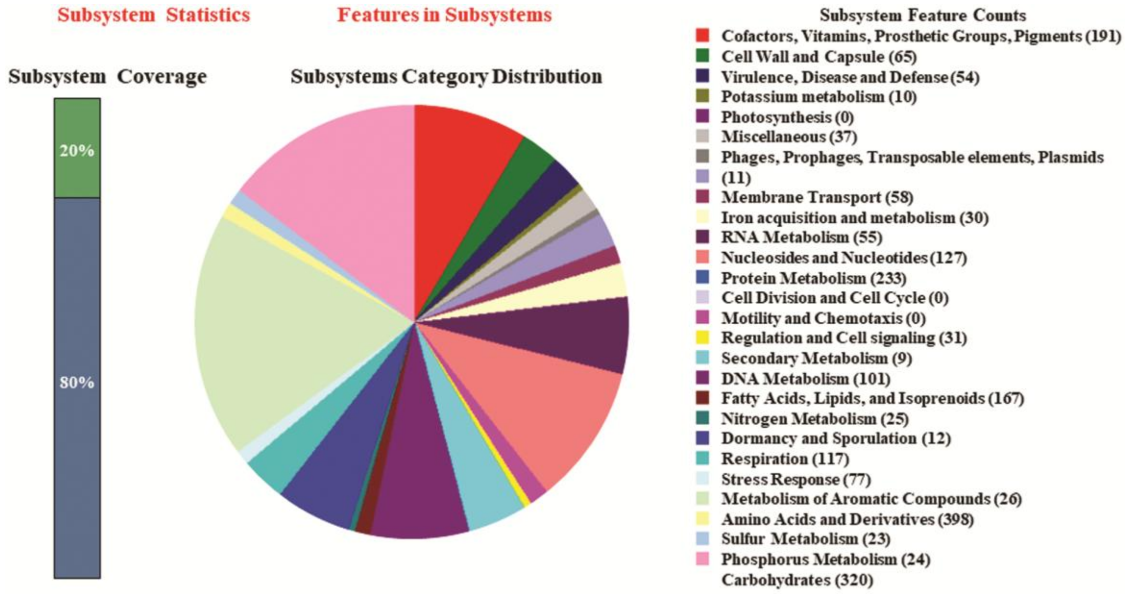
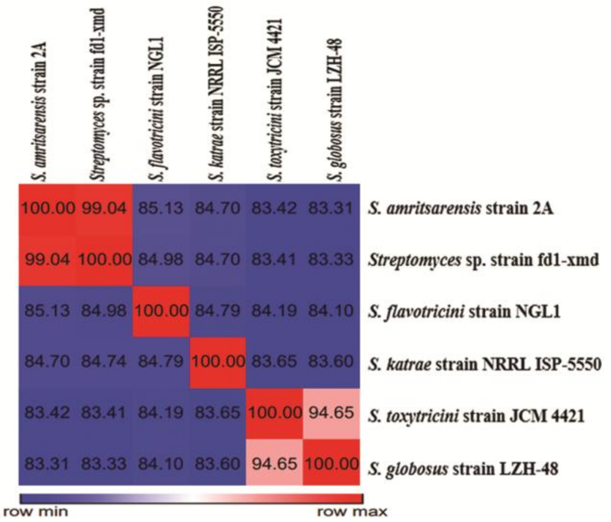


Fig. 1 — Analysis of *S. amritsarensis* 2A genome sequence using RAST144 server[20]

S. flavotricini strain NGL1 (NZ_JAINUL010000045.1), *S. katrae* strain NRRL ISP-5550 (NZ_JZWV01001871.1), *S. toxytricini* strain JCM 4421 (NZ_BMTY01000053.1) and *S. globosus* strain LZH-48 (NZ_CP030862.1) displayed an identity of 99.04, 85.13, 84.70, 83.42 and 83.31%, respectively (Fig. 2). Further, secondary metabolite biosynthetic gene clusters of *S. amritsarensis* 2A and its closely related strains were predicted using antiSMASH. The nucleotide sequence identity (%) of various biosynthetic clusters of closely related strains shows variation when compared with *S. amritsarensis* (Table 1). Moreover, analysis unveiled four complete biosynthetic gene clusters responsible for synthesizing various known antimicrobials: alkylresorcinol, desferrioxamin B (siderophore), coelichelin and melanin, present in *S. amritsarensis* Genome. The putative biosynthetic clusters encoding known antimicrobials, their regions, and sequence length is shown in Table 2.

