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Lipopeptide antibiotic production by *Bacillus velezensis* KLP2016

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ABSTRACT

A bacterial strain isolated from a soil sample collected from Tamil Nadu Agriculture University, Coimbatore (India) was characterized as *Bacillus velezensis* KLP2016 by 16S rRNA molecular typing (Accession number KY214239). The extracellular lipopeptide was extracted from the cell-free fermentation medium of *B. velezensis* by acid-precipitation method. Optimized culture conditions enhanced the lipopeptide yield (2506 mg/L) from an earlier value of 1852 mg/L in the fermentation broth of *B. velezensis* KLP2016. The crude lipopeptide of *B. velezensis* showed 45.0 ± 1.2% and 70.6 ± 2.4% growth inhibition of *Aspergillus niger* and *Mucor* sp., respectively by the Agar well-diffusion assay on Potato Dextrose Agar (PDA) plates. The lipopeptide nature of the acid-precipitated fraction of *B. velezensis* was confirmed by the TLC and FTIR studies. The lipopeptide of *B. velezensis* exhibited strong (~90%) cytotoxic activity against Hep2-C human cervical carcinoma cells by MTT assay. The *B. velezensis* strain KLP2016 appeared to be a novel bacterium capable of producing potent antifungal and anticancer extracellular lipopeptide(s) in the fermentation broth.

INTRODUCTION

The indiscriminate and excessive use of xenobiotics to control the plant diseases has disturbed the ecological balance of the soil leading to the development of resistant strains of pathogens, groundwater contamination and health risks to humans (Meena *et al.*, 2014). Today one of the major challenges being faced by the biologists is the development of environmental friendly alternatives to the use of chemical pesticides to counter a variety of crop diseases (Ongena and Jacques, 2007; Meena and Kanwar, 2015). The increasing drug resistance in microorganisms has prompted a need to search potent alternative antimicrobial molecules like lipopeptides (LPs) with a new mode of action for use in agriculture, preservation of food and dairy products (Mandal *et al.*, 2013). The LPs are formed by cyclic or short linear peptide linked to a lipid tail or other lipophilic molecule. The demand of LPs is surging by leaps and bound due to their utility in agriculture practices and human welfare (Meena *et al.*, 2016). Polymyxin A (a type of LP) was discovered in year 1949 from a soil bacterium *Bacillus polymyxa* (Jones, 1949). Daptomycin was the first cyclic LP antibiotic which was approved in USA by Food and Drug Administration (FDA) for the treatment of blood and skin infections caused by some selected Gram-positive microorganisms (Nakhate *et al.*, 2013). *Bacillus* genus members are considered as dedicated microbial factories for the large scale production of such type of bioactive molecules (Roongswang *et al.*, 2011; Wang *et al.*, 2015; Dhiman *et al.*, 2016). The LPs containing higher carbon atoms, such as ≥14 in lipid tail exhibit higher antifungal activity in addition to antibacterial activity (Mandal *et al.*, 2013). *Actinobacteria* species of the genus *Streptomyces* are reported rarely to produce antimicrobial lipopeptide(s) molecules with their applications in pharmaceutical industries (Sharma *et al.*, 2014). The 'Polymyxin' LP binds to lipopolysaccharides of microbial cell membrane by electrostatic interaction through its *N*-terminal fatty acyl tail (Deris *et al.*, 2014). The wide applications of the Surfactin make it a potent candidate drug to address a number of global issues in medicine (Banat *et al.*, 2014; Singla *et al.*, 2014), industry (Nitschke and Costa, 2007; Abdel-Mawgoud, 2008) and in environmental protection (Mulligan, 2009; Pereira *et al.*, 2013; Gudina *et al.*, 2015). In the present study, we attempted the production of higher

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titre of lipopeptide in the fermentation broth of *B. velezensis* KLP2016. The cytotoxicity of the bacterial lipopeptide was also checked on the human cancer cell line.

MATERIALS AND METHODS

Collection of soil sample

A soil sample was collected from tea plantation soil from the campus of Tamil Nadu Agricultural University (TNAU), Coimbatore (India) in sterile Oak-ridge tubes.

