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# Isolation and characterization of halophilic *Bacillus* sp. BS3 able to produce pharmacologically important biosurfactants

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ABSTRACT

**Objective:** To characterize the pharmacological importance of biosurfactants isolated from halophilic *Bacillus* sp BS3. **Methods:** Halophilic *Bacillus* sp. BS3 was isolated from solar salt works, identified by 16S rRNA sequencing and was used for screening their biosurfactant production. Characters of the biosurfactant and their anticancer activity were analyzed and performed in mammary epithelial carcinoma cell at different concentrations. **Results:** The biosurfactant were characterized by TLC, FTIR and GC-MS analysis and identified as lipopeptide type. GC-MS analysis revealed that, the biosurfactant had various compounds including 13-Docosenamide, (Z); Mannosamine, 9- and N,N,N′,N′-tetramethyl. Surprisingly the antiviral activity was found against shrimp white spot syndrome virus (WSSV) by suppressing the viral replication and significantly raised shrimp survival ($P<0.01$). Anticancer activity performed in the mammary epithelial carcinoma cell at different concentrations of biosurfactants, among the various concentrations of biosurfactants such as 0.000 25, 0.002 5, 0.025, 0.25 and 2.5 μg, the 0.25 μg concentration suppressed the cells significantly ($P<0.05$) to 24.8%. **Conclusions:** Based on the findings, the present study concluded that, there is a possibility to develop eco-friendly antimicrobial and anticancer drugs from the extremophilic origin.

## 1. Introduction

Biosurfactants produced by microorganisms[1], are amphipathic surface active molecules containing hydrophilic and hydrophobic moieties that act by emulsifying hydrocarbons, increasing their solubilisation and subsequently rendering them available for microbial degradation[2]. They can be glycolipids, lipopeptides, lipopolysaccharides, polysaccharide protein complexes, fatty acids and lipids[3]. Bioemulsifiers generally include low-molecular-weight compounds, such as lipopeptides and glycolipids and bioemulsans include high-molecular-weight polymers of polysaccharides, lipopolysaccharides, proteins or lipoproteins[4]. Biosurfactants are the most important and valuable products of biotechnology for industrial and medical applications. In recent years, these biomolecules were also found to possess several interesting properties of therapeutic and biomedical importance[5]. They have several applications including agriculture, bioprocessing, pharmaceuticals, food industry, dermatology and cosmetics industry. In pharmacological field, the biosurfactants act as antibacterial, antifungal, antiviral, anticancer, immunomodulator, anti-adhesive, antioxidants, stimulate dermal fibroblasts, vaccines and gene therapy[6].

Microbes from extreme environments have attracted considerable attention in recent years. This is primarily due to the secret that they hold about the molecular

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evolution of life and stability of the macromolecules[7]. They are often under extreme conditions of pressure, temperature, salinity, and depletion of micronutrients, with survival and proliferation often depending on the ability to produce biologically active compounds. The diversity of marine environments has exerted a driving force on bacteria selection leading to new adaptive strategies and the synthesis of new metabolites[8]. Microbial secondary metabolites have been recognized as a major source of compounds endowed with ingenious structures and potent biological activities. Marine microorganisms have been continuously found with unrivalled capacity to synthesize bioactive secondary metabolites. It has been reported that the number of novel marine natural compounds in 2008 exceeded 1 000, most of which were isolated from indigenous or symbiotic microorganisms[9].

The cyclic lipopeptide surfactin produced by *Bacillus subtilis* is one of the most powerful biosurfactant known today[10]. They are active at extreme temperatures, pH and salinity as well, and can be produced from industrial wastes and from by-products[11]. The lipopeptide biosurfactant are most prominently known as surfactin produced mostly by the *Bacillus* sp. Despite similar global structures, surfactins, iturins and fengycins differ in some aspects regarding their biological activities[10].

The search for novel biosurfactants in extremophiles seems to be particularly promising since they have particular adaptations to increase stability in adverse environments and the microbial products are highly stable and important in medical biotechnology. The present study point out the screening, characterization and its pharmaceutical importance of biosurfactants extracted from the halophilic *Bacillus* sp BS3 which was isolated from solar salt works in southern India.

## 2. Materials and methods

### 2.1. Sampling

Condenser water had the salinity of 155‰ was collected from the solar salt works in Thamaraikulam, Kanyakumari district, Tamilnadu, India (Lat. 8° 11′ N and Long. 77° 29′ E). Samples were collected in sterile polythene bags, transported to the laboratory aseptically and stored at 4 ℃ for further use.

