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ORIGINAL PAPER

Isolation and partial characterization of antibacterial lipopeptide produced by *Paenibacillus polymyxa* HKA-15 against phytopathogen *Xanthomonas campestris* pv. phaseoli M-5

Vellaichamy Mageshwaran · Suresh Walia · Kannepalli Annapurna

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Abstract An antibacterial metabolite was isolated from *Paenibacillus polymyxa* HKA-15, a soybean bacterial endophyte. The purification of the crude metabolite from *Paenibacillus polymyxa* HKA-15 was done by column chromatography. In TLC, a spot with an R_f value of 0.86 (±0.02) from the purified fraction showed bioactivity against *Xanthomonas campestris* pv. phaseoli M-5. In SDS-PAGE, the purified antibiotic was separated in the molecular weight range of 3.5 kDa. The exact molecular weight of the active compound was identified as 1,347.7 Da using MS-MS analysis. Infra red spectrum and ^{1}H NMR analysis showed the presence of amino acids and fatty acids in the active compound. The characterization of the antibacterial compound revealed its lipopeptide nature. In an agar diffusion assay, the crude metabolite showed a broad spectrum of activity, being able to inhibit the growth of the fungal pathogen, *Rhizoctonia bataticola, Macrophomina phaseolina* and *Fusarium udum*. A stronger inhibition was observed against bacterial pathogens *viz., X. campestris pv.*phaseoli M-5, *X. campestris pv.* phaseoli CP-1-1, *Xanthomonas oryzae*, *Ralstonia solanacearum* and *Micrococcus luteus.*

V. Mageshwaran (✉)
Chemical and Biochemical Processing Division, Central Institute for Research on Cotton Technology, Adenwala Road, Matunga, Mumbai 400019, India
e-mail: mageshbioiari@gmail.com

S. Walia
Division of Agricultural Chemicals, Indian Agricultural Research Institute, Pusa, New Delhi 110012, India

K. Annapurna
Division of Microbiology, Indian Agricultural Research Institute, Pusa, New Delhi 110012, India

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Introduction

Indiscriminate use of chemicals for the control of plant diseases results in environmental degradation, food poisoning and mammalian toxicity. Researchers are focusing on the alternative ways to manage disease, and biocontrol using microorganisms to control plant pathogens offers best alternative to chemical control, since they are eco-friendly, effective control agents and cause less mammalian toxicity. *Bacillus* sp. and its related genera have been identified as potential biocontrol agents, as they produce wide range of cyclic lipopeptides active against various microorganisms (Kim et al. 2003; Pueyo et al. 2009; Yoshida et al. 2000; Safdi et al. 2002; Hyun et al. 1999). Based on the chemical nature, the large number of peptide antibiotics produced by *Bacillus* and *Paenibacillus* may be classified into two broad groups, cyclic and linear peptides. The identification and characterization of these peptides from different strains showed that they may be bacillopeptins (Kajimura et al. 1995), members of the fusaridicin group of peptides (Beatty and Jensen 2002), matacin (polymyxin M) (Martin et al. 2003), bacilysocin (Tamehiro et al. 2002), bacillomycinF (Mhammedi et al. 1982) gaversin and saltavalin (Pichard et al. 1995) and polymyxin B (Selim et al. 2005).

The antimicrobial metabolites produced by *Paenibacillus* sp. are low molecular weight compounds with an approximate molecular weight of 1 kDa and they are resilient to a wide range of physico-chemical stresses (Tendulkar et al. 2007; Weid et al. 2003; Beatty and Jensen

2002; Lee et al. 2008). A large number of studies have been done on structural elucidation of antifungal peptide antibiotics produced by *Paenibacillus* strains (Selim et al. 2005; Lee et al. 2008). *X. campestris* pv. phaseoli causing bacterial common blight disease is an important pathogen in French beans and naturally infects other legumes and is a pathogen hard to control, since it shows an increasing resistance to chemical control (Zanatta et al. 2007). Earlier work done in our laboratory showed that *Paenibacillus polymyxa* HKA-15 was active against *R. bataticola* causing charcoal rot disease in soybean (Senthilkumar et al. 2007a). The antifungal property of this strain was found to be due to peptide antibiotics, since it failed to produce siderophores, chitinase, protease and HCN. However, the antibacterial activity of the metabolite produced by *Paenibacillus polymyxa* HKA-15 against phytopathogen *Xanthomonas campestris* pv. phaseoli was unknown. Hence this study was undertaken to characterize the antibacterial metabolite produced by *Paenibacillus polymyxa* HKA-15 against *X. campestris* pv. phaseoli M-5.

Materials and methods

Microorganisms and culture conditions

Soybean bacterial endophyte *Paenibacillus polymyxa* HKA-15 obtained from Division of Microbiology, IARI, New Delhi. The test plant pathogenic strains including *X. campestris* pv. phaseoli M-5 were obtained from Division of Plant Pathology, IARI, New Delhi. The test human pathogenic strains were obtained from Delhi University South Campus, New Delhi. Bacterial strains were grown in nutrient broth at 30°C and preserved with 40% (vol/vol) glycerol at −20°C. Fungal strains were grown in potato dextrose agar (PDA) and preserved in PDA slants at 4°C.