Isolation of extracellular LPs producing bacterial strain

One gram of soil was serially diluted 10^{-1} to 10^{-10} times in autoclaved Nutrient broth, pH 7.2. Thirty microliters of each of the dilutions was spread over Nutrient agar (pH 7.2) plates. Seven discrete bacterial colonies (LP-1, LP-2, LP-3, LP-4, LP-5, LP-6 and LP-7) from Nutrient agar plate were screened for antifungal activity and one of the bacterial isolates (LP-3) exhibiting highest zone of growth inhibition against fungal growth of *Aspergillus niger* and *Mucor* sp was selected to conduct further experiments. For production and extraction of LP, the selected bacterial culture was grown at 30ºC for 72 h in Erlenmeyer flasks (250 mL capacity) containing 100 mL of Luria Bertani (LB) broth (pH 7.0) at 200 rpm in a shaking incubator. The bacterial cells were removed from the fermented LB broth by centrifugation (10,000 g for 10 min at 4ºC). The cell-free broth was collected and its pH was adjusted to 2.0 by adding 6 N HCl. The acid precipitate recovered by centrifugation (12,000 g for 15 min at 4ºC) was extracted with methanol (Gover *et al.*, 2010). The methanol (1 mL) extracted LP-fraction was bio-assayed for antifungal activity against both *Mucor* sp. and *A. niger*.

Antifungal activity assay of isolated lipopeptide(s)

The lipopeptide extract (50 µL) was bio-assayed on Potato Dextrose Agar (PDA) medium against *A. niger* and *Mucor* sp. by Agar well-diffusion method (Tagg and McGiven, 1971). The test fungus culture was transferred in the middle of the PDA plate and at the periphery, an appropriate amount of methanol extracted lipopeptide preparation was loaded aseptically in the well (diameter 6 mm). The Petri plates were incubated at 30ºC and the zones of inhibition (%) against each of the fungal pathogens were recorded after 3 days using the following equation;

$$\frac{Dt - Dc}{Dc} X\ 100$$

Where Dc: Average diameter of control mycelial colony; Dt: Average diameter of mycelial colony treated with LP.

Differential staining and 16S rRNA sequencing of the selected bacterial isolate LP-3

Gram's staining and endospore staining of the selected bacterial isolate was performed by using commercial kits (Hi Media, Mumbai, India). The selected bacterial isolate 'LP-3' was also characterized by 16S rRNA analysis. The gene sequences of related bacterial strains were obtained from the server through the BLAST with the help of forward and reverse sequence for alignment using CLUSTAL-W program of MEGA software version 6.0 (Tamura *et al.*, 2007). An evolutionary dendrogram was deduced by using the Neighbour-Joining method. The Bootstrap consensus tree generated from 500 replicates was taken to present the dendrogram and evolutionary history of the analysed taxa (Felsenstein, 1985).

Optimization of nitrogen and carbon source for production of lipopeptide by the *B. velezensis* KLP2016

The effect of a few nitrogen sources on the production of lipopeptide by *B. velezensis* was studied by adding 1% (w/v) of chosen (ammonium sulphate, peptone, sodium nitrate, ammonium nitrate, beef extract, ammonium chloride or yeast extract) nitrogen source to the production broth (pH 7.0). Similarly, the effect of the selected carbon source(s) on the production of LP by the bacterial isolate was studied by adding 1% (w/v) of chosen (glucose, sucrose, fructose, maltose, sorbitol and starch) carbon source to the LB broth supplemented with 1% beef extract (pH 7.0). The inoculated broths (4% v/v of 10 h seed culture) were incubated for 48 h at 30ºC under shaking at 200 rpm. Results were recorded as yield of the LP in fermentation broth.

Optimization of production time, temperature and pH

The effect of incubation time (24, 48, 72 and 96 h) was tested to improve the production of LP by the bacterial isolate. The inoculated broths (pH 7.0; 4% v/v of 10 h old seed culture) were kept at 30ºC for 24, 48, 72 and 96 h under shaking (200 rpm). The effect of incubation temperature (25, 30, 35, 37, 40 and 45°C) was tested on the LP production by *B. velenzensis* under continuous shaking. To study the effect of pH (4, 5, 6, 7, 8 and 9) of LB broth, the broths were individually set to the selected pH value followed by inoculation as mentioned above. The crude lipopeptide was extracted and analysed for yield of LP at above parameters.

SDS-PAGE of the crude lipopeptide preparation

The crude lipopeptide preparation was analysed to determine its molecular weight by denaturing SDS-PAGE (16%) in comparison to Iturin and Surfactin used as reference lipopeptide molecules. Silver stain was used to visualize the protein bands on SDS-PAGE.

Thin layer chromatography (TLC) analysis of LP

A mobile phase consisting of chloroform: methanol: water (65:25:4) was prepared. The 0.22 micron membrane sterilized LP was applied (5 µL) on to the TLC plate. After sample loading, the TLC plate (SDFCL, Silica gel 60/UV_{254}, 0.5 cm × 20 cm and thickness: 0.2 mm) was transferred into the solvent/mobile phase. The developed TLC plate was removed and allowed to air dry. The TLC plate was sprayed uniformly with ninhydrin solution (0.25% in ethanol) and placed in an oven at 110ºC for 20 min to visualize the LP spots. To test the presence of the lipid moiety, the TLC plate was sprayed with water. R_f value of the extracted LP was calculated by the following formula (Razafindralambo *et al.*, 1993);

$$Rf = \frac{\text{Distance travelled by the solute from origin (cm)}}{\text{Distance travelled by the solvent from origin (cm)}}$$

Partial characterization of LPs by the spectrophotometry (FTIR)

The chemical nature of the acid precipitated and methanol extracted LP of *B. velezensis* KLP2016 was confirmed by FTIR analysis (Perkin Elmer; SAIF, Panjab University, Chandigarh). FTIR spectra of lipopeptide were observed at 400–4000 wave numbers (cm^{-1}). Structure-property relationship of the isolated lipopeptide was partially determined by the FTIR spectra to confirm the insertion reactions and fictionalization.