### 2.2. Isolation and phenotypic identification

Water samples were serially diluted from $10^{-1}$ to $10^{-8}$ in distilled water and 100 μL of each dilution was spread onto sterile nutrient agar plates containing 5% to 20% NaCl. After incubation at 37 ℃ for 7 d, morphologically different colonies were identified by Gram staining, motility and biochemical confirmations.

### 2.3. Genomic identification by 16S rRNA sequencing

Genomic DNA (100 ng) isolated from halophilic *Bacillus* sp. BS3 strain was amplified by PCR using 16S rRNA universal primers (Forward: 5′ CAGGCCTAACACATGCAAGTC 3′; Reverse: 5′ GGGCGGWGTGTACAAGGC 3′). The PCR product was cloned into the vector pTZ57R and used to transform *Escherichia coli* DH5 α as described by Maciel *et al*[12]. The transformants were sequenced using an ABI 3700 automated DNA sequencer. Sequences were compared with other 16S rRNAs obtained from GenBank using the BLAST program. The phylogenetic tree was constructed by MEGA5 software and evolutionary history was inferred using the UPGMA method[13]. The evolutionary distances were computed using the Maximum Composite Likelihood method[14] and are in the units of the number of base substitutions per site. The optimal tree with the sum of branch length = 0.316 626 28 is shown.

### 2.4. Biosurfactant screening

Different biosurfactant screening methods were done for the potential biosurfactant producing halophilic *Bacillus* sp. BS3. The methods adopted were (a) drop-collapse test by adding mineral oil in 96-well microtitre plates[15]; (b) Oil spreading technique by adding weathered crude oil[16]; (c) Emulsification activity by adding kerosene and equal volume of cell free supernatant[17] and (d) Hemolytic activity in 5% blood agar plate.

### 2.5. Biosurfactant extraction

The biosurfactant was extracted from cell-free broth at 72-h grown cells by step-by-step purification of acid precipitates using adsorption chromatography. Bacterial cells were removed from surfactant-containing medium by centrifugation (10 000 rpm for 20 min). The supernatant was subjected to acid precipitation by adding 6 N HCl to achieve a final pH of 2.0 and allowing the precipitate to form at 4 ℃. The precipitate was pelleted at 10 000 rpm for 20 min, re-dissolved in distilled water, adjusted to pH 7.0, freeze-dried, and weighed. The dried surfactant was extracted with acetone and dried with the aid of a rotary evaporator under vacuum[18].

### 2.6. Partial purification and structural elucidation

#### 2.6.1. Thin-layer chromatography (TLC)

The dried biosurfactants obtained from acid precipitation method was dissolved with distilled water and spotted on TLC (Merck) sheets and run with $CHCl_3/CH_3OH/H_2O$ (65:15:1) as mobile phase. The chromatogram was developed under

short UV light as well as exposing iodine vapour. The Rf value was calculated as per the standard database of biosurfactants[19].

#### 2.6.2. Fourier transmission infrared spectroscopy (FT-IR)

The basic functional groups of the purified Biosurfactants from halophilic *Bacillus* sp. BS3 were analyzed qualitatively by Fourier Transform Infra Red (FTIR)[20].

#### 2.6.3. Gas chromatography- Mass spectrometry (GC-MS)

GC-MS analysis of partially purified biosurfactants were analysed individually using Agilent GC-MS 5975 Inert XL MSD (United States) gas chromatography equipped with J and W 122-5532G DB-5 ms 30 mm × 0.25 mm × 0.25 μm and mass detector (EM with replaceable horn) was operated in EMV mode. Helium was used as carrier gas with the flow rate of 1.0 mL/ min. The injection port temperature was operated at 250 ℃. The column oven temperature was held at 80 ℃ for 2 min then programmed at 10 ℃ per minute to 250 ℃, which was held for 0 min, and then at 5 ℃ per minute to 280 ℃ which was held for 9 min. Electron impact spectra in positive ionization mode were acquired between m/z 40 and 450.

