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Short Communication

In-vitro studies on a natural lantibiotic, paenibacillin: A new-generation antibacterial drug candidate to overcome multi-drug resistance

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ABSTRACT

The alarming burden of antibiotic resistance in nosocomial pathogens warrants the discovery and development of new and effective antimicrobial compounds. Small cationic antimicrobial peptides seem to be a promising therapeutic alternative to fight multi-drug resistance. This study investigated the in-vitro potential of a previously reported lantibiotic, paenibacillin, from the clinical perspective. An antimicrobial peptide, M152-P4, was isolated, purified and characterized from a mud isolate, and its susceptibility was determined in clinical isolates of *Staphylococcus aureus* and *Enterococcus* spp. Time-kill kinetics, resistance, probable mode of action, haemolytic activity and mammalian cytotoxicity were investigated. M152-P4 was identified as paenibacillin based on mass spectroscopy data, amino acid analysis and biosynthetic gene cluster analysis. It had potent antibacterial activity against the Gram-positive pathogens tested, with minimum inhibitory concentrations from 0.1 to 1.56 µM. It appeared very challenging for *S. aureus* to develop resistance to this compound. Also, paenibacillin penetrated the outer layer of bacteria, and depolarized the membrane completely by creating pores in the plasma membrane with better potential than nisin. Paenibacillin showed no haemolysis up to 60 µM, and the half maximal inhibitory concentration on mammalian cell lines was >100 µM. These results highlight the excellent antibacterial properties of paenibacillin in clinically relevant pathogens. It is stable in the presence of serum, and non-haemolytic and non-cytotoxic even above the therapeutic concentration. Further research efforts regarding toxicity and in-vivo efficacy are necessary to develop paenibacillin as a next-generation therapeutic drug to overcome multi-drug resistance in Gram-positive pathogens.

1. Introduction

Antibiotic-resistant pathogens pose a looming challenge to the world which, if not resolved, may lead to a post-antibiotic era. The World Health Organization recently announced a list of global priority pathogens to pave the way for the discovery and development of new antimicrobial compounds [1]. Among them, the ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter* spp.), which cause major hospital-acquired and community infections worldwide, were ranked as top priority [2].

With resistance emerging against the last-resort antibiotics and failure to develop sufficient new antibiotics using traditional small-molecule-based therapeutics, the research focus has moved towards short antimicrobial peptides (AMPs) and host-defence peptides [3,4]. These peptides are present in almost every form of life as nature's antibiotics. Over the last few years, several AMP-based therapeutics have reached advanced clinical trials, but none have been approved by the Food and Drug Administration to date because of their toxicity, limited or no advantage over current antibiotics, haemolytic activity, poor solubility, or low stability under in-vivo conditions [5].

Antimicrobial peptides produced by bacteria, called 'bacteriocins', have been studied for their use in food preservation or as anti-infective agents [6]. They mimic host-defence peptides, and display similar structures and activity against various human pathogens. Lantibiotics, a subclass of bacteriocins, are produced exclusively by Gram-positive bacteria such as *Bacillus* spp., *Paenibacillus* spp., *Lactobacillus* spp. and *Lactococcus* spp., and show antibacterial activity mainly against Gram-positive pathogens [7].

One of the natural lantibiotics, paenibacillin, was discovered from a *Paenibacillus polymyxa* strain in 2007 by He et al., and was investigated as an alternative to nisin for use against food-borne

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pathogens [8,9]. The present study reports the isolation, purification and characterization of paenibacillin from *Paenibacillus jamilae* M-152 strain. This lantibiotic was studied for its clinical potential to tackle the burden of multi-drug resistance in Gram-positive bacteria. To the authors' knowledge, this is the first study on the clinical relevance, probable mode of action and haemolytic activity of paenibacillin for development as a next-generation antimicrobial drug candidate.