Effect of the LP on a human Hep2C cervical cancer cell line by the MTT assay

The viability of human cervical carcinoma Hep2C cells was detected using the MTT dye which forms formazan crystals of the blue colour by a reduction process by the mitochondrial dehydrogenase present in living mammalian cells. The Hep2-C cells suspended at a final concentration of 1.2×10^4 cells/mL in the Dulbecco's Modified Eagle's Medium (DMEM) containing 5% Foetal bovine serum were seeded in 96-well plates. Plates were kept for growth of Hep2C cells for 24 h in a CO_2 incubator (37ºC; 4% CO_2). Thereafter, the cells were treated with the 0.22 micron membrane sterile LP sample at varying concentrations (5, 15, 25, 35, 45, 55, 65 and 75 µg/mL). After 24 h of LP treatment, MTT (5 mg/mL in distilled water) was added to each of the wells of 96-well plate followed by gently mixing and incubation for 1 h in dark (Wang *et al.*, 2007; Sharma *et al.*, 2017). The DMEM was completely decanted and intracellular formazan crystals were dissolved in DMSO (100 µL). The absorbance of each well was measured at 570 nm with an automated plate reader (MULTISCAN EX, Thermo Electron Corporation, China). The LP-treated cells were compared with the placebo (cells treated with phosphate buffered saline pH 7.2) saline.