Production, purification and characterization

Culture filtrate broth (CFB) obtained from four days fermented broth of *S. amritsarensis* 2A in SCN broth displayed promising activity against test strains including *B. subtilis* (MTCC 619), *S. aureus* (MTCC 96), *E. coli* (MTCC 1885), *K. pneumoniae* sub sp. *pneumoniae* (MTCC 109), *P. aeruginosa* (MTCC 1688) and *C. albicans* (MTCC 3017). The active compound from CFB was recovered using XAD-4 (5% v/v) resin, purified by size exclusion chromatography (Toyopearl resin HW-40) and reverse phase-HPLC. The HPLC purified peptide showed retention peak at 19.8 min (Fig. 3A).



	S. amritsarensis strain 2A	*Streptomyces* sp. strain fd1-xmd	*S. flavotricini* strain NGL1	*S. katrae* strain NRRL ISP-5550	*S. toxytricini* strain JCM 4421	*S. globosus* strain LZH-48
S. amritsarensis strain 2A	100.00	99.04	85.13	84.70	83.42	83.31
Streptomyces sp. strain fd1-xmd	99.04	100.00	84.98	84.70	83.41	83.33
S. flavotricini strain NGL1	85.13	84.98	100.00	84.79	84.19	84.10
S. katrae strain NRRL ISP-5550	84.70	84.74	84.79	100.00	83.65	83.60
S. toxytricini strain JCM 4421	83.42	83.41	84.19	83.65	100.00	94.65
S. globosus strain LZH-48	83.31	83.33	84.10	83.60	94.65	100.00

Fig. 2 — Heat map showing average nucleotide identities (ANI) of *S. amritsarensis* 2A and closely related strains genomes. The colour cut-off for lower identity (blue) is 83.31

Further characterization of purified fraction revealed presence of a single band with approximate molecular weight of approximately 1 kDa in Tricine SDS-PAGE. The single band indicates that the peptide purified to homogeneity. The peptide activity confirmed by overlaying the unstained part of the gel containing purified peptide through in-gel activity assay using *B. subtilis*. After incubation, inhibitory zone was observed at the same position that was visualized in the stained gel (Fig 3A). MALDI-TOF

Table 1 — Different secondary metabolites clusters present in *S. amritsarensis* 2A and its closely related spp.

Antimicrobial Peptides	Secondary metabolite cluster representation	Mol wt (Da)	Nucleotide sequence identity (%) Strains						Reference
			1	2	3	4	5	6	
Alkylresorcinol	Type III Polyketide synthesae	236	100	100	-	-	100	100	37
Desferrioxamin B (siderophore)	Cluster 15 BCGs	560	100	100	100	100	-	-	3
Coelichelin	NRPS	390	100	100	-	-	-	-	38
Melanin	Type-I PKS clusters	318.2	100	100	100	100	28	28	39
Tunicamycin B1	N-acetylglucosamine	870	85	85	92	-	-	-	8
Deimino-antipain	NRPS	605.7	66	66	-	-	-	-	40
Streptothricin	NRPS	502.5	66	87	-	-	-	-	41
Carotenoid	Tarpene	459.6	63	63	-	-	-	-	42
Hopene	Tarpene	410.7	61	61	61	-	61	23	43
Butyrolactone	Regulator of antibiotic biosynthesis clusters	424.4	36	36	-	-	-	-	29
Toxoflavin	Cryptic biosynthetic intermediate	193.16	14	14	-	-	-	-	8
Chloramphenicol	NRPS	323.13	11	11	-	-	-	-	29
Himastatin	NRPS	1485.7	24	52	-	-	-	12	8

[1; *S. amritsarensis* 2A, 2; *Streptomyces* sp. strain fd1-xmd, 3; *S. toxytricini* strain JCM 4421, 4; *S. globosus* strain LZH-48, 5; *S. flavotricini* strain NGL1, *S. katrae* strain NRRL ISP-5550,-; Represents absence of the nucleotide cluster in particular strain]

Table 2 — Putative biosynthetic clusters encoding known antimicrobials using antiSMASH bioinformatics tool

Compound	Mol. wt. (Da)	antiSMASH Region	Nucleotide sequence (From – to)
Alkylresorcinol	236	102.1	1-23 – 214
Desferrioxamin B	560	178.1	50303 – 62084
Coelichelin	390	145.1	3637 – 46894
Melanin	318.2	199.2	77776 – 104029