2. Materials and methods

2.1. Antimicrobial screening and purification of antimicrobial compound M152-P4

The bacteria were isolated from mud samples from a village situated in Haryana state, India. The isolates were grown in tryptic soy broth (HiMedia Labs, Mumbai, India) for 48–96 h, and the crude extracts were prepared using Diaion HP 20 resin (Sigma-Aldrich Corp., St Louis, MO, USA), as described previously [10]. The antimicrobial activity of cell-free supernatants and extracts was assessed using an agar well diffusion assay with the test strain (10^6 colony-forming units/mL) seeded in the molten agar [10]. Positive isolates showing antimicrobial activity against at least one indicator strain were identified based on their 16S rRNA gene sequencing. Approximately 450 strains were screened for their ability to inhibit the indicator pathogens viz. *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 29665, *Staphylococcus aureus* ATCC 25923 and *Candida albicans* ATCC 10231. The isolate M-152 strain displayed good activity against both Gram-positive (*S. aureus* ATCC 25923) and Gram-negative bacteria (*E. coli* ATCC 25922, *K. pneumoniae* ATCC 29665). The isolate was inoculated in a 5.6-L batch consisting of eight 2-L flasks containing 700 mL sterile Mueller-Hinton broth. Fermentation lasted for 24 h and the culture was harvested by centrifugation. The crude extract was loaded on cation-exchange chromatography: SP-sepharose column (bed height 5×15 cm, column volume 300 mL), flow rate 10 mL/min, 10 mM ammonium acetate buffer with pH 5.0±0.1. 1 M NaCl in the same buffer was used as an eluent solution. A two-stage linear gradient of 0–0.5 M NaCl in 900 mL and 0.5–1 M in 300 mL was used to elute the bound components. The active fractions were further purified by reverse-phase high-performance liquid chromatography (HPLC) on C18 column (25×250 mm, 5 µm) (Phenomenex, Torrance, CA, USA) using water and acetonitrile (both containing 0.075% trifluoroacetic acid) as Solvents A and B, respectively. One prominent peak, initially named M152-P4, was active against *S. aureus* and was further purified by a second round of HPLC on C4 column with Solvent A as 20% acetonitrile and 80% 5 mM ammonium acetate buffer (pH ~5.2) and Solvent B as 100% acetonitrile. The pure peak was lyophilized after desalting, and further studies were conducted with HPLC purified compound.

2.2. Mass spectrometry and amino acid analysis

The purified compound was subjected to matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) [AB Sciex, Warrington, UK; 5800 MALDI time of flight (TOF)]. Tandem MS spectrum was acquired with a MALDI-TOF analyser. For amino acid composition analysis, 30 µL of the sample (1 mg/mL concentration) was hydrolysed in accordance with the manufacturer's protocol (PICO-TAG amino analysis system, Waters Corp., Milford, MA, USA). The hydrolysate was analysed on HPLC after derivatization.

2.3. Whole-genome sequencing and biosynthetic gene cluster analysis

Genomic DNA was extracted from strain M-152 using a ZR Fungal Bacterial DNA Miniprep kit (Zymo Research, Irvine, CA, USA) in accordance with the manufacturer's instructions. The DNA samples were submitted to Genotypic India Pvt. Ltd, Bangalore, India for complete genome sequencing using Illumina and Nanopore technology. The complete hybrid assembly was obtained, the sequence was uploaded on antiSMASH (Version 3.0) [11], and biosynthetic gene clusters were identified.

2.4. Minimum inhibitory concentration determination, time-kill kinetics and resistance studies

Minimum inhibitory concentrations (MICs) were calculated using a microbroth dilution assay in cation-adjusted Mueller-Hinton broth according to the guidelines of the Clinical Laboratory Standards Institute [12]. To study the time-dependent killing phenomenon of M152-P4, *S. aureus* ATCC 25923 was incubated with 1xMIC and 2xMIC of the compound, and samples were spread plated on Mueller-Hinton agar plates at different time intervals. To study the tendency of pathogens to develop acquired resistance towards M152-P4, sequential culturing of *S. aureus* ATCC 25923 was performed in the presence of subinhibitory concentrations of M152-P4 or ciprofloxacin as an experimental control.