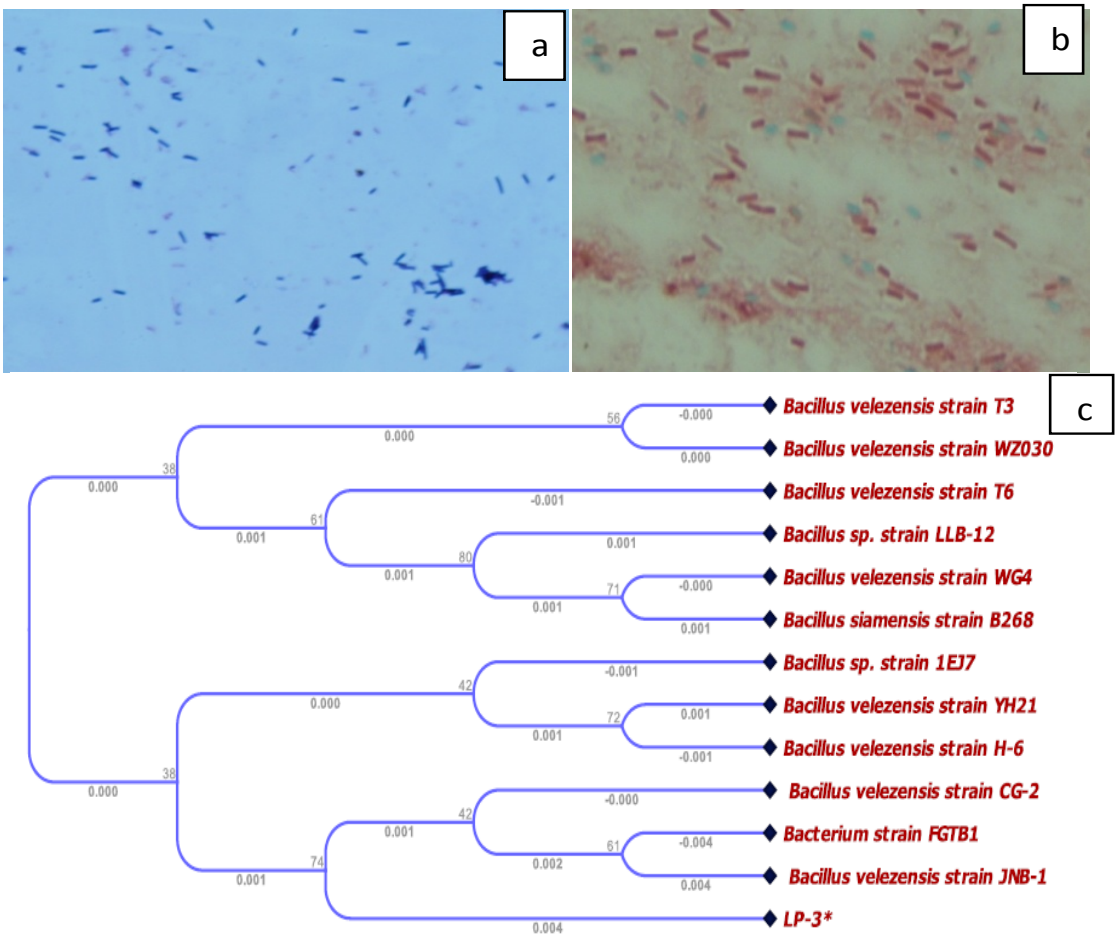

Fig. 1: Bacterial isolate LP-3. (a) Gram's staining image showed rod shaped bacteria; (b) Green colour rod shaped endospore and (c) Phylogenetic tree of *B. velezensis* KLP2016 (mentioned as isolate LP-3) generated by software MEGA 6.0 version showed prominent similarity of the investigated amplified DNA (for r-RNA) to 12 nearest neighbours.

RESULTS

Isolation and characterization of extracellular LP-producing bacterial isolate

The LPs producing bacterial colonies were isolated from the soil sample. The isolation was done in a Nutrient broth at pH 7.2. Seven bacterial colonies were isolated, maintained as pure culture on Nutrient agar plates and screened individually for their antifungal activities against *A. niger* and *Mucor* sp. One of the isolates (LP-3; Figure 1a), which was Gram positive, rod shaped

bacterium possessed highest antifungal activity was selected for extraction of LPs. The old culture of this bacterium on staining (with malachite green and counterstaining with safranin) also showed presence of green coloured endospores among vegetative cells (Figure 1b).

Identification of bacterial isolate by 16S r-RNA sequencing

The bacterial isolate 'LP-3' was characterized by 16S r-RNA analysis. The gene sequences of closely related bacterial strains were retrieved from a server through the BLAST with the help of forward and reverse sequence using CLUSTAL-W program of MEGA software version 6.0. The Bootstrap consensus tree generated from 500 replicates was taken to present the dendrogram and evolutionary history of the analysed taxa (Figure 1c). The bacterial isolate 'LP-3' was identified as *B. velenzensis* strain KLP2016 (Accession number KY214239).

Antifungal activity of extracellular lipopeptide fraction of *B. velezensis*

Antifungal activity of the extracted LP of *B. velezensis* was tested against the growth of *A. niger* and *Mucor* sp on PDA Petri plates. The extracted LP preparation of *B. velezensis* showed the highest antifungal activity of 70.6 ± 2.4% against *Mucor* sp. (Figure 2).

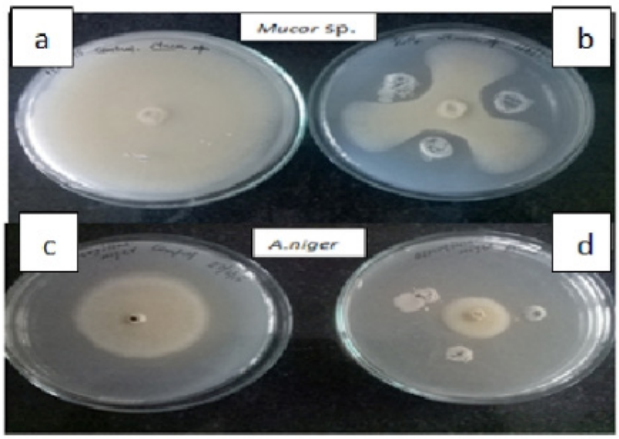

Fig. 2: Antifungal properties of the crude lipopeptide(s) preparation extracted from the *B. velezensis* against *Mucor* sp. (b): 70.6 ± 2.4%) and *Aspergillus niger* (d): 45.0 ± 1.2%), respectively. Separate placebo controls were kept for monitoring *Mucor* sp. (a) and *Aspergillus niger* (c) growth-inhibition assay.

Optimization of nitrogen and carbon source for production of LPs by *B. velezensis*

The effects of various selected nitrogen and carbon sources on the production of extracellular LP by *B. velezensis* KLP2016 were studied. The recorded results indicated that beef-extract was the best nitrogen source for the optimal yield of lipopeptides (1852 mg/L; Figure 3a) by *B. velezensis* KLP2016. Among various carbon sources (sucrose, fructose, sorbitol, glucose, starch and maltose), the sorbitol was the most preferred carbon source for LP production by *B. velezensis* KLP2016 (1900 mg/L; Figure 3b).