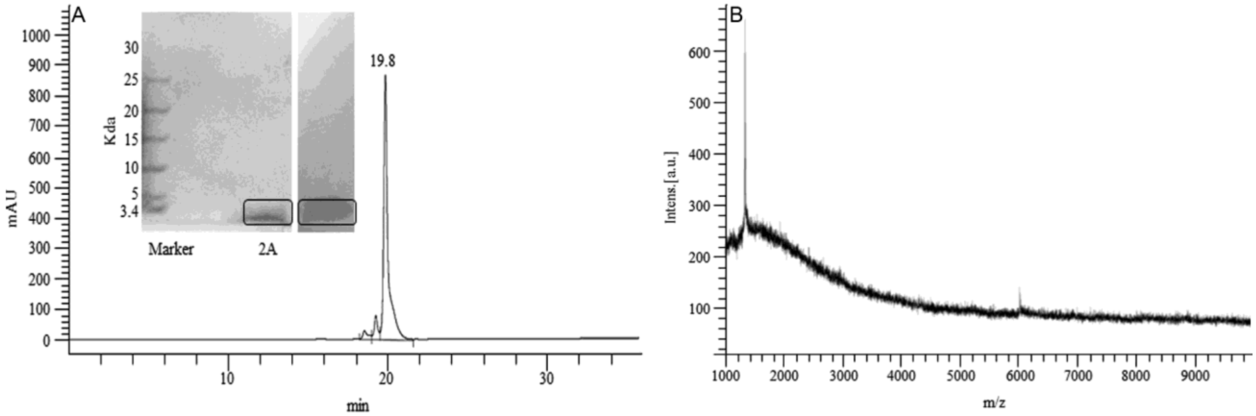


Fig. 3 — Purification and characterization of antimicrobial peptide (A) RP-HPLC profile of peptide 2A inset showing Tricine SDS-PAGE of peptide and bioautography demonstrating a clear inhibition zone against *B. subtilis*. (B) MALDI analysis of peptide 2A

revealed that the active peptide has molecular mass of 1341.7 Da (Fig 3bB). The *de novo* sequence hypothesized for peptide leader/core peptide using antiSMASH represented in Fig 4A. Further, the lanthipeptide 2A sequence cleavage site, and similarity search with closely related lanthipeptides revealed 60–37 % similarity with other lanthipeptide (Fig 4B) using RiPPMiner database.

MIC and stability profile

The purified peptide demonstrated antimicrobial activity against all test strains used. The MIC assay using micro-titer plates in triplicates revealed that *B. subtilis* (MTCC 619) has lowest and *C. albicans* (MTCC 3017) has highest MIC values i.e. 2.2µg/mL and 30.8µg/mL, respectively (Table 3). The results of heat stability assay for lanthipeptide 2A demonstrated

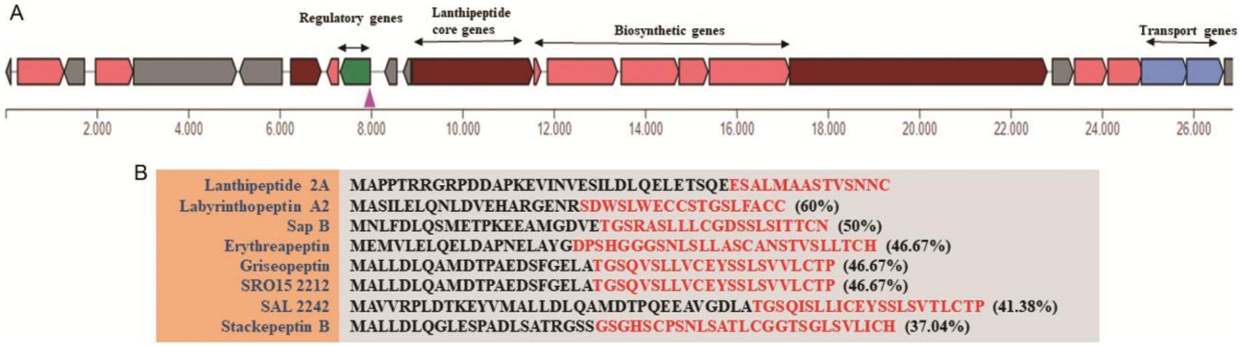


Fig. 4 — (A) Gene cluster of peptide from *S. amritsarensis* 2A genome sequence involved in biosynthesis of lanthipeptide 2A; (B) Lanthipeptide 2A sequence similarity with closely related lanthipeptide sequences

Table 3 — MIC values of lanthipeptide 2A against various test strains

Test organism	Reference number	Ampicillin (μg/mL)	Fluconazole (μg/mL)	Lanthipeptide 2A (μg/mL)
S. aureus	MTCC 96	0.25	-	8.2
B. subtilis	MTCC 619	0.50	-	2.2
E. coli	MTCC 1885	1.0	-	16.4
P. aeruginosa	MTCC 1688	1.5	-	20.2
K. pneumoniae	MTCC 109	1.0	-	16.6
C. albicans	MTCC 3017	-	20	30.8

that the peptide was highly stable at 100°C for 15 min. However, reduction in antimicrobial activity was observed at 121°C for 15 min (autoclaving). There was no reduction in peptide antimicrobial activity between *p*H 4.0 –10.0, though, the activity was reduced at *p*H 11.0 (Table 4). Lanthipeptide 2A retained more than 90% antimicrobial activity even after 6 h incubation with trypsin and proteinase K (Table 4).