2.5. Membrane permeabilization and depolarization assays

An *N*-phenyl-1-naphthylamine (NPN) assay was performed to determine outer surface disruption, as explained previously [10]. An ortho-nitrophenyl-β-galactoside (ONPG) assay was employed with some modifications to determine inner membrane permeabilization [13]. *E. faecium* ATCC 51559 was grown in minimal medium supplemented with 2% lactose. The cells were incubated with 1.5 mM ONPG and M152-P4 or nisin (10 µM) in a 96-well plate at 37°C. Absorbance was measured on a microplate reader (BioTek Instruments Ltd, Sandy, UK) at 420 nm from 0 to 42 min with 3-min intervals. Cells without peptide were considered as the negative control. Cytoplasmic membrane permeabilization was studied using a fluorescent dye, propidium iodide (PI) (Thermo Fisher Scientific, Pune, India). *E. faecium* cells were treated with two different concentrations of M152-P4 (10 µM and 20 µM) or nisin (20 µM) in 96-well plates. The dye was added to all samples at a final concentration of 4 µM and incubated in the dark for 20–30 min. Fluorescence was measured on a fluorescence microplate reader with excitation at 535 nm and emission at 617 nm. The fluorescent probe 3, 3-dipropylthiacarbocyanine [$DiSC_3(5)$; Sigma-Aldrich Corp.] was used to investigate alterations in membrane potential across the plasma membrane, as reported previously [14].

2.6. Haemolytic activity

To conduct the haemolysis experiment, fresh blood was collected from a rabbit. All the protocols and procedures were in compliance with the ethical standards of the Institutional Animal Ethics Committee of the Institute of Microbial Technology (Approval No. IAEC/17/11). The effect of M152-P4 on erythrocytes was determined as explained by Stark et al. [15].

2.7. Mammalian cytotoxicity

Cytotoxicity assays were performed on two different cell lines: human embryonic kidney cell line (HEK293) and mouse macrophage cell line (J774) in 96-well plates. Approximately 2×10^4 cells were seeded per well and after a 24-h incubation period, cells were treated with different concentrations of the peptide and incubated for another 24 h. Next, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was added and incubated for 3–4 h. Formazan crystals were dissolved by adding 20% sodium dodecyl sulphate solution in 50% dimethylformamide, and cell growth