Optimization of incubation time, temperature and pH for the LPs production by the *B. velezensis*

The recorded results showed that a maximum yield of LPs (2075 mg/L; Figure 3c) in the fermentation broth by *B. velezensis* KLP2016 was observed after 72 h. Thus the LP preparation extracted after 72 h was considered most suitable to harvest antifungal LPs from *B. velezensis* KLP2016. LPs production was also analysed at different growth temperatures and the maximum lipopeptide yield (2135 mg/L; Figure 3d) was observed at 30ºC. Study of the effect of the different pH (4, 5, 6, 7, 8 and 9) of the production broth [Luria Bertani broth containing 1% beef extract and 1% sorbitol] on LPs production by *B. velezensis* KLP2016 showed that a pH 7 of the broth supported the maximum LP (2506 mg/L; Figure 3e) production under shake flask culture.

SDS-PAGE and TLC analysis of the crude lipopeptide of *B. velezensis* strain

The analysis of crude lipopeptide preparation of *B. velezensis* KLP2016 under denaturing SDS-PAGE (16%; Figure 4a) in comparison to reference Iturin and Surfactin revealed that the extracted bacterial lipopeptide possessed a protein band at the same position as that of the Iturin and Surfactin reference compounds (~1.1 kDa). On the basis of obtained results, the extracted LP preparation appeared to be Iturin- or Surfactin-type of lipopeptide.

The TLC plate containing lipopeptide fraction was sprayed with ninhydrin for the detection of peptide/amino acid residues, and water for the presence of a lipid moiety in the LP (Figure 4b & c). The R_f values were recorded as 0.53 and 0.79 when TLC plate was sprayed with the ninhydrin for protein detection, while R_f of 0.62 was observed when TLC plate was sprayed with water for presence of lipid moiety.

FTIR spectrum of the isolated lipopeptide preparation

The chemical nature of the isolated LP fraction was confirmed by FTIR spectra (Figure 5). The IR spectrum showed a peak at 3284.32 cm^{-1} which was due to the N-H stretching vibrations, a typical characteristic of carbon containing compound with amine group. The peak recorded at 3052.32 cm^{-1} was due to a long aliphatic chain that showed the presence of a long carbon chain. A peak observed at 2958.30 cm^{-1} signified the presence of a long stretch of C–CH_3 bond or long alkyl chains. Other significant peaks at 2871.62 cm^{-1} in the spectra corresponded to C-H in long alkyl chain. The peaks observed at 1658.30 cm^{-1} revealed the presence of CO-N stretching in the peptide bond in the lipopeptide molecule. Peaks at 1408.42 cm^{-1} were due to the aromatic group in the lipopeptide preparation. Peak at 1233.45 cm^{-1} showed the C-O deformation vibration. This FTIR spectrum of the extracted LP of *B. velezensis* KLP2016 thus confirmed the lipopeptide nature of the acid-precipitated fraction of cell-free fermentation broth.