Toxicity testing and time-kill assay

Lanthipeptide 2A did not demonstrate any hemolytic activity at 2× and 5× MIC concentration (Fig. 5A). Bactericidal kinetic studies of lanthipeptide 2A (2× MIC) using *S. aureus* (MTCC 96) cells showed complete reduction in OD after 2 h. However, bacterial load was completely reduced after treatment for 90 min at 5× MIC (Fig 5B). CFU count for negative controls did not reveal any reduction in bacterial load.

Discussion

Resistance among pathogenic bacteria has increased[25,26] in the last two decades. Presently available antibiotics are becoming less efficient against these resistant pathogens[27]. Consequently, screening and characterization of the novel antimicrobial compounds especially small peptides with distinctive scaffolds and innovative mechanisms of action. These compounds have the potential to serve as the foundation for new antibiotic classes. Members of the genus *Streptomyces* known producers of antibiotics, antimicrobial peptides, lipopeptides and lanthipeptides[28]. Lanthipeptides belongs to ribosomally encoded and post-translationally modified peptides (RiPPs) group, produced by a large variety of microorganisms[29]. Genus *Streptomyces* synthesizes approximately 80% of the known actinobacterial compounds. Among these, around 10,000 exhibit antibacterial activity. Despite this, there remains significant potential for the discovery of novel

Table 4 — Factors affecting lanthipeptide 2A activity

Treatment	Reaction Duration/ Condition	Residual activity (%)
*p*H–3.0	1h/ 24 ºC	68.2
*p*H–5.0	1h/ 24 ºC	100
*p*H–6.0	1h/ 24 ºC	100
*p*H–7.0	1h/ 24 ºC	100
*p*H–8.0	1h/ 24 ºC	100
*p*H–10.0	1h/ 24 ºC	94.1
*p*H–11.0	1h/ 24 ºC	64.2
50 ºC	1h	100
70 ºC	1h	100
80 ºC	1h	100
100 ºC	15 min	100
100 ºC	30 min	87.5
100 ºC	45 min	68.7
100 ºC	1h	0
121 ºC	15 min (304 KPa)	50
Proteinase k	6h/37 ºC./1mg/mL	90.3
Trypsin	6h/37 ºC./1mg/mL	93.5

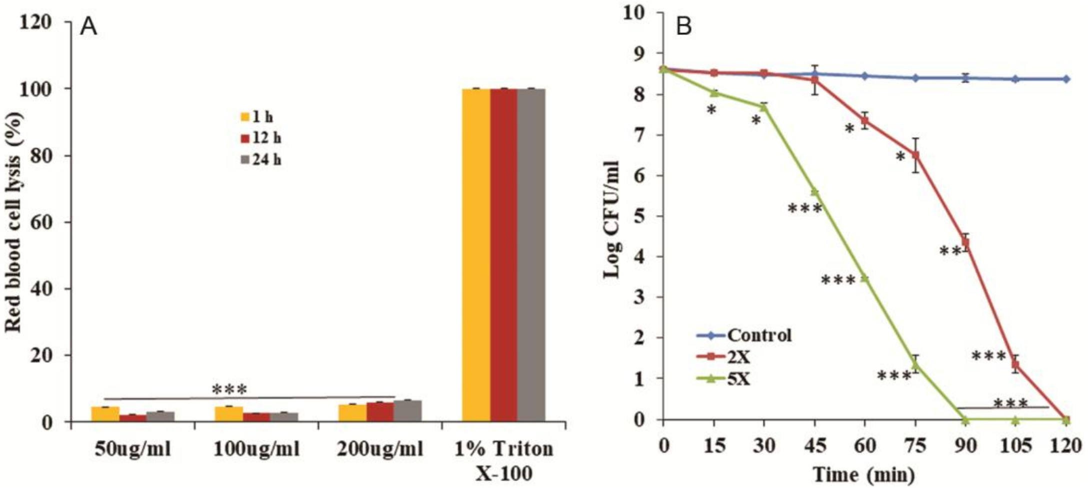


Fig. 5 — (A) Hemolytic activity of lanthipeptide 2A; (B) Killing kinetics of lanthipeptide 2A (2× and 5× MIC) against *S. aureus* (MTCC 96)