Size exclusion chromatography and hydrophobic interaction chromatography

48-h-old cultures of *Paenibacillus polymyxa* HKA-15 grown in 5 l of nutrient broth supplemented with 0.5% glucose at 30°C under shaking conditions (180 rev/min) was extracted with an equal volume of *n*-butanol. The butanol phase was separated and concentrated to dryness by vacuum evaporation. The dried residue was dissolved in methanol and called the crude metabolite or crude extract. 5 ml of crude extract (13.7 mg ml^{-1}) in methanol was applied to a methanol-equilibrated Sephadex LH20 column (1.5 × 35 cm) and eluted with methanol at a flow rate of 0.2 ml min^{-1} in Size Exclusion Chromatography (SEC). 1 ml fractions were collected in vials and their absorbance at 214 nm and 280 nm were read. The antibacterial activity of fractions was located by agar diffusion assay. In the agar diffusion assay, NA plates were spread with 100 µl of 0.5 OD pathogenic culture *X. campestris* pv. phaseoli M-5. Wells of diameter 0.5 cm were made using the reverse of sterile 100 µl microtips and the bases of the wells were sealed with 0.8% plain agar. 30 µl of the fraction was placed into these wells and plates were incubated at 30°C for 48 h and observed for the presence of inhibition zone. Bioactive fractions were pooled and reduced to dryness. Dried antibacterial compounds were redissolved in 1 ml of HPLC-grade methanol and designated SEC-purified fractions. The SEC-purified fractions were further purified by Hydrophobic Interaction Chromatography (HIC). One ml of SEC-purified compounds in 20% methanol was applied to a Sephadex LH20 column (1.5 × 8 cm, equilibrated with 20% methanol) and eluted isocratically with 20, 50 and 100% methanol at a flow rate of 0.2 ml min^{-1}. One ml fractions were collected in vials and their absorbance at 214 nm was read. Fractions were collected for each concentration of methanol until the absorbance at 214 nm became zero. The antibacterial activity of collected fractions was located by agar diffusion assay. Bioactive fractions, eluted with different concentrations of methanol were pooled separately and reduced to dryness. Dried antibacterial compounds were dissolved in 1 ml of HPLC grade methanol and referred to as SEC-HIC purified fractions.

Thin layer chromatography

Crude antibiotic and SEC-HIC purified fractions were spotted on TLC silica gel 60 F_{254} from Merck. After drying, TLC plates were developed with a solvent system of water-saturated chloroform and methanol (6.5:3.5 v/v). The spots were developed with iodine vapor. Retention values (R_f) were calculated based on distance moved by the compound divided by distance moved by the solvent front. The R_f value for each TLC-positive band of the antagonistic substances was calculated. Each band was then extracted with methanol and filtered through a 0.45 µm filter to remove silica debris. The TLC fractions were then tested for antibacterial activity against *X. campestris* pv. phaseoli M-5.

Analytical high perfomance liquid chromatography

The purity of the bioactive fraction was tested by analytical HPLC (Waters model 600), with photodiode array (PDA) detector (Waters 2996), equipped with C 18 reverse phase column (250 × 4.6 mm dia.). 20 µl of the sample was injected into the HPLC system under isocratic condition and the mobile phase consisted of acetonitrile and 0.2% disodium orthophosphate in the ratio of 20:80. The flow rate was 0.4 ml min^{-1} and the reading was monitored at 214 nm.

Physical and chemical characterization

The effects of pH, enzymes, surfactants and organic solvents on SEC-HIC active fractions were examined. To determine the effect of pH, sample (90 µl) mixed with 10 µl of 100 mM HCl to give pH 2 (10 mM HCl) and 90 µl of sample added with 10 µl of 1 M NaOH to give pH12 (100 mM NaOH). To test the effect of enzymes on active fractions, 23 µl of 21.4 mg ml^{-1} of proteinase K (0.5 mg ml^{-1}) was added with 77 µl of sample and Pepsin at final concentration of 0.5 mg ml^{-1} was added with 100 µl of sample. The effects of glycerol (5%), Triton X100%, and SDS (1%) on purified fractions were tested. Methanol, ethanol and chloroform at 10% (v/v) were added to the sample and tested for bioactivity. To analyse the thermal stability, the samples were exposed to autoclaving temperature (+121°C for 15 min) and at −20°C for 3 h. The treated samples were incubated at 37°C for 3 h and tested for bioactivity against *X. campestris* pv. phaseoli M-5. A control was maintained with no addition and incubated at 37C for 3 h. In enzymatic treatment, the samples were boiled for 5 min to stop the activity of enzymes after incubation at 37°C for 3 h.

SDS-PAGE, mass spectrometry, FT-IR spectrum and NMR spectroscopy

To find out the molecular weight range of the compound SDS-PAGE was performed with 14% polyacrylamide gel. 4 µl of active fraction was mixed with 5 µl of sample loading buffer and made up to 20 µl with distilled water, boiled for 2 min and spun it for few seconds to settle the debris. The sample was run in a vertical gel electrophoresis as described by Laemmli (1970) and stained with Coomassie Brilliant Blue R250. To study the exact molecular weight of the active compound, ESI-MS was performed with an LCQ ion trap mass spectrometer (Finnigan MAT, San Jose, CA) equipped with an electrospray ionization (ESI) interface operated in the positive-ion mode. Full MS acquisition was recorded over the mass range of 150–1,500 amu. To know the infra red spectrum, the active compound was processed with KBr to make a pellet. Spectra of the pellet were recorded in the transmission mode between wave numbers of 4,000 and 600 cm^{-1} using a resolution of 4 cm^{-1} 20-kHz scan speed in Perkin-Elmer FTIR spectrophotometer (model 128). One dimensional ^{1}H NMR spectra were recorded at 297.1 K on an AMX 300 NMR Spectrometer (Bruker, Karlsruhe, Germany; 700 MHz) equipped with an Aspect 3000 computer (Bruker) locked to the deuterium resonance of the solvent $CDCl_3$, with out spinning data were recorded at 32 k (the number of data points per ppm of the plot).