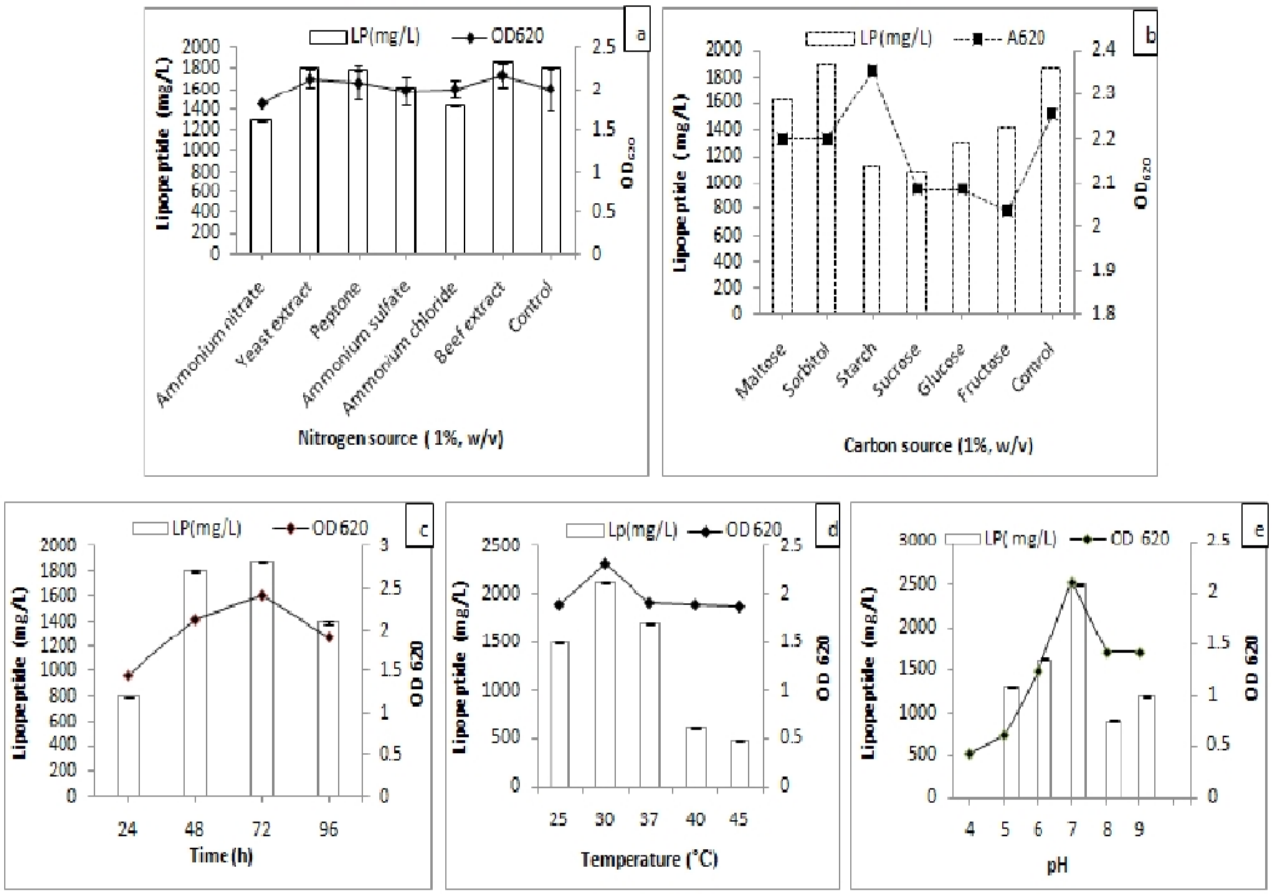

Fig. 3: Optimization of different nitrogen and carbon source for LPs production by the *B. velezensis*. (a) Nitrogen source optimization; (b) Carbon source optimization; (c) Optimization of the incubation time; (d) Optimization of the temperature and (e) Optimization of pH of fermentation broth.

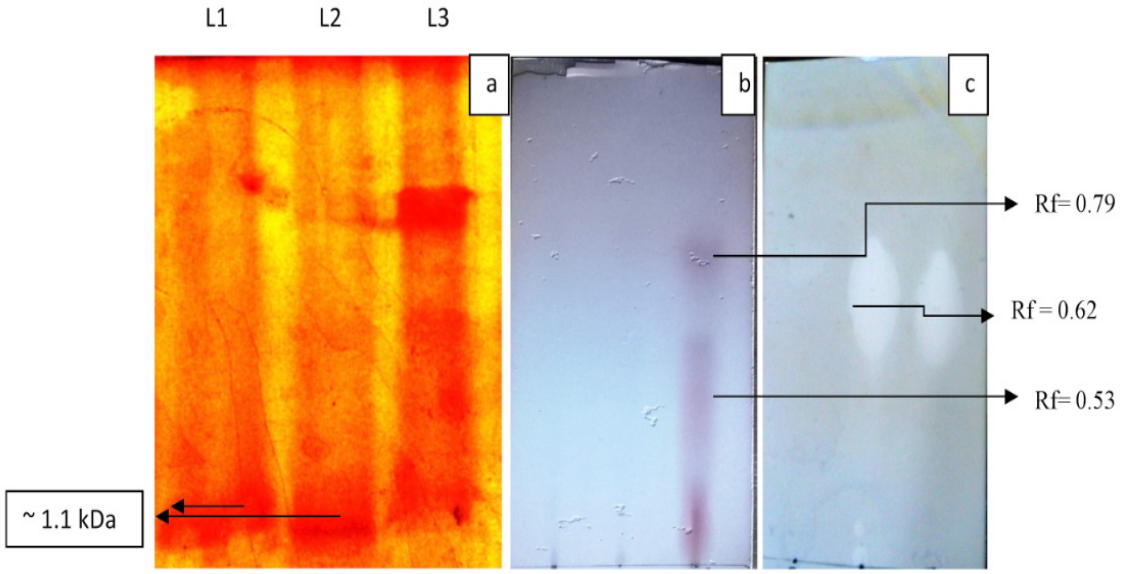

Fig. 4: SDS-PAGE and TLC of the crude lipopeptide preparation with reference to authentic Surfactin and Iturin. (a) L1: Band of authentic Surfactin, L2: Band of authentic Iturin A and L3: Bands of crude lipopeptide preparation. (b) TLC plate sprayed with ninhydrin produced blue violet spots indicating presence of the peptide moiety and (c) TLC plate sprayed with water showed white spots indicating the presence of lipid moiety in the acid-precipitated fraction of *B. velezensis* KLP2016 cell-free broth.