### 2.7. Pharmacological potential of halophilic Bacillus sp Biosurfactants

#### 2.7.1. Antibacterial and antifungal activity

*In vitro* antibacterial activity was performed by the partial purified biosurfactants against human pathogens (*Escherichia coli*, *Pseudomonas aerugenosa*, *Salmonella typhi* and *Staphylococcus aureus*) using agar diffusion method and agar over layer method. For anti fungal activity, 20 μL of extract was poured into the well of the Potato Dextrose Agar plates and fungal spores were inoculated onto the plates and incubated at 35 ℃ for 48 h. The zone of inhibition was recorded.

#### 2.7.2. Antiviral activity

Antiviral activity was performed against white spot syndrome virus (WSSV) following the method of Balasubramanian *et al*[21]. Five micro litre of purified WSSV suspension (300 μg of total protein) was mixed with different percentages of biosurfactants (25%, 50%, 75% and 100%) and incubated at 29 ℃ for 3 h. After incubation period, the mixture was injected intramuscularly to *Fenneropenaeus indicus* and had the average weight of (10±1) g. Three replicates were ($n$= 10×3= 30) maintained in all treatments. Mortalities were recorded daily and the experiment was carried out up to 10 d. Control shrimps were injected with a mixture of 10 μL NTE buffer and 5 μL viral suspensions. Haemolymph samples were collected from all injected shrimps and checked by WSSV diagnostic PCR using VP 28 primer designed by Yoganandhan and Sahul Hameed[22] The DNA extraction and PCR amplification were carried out by following the method described by Islam *et al*[23]. Haemolymph samples of experimental and control shrimps were tested by the first step PCR. The negative samples detected in the first step were further subjected for second step PCR analysis.

#### 2.7.3. Anticancer activity

The anticancer activity was performed in tumor mammary epithelial carcinoma cell lines with the partial purified biosurfactants extracted from halophilic *Bacillus* sp. following the method of Freshney[24] and the activity was monitored after 48 h.

## 3. Results

### 3.1. Identification of halophilic Bacillus sp.

Among the various isolates from the solar salt works, the effective biosurfactant producing bacteria was confirmed as *Bacillus* sp. by morphological, biochemical and 16S rRNA sequencing. The phenotypic confirmation revealed that, the *Bacillus* sp. BS3 are gram positive, rod shape, non motile and able to ferment carbohydrate such as glucose, sucrose and galactose (Table 1). Phylogenetic and evolutionary analysis of the 16S rRNA sequence revealed that, the halophilic *Bacillus* sp. BS3 was more than 90% similarity to the other *Bacillus* sp. such as *Bacillus cereus*, *Bacillus anthracis*, *Bacillus thuringiensis* and other uncultured bacterium (Figure 1).

**Table 1**

Phenotypic identification of biosurfactant produced halophilic *Bacillus* sp. BS3 isolated from Thamaraikulam solar salt works in India.

| Sl. No | Confirmative test | *Bacillus* sp. BS3 |
|---|---|---|
| 1 | Gram staining | + |
| 2 | Cell shape | rod |
| 3 | Motility | - |
| 4 | Indole | - |
| 5 | Methyl red | - |
| 6 | VP | - |
| 7 | Citrate | + |
| 8 | Oxidase | - |
| 9 | Catalase | + |
| 10 | Nitrate | + |
| 11 | Urease | + |
| 12 | TSI | - |
| 13 | Gelatin hydrolysis | + |
| 14 | Starch hydrolysis | + |
| 15 | CHO fermentation | Glucose |
| | | Sucrose |
| | | Galactose |
| | | Lactose |

**Figure 1.** Evolutionary relationships of biosurfactant producing halophilic *Bacillus* sp. BS3′s 16S rRNA sequence compared with other taxa.

### 3.2. Biosurfactant screening

Of the different biosurfactant screening tests such as drop collapse test, oil spreading test, emulsification activity and haemolytic activity, the halophilic *Bacillus* sp. BS3 was able to produce biosurfactants. The halophilic *Bacillus* sp. was highly positive in those tests, whereas very low or no activities were observed in compared with the *Bacillus subtilis* isolated from back water and MTCC strains respectively (Table 2).