Antimicrobial spectrum of crude metabolite

The inhibition spectrum of crude metabolite was determined as follows: 30 µl of crude metabolite was placed in a well made in NA medium spread with the test bacterium. For antifungal assay, a disc of test fungus was placed in the centre of a PDA plate containing crude metabolite of HKA-15 cells. The plates were incubated 1 week for fungi and 24–48 h for bacteria.

Results

Isolation and purification of antibacterial lipopeptide

Initially the crude antibiotic was purified by size exclusion chromatography. Out of 34 fractions collected from SEC, fractions 8–22 showing antibacterial activity against *Xanthomonas campestris* pv. phaseoli M-5 were pooled and concentrated to dryness. A_{214} and A_{280} values of each fractions were plotted (Fig. 1) along with their antibacterial activity (ZOI in mm). The peak for antibacterial activity was merged with the peak of A_{214}. The active fractions showed maximum absorbance in A_{280} and A_{214}, respectively. In HIC, the metabolite was eluted with 20, 50 and 100% methanol. The absorbance was read at 214 nm. The fractions eluted with 20% methanol and 100% methanol showed absorption peaks (Fig. 2). The fractions eluted with 20% methanol (Fraction no. 5–25) showing antibacterial activity against *Xanthomonas campestris* pv. phaseoli M-5 were pooled and concentrated to dryness and called SEC-HIC purified fractions.

There were three distinct bands obtained with R_f values of 0.28, 0.44 and 0.88, respectively when crude antibiotic were spotted on TLC plates whereas a single band with

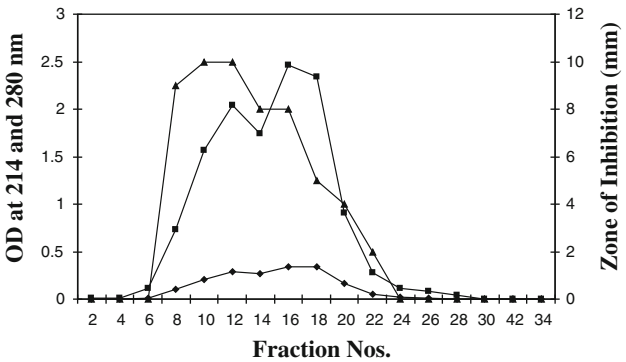


Fig. 1 Size exclusion chromatography fractions on bioactivity against *Xanthomonas campestris* pv. phaseoli M-5 (*filled square*) OD at 214 nm; (*filled lozenge*) OD at 280 nm; (*filled triangle*) Zone of Inhibition (mm)

R_f value 0.86 was obtained when SEC-HIC active fractions were spotted on TLC plates (Fig. 3a). The material with an R_f value of 0.86 (±0.02) from crude antibiotic and SEC-HIC purified fractions showed antagonistic activity against *Xanthomonas campestris* pv. phaseoli M-5 (Fig. 3b). Analytical HPLC analysis showed two peaks one major peak (peak I) with retention time value of 4.60 and one minor peak (peak II) with a retention time value of 6.48, respectively. Best separation was achieved with a mobile phase consisted of acetonitrile and 0.2% disodium orthophosphate in the ratio of 20:80 (v/v). The percentage area of peak I and peak II were 85.02 and 14.98, respectively (Fig. 4).

Characterization of antibacterial lipopeptide

As shown in Table 1, no loss of activity was observed when the purified fraction was exposed to temperatures −20 and 121°C for 15 min. The compound retained full activity when they subject to pH 2 and partially lost the activity at pH 12 at 37°C at overnight incubation. Treatment with pepsin resulted in loss of activity. Treatment with organic solvents such as Acetone, Ethanol, Methanol and Chloroform showed no loss of activity and it was found that they are soluble in these solvents. Anti bacterial compound treated with Glycerol (5%) resulted in half a reduction of activity. Treatment with Triton X100 (1%) showed that half a reduction of activity in SEC-HIC active fractions. No loss of activity was observed when antibacterial compound was treated with SDS (1%).

In SDS-PAGE, the purified metabolite was separated in the molecular weight range of 3.5 kDa (Fig. 5). The accurate molecular weight of the purified compound was determined

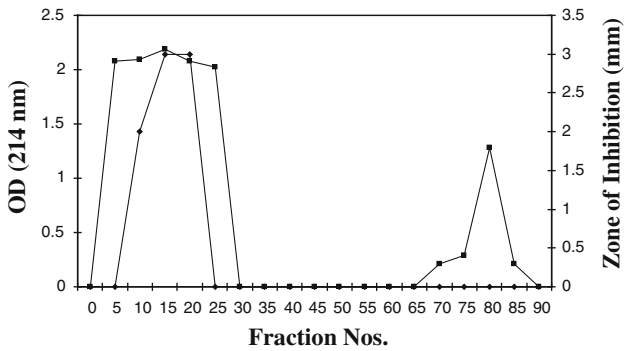


Fig. 2 Hydrophobic interaction chromatography fractions on bioactivity against *Xanthomonas campestris* pv. phaseoli M-5 (*filled square*) OD at 214 nm; (*filled lozenge*) Zone of Inhibition (mm)

