

## Production of biosurfactant from *Bacillus subtilis* (MF 582633) and its evaluation for antimicrobial, antioxidant, larvicidal and antitermite activities

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Present study evaluates the production and characterisation of biosurfactant for its antimicrobial, antioxidant, larvicidal and antitermite activities. A bacterium has been isolated from fatty acid contaminated soil and identified as biosurfactant producer, Gram positive *Bacillus subtilis* (MF582633) subspecies *inaquosorum* based on 16S rRNA sequencing and unique QR codes. The bacterium produced 2.03 g/L of biosurfactant using 3% glycerol as a carbon source. Among the tested five organisms *K.pneumoniae* revealed (20.3 mm) and *E coli* (10 mm) zone of inhibition as compared to standard antibiotic ampicillin (40 µg/mL) when biosurfactant concentration is reported to be 40 µg/mL. Antioxidant activity in terms of IC<sub>50</sub> value at 0.4 mg/mL is recorded. The 100% larvicidal activity is recorded at 50 mg/mL concentration of biosurfactant while 40 mg/mL concentration in 48 h revealed 100% antitermite activity. Biosurfactant is purified through silica gel column chromatography. Purified biosurfactant has been characterised using FTIR and <sup>1</sup>H NMR analysis and revealed surfactin type of lipopeptide biosurfactant. Experimental results may add its value in the development of antibiotics and biopesticides to stop the menace of UTI infections, *Culex* larvae and termites.

**Keyword:** Biosurfactant, Biocides, Spectroscopy, Urinary Tract Infection

Biosurfactant are the surface active, amphiphilic compounds produced by variety of microorganisms. The unique property of biosurfactant is to accumulate between fluid phases and to reduce the surface and interfacial tension<sup>1</sup>. Surfactants are widely used in agricultural, food processing, cosmetics and pharmaceutical industries. Most of the surfactants used in these sectors are synthesized by chemical methods. Currently various microorganisms have been also reported to produce biosurfactants. The well-studied members belongs to bacterial genera like *Pseudomonas*, *Bacillus*, *Acinetobacter*, *Alcaligenes*, *Rhodococcus*, *Corynebacterium* etc<sup>2,3</sup>. Due to recalcitrant and persistent nature, over and unlimited use of surfactant leads to environmental and toxicological problems<sup>4</sup>. Therefore researchers are looking for new ecofriendly biosurfactant production technologies using microorganisms.

There are reports on the use of *Bacillus subtilis* and *Bacillus licheniformis* as a source of biosurfactant with properties like enhancement of the biodegradation of hydrocarbons and chlorinated pesticides; antimicrobial and antifungal activities, inhibition of fibrin clot formation, haemolysis and

formation of ion channels in lipid membranes and anticancer activities<sup>5,6</sup>. Now a day's drug resistance acquired by microorganisms and insect pests is a challenging problem in pharmaceutical and agro based industries. Both the industries therefore, warrants to search a new cost effective antimicrobials and safe bio pesticides. Another problem in the health sectors is the menace of mosquitoes which are reported to nurse the plethora of viruses causing number of diseases. Currently available synthetic insecticides are found to be ineffective and inhibit non-target beneficial insects. In addition to this contamination of food and drinking water sources results in the damage to beneficial flora and fauna of natural sites<sup>7</sup>. To control the disease spread, conventional treatments are inadequate therefore warrants to develop novel and efficient control strategies by formulating new efficient mosquitocidal agents.

In agricultural fields, termites causes enormous economic losses to many crop plants, tree species and building materials etc. by infesting at various stages<sup>8</sup>. There are few reports on the use of biosurfactants to treat Urinary Tract Infection (UTI) caused by

members of *Enterobacteriaceae* family. In addition to this use of biosurfactant as a larvicidal agent is reported elsewhere while no report is available on antitermite activity of biosurfactant.