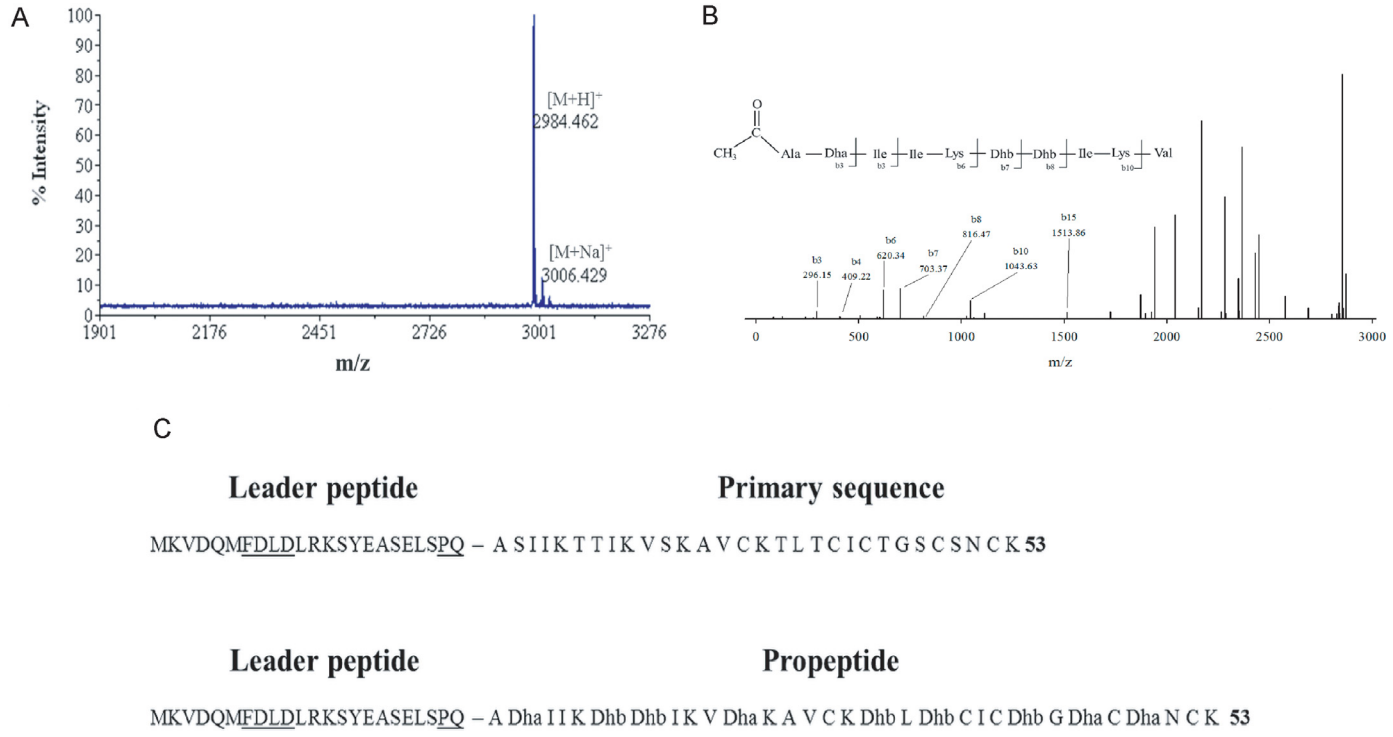

Fig. 1. (A) Matrix-assisted laser desorption/ionization mass spectrometry analysis of purified compound M152-P4 showing an m/z of 2984.462 $[M+H]^+$ along with sodium-adduct ion. (B) MS/MS spectrum and de-novo sequencing showing partial amino acid sequence at N-terminus. The observed b-ions were assigned upon fragmentation, corresponding to the partial amino acid sequence shown in the figure (Dha, didehydroalanine; Dhb, didehydrobutyrine). (C) The amino acid sequence of M152-P4 obtained from whole-genome analysis. The top panel shows the leader peptide (conserved motif underlined) along with the primary unmodified sequence encoded by the biosynthetic gene. The lower panel shows the propeptide sequence after dehydration of all serine and threonine residues. The sequence is consistent with the partial de-novo sequence obtained from the MS/MS spectrum.

was quantified by measuring absorbance at 570 nm in a microplate reader. Toxicity was calculated with respect to control live cells.

3. Results and discussion

3.1. Identification of the antimicrobial compound M152-P4

The M-152 extract showed consistent antimicrobial activity, and the strain was identified as *Paenibacillus* spp. based on 16S rRNA gene sequencing (Genbank accession number MK156348), MALDI-TOF analysis and whole-genome analysis. The complete genome of this bacterium has been submitted to the National Center for Biotechnology Information under accession number CP034141. Antimicrobial activity of this micro-organism had not been explored previously. One draft genome of the same species (but a different strain) [16] was found; this showed the presence of a polymyxin B gene cluster when uploaded on antiSMASH. This explained the activity against Gram-negative bacteria. Therefore, the authors switched to the isolation of anti-Gram-positive compounds, and used different chromatographic techniques to purify the antibacterial compound M152-P4 (Fig. S1, see online supplementary material). M152-P4 appeared as an m/z of 2984.4 $[M+H]^+$ on MALDI-MS (Fig. 1A). In the MS/MS spectrum, limited fragmentation was observed (Fig. 1B). De-novo sequencing yielded a partial amino acid sequence which was consistent with amino acid analysis (Fig. S2, see online supplementary material). The whole-genome data analysis yielded one lantibiotic gene cluster showing 100% identity with paenibacillin [17]. The molecular weight and primary sequence of M152-P4 were consistent with paenibacillin. The complete comparative analysis of both clusters is given in Table S1 (see online supplementary material). Together, mass spectroscopy data, amino acid composition and biosynthetic gene cluster analysis confirmed that M152-P4 is paenibacillin. The pro-peptide sequence and leader peptide are shown in Fig. 1C.