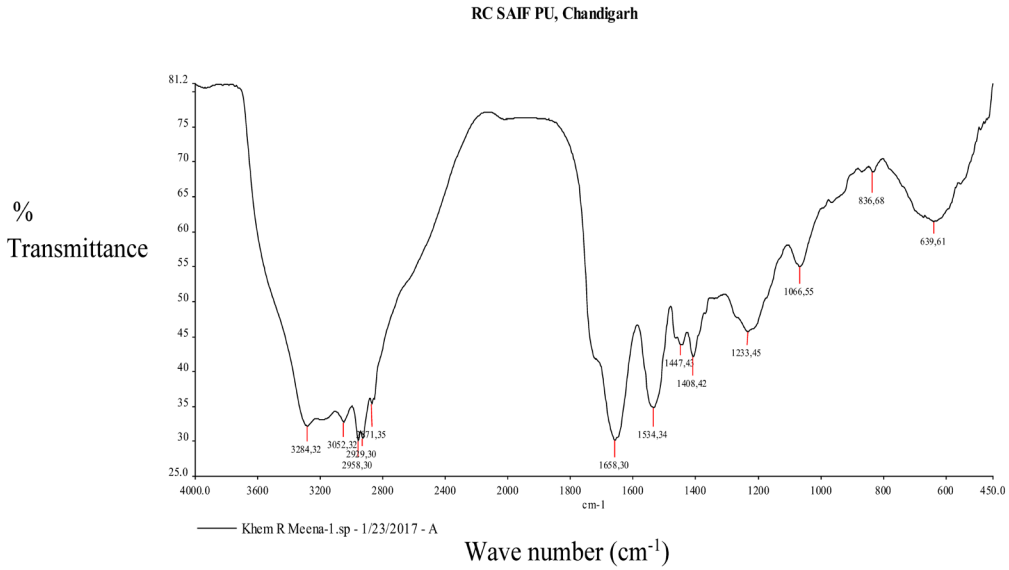

Fig. 5: FTIR spectra of the LP extracted from the fermentation broth of *B. velezensis* KLP2016.

MTT assay

To establish the concentration of LP fraction necessary to inhibit the growth/proliferation of Hep2-C cells, these cells plated in 96-well plates were treated with LP fraction at concentration varying from 5 to 75 µg/mL (Table 1). The maximum growth inhibition (94.24 ± 2.3%) of human cervical Hep2-C cancer cells was observed at 75 µg/mL concentration of LP of *B. velezensis* KLP2016.

Table 1: Killing of Hep2-C cells by lipopeptide of *Bacillus velezensis* KLP2016.

Lipopeptide (µg/mL)	Hep2C cell death (%)
5	45.84 ± 3.24
15	51.07 ± 2.45
25	84.23 ± 2.13
35	91.01 ± 1.52
45	88.01 ± 2.42
55	84.82 ± 3.17
65	86.37 ± 2.41
75	91.24 ± 3.43

DISCUSSION

The LPs are considered as the versatile bio-weapons due to their antibacterial, antifungal, anticancer or antitumor applications. A bacterial strain isolated from the oil contaminated desert site in Gujarat (India) has been previously reported for the lipopeptide production to establish its biosurfactant application in microbial-enhanced oil recovery (Sujata *et al.*, 2016). The coastal sea-sediment samples from three different contaminated locations of Nagapattinam fishing harbor, Tamil Nadu, India have been reported for isolation of lipopeptide-producing microbes (Panagal *et al.*, 2016). In our study, a bacterium KLP2016 was isolated from the soil collected from the TNAU, Coimbatore. The bacterial isolate characterized by the 16S rRNA sequencing was found to be a *Bacillus velezensis* strain which has not been reported earlier for the LP production.

The selection of suitable carbon and nitrogen source are among most important growth nutrients/factors which might affect the lipopeptide production by bacterial cells. The basal salt medium containing urea has been reported to produce 720 mg/L LPs from *B. subtilis* SPB1 strain after the 48 h of incubation. The production of these LPs was enhanced to 750 mg/L when the concentration of urea was kept 5.0 g/L, and the medium contained glucose (40 g/L) as a carbon source (Abushady *et al.*, 2005). A medium containing 20.0 g/L carbon source, 2.0 g/L nitrogen source, 3.0 g/L $K_2HPO_4.3H_2O$, 10 g/L $NaH_2PO_4.2H_2O$, 0.2 g/L $MgSO_4.H_2O$, 0.002 g/L $MnCl_2.4H_2O$ and 0.2 g/L yeast extract enhanced the Surfactin production (Liu *et al.*, 2012). Sucrose (a carbon source) enabled the *Bacillus* sp. to reach a maximum yield of 102.0 mg/L C15-Surfactin while ammonium nitrate (a nitrogen source) also enhanced the yield of Surfactin (99.6 mg/L). A *B. subtilis* produced 720 to 2040 mg/L of biosurfactant at the end of the fermentation (Dhouha and Semia, 2011). However, in our study, among various nitrogen sources (ammonium nitrate, yeast extract, peptone, beef extract, ammonium chloride *etc.*), beef extract (1%; w/v) was the best nitrogen source for extracellular production of LPs (1812 to 1852 mg/L). The least positive effect was observed in case of use of ammonium nitrate in the broth (1292 mg/L) in comparison to use beef extract. When concentration of beef extract was optimized (1%; w/v), the lipopeptide yield reached 1864 mg/L. In the present study, among various carbon sources (maltose, sorbitol, starch, sucrose, glucose and fructose), recorded results indicated that sorbitol (1%) was best carbohydrate to produce extracellular LPs by *B. velezensis* KLP2016 (1900 mg/L; Figure 3b).