In the present investigation an attempt has been made to produce and characterise biosurfactant from *B. subtilis* (MF 582633) and to evaluate its antimicrobial activity against UTI pathogens, larvicidal, antitermite and antioxidant activities. Figure. 1 shows Phylogenetic tree for *Bacillus subtilis* (MF582633)

## Experimental Section

### Sample collection and Microorganism isolation

Soil samples were collected in sterile bags from local oil industries of Nanded (MS), India. About 1.0 gm of above soil sample was enriched in 100 mL sterile nutrient broth having diesel as a carbon source. Flask containing this medium was kept in shaking incubator at 120 rpm at 37°C for 48 h. A loopful of enriched culture was spread on nutrient agar plates. Plates were kept in incubator at 37°C for 24 h so as to obtain pure cultures.

Isolated colonies were selected for further screening on nutrient agar with Methylene blue stain as a secondary screening to check the viability of cells. Selected screened organisms were carry forward for NaCl tolerance test at different NaCl concentrations (3,6,9 and 12%) with nutrient agar as a medium<sup>9</sup>. The plates thus prepared were incubated for 24 h at 37°C in incubator. The screened pure culture colonies were stored in a nutrient agar slants at 4°C in refrigerator for further study.

### Screening for Potential Biosurfactant Producing-strain

Mineral Salt Medium (MSM) (g/L D/W) containing 5 K<sub>2</sub>HPO<sub>4</sub>, 2 KH<sub>2</sub>PO<sub>4</sub>, 2 MgSO<sub>4</sub>, 0.1 NaCl, 5 NaNO<sub>3</sub>, 3 yeast extract and 15 mL glycerol were used to screen the potent biosurfactant producing strain<sup>10</sup>. The flasks containing 100 mL MSM medium inoculated with pure culture slants diluted with 5 mL saline and was incubated for 3 days at 37°C in shaking incubator at 160 rpm. Growth kinetics of microorganism and biosurfactant production was monitored for 3 days. During fermentation period different process parameters such as growth, pH and biosurfactant concentration were determined at regular intervals. All these parameters were checked in triplicate and did not vary more than 5%.

### Para film M test

This test was used to check the presence of biosurfactant in a sample. One drop of Bromophenol blue indicator was added to 2 mL of above pure culture supernatant sample. About 10 µL of sample was carefully placed like a drop on para film-M. The shape of this drop on the surface was inspected after 5 min. If the drop becomes flat it indicates presence of biosurfactant and if it remains in drop shape, it indicates absence of biosurfactant<sup>11</sup>.

### Emulsification index (E<sub>24</sub>)

About 2 mL supernatant obtained from above fermentation medium was diluted with 2 mL of substrate (diesel) in a test tube. The mixture was vigorously shaken in a vortex mixer at high speed for 10 min. The emulsion stability was determined after