3.2. Minimum inhibitory concentration, time-kill kinetics and resistance studies

M152-P4 had excellent antimicrobial activity within the range 0.1–1.56 μM against all of the Gram-positive pathogens tested (Table 1). The study also included multi-drug-resistant clinical isolates of *S. aureus* and *Enterococcus* spp., which were all sensitive to M152-P4. This peptide was not effective against Gram-negative bacteria. The MIC was also checked in the presence of fetal bovine serum, and no difference in value was found; this supports the in-vivo stability of this peptide at this preliminary stage. In time-kill experiments, the peptide killed the bacteria completely in 4–6 h at very low concentrations (1xMIC and 2xMIC), which depicted its bactericidal nature. As seen in Fig. 2A, there was no regrowth after 24 h. *S. aureus* was also grown in the subinhibitory concentration of M152-P4 to allow the development of resistance, but the change in MIC of M152-P4 after 25 days of sequential passaging was four to eight fold (Fig. 2B). Moreover, there was no increase in MIC for the first week. Ciprofloxacin was used as an experimental control against which bacteria successfully developed resistance, with a maximum increase in MIC of 128 fold. There was no contamination during the 25 days of sequential passaging which might be responsible for the increase in MIC value. This was confirmed by MALDI-TOF analysis after 25 days, and both cultures were found to be *S. aureus* with Biotyper scores >2.4 (reliable for species-level identification).