compounds [5, 27]. Considering the fact that *Streptomyces* are potent producers of antimicrobials, *S. amritsarensis* 2A exhibiting strong antimicrobial activity was consider for study, as a source of new solution for combating antimicrobial resistance. Strains of *Streptomyces* and related genera harbor an extensive reservoir of biosynthetic gene clusters that encode secondary metabolites. These metabolites have great potential, could be used as novel antimicrobials and detailed information is available in the antiSMASH database[18]. Biosynthetic gene clusters studies through genome mining has revealed that actinobacteria can produce lanthipeptides that hold great potential as therapeutics[29]. In the present study, we analysed genome of *S. amritsarensis* 2A in detail, and compared it with its closely related strains. Biosynthetic gene clusters encoding secondary metabolites were identified and analysed using the antiSMASH database. The genome studies revealed that the ANI values of *S. amritsarensis* 2A and its closely related strains varied from 99.04- 83.31%. *S. amritsarensis* 2A and *Streptomyces* sp. strain fd1-xmd displayed ANI 99.04% similarity, representing the same species. However, others displayed ANI <90% illustrating that they are different species[30].

RAST and bioinformatics analyses of genome sequence revealed the ability of *S. amritsarensis* 2A to produce multiple antimicrobials. Whole-genome analysis using the antiSMASH database revealed the presence of multiple NRPS and PKS biosynthetic gene clusters producing various antimicrobials. Four biosynthetic clusters showed 100% nucleotide sequence identity with already known clusters and they includes alkylresorcinol, Desferrioxamine B, coelichelin and melanin (Table 1). The active antimicrobial compound produced, purified and characterized using chromatographic techniques. MALDI-TOF data revealed that the active peptide has molecular mass of 1341.7 Da. However, molecular weight of the closely related lanthipeptide such as SapB and LabyA1 is 2027.8 Da and 2073.7 Da, respectively[31,32]. According to antiSMASH database analyses, Lanthipeptide 2A is a novel peptide, belonging to the Class III lanthipeptides. The members of class III lanthipeptides include erythreapeptin, avermipeptin, griseopeptin, labyrinthopeptin A2 (LabA2) and NAI-112 isolated from *Actinomadura namibiensis* DSM 6313 and *Actinoplanes* DSM24059, respectively[33,34].

Lanthipeptides, a fascinating class of ribosomally synthesized and post-translationally modified peptides (RiPPs), undergo extensive post-translational modifications that significantly enhance their stability[35]. Lanthipeptides precursor peptide contains a leader and a core peptide. They contain characteristic lanthionine or methyl-lanthionine residues, which contribute to their robustness. These modifications restrict their conformational flexibility, making them highly stable even under challenging conditions like high temperatures and proteolytic enzyme activity. Similar, stability results were observed when lanthipeptide 2A was subjected to varying temperatures, *p*H levels and proteolytic enzymes. AntiSMASH data reveals that it comprises of 14 amino acids, serine (Ser) and threonine (Thr) residues within lanthipeptide 2A undergo dehydration forming dehydroalanine (Dha) and dehydrobutyrine (Dhb). Notably, lanthipeptide 2A exhibits potent antimicrobial activity against various test strains, with MIC values range from 2.2 to 30.8 µg/mL. Remarkably, even at 5× MIC concentration, it remains non-hemolytic and effectively reduces bacterial load after 2 h of incubation. Natural antimicrobial peptides (AMPs) hold great promise as substitutes or adjuvants for treating infections. However, they do come with certain drawbacks, including toxicity, limited resistance to proteolysis, and the costly process of isolation and purification[18,36]. Lanthipeptide 2A exhibits broad activity spectrum, low MIC values, thermos-stability and non-haemolytic characteristics, making it a promising candidate for therapeutic applications.

Conclusion

Streptomyces amritsarensis 2A (MTCC 11845, JCM 19660) produces a novel potent lanthipeptide 2A with a low molecular mass of 1341.7 Da. Lanthipeptide 2A exhibits a broad activity spectrum, low MIC values, thermos-stability, and non-hemolytic characteristics. These attributes make it a promising candidate for therapeutic applications. Furthermore, *in vivo* experiments would be performed to explore its potential applications in humans.

Authors Contribution:

DS designed the experiments. DS and KK performed experiments. DS, KK, and RK analyzed data. DS, KK, and RK prepared the manuscript.

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Conflict of interest

The authors have no conflicts of interest to declare.

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