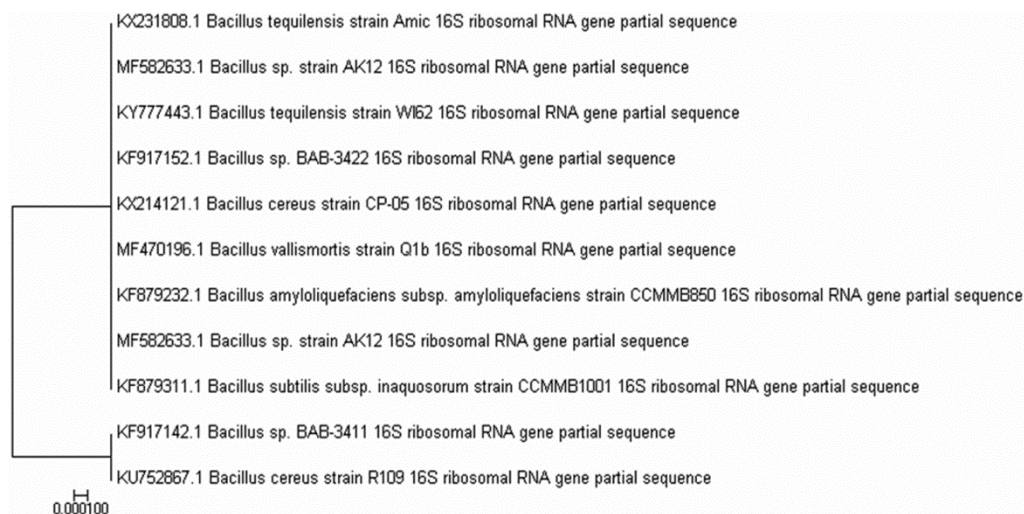


Fig.1 — Phylogenetic tree for *Bacillus subtilis* (MF582633)

24 h and activity was determined after an hour. Emulsification index  $E_{24}$  (%) was determined by using the standard formula<sup>12</sup>.

$E_{24}$  (%) = The height of emulsion layer / (The height of total solution)  $\times 100$

#### Molecular characterization and generation of QR codes

Selected screened isolate was incubated for 24 h at 37°C in Mineral Salt Medium (MSM) agar for 16S r RNA sequencing. Based on 16S rRNA sequencing results a unique QR codes was generated, GC content was determined along with CGR and FCGR codes for accurate identification of isolate. Selected isolate nucleotide sequence was submitted to NCBI under the accession number MF 582633.

#### Production, Extraction and Purification of biosurfactant

MSM (g/L D/W) containing Mineral Salt Medium (MSM) (g/L D/W) containing 5 K<sub>2</sub>HPO<sub>4</sub>, 2 KH<sub>2</sub>PO<sub>4</sub>, 2 MgSO<sub>4</sub>, 0.1 NaCl, 5 NaNO<sub>3</sub>, 3 yeast extract and 15 mL glycerol were used for biosurfactant production. The flasks containing 100 mL MSM medium was inoculated with pure culture slant (fresh cultured 2 mL dilution with saline) and was incubated for 3 days at 37°C in shaking incubator at 160 rpm. During fermentation period different process parameters like growth, pH and biosurfactant concentration were determined at regular intervals in triplicates. Different carbon sources like 3% glycerol, glucose and diesel were tested for production of biosurfactant separately.

The cell free supernatant (CFS) was centrifuged at 10,000 rpm for 10 min at 4°C. CFS was acidified with drop wise addition of 6 N HCl to adjust the pH 2.0 and kept overnight at 4°C. The biosurfactant thus precipitated, was extracted by equal volumes of ethyl acetate. The organic phase was subjected to rota-evaporation to obtain the dried crystals. Carbohydrate content of the biosurfactant was determined by the Phenol-Sulphuric acid method using D-glucose as a standard<sup>13</sup>.

About 1.0 gm of crude biosurfactant was dissolved in 1 mL of chloroform/methanol (2:1) and added to the column (40 cm $\times$ 5 cm size). Sample was eluted by admixing chloroform, methanol and an aqueous solution of 28% (v/v) ammonium hydroxide with increasing polarities like (80:20:4) (v/v/v), (75:25:4) and (65:35:5). Presence of biosurfactant in each eluted 1 mL sample was identified by spotting a drop on pre-coated silica gel 60 TLC plate (Merck). Chloroform, methanol and an aqueous solution of

28% (v/v) ammonium hydroxide in the proportion of (65:35:5) was used as a mobile phase. The TLC plates were developed by spraying distilled water and dried at 100°C to check the presence of the biosurfactant<sup>14</sup>. For the confirmation of lipids and peptides standard methods were used<sup>15</sup>. The R<sub>f</sub> value of the purified biosurfactant exactly matches with the standard surfactin (Sigma).