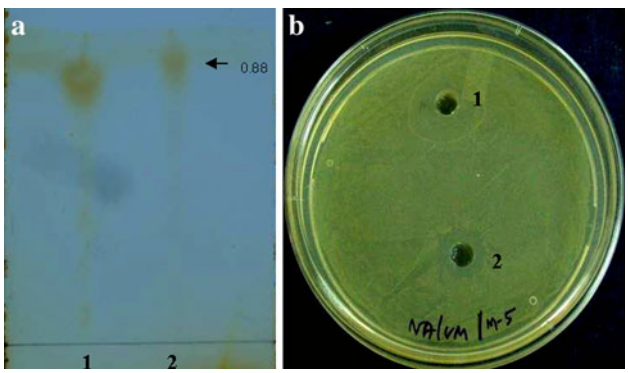


Fig. 3 TLC and its bioassay of purified antibiotic **a** TLC chromatogram (*1*) Crude metabolite (*2*) Purified antibiotic; **b** Bioassay against *Xanthomonas campestris* pv. phaseoli M-5 (*1*, Control (Methanol); *2*, purified antibiotic showing Zone of Inhibition)

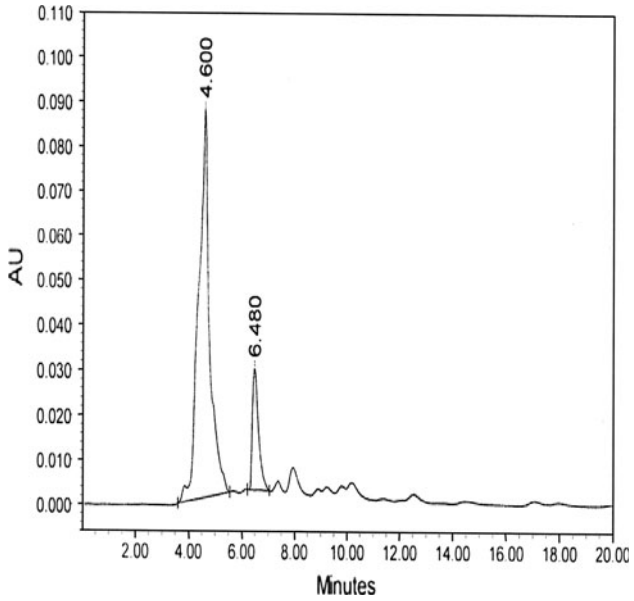
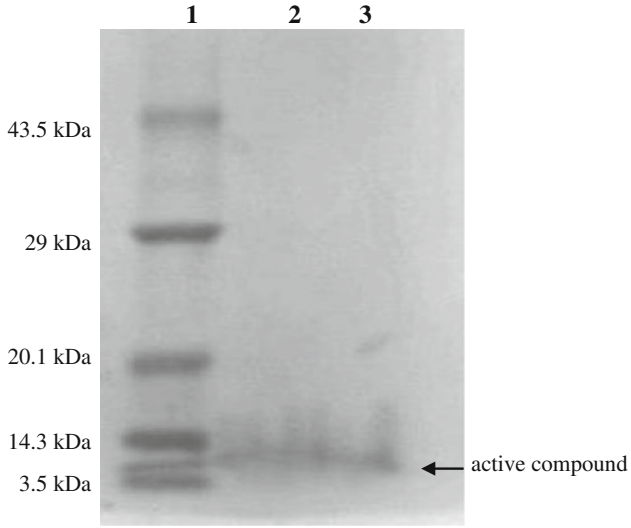


Fig. 4 Analytical HPLC chromatogram of purified antibiotic

Table 1 Physical and chemical characterization of purified antibiotic against *Xanthomonas campestris* pv. phaseoli M-5

Treatment	Zone of Inhibition (mm)
Control (none added) 3 h at 37°C	15
−20°C.	15
121°C	15
pH 2	15
pH 12	8
Proteinase K (0.5 mg/ml)	15
Pepsin (0.5 mg/ml)	–
Glycerol (5%)	8
Triton X-100 (1%)	8
SDS (1%)	15
Methanol	15
Chloroform	15
Ethanol	15

by means by using Full Electro Spray Ionization (ESI) mass spectrometry analysis. The molecular weight of the active compound was identified as 1,347.7 Da (Fig. 6). The infrared spectrum of the purified sample showed the major peaks at wavelength (cm^{-1}) 3,267, 2,959, 2,927, 1,644 and 1,454



1 - Low molecular weight protein marker , 2,3 – bioactive compound

Fig. 5 SDS-PAGE

(Table 2; Fig. 7). Results obtained with 700 MHz for ^{1}H NMR (Fig. 8) clearly indicate that the molecule being studied is a lipopeptide. The peak at 1.3 ppm indicates the presence of methyl groups $(CH_3)_n$. The spectral peak at 3.4 ppm indicates the presence of an acyl group ($–COCH_3$) and also the presence of an amide linkage ($–NH–CH_2–$) was indicated by the spectral peak at 4.9 ppm.