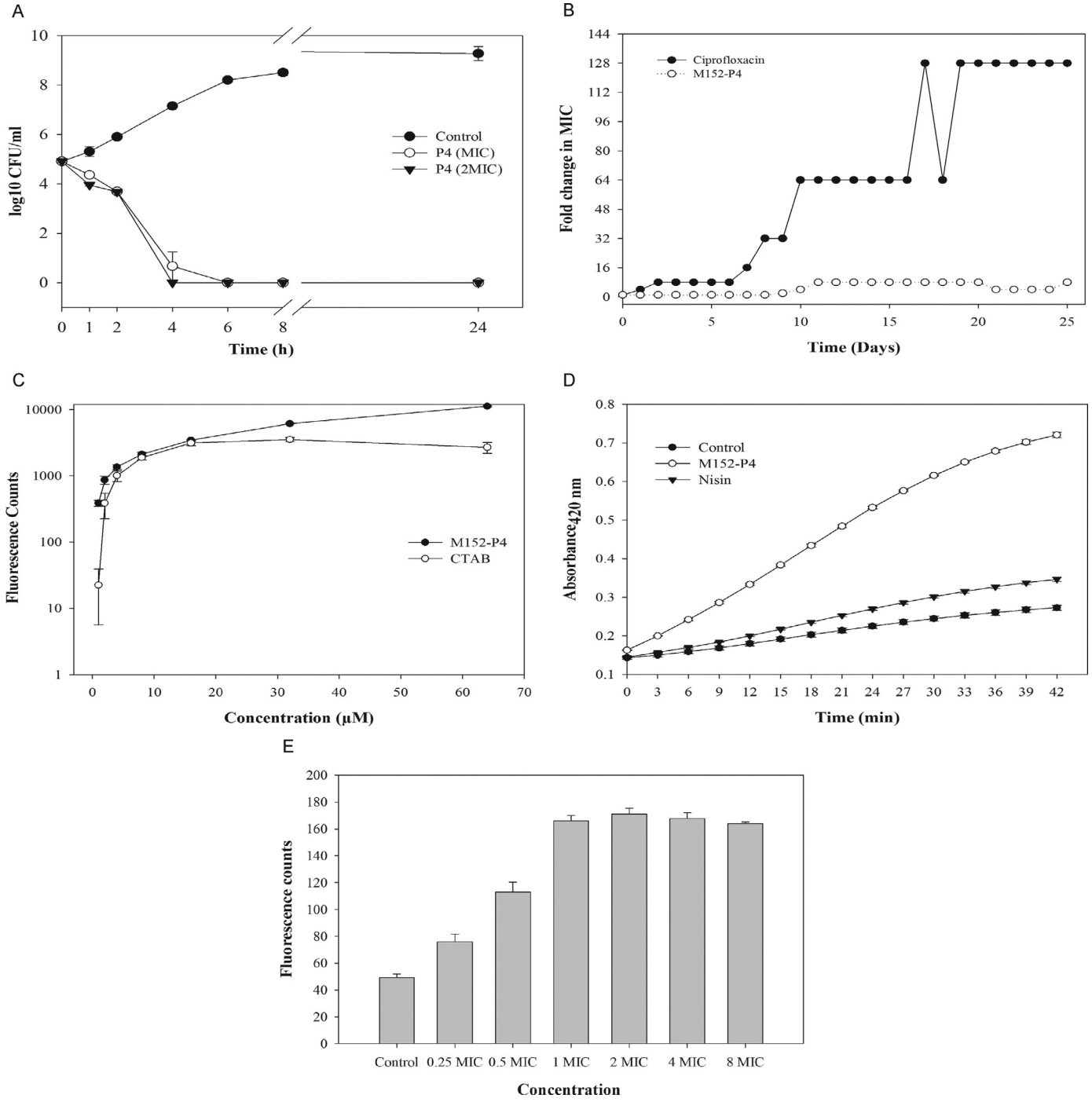

Fig. 2. Resistance development and mode of action studies with M152-P4. (A) Time-kill kinetics of M152-P4 against *Staphylococcus aureus*. No regrowth was observed after 24 h, even in the presence of a low concentration of the antimicrobial compound. The experiment was performed in triplicate and data are plotted as mean±standard deviation (SD). (B) Resistance study by sequential passaging of *S. aureus* ATCC 25923 in the presence of a sublethal concentration of M152-P4 or ciprofloxacin. A very low level of resistance was developed towards M152-P4, while the minimum inhibitory concentration (MIC) of ciprofloxacin increased up to 128 fold. (C) *N*-phenyl-1-naphthylamine (NPN) assay with *S. aureus* ATCC 25923 to show permeabilization of the outer surface. Fluorescence intensity increases in a concentration-dependent manner. NPN shows strong fluorescence in a hydrophobic environment, which is bacterial periplasm in this case. Data plotted are mean±SD of three replicates. (D) Ortho-nitrophenyl-β-galactoside (ONPG) assay with *Enterococcus faecium* ATCC 51559 with M152-P4 or nisin (both 10 μM) to show inner membrane permeabilization. M152-P4 shows time-dependent permeabilization and absorbance intensity reached saturation in 42 min. The difference between the permeabilization of nisin and M152-P4 is more than two fold. The experiment was conducted in triplicate and the data are representative of three independent experiments. (E) Membrane depolarization assay with $DiSC_3(5)$ dye. The control bar is without any peptide treatment. When hyperpolarized *S. aureus* cells were treated with M152-P4 at different concentrations, cells became depolarized, which is denoted by an increase in fluorescence. At a concentration equal to the MIC, complete depolarization was noticed. Data are plotted as mean±SD of three replicates. CFU, colony-formin gunits; MIC, minimum inhibitory concentration.

Table 1
Antibacterial activity of M152-P4 against different pathogens.

Strain	MIC (μM)	Strain	MIC (μM)
S. aureus ATCC 25923 (MSSA)	1.56	*E. faecium* ATCC 51559 (MDR[a])	0.1
S. aureus ATCC 43300 (MRSA)	0.2	*E. faecium* 25KH25	0.1
S. aureus ATCC 33591 (MRSA)	0.39	*E. faecium* 24FH29	0.2
MRSA 831	1.56	*E. faecalis* ATCC 51299 (VRE)	0.78
MRSA 839	1.56	*E. faecalis* ATCC 29212	0.39
MRSA 1	0.39	*Staphylococcus epidermidis* MTCC 3615	0.39
MRSA 2	0.39	*Klebsiella pneumoniae* ATCC 29665	>25
MRSA 3	0.39	*Escherichia coli* ATCC 25922	>25
MRSA 4	0.39	*Pseudomonas aeruginosa* ATCC 27853	>25
Bacillus subtilis ATCC 6633	0.2	*Acinetobacter baumanii* ATCC 19606	12.5
E. faecium NCTC 7171	0.1		

MIC, minimum inhibitory concentration; *S. aureus, Staphylococcus aureus*; MRSA, meticillin-resistant *S. aureus; E. faecium, Enterococcus faecium; E. faecalis, Enterococcus faecalis*; MDR, multi-drug-resistant (resistant to ampicillin, ciprofloxacin, gentamicin, rifampin, teicoplanin and vancomycin); VRE, vancomycin-resistant enterococci.