#### Antimicrobial activity

The purified biosurfactant was dissolved in distilled water (1 mg/mL) and kept in a presterilized glass test tube. The resulting stock solution was used to check antimicrobial activity against the isolated human urinary tract infecting (UTI) pathogens like *E. coli*, *K. pneumoniae*, *S. paucimobilis*, *S. aureus* and *P. aeruginosa* using actively growing UTI pathogens on Muller Hinton Agar (MHA) plate using well diffusion method. All the test UTI pathogens inoculated in to nutrient broth medium and incubated at 37°C for overnight. The absorbance at 480nm was correlated to 0.1 (10<sup>8</sup> CFU/mL) according to MacFarland standard. The test strains were spread on MHA plates, using sterile cork borer the wells were made at the centre of each plate and it was filled with 20  $\mu$ L of various concentrations of biosurfactant (30,40,50  $\mu$ g/mL). Control plates were added with ampicillin (40  $\mu$ g/mL). All the plates were incubated for 24 h at 37°C. The diameter of clear zone formed in plates were recorded in mm<sup>16</sup>. All the results are represented as the average of three replicates.

#### DPPH Scavenging activity

The DPPH scavenging activity experiment was performed by the standard method<sup>17</sup> with slight modifications using ascorbic acid as a standard. Stock solution of purified biosurfactant was diluted to a final conc. of 10-100 mg/mL in methanol. DPPH methanolic solution (3 mL, 3Mm) was added at a different concentrations of prepared biosurfactant solutions and allowed to react in dark condition for 30 min. at room temperature. Absorbance was recorded at 517 nm and converted to % antioxidant activity, ascorbic acid was used a standard. Scavenging activity was appeared as % inhibition and calculated using the formula,

$$I \% (\text{DPPH radical}) = [(Ac - As / Ac)] \times 100$$

where As- represents the absorbance of the biosurfactant sample while, Ac represent the absorbance of the control. IC<sub>50</sub> (half maximal inhibitory concentration of biosurfactant) were calculated<sup>18</sup>.

### Larvicidal activity

*Culex* larvae were collected from the open uncovered septic tanks. Larvae were identified by standard classification manually and reared under laboratory conditions<sup>19</sup>. The larvicidal assay was performed by using WHO guidelines. Briefly fourth instar *Culex* larvae were taken in triplicate set each of 25 in 50 mL of distilled water in a closed test tubes having air space of its 2/3 volume. To each tube ~0.2 mL of biosurfactant (20-50 mg/mL) was added separately and maintained at 25°C. Control tube contain 50 mL distilled water only. During incubation period (12-48 h) when larvae indicated with no sign of swimming and completely inactive were treated as dead. The mortalities were calculated using Abbott's formula,

$$\text{Mortality (\%)} = [(X - Y)/X] \times 100$$

where

X = % survival in control and Y = % survival in treated larvae<sup>20</sup>.

### Anti-termite activity

Indian white termites *Odontotermes obesus* (family- Odontotermitidae) were collected from the decayed logs from SRTM University campus Nanded, India. The toxicity of biosurfactant (30-50 mg/mL) was assayed according to the standard method. Biosurfactant concentrations of 30,40 and 50 mg/ mL were prepared in distilled water and applied on the Whatman paper strips separately and dried in a

laminar air flow for 30 min. The precoated paper strips were placed exactly in the centre of petridish as a test and uncoated as a control. About 30 active termites of *O. obesus* were released in each petridish. Pieces of moist paper were supplied as a food to both the test and control termites. All petridishes were incubated at room temperature. The mortality of the termites were recorded after 12, 24, 36 and 48 h<sup>21,22</sup>.

## Results and Discussion

### Isolation, Molecular identification of biosurfactant producing bacterium

A Gram positive even rods, endospore forming with dry colonies morphology was isolated from local oil industry of Nanded, India. The sequence of 16S rRNA of isolated bacterium demonstrated a high degree of similarity (99%) with *Bacillus subtilis* subspecies *inaquosorum* (Fig. 1). The unique QR code (Fig. 2 A,B,C,D) was generated using online freeware for identified bacterium which contain GC ratio about 65:45. CGR codes revealed number of dots which are concentrated in each portion of the square while FCGR codes indicates the number of base pairs repeated is directly proportional to the darkness of the square<sup>23</sup>.