### 3.3. Partial purification and structural elucidation

The extracted biosurfactants′ relative front ($R_f$) was calculated 0.68 of the chromatogram developed in TLC. Based on the $R_f$ value database, it was concluded that, the extracted biosurfactant from halophilic *Bacillus* sp belongs to lipopetide type (Figure 2). The different peak of the IR spectral analysis revealed that, the stretch, 3 429 $cm^{-1}$ denoted as N-H group. The presence of this peak reveals the fact that the sample contains a primary or secondary amine or an amide or substituted amide group in the sample. The peak at 2 084 $cm^{-1}$ corresponds to cumulated system like R2C=N=N in the sample. The presence of the peak at 1 641 $cm^{-1}$ confirms the presence of olefininc band (C=C) is unsaturation and the peak at 406 $cm^{-1}$ is due to C-I (Carbon -Iodine) bond (Figure 3). The various retention times of the biosurfactants′ GC-MS and NIST 98 and 2005 database analysis revealed that, the peak from different retention times such as 29.57, 29.59, 29.60, 30.58, 13.51, 16.09 and 16.22 confirmed as 13-Docosenamide, (Z); Mannosamine, 9-Octadecenamide, (Z), 2-Octanol, 2-methyl-6-methylene, Cylohex-1,4,5-triol-3-one-1-carbo, 2-Butanamine, 2-methyl- and 1,2-Ethanediamine, N,N,N′,N′-tetramethyl- respectively (Table 3).

**Table 2**

Biosurfactant screening halophilic *Bacillus* sp. isolated from Thamaraikulam solar salt works in India.

| Bacterial strains | Drop collapse test | Oil spreading test | Emulsification activity | Haemolytic activity |
|---|---|---|---|---|
| Halophilic *Bacillus* sp.* | ++++ | +++ | ++++ | ++++ |
| *Bacillus subtilis* (MTCC)** | - | - | - | - |
| *Bacillus subtilis*** | ++ | + | + | + |

* Biosurfactant produced *Bacillus* sp. BS3 isolated from solar salt works; ** *Bacillus subtilis* from MTCC strain (IMTECH, Chandigar, India) and *** *Bacillus subtilis* from isolated from back water of Rajakkamangalam, India.

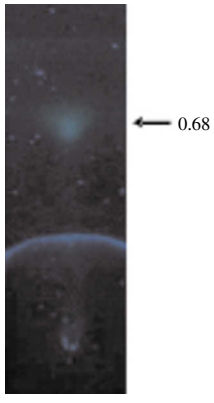


**Figure 2.** Qualitative analysis of partially purified biosurfactants by TLC.

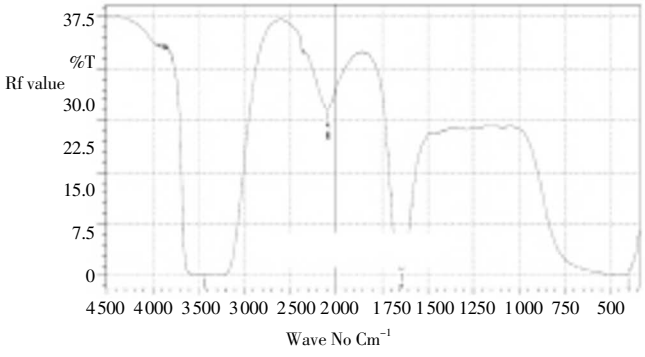


**Figure 3.** FTIR analysis for the partially purified biosurfactant from halophilic *Bacillus* sp. BS3.

### 3.4. Pharmacological potential of biosurfactants

The *Bacillus* sp. BS3′s biosurfactants were effectively

**Table 3**

Major chemical compounds identified from the partial purified biosurfactants from halophilic *Bacillus* sp. BS3 by GCMS analysis.

| Retention time | Name of compounds | Molecular formula | Molecular weight | Quality % |
|---|---|---|---|---|
| 29.57 | 13-Docosenamide, (Z) | $CH_3(CH_2)_7CH=CH(CH_2)_{11}CONH_2$ | 337.58 | 93 |
| 29.59 | Mannosamine | $C_6H_{13}NO_5 \cdot HCl$ | 215.60 | 45 |
| 29.60 | 9-Octadecenamide, (Z) | $C_{18}H_{35}NO$ | 281.40 | 72 |
| 30.58 | 2-Octanol, 2-methyl-6-methylene | $C_{12}H_{22}O_2$ | 198.30 | 43 |
| 13.51 | Cylohex-1,4,5-triol-3-one-1-carbo | $C_5H_8FN_3$ | 129.13 | 64 |
| 16.09 | 2-Butanamine, 2-methyl- | $C_5H_{13}N$ | 87.16 | 38 |
| 16.22 | 1,2-Ethanediamine, N,N,N′,N′-tetramethyl- | $C_6H_{16}N_2$ | 116.20 | 80 |