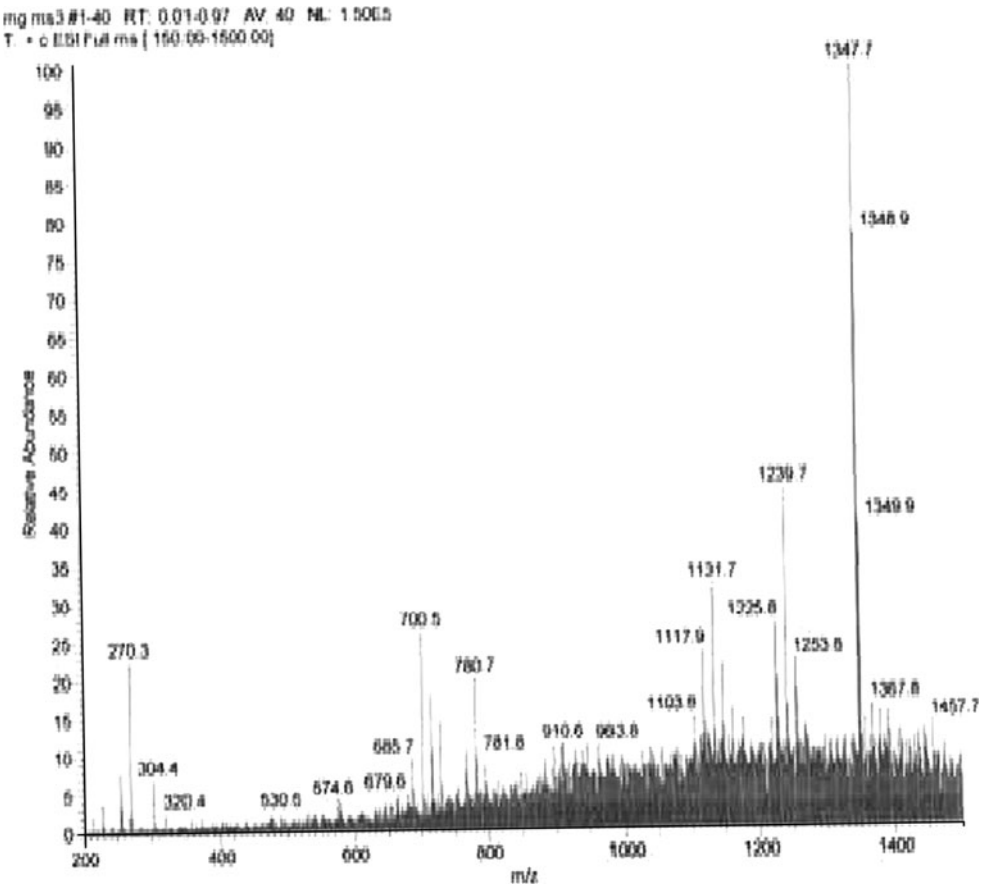
Antimicrobial spectrum of the crude metabolite

In agar diffusion assay, the metabolite was able to inhibit fungal pathogens viz., *Rhizoctonia bataticola, Macrophomina phaseolina and Fusarium udum, Fusarium* sp., and *Fusarium oxysporum* and bacterial pathogens viz., *X. campestris pv.*phaseoli CP-1-1, *X. campestris pv.*phaseoli M-5, *Xanthomonas oryzae*, *Ralstonia solanacearum, Micrococcus luteus, Listeria monocytogenes* and *Vibrio cholerae* VC 20 (Tables 3, 4).

Discussion

In our study, the antibacterial lipopeptide from *Paenibacillus polymyxa* HKA-15 was isolated, purified and partially characterized. A small scale extraction of crude metabolite

Fig. 6 Mass spectrometry chromatogram of purified active compound



was carried out using *n*-butanol from 5 l of culture broth of *Paenibacillus polymyxa* HKA-15 grown at 30°C for 48 h under shaking conditions. The crude metabolite was then subjected to purification followed by bioactivity testing against *X. campestris* pv. phaseoli M-5. The crude extract was purified using size exclusion chromatography and hydrophobic interaction chromatography. The purified fractions are called SEC-HIC active fractions. TLC analysis of crude antibiotic and SEC-HIC purified fractions gave three and one distinct spots, respectively. The bioassay of TLC spots revealed that the spot with R_f value of 0.88 showed antagonistic activity. Bioassay performed on seeded *Cladosporium* TLC plate demonstrated that *B.cereus* X16 produces more than one antibiotic (Safdi et al. 2002). HPLC analysis showed two peaks one major peak with Retention Time value of 4.60 and one minor peak with Retention Time value of 6.48, respectively.