### Screening and evaluation of biosurfactant production

*B. subtilis* showed highest emulsification index (77%) with positive results in parafilm M oil test

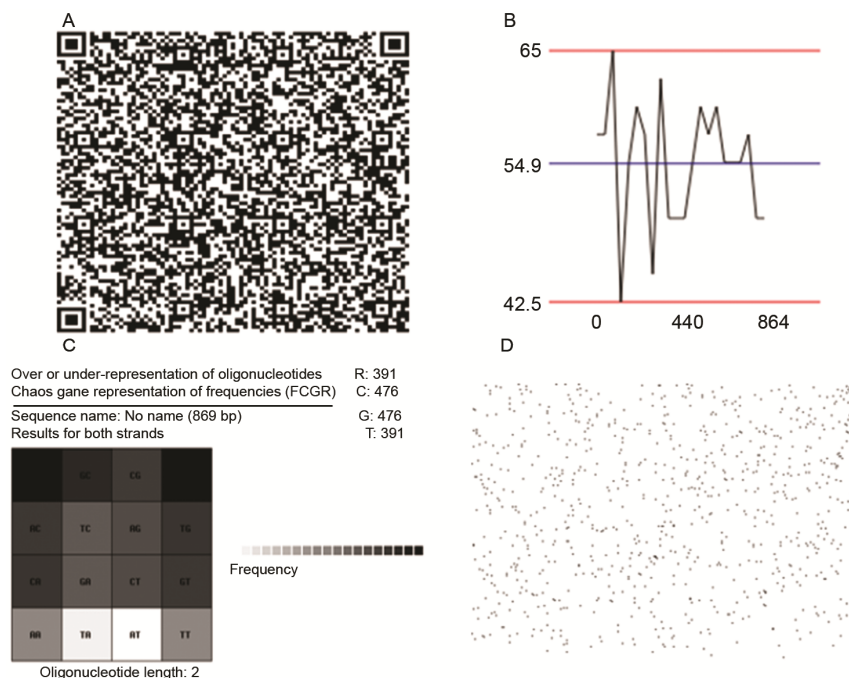


Fig. 2 — ( A,B,C,D) QR codes of *Bacillus subtilis* (MF582633)

having more than 6% NaCl tolerance. This method is better predicted biosurfactant production than the drop collapse method because it is very sensitive for detection<sup>24</sup> and it has several advantages in requiring a small volume of samples. They are rapid and easy to be carried out, and do not require specialized equipment<sup>25</sup>. Two types of carbon sources i.e. water-insoluble (diesel) and water-soluble (glycerol) were used<sup>26</sup>. Only 3% glycerol showed optimum production i.e. 2.03 g/L at pH 6.8 in 72h of incubation (Fig. 3). Higher percentage of glycerol revealed negative results. It may contribute to inhibit the growth of microorganism which might be results in lower the production of biosurfactant. The results obtained in the study with 3 % diesel yields 1.78 g/L biosurfactant, here water insoluble substrates (diesel) may slowly transport the nutrients to the microorganisms which results in the lesser yields as compared to water soluble substrates. The results indicate at 96 h production yield was getting decreased sharply. 5% inoculums size will be better at 72 h for optimum production. Inoculums size may influence the duration of lag phase, specific growth rate, biomass yield and quantity of the final product<sup>27</sup>.