inhibited the growth of the pathogenic bacteria as well as fungi (Table 4). The antibacterial activity observed was 16.00, 14.06, 13.10 and 10.10 mm of zone of inhibition against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi*, respectively. Also higher antifungal activity observed against *Trichophyton rubrum*, *Aspergillus niger* less activity against *Aspergillus flavus* and *Fusarium* sp. respectively. The antiviral effect of the biosurfactant against WSSV revealed that, the higher percentages (50%, 75% and 100%) of biosurfactant were effectively suppressed the WSSV control (Figure 4). The WSSV injected shrimp succumbed to death cent percent at 5 d whereas the surfactant treated groups had prolonged survival and less mortality of 70% and 90% at the end of experiment ($P<0.05$). One step PCR detection also supported the higher percentages of biosurfactants. There no positive PCR signals observed in 75% and 100% of biosurfactants due to cent percent of viral suppression found (Figure 5). Various concentrations of biosurfactants treated mammary epithelial carcinoma cell were given in the Figure 6. The concentrations of 0.000 25 μg suppressed the cells of 7.42%; 0.002 5 at 16.44%; 0.025 at 21.66% and 0.25 at the maximum of 24.8% and significantly varied ($P<0.05$).

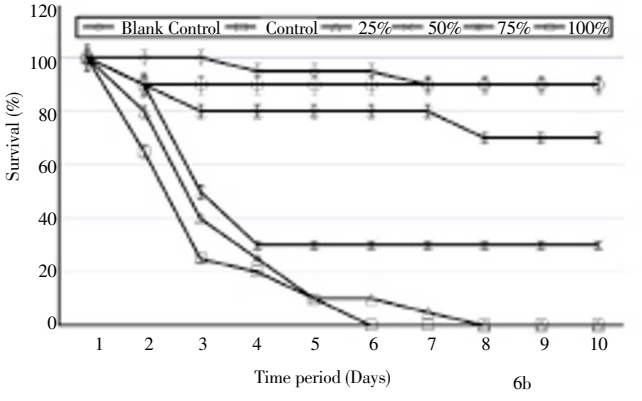


**Figure 4.** Survival of *Penaeus monodon* after injection with partially purified biosurfactant of halophilic *Bacillus* sp. BS3 incubated with WSSV.

**Table 4**

*In vitro* antibacterial and antifungal activity of partial purified biosurfactants from halophilic *Bacillus* sp BS3.

| Sample No. | Antibacterial activity | | Antifungal activity | |
|---|---|---|---|---|
| | Bacterial pathogens | Activity (mm of zone of inhibition) | Fungal pathogens | Activity |
| 1 | *E. coli* | 16.00 ± 0.15 | *T. rubrum* | ++++ |
| 2 | *S. aureus* | 14.06 ± 0.12 | *A. niger* | ++++ |
| 3 | *P. aeruginosa* | 13.10 ± 0.12 | *A. flavus* | ++ |
| 4 | *S. typhi* | 10.10 ± 0.13 | *Fusarium* sp. | ++ |

++++: higher activity; ++: medium activity; +: less activity.

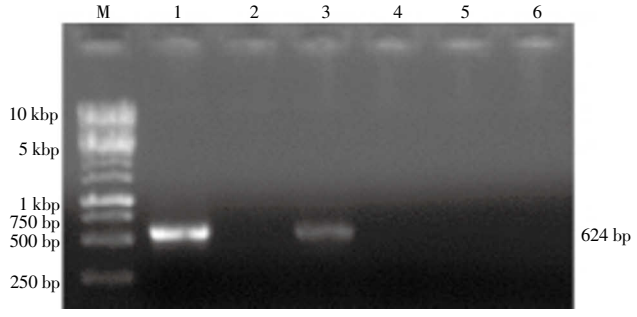


**Figure 5.** Antiviral screening of partially purified biosurfactant of halophilic *Bacillus* sp BS3 against WSSV by PCR detection.
Lanes M-Marker; 1-Positive control; 2-Negetive control; 3%-25%; 4%-50%; 5%-75% and 6%-100% biosurfactant incubated with WSSV and injected to *Penaeus monodon*.