The physical, chemical and enzymatic characterization of the active SEC-HIC fractions showed the purified antibiotic is highly resistant to wide range of temperature, pH, organic solvents and surfactants. Earlier work done on characterization of peptides showed that the peptide antibiotic isolated from different bacterial strains was highly resistant to different physical and chemical stresses. (Wu et al. 2005; Senthilkumar et al. 2007b; Lee et al. 2008; Fontoura et al. 2009). The partial loss of activity to

Table 2 Fourier transform infra-red (FT-IR) spectroscopy absorption bands of antibacterial metabolite from *Paenibacillus polymyxa* HKA-15

Frequency (cm^{-1})	Possible assignment*
3,267	H bonded OH groups NH2 stretching
2,960–2,927	Aliphatic C–H stretching fatty acids
1,664	NH2 bending, C = O, C = N stretching
1,454	C–H deformations in aliphatics
1,238	Aromatic alcohols

* Assignment according to Bizani et al. (2005a, b), Motta et al. (2007), Lee et al. (2008)

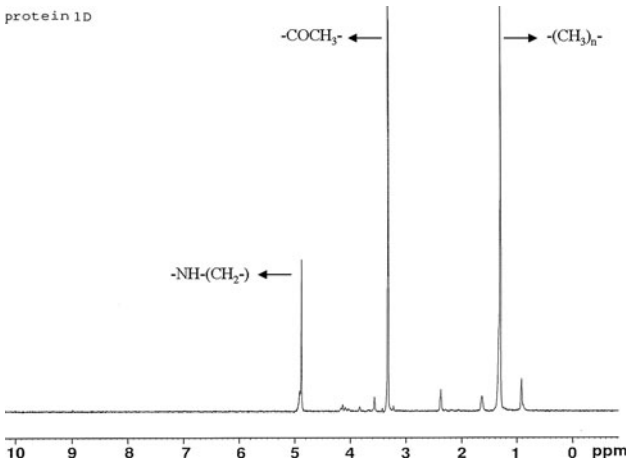


Fig. 8 ^{1}H NMR Spectoscopic analysis of antibacterial peptide

Fig. 7 Infra red spectrum analysis of purified antibiotic

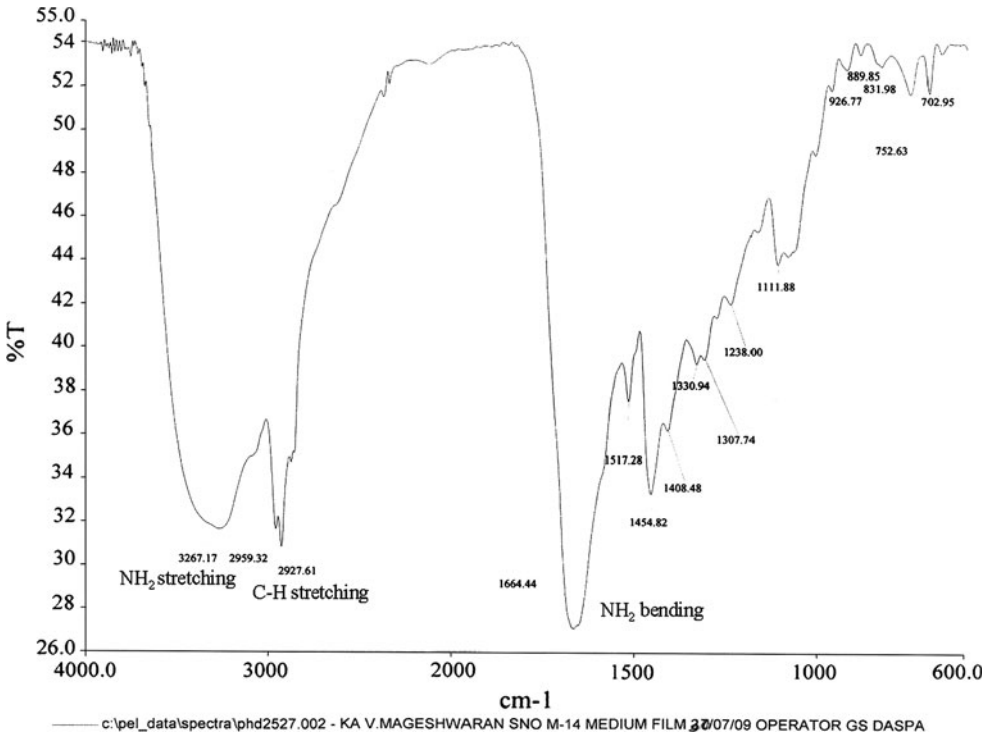


Table 3 Inhibitory spectrum of metabolite produced by *Paenibacillus polymyxa* HKA-15 against fungi

S. no.	Fungal pathogens	Disease caused/characteristic feature	Antifungal activity
1	*Rhizoctonia bataticola*	Charcoal rot	++
2	*Fusarium spp*	Fusarium root rot	+
3	*Sclerotium rolfsii*	Southern blight	−
4	*Fusarium oxysporum*	Wilt	+
5	*Macrophomina phaseolina*	Charcoal rot	++
6	*Aspergillus flavus*	Aflatoxin	−
7	*Pyricularia grisea*	Rice blast	−
8	*Fusarium udum*	Wilt in red gram	++
9	*Rhizoctonia solani*	Sheath blight in rice	−
10	*Colletotrichum dematium*	Anthracnose	−

The anti fungal activity was tested using the agar-well diffusion assay in Potato Dextrose Agar medium. Activity was scored after three independent experiments as follows: − no suppression, + weak suppression (with narrow clear zone around the well), ++ medium suppression or +++ strong suppression (with large, clear zones around the well)

Table 4 Inhibitory spectrum of metabolite produced by *Paenibacillus polymyxa* HKA-15 against bacteria