#### Extraction and purification of biosurfactant

At acidic pH biosurfactant was precipitated out and dissolved in ethyl acetate. At pH 2.0, biosurfactant

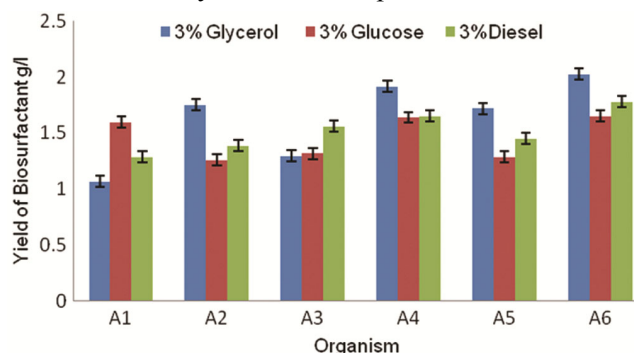


Fig.3 — Yield of Biosurfactant g/ l using 3% Glycerol, 3% Glucose and 3% Diesel respectively in bars

converted in to a protonated form, making it less soluble in water and favours easy recovery. Biosurfactant collected from organic phase was purified by silica gel column chromatography and each fraction were analyzed by TLC. The chromatogram was visualized by spraying distilled water, ninhydrin and iodine vapours which indicates presence of biosurfactant, peptides and lipids respectively. The R f value were compared with standard surfactin (Sigma).

#### Antimicrobial activity

Antibacterial activity of surfactin extracted from *Bacillus subtilis* (MF 582633) was analyzed against the urinary tract infecting (UTI) bacterial strains. The zones of inhibition revealed by biosurfactant against bacterial strain were evaluated in mm. Overall antibacterial activity (Table 1) was observed in all bacteria under study with varying zone diameters of the inhibition. Biosurfactant (40µg/mL) revealed minimum zone of inhibition against *E.coli* (10 mm) while highest with *K.pneumoniae* (20.3 mm) as compared to standard antibiotic used in the study. An average zone of inhibition (15.02 mm) were recorded for all the UTI bacterial strains. It is noticed that when antimicrobial assay was performed against the UTI pathogen using 1 mg/mL biosurfactant with varying surfactant concentration 30,40 and 50 µg/mL, the inhibition zone was comparable. Literature indicates that approximately 5% of genome of *Bacillus* species encodes for the synthesis of antimicrobial compounds<sup>28</sup>. Surfactant with lipopeptide nature revealed antimicrobial activities<sup>29</sup>.

Present findings are in accordance with findings of earlier research<sup>30,31</sup> (Table 1). *Bacillus subtilis* MF582633 shows the 40 µg/ mL ideal concentration to inhibit the growth of both Gram positive and Gram negative microorganisms. The diverse structure of biosurfactants confers them the ability to display versatile performance. By its structure, biosurfactants exerts its toxicity on the cell membrane permeability

Table 1 — Antimicrobial activity against UTI pathogens by agar well diffusion method

Test organism	Zone of inhibition in mm		
	30 µg/mL	40 µg/mL	50 µg/mL
<i>E.coli.</i>		Biosurfactant	Ampicillin
	7	10	9.6
<i>S. aureus</i>	9	14.4	13.2
<i>P. aeruginosa</i>	6	12.6	11.3
<i>S. paucomobilis</i>	11	17.8	16.9
<i>K. pneumoniae</i>	13	20.3	19.4

bearing the similitude of a detergent like effect<sup>32</sup>. Similar type of results for antimicrobial activity of iturin biosurfactant obtained from catheter associated *Bacillus subtilis* P1a strain is reported elsewhere<sup>33</sup> with respect to UTI pathogens. Lipopeptides type of biosurfactants may disturb the integrity of cytoplasmic membranes thereby resulting entry of biosurfactants inside the cell and inhibit the growth of UTI pathogens.