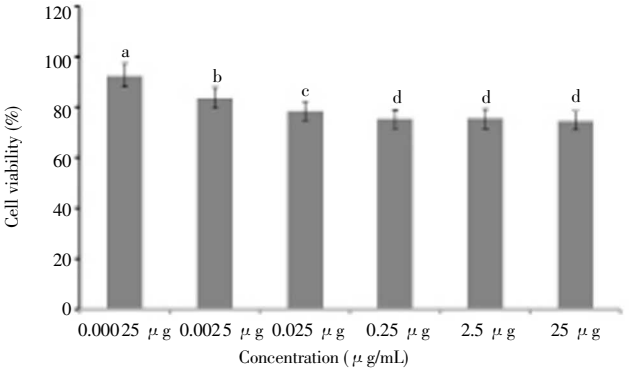


**Figure 6.** Anticancer activity performed in tumor mammary epithelial carcinoma cell lines with partial purified biosurfactants extracted from halophilic *Bacillus* sp. BS3.

## 4. Discussion

Besides their role in hydrocarbon bioremediation and microbial enhanced oil recovery[25], these biological products have potential uses in agriculture and the cosmetic, pharmaceutical, detergent, food, textile, paper and paint industries[26]. The biosurfactants of halophilic *Bacillus* sp. BS3 seems to increasing stability in the harsh environments and the strain grow well, higher emulsification stability in higher salinity, temperature and pH. Seghal Kiran *et al*[10] demonstrated that the biosurfactant produced by *Bupleurum aureum* MSA13 was stable even at autoclaving and the biosurfactant produced by marine actinobacterium was stable at high NaCl. Such extreme stability was reported by Abdel- Mawgoud *et al*[27] for the *Psudomonas aeruginosa* strain.

The lipopeptide biosurfactant are most prominently known as surfactin produced mostly by the *Bacillus* sp. Despite similar global structures, surfactins, iturins and fengycins differ in some aspects regarding their biological activities. For instance, iturins and fengycins display a strong antifungal activity, while surfactins are not fungitoxic by themselves. Surfactins show weak antibacterial properties but iturins may be strongly inhibitory to the growth of some gram-positive bacteria[10]. In the present study different screening methods such as drop collapse test, oil spreading test, emulsification activity and haemolytic activity were the simplest and powerful tools for the primary screening for biosurfactants. The methods supported for positive production of biosurfactants in the halophilic *Bacillus* sp. BS3 in compare with other *Bacillus subtilis* sp. The anti-adhesion activity of lipopeptide biosurfactants from *Bacillus* spp. was shown to prevent biofilmformation by bacterial human pathogens[28]. The cyclic lipopeptide surfactin, produced by *Bacillus subtilis* ATCC 21332, is one of the most powerful biosurfactants. Lakshmipathy *et al*[29] screened the amphipathic extracellular lipopeptides biosurfactant production in marine actinomycetes, *Streptomyces* spp. VITDDK3 by conventional screening methods, including hemolytic, drop collapsing and lipase production activity.

*Bacillus* sp. has been reported as the major producer of biosurfactants including lipopeptides, glycolipids, surfactin and halobacillin *etc*. The present study TLC analysis revealed that, the $R_f$ value of 0.68 was confirmed as lipopeptide[30]. The lipopeptide biosurfactant was characterized from *Bacillus circulans* by FTIR and TLC analysis[5]. The GC-MS analysis revealed that, the biosurfactant of halophilic *Bacillus* sp. BS3 contain glycolipids, polymers and other compounds including 13-Docosenamide, (Z); Mannosamine, 9- and N,N,N′,N′-tetramethyl *etc*. The ether extract of endophytic fungus *Paecilomyces* sp. contain 13-Docosenamide, (Z) had significant antifungal and antitumor properties. The other compound, mannosamine is responsible for the formation of saccharides in different species of *Bacillus*. The present study, the biosurfactant contain mannosamine at the quality of 45% and 9-Octadecenamide, (Z) at 72% quality level. D-mannosamine and D (+)-mannose which was isolated from the ovary of cobia *Rachycentron canadum* had antibacterial activity against *Escherichia coli* and mitogenic activity reported by Ngai and Ng[31]. The sponge associated actinomycetes, *Nocardiopsis dassonvillei* MAD08 contain 9-octadecenamide, (Z) had the broad range of antimicrobial activity including anticandid activity[32]. 1,2-Ethanediamine, N,N,N′,N′-tetramethyl- is a polymeric biosurfactant also present in the biosurfactant of the *Bacillus* sp. BS3 at high quality level. 1,2-Ethanediamine, N,N,N′,N′-tetramethyl- had a broad pharmacological activities including anti tumor, antifungal activities, fouling in aqueous system and shown to be microbicidal and preventing adhesion of bacteria. *Bacillus subtilis* lipopeptide biosurfactant was also used for control of *Culex quinquefasciatus*[33], *Anopheles stephensi*[34] and *Aedes aegypti*[35]. Interestingly, *Bacillus subtilis* has been reported as a biological control agent against *Spodoptera littoralis*[36].