S. no.	Bacterial pathogens	Disease caused/characteristic features	Anti bacterial activity
1	*X. campestris pv.phaseoli* CP-1-1	Common blight	+++
2	*X. campestris pv.phaseoli* M-5	Common blight	+++
3	*Xanthomonas oryzae*	Rice leaf blight	+++
4	*Ralstonia solanaecerum*	Vascular wilt	+++
5	*Aeromonas hydrophila 1739*	Gastroenteritis	+
6	*Enterococcus faecalis 439*	Endocarditis	+
7	*Salmonella typhimurium*	Typhoid	−
8	*Shigella flexneri*	Shigellosis	−
9	*Listeria monocytogenes*	Listeriosis	++
10	*Vibrio cholerae VC 20*	Cholera	++
11	*Bacillus subtilis 168*	Soil bacterium	−
12	*Pseudomonas fluroscens PS* 2-3	Bio control agent	+
13	*Escherichia coli DH5α*	Common intestinal bacteria	−
14	*Azotobacter chroococcum*	Free living N2 fixers	−
15	*Bradyrhizobium japonicum* T2-3	Symbiotic N2 fixers	−
16	*Micrococcus luteus*	Common skin flora	+++
17	*Lactobacillus plantarum*	Common gut flora	−

The anti bacterial activity was tested using the agar-well diffusion assay in Nutrient Agar medium. Activity was scored after three independent experiments as follows: − no suppression, + weak suppression (with narrow clear zone around the well), ++ medium suppression or +++ strong suppression (with large, clear zones around the well)

Proteinase K and complete loss of activity to protease like pepsin reveal the peptide nature of the metabolite isolated from the *Paenibacillus polymyxa* HKA-15. Similar results were obtained during characterization of other peptide antibiotics (Bizani et al. 2005a; Wu et al. 2005). The molecular mass of the peptide antibiotic on 14% SDS-PAGE was observed to be 3.5 KDa suggesting the homogeneity of purified metabolite. These results are in agreement with earlier reported work (Bizani et al. 2005a; Motta et al. 2007; Lee et al. 2008). The mass spectrum analysis of the sample showed the molecular weight of the compound is 1,347.7 Da. This discrepancy of result from SDS-PAGE can be explained on the basis of the abnormal behaviour of some highly hydrophobic proteins in SDS-PAGE (Kaufman et al. 1984; Motta et al. 2007). The main type of peptide antibiotics produced by *P. polymyxa* come under the polymyxin-colistin-circulin family and the molecular weight of the peptide antibiotics produced by *P. polymyxa* has been well reviewed (Raza et al. 2008). Similarly Wu et al. (2005) reported that the molecular weight of the subpeptin JM4-A and subpeptin JM4-B produced by *Bacillus subtilis* strain JM4 were 1,422.71 and 1,422.65 Da,

respectively. The genera *Bacillus* and *Paenibacillus* produce a wide range of non-ribosomal peptide antibiotics such as iturins, surfactins, polymyxins, fengycins, fusaridicin etc. with a range of molecular weights due to changes in the number and substitution of amino acids in their structure (Selim et al. 2005; Wu et al. 2005; Pueyo et al. 2009).

The infra-red spectrum analysis of the antibiotic offers valuable information about peptide structure. Analysis of the spectrum of our compound showed typical absorption bands corresponding to N–H stretching of proteins and peptide bonds and presence of aliphatic C–H stretching showed concrete evidence that the substance is peptide in nature containing fatty acids in their structure. The results clearly showed the hydrophobic nature of peptide antibiotics and are in agreement with previous work on peptide antibiotics (Bizani et al. 2005a and b; Motta et al. 2007; Lee et al. 2008). Further evidence of the lipopeptide nature of the antibacterial metabolite was given by ^{1}H NMR analysis where spectral peaks were obtained corresponding to methyl group, acyl group and amide linkages. The results are in agreement with the earlier work on protein characterization using ^{1}H NMR analysis such as surfactant produced by *Bacillus subtilis* (Makkar and Cameotra 1999) and bacteriocin produced by *Lactobacillus lactis* (Shufie et al. 2008). The agar diffusion assay with wide range of pathogenic fungi and bacteria shows the antimicrobial spectrum of metabolite produced by *Paenibacillus polymyxa* HKA-15.

Conclusion

The present study revealed the lipopeptide antibiotic produced by *Paenibacillus polymyxa* strain HKA-15 has a wide antimicrobial spectrum and showed strong antagonism against the phytopathogen *Xanthomonas campestris* pv. phaseoli M-5. Further work on biocontrol efficacy of this antibiotic under field conditions will help in development of newer antibiotic for sustainable control of bacterial common blight disease in beans.

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References

Beatty PH, Jensen SE (2002) *Paenibacillus polymyxa* produces fusaricidin-type antifungal antibiotics active against *Leptosphaeria maculans*, the causative agent of blackleg disease of canola. Can J Microbiol 48:159–169

Bizani D, Dominguez APM, Brandelli A (2005a) Purification and partial chemical characterization of the antimicrobial peptide cerein 8A. Lett Appl Microbiol 41:269–273

Bizani D, Motta A, Morrissy JAC, Terra RMS, Souto AA, Brandelli A (2005b) Antibacterial activity of cerein 8A, a bacteriocin like peptide produced by *Bacillus cereus*. Int Microbiol 8:125–131

Fontoura R, Spada JC, Silveira ST, Tsai SM, Brandelli A (2009) Purification and characterization of an antimicrobial peptide produced by *Pseudomonas* sp. strain 4B. World J Microbiol Biotechnol 25:205–213

Hyun JW, Kim YH, Lee YS, Park WM (1999) Isolation and Evaluation of protective effect against *Fusarium* wilt of sesame plants of antibiotic substance from *Bacillus polymyxa* KB-8. Plant Pathol J 15:152–157