The bioprocess/fermentation temperature also influences the composition of LPs produced by *B. subtilis* RB14, with Iturin A predominantly being synthesized at 25°C while Surfactin production was favoured at 37°C (Ohno *et al.*, 1995). About 1900 mg/L of LPs were produced at temperature 40ºC by *B. subtilis*. Another *B. subtilis* K1 strain exhibited maximum growth at the pH range of 7.0 to 9.0 with maximum production of antifungal activity at pH 9.0 (Mizumoto and Shoda, 2007). In our study, the results showed that a maximum yield of LPs by *B. velezensis* KLP2016 was observed after 72 h (2075 mg/L; Figure 5A). Thus the lipopeptides acid-precipitated in cell-free broth of *B. velezensis* KLP2016 after 72 h of fermentation was considered most productive to extract LPs for potent antifungal activity. Lipopeptide(s) production was also analyzed at different fermentation temperatures (25, 30, 35, 37, 40 and 45°C), and interestingly the maximum LPs yield (2135 mg/L; Figure 3d) was observed at 30ºC. At the temperature above of 37ºC (40ºC and 45ºC), the *B. velezensis* KLP2016 didn't show much growth as well as lipopeptide yield. The study of the effect of the pH (4, 5, 6, 7, 8 and 9) of the optimized fermentation broth showed that a pH 7 was the best for lipopeptide (2506 mg/L; Figure 3e) production *B. velezensis* KLP2016. After optimization of all the bioprocess/fermentation conditions, the LPs yield in the production broth enhanced from an initial value of 1852 mg/L to a maximum of 2506 mg/L, which thus indicated approximately 35% higher yield of LPs over the initial unoptimized broth and fermentation conditions.

In a recent report, a *Bacillus* sp. CCMI 1051 exhibited antifungal activity 25.7 ± 1.00, 23.1 ± 0.93 and 22.1 ± 0.19 mm against *Mucor* sp., *Alternaria* sp. and *Cladosporium* sp., respectively (Silva *et al.*, 2016). In our study, lipopeptide fraction of *B. velezensis* KLP2016 inhibited the growth of *A. niger* and *Mucor* sp. on PDA by 45.0 ± 1.2 and 70.6 ± 2.4%, respectively. The lipopeptide fraction of *B. velezensis* KLP2016 was also checked for the anticancer activity against the human Hep2-C cell line (A derivative of HeLa cells). The outcome was startling as 91.2% cells death was observed at a concentration of 75 µg/mL of LPs of *B. velezensis* KLP2016. The SDS-PAGE of the LP fraction of *B. velezensis* KLP2016 showed presence of a protein of ~1.1 kDa which was similar to the molecular size of Surfactin and Iturin reference molecules. Presence of both the lipid and protein moiety in the lipopeptide of *B. velezensis* KLP2016 was confirmed by the TLC. The FTIR spectra of the crude lipopeptide also confirmed the lipopeptide nature of the acid-precipitated cell-free fermentation broth of *B. Velezensis* KLP2016.

CONCLUSION

The selected bacterium *B. velezensis* KLP2016 (Accession number KY214239) was found to produce extracellular LP having potent antifungal and anticancer activities. The lipopeptide preparation however, showed strongest antifungal (70.6 ± 2.4% growth inhibition) activity against *Mucor* sp. Optimization of the bioprocess conditions resulted in an enhanced lipopeptide yield (2506 mg/L) in the fermentation broth of *B. velezensis* KLP2016 from an earlier value of 1852 mg/L. The MTT assay was performed on human Hep2C cells to check the cytotoxicity of the lipopeptide fraction. The extracted LP preparation appeared to contain Iturin and Surfactin on the basis of FTIR and SDS PAGE analysis. This is a novel bacterium, which has not been reported in the literature earlier for the production of lipopeptides.

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CONFLICT OF INTEREST

There is no conflict of interest among the authors or with their parent institution.

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