3.3. Mechanism of action studies

To study the probable mode of action of M152-P4, an NPN assay was performed initially; this assay is mainly used to determine the outer membrane permeabilization of Gram-negative bacteria [18]. As lantibiotics canonically target lipid II present in the periplasm of bacteria [7], the authors examined the tendency of M152-P4 to cross the peptidoglycan layer and reach the periplasm. NPN is a fluorescent dye that exhibits very strong fluorescence in a hydrophobic environment (periplasm in this case) and negligible fluorescence in aqueous condition (surroundings of bacteria). When the *S. aureus* cells were treated with varying concentrations of M152-P4, a concentration-dependent increase in the fluorescence of NPN was observed (Fig. 2C); this clearly indicates that the outer surface of the bacteria has been compromised. A cation detergent, CTAB, was used as a positive control in this experiment because of its surface-acting properties. Nisin, the most studied lantibiotic, was also used in this experiment, and similar results were obtained (data not shown). For inner membrane permeabilization, two different assays were performed with membrane impermeable substrates: ONPG assay and PI uptake assay. In the ONPG assay (Fig. 2D), *S. aureus* was selected initially, but *S. aureus* ATCC 25923 and *S. aureus* MTCC 96 did not produce β-galactosidase enzyme as visualized by X-gal screening assay. As such, *E. faecium* ATCC 51559 was chosen, and this gave positive results with X-gal. *E. faecium* cells showed time-dependent permeabilization of plasma membrane when treated with M152-P4 at 10 μM, and saturation was reached in 42 min. On the contrary, nisin showed very weak permeabilization in this assay, presumably due to the higher MIC of nisin against this bacterium and the fact that nisin is mainly active against food-borne pathogens. Membrane permeabilization was also studied using PI, and similar results were obtained (data not shown). These data suggest that M152-P4 can cause cytoplasmic membrane permeabilization. Depolarization was studied using membrane-potential sensitive dye $DiSC_3$, and concentration-dependent fluorescence leakage was observed upon peptide treatment; this reached saturation above a certain concentration (Fig. 2E). This finding indicates that M152-P4 caused complete cytoplasmic membrane depolarization in bacteria.

3.4. Haemolytic activity and mammalian cytotoxicity

The tendency of M152-P4 to cause haemolysis was tested using fresh rabbit red blood cells (RBCs) (Fig. 2F). 0.1% Triton X-100 was used as a positive control, which caused immediate lysis of RBCs. This was considered as 100% lysis, and the haemolytic activity of M152-P4 was calculated relative to this result. Haemolytic activity was evaluated at six different concentrations of M152-P4 (10–60 μM). M152-P4 did not cause any lysis at concentrations up to 60 μM. This concentration is almost 40 times the MIC of M152-P4 against *S. aureus* ATCC 25923. The toxicity of M152-P4 was evaluated on two different cell lines. The half maximal inhibitory concentration (IC_{50}) was not achieved below 100 μM, which was the highest concentration studied. The difference between average MIC values against clinical pathogens and IC_{50} in cell lines is more than 100 fold, which is significant from the pharmacological perspective.

4. Conclusion

This study investigated the in-vitro potential of an antibacterial peptide, paenibacillin, against clinically relevant human pathogens. Paenibacillin showed potent activity against all pathogens tested with very low MIC values. It is highly active against multi-drug-resistant isolates of meticillin-resistant *S. aureus* and vancomycin-resistant enterococci. Paenibacillin slowed down the de-novo evolution of acquired resistance in bacteria, and was stable in the presence of serum. Moreover, paenibacillin exhibited no haemolytic activity or cytotoxicity above the therapeutic concentration which is desirable for drugs to be used systemically. However, further studies are needed to investigate its precise target in the bacteria and its mechanism of action. In-vivo efficacy and toxicity studies of this lantibiotic are needed. Murepavadin is a novel antibacterial compound currently in phase III clinical trials against hospital-acquired bacterial pneumonia and ventilator-associated bacterial pneumonia caused by *P. aeruginosa* [19]. Murepavadin was designed based on the natural host defence AMP, protegrin I [20]. This supports the authors' hypothesis that antimicrobial peptides such as paenibacillin could serve as a scaffold for further development as a next-generation antibacterial drug to fight difficult-to-treat pathogens.

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Competing interests

None declared.