Kajimura Y, Sugiyama M, Kaneda M (1995) Bacillopeptins, new cyclic lipopeptide antibiotics from *Bacillus subtilis* FR-2. J Antibiot 48:1095–1103

Kaufman E, Geisler N, Weber K (1984) SDS-PAGE strongly overestimates the molecular masses of the neurofialmental proteins. FEBS Lett 170:81–84

Kim HS, Park J, Choi SW, Choi KH, Lee GP, Ban SJ, Lee CH, Kim CS (2003) Isolation and Characterization of *Bacillus* strains for biological control. J Microbiol 41:196–201

Laemmli LK (1970) Cleavage of structural proteins during the assembly of head of bacteriophage T_4. Nature 227:680

Lee H, Churey JJ, Worobo RW (2008) Purification and structural characterization of bacillomycin F produced by a bacterial honey isolate active against *Byssochlamys fulva* H25. J Appl Microbiol 105:663–673

Makkar RS, Cameotra SS (1999) Structural characterization of a biosurfactant produced by *Bacillus sutilis* at 45°C. J Surf Det 2:367–372

Martin NL, Hu H, Moake M, Churey JJ, Whittal R, Worobo RW, Vederas JC (2003) Isolation, structural characterization, and properties of mattacin (Polymyxin M), a cyclic peptide antibiotic produced by *Paenibacillus kobensis* M. J Bio Chem 278:13124–13132

Mhammedi A, Peypoux F, Besson F, Michel G (1982) Bacillomycin F, a new antibiotic of iturin group; isolation and characterization. J Antibiot 35:306–311

Motta AS, Lorenzini DM, Brandelli A (2007) Purification and partial characterization of an antimicrobial peptide produced by a novel *Bacillus* sp. isolated from the amazon basin. Curr Microbiol 54:282–286

Pichard B, Larue JP, Thouvenot D (1995) Gavesrin and saltavalin, new peptide antibiotics produced by *Bacillus polymyxa*. FEMS Microbiol Lett 133:215–218

Pueyo MT, Bloch CJ, Carmona RAM, Masico P (2009) Lipopeptides produced by a soil *Bacillus megaterium* strain. Microb Ecol 57:367–378

Raza W, Yang W, Shen QR (2008) *Paenibacillus polymyxa*: Antibiotics, hydrolytic enzymes and hazard management. J Plant Pathol 90:419–430

Safdi N, Cherif M, Hajlaui MR, Boudabbous A, Belanger R (2002) Isolation and partial purification of antifungal metabolites produced by *Bacillus cereus*. Ann Microbiol 52:323–337

Selim S, Negrel J, Govaerts C, Gianinazzi S, Tuinen DV (2005) Isolation and partial characterization of antagonistic peptides produced by *Paenibacillus* sp. Strain B2 isolated from the sorghum mycorhizosphere. Appl Environ Microbiol 71:6501–6507

Senthilkumar M, Govindasamy V, Annapurna K (2007a) Role of antibiosis in suppression of charcoal rot disease by soybean endophyte *Paenibacillus* sp. HKA-15. Curr Microbiol 55:25–29

Senthilkumar M, Govindasamy V, Dureja P, Annapurna K (2007b) Purification and partial characterization of antifungal peptides from soybean endophyte-*Paenibacillus* sp strain HKA-15. J Plant Biochem Biotech 16:131–134

Shufie EHA, Ibrahim N, El-Sabour HA, Mostafa YA (2008) Purification and Characterization of bacteriocin produced by

isolated strain of *Lactococcus lactis*. J Appl Sci Res 4: 1315–1321

Tamehiro N, Okamoto HY, Okamoto S, Ubukata M, Hamada M, Naganawa H, Ochi K (2002) Bacilysocin, a Novel Phospholipid antibiotic produced by *Bacillus subtilis* 168. Antimicrob Agents Chemother 46:315–320

Tendulkar SR, Saikumar YK, Patel V, Raghotama S, Munshi TK, Balaram P, Chatoo BB (2007) Isolation, purification and characterization of an antifungal molecule produced by *Bacillus licheniformis* BC98, and its effect on phytopathogen *Magnoporthe grisea*. J Appl Microbiol 103:2331–2339

Weid VD, Alviano DS, Santos ALS, Soares RMA, Alviano CX, Seldin L (2003) Antimicrobial activity of *Paenibacillus peoriae* strain NRRL BD-62 against a broad spectrum of phytopathogenic bacteria and fungi. J Appl Microbiol 95:1143–1151

Wu S, Jia S, Sun D, Chen M, Chen X, Zhong J, Huan L (2005) Purification and characterization of two novel antimicrobial peptides subpeptin JM4-A and subpeptin JM4-B produced by *Bacillus subtilis* JM4. Curr Microbiol 51:292–296

Yoshida S, Hiradate S, Tsukamot T, Hatakeda K, Shirata A (2000) Antimicrobial activity of culture filtrate of *Bacillus amyloliquefaciens* RC-2 isolated from mulberry leaves. Biocontrol 91: 181–187

Zanatta ZGCN, Moura AB, Maia LC, Santos AS (2007) Bioassay for selection of biocontroller bacteria against bean common blight (*Xanthomonas axonopodis* pv. phaseoli). Braz J Microbiol 38:511–515