Lipopeptides can act as antibiotics, antiviral and antitumour agents, immunomodulators or specific toxins and enzyme inhibitors[15]. The lipopeptides showed higher activity against gram-positive cocci than against gram-negative bacilli[37]. The present results revealed that, the biosurfactants extracted from halophilic *Bacillus* BS3 were able to suppress the bacterial sp of *Escherichia coli*, *Staphylococcus aureus*, *Psudomonas aeruginosa* and *Salmonella typhi* at more than 10 mm of zone of inhibition. The biosurfactant may inhibit the cell wall synthesis and inhibit the protein synthesis of the bacteria. Mukherjee *et al*[38] purified a biosurfactant from marine *Bacillus circulans* exhibit enhanced surface and antimicrobial activities. It is well known that some types of biosurfactants produced by several *Bacillus* species exhibit antimicrobial activity against many bacteria, including pathogens[5]. *Bacillus amyloliquefaciens* ES-2 from *Scutellaria baicalensis* coproduces surfactins and fengycins with antimicrobial activities against some phytopathogenic food-borne pathogenic and spoilage bacteria and fungi[39]. Surfactin from *Bacillus subtilis* was reduce the adhesion and to disrupt biofilms of food-borne pathogenic bacteria. These molecules were strong surface activity, emulsion forming ability and has shown antimicrobial properties[40,41]. The antifungal activity against phytopathogenic fungi has been demonstrated for glycolipids, such as cellobiose lipids[42], rhamnolipids[43], and cyclic lipopeptides[44]. Arena *et al*[45] reported novel type of EPS-1 polysaccharide had an antiviral and immunomodulatory effect which was produced by thermo tolerant strain *Bacillus licheniformis*. The characteristic structural element in lipopeptidesis a specific fatty acid, which was combined with an amino acid moiety. As a consequence of this amphiphilic structure, lipopeptides have various interesting biologicalproperties. They exhibit antifungal properties, moderate antibacterial and hemolytic properties, induce the formationof ion channels in lipid bilayer membranes, and exhibit antitumorand anti-viral activities[46]. The biosurfactant incubated WSSV injected shrimps showed higher survival rate compared with the control. The 100% biosurfactant treated group had increased

the survival rate (3 times). The biosurfactants may suppress the viral transcription and replication. Surfactin is one of the most powerful biosurfactants and is known to have anti-inflammatory, antibiotic and anti-tumour functions[47]. Cao *et al*[48] demonstrated that surfactin induces apoptosis in human breast cancer MCF-7 cells through a ROS/JNK-mediated mitochondrial/caspase pathway. An antitumour lipopeptide biosurfactant purified from *Bacillus natto* TK-1 was able to inhibit the proliferation of MCF-7 human breast-cancer cells[49]. Kim *et al*[50] reported that lipopeptide shows an antiproliferative effect on human colon carcinoma LoVo cells via apoptosis induction and cell-cycle arrest at $G_1$-phase. Antimicrobial lipopeptides produced by *Bacillus subtilis* FMBJ inactivated the cell-free virus of porcine parvovirus, pseudorabies virus, newcastle disease virus and bursal disease virus, while it effectively inhibited replication and infectivity of the Newcastle disease virus and bursal disease virus[51]. Also a rhamonolipid and its complex with alginate, both produced by a *Pseudomonas* sp. strain, showed significant antiviral activity against herpes simplex virus types 1 and 2[52]. The present findings revealed that the biosurfactant isolated from the halophilic *Bacillus* sp. BS3 had wider pharmacological activities and this will helps to develop novel drugs. Further studies are needed to improve the biosurfactant production and purification of the various compounds such as lipopeptides, glycolipids and polymers from the *Bacillus* sp. BS3.

## Conflict of interest statement

We declare that we have no conflict of interest.

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