Ethical approval

The protocol was approved by the Institutional Animal Ethics Committee of the Institute of Microbial Technology (Approval No. IAEC/17/11).

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Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2019.03.020.

References

[1] World Health Organization. Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. Geneva: World Health Organization; 2017.
[2] Santajit S, Indrawattana N. Mechanisms of antimicrobial resistance in ESKAPE pathogens. BioMed Res Int 2016;2016:2475067.
[3] Steckbeck JD, Deslouches B, Montelaro RC. Antimicrobial peptides: new drugs for bad bugs? Expert Opin Biol Ther 2014;14(1):11–14.
[4] Pfalzgraff A, Brandenburg K, Weindl G. Antimicrobial peptides and their therapeutic potential for bacterial skin infections and wounds. Front Pharmacol 2018;9:281.
[5] Boto A, Pérez de la Lastra JM, González CC. The road from host-defense peptides to a new generation of antimicrobial drugs. Molecules 2018;23:311.
[6] Cotter PD, Ross RP, Hill C. Bacteriocins – a viable alternative to antibiotics? Nat Rev Microbiol 2013;11:95.
[7] Chatterjee C, Paul M, Xie L, Van Der Donk WA. Biosynthesis and mode of action of lantibiotics. Chem Rev 2005;105:633–84.
[8] He Z, Kisla D, Zhang L, Yuan C, Green-Church KB, Yousef AE. Isolation and identification of a *Paenibacillus polymyxa* strain that coproduces a novel lantibiotic and polymyxin. Appl Environ Microbiol 2007;73:168–78.
[9] Gerst MM, Huang E, Zhang L, Yousef AE. Development of a new paenibacillin-producing strain and testing its usability in improving food safety. J Food Sci 2015;80:M1538–43.
[10] Jangra M, Randhawa HK, Kaur M, Srivastava A, Maurya N, Patil P, et al. Purification, characterization and in vitro evaluation of polymyxin A from *Paenibacillus dendritiformis*: an underexplored member of the polymyxin family. Front Microbiol 2018;9:2864.
[11] Weber T, Blin K, Duddela S, Krug D, Kim HU, Bruccoleri R, et al. antiSMASH 3.0 – a comprehensive resource for the genome mining of biosynthetic gene clusters. Nucleic Acids Res 2015;43:W237–43.
[12] Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: Twenty Fifth International Supplement M100-S25. Wayne, PA: CLSI; 2015.
[13] Sun C, Li Y, Cao S, Wang H, Jiang C, Pang S, et al. Antibacterial activity and mechanism of action of bovine lactoferricin derivatives with symmetrical amino acid sequences. Int J Molec Sci 2018;19:2951.
[14] Cheng M, Huang JX, Ramu S, Butler MS, Cooper MA. Ramoplanin at bactericidal concentrations induces bacterial membrane depolarization in *Staphylococcus aureus*. Antimicrob Agents Chemother 2014;58:6819–27.
[15] Stark M, Liu L-P, Deber CM. Cationic hydrophobic peptides with antimicrobial activity. Antimicrob Agents Chemother 2002;46:3585–90.
[16] Midha S, Bansal K, Sharma S, Kumar N, Patil PP, Chaudhry V, et al. Genomic resource of rice seed associated bacteria. Front Microbiol 2016;6:1551.
[17] Huang E, Yousef AE. Biosynthesis of paenibacillin, a lantibiotic with N-terminal acetylation, by *Paenibacillus polymyxa*. Microbiol Res 2015;181:15–21.
[18] Wang J, Chou S, Xu L, Zhu X, Dong N, Shan A, et al. High specific selectivity and membrane-active mechanism of the synthetic centrosymmetric α-helical peptides with Gly-Gly pairs. Sci Rep 2015;5:15963.
[19] Martin-Loeches I, Dale GE, Torres A. Murepavadin: a new antibiotic class in the pipeline. Exp Rev Anti-Infect Ther 2018;16:259–68.
[20] Srinivas N, Jetter P, Ueberbacher BJ, Werneburg M, Zerbe K, Steinmann J, et al. Peptidomimetic antibiotics target outer-membrane biogenesis in *Pseudomonas aeruginosa*. Science 2010;327:1010–13.