#### DPPH scavenging activity

It is well known fact that antioxidants are able to seize the free radical chain of oxidation and form stable free radicals, which would not initiate further oxidation. 1,1-diphenyl-2-picrylhydrazyl (DPPH) has been used extensively as a free radical to evaluate reducing activity of substances. The DPPH scavenging activity of produced biosurfactant and ascorbic acid as a control were summarized (Fig. 4). Linear response curves were obtained and the IC<sub>50</sub> values were estimated at 0.2 mg/mL for ascorbic acid while to obtain the same IC<sub>50</sub> value the concentration of biosurfactant was 0.4 mg/mL. The biosurfactant is observed to be lipopeptide in nature and exhibited effective antioxidant activity against DPPH in a dose dependent manner. The results obtained from *Bacillus subtilis* (MF582633) biosurfactant antioxidant activity

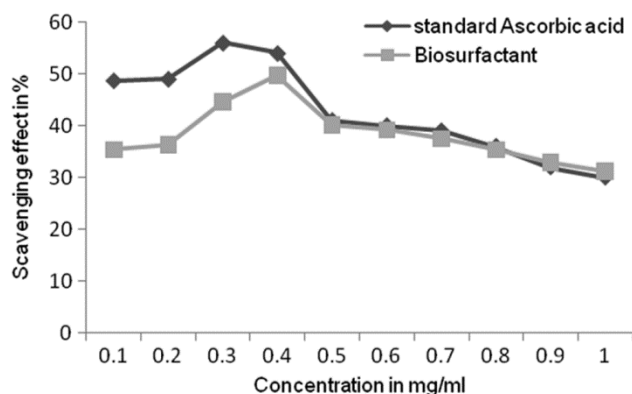


Fig. 4 — Scavenging effect of standard Ascorbic acid and Biosurfactant

revealed less antioxidant potential as compared to standard ascorbic acid used in the study. Increasing in the concentration of biosurfactant may show higher scavenging effect due to the capacity to donate hydrogen.

#### Larvicidal and Antitermite Activity

Larvicidal and antitermite activity data of biosurfactant is depicted (Table 2). It has been noticed that in 24 to 48 h incubation (with the specific concentration of biosurfactant indicates) the 100% larvicidal effect was observed at 50 mg/mL concentration of biosurfactant. As time of exposure increases the mortality rate was also increases. These findings were matched with the earlier findings. *Bacillus* genus is the most extensively studied genus for the mosquitocidal properties. *Bacillus thuringiensis* and *B. sphaericus* have been used for mosquito control<sup>34,35</sup>. Other bacilli such as *B. alvei* and *B. brevis*, *B. circulans*<sup>36</sup> and *B. subtilis* also has been reported to produce mosquitocidal toxins<sup>37</sup>. From the results obtained surfactin can be exploited in the formulation of a safer, biopesticide for effective control of mosquito larvae.

This was the first report, testing the antitermite activity of obtained biosurfactant. As reported elsewhere<sup>38</sup> antitermite activity was depend upon extract yield and the nature of extracting solvent for the wood species. Due to the presence of different antitermitic compounds of varied chemical characteristics and polarities may or may not be soluble in a particular solvent, might be the reasons why termites respond differently. In the above attempts this might be the reason of chemical structure and concentration of biosurfactant responsible for the antitermite activity. Treatment at 40-50 mg/mL concentration of biosurfactants leads to the complete mortality at of termites. Biosurfactant was prepared in the distilled water and used for the experiments while in case of control only distilled water was used. Rather than using harmful chemical pesticides are extensively applied for controlling the termite attack<sup>39,40</sup> which

Table 2 — Larvicidal and Antitermite activity of biosurfactant from *Bacillus subtilis* (MF582633)

Incubation	12 h			24h			36h			48h			Control			
	30	40	50	30	40	50	30	40	50	30	40	50	12	24	36	48h
Concentration of biosurfactant in mg/mL	30	40	50	30	40	50	30	40	50	30	40	50	-	-	-	-
Larvi Mortality rate in %	20	35	42	25	40	45	25	40	45	50	90	100	5	10	15	20
Termites mortality rate in %	25	30	35	50	60	70	75	90	100	90	100	-	10	20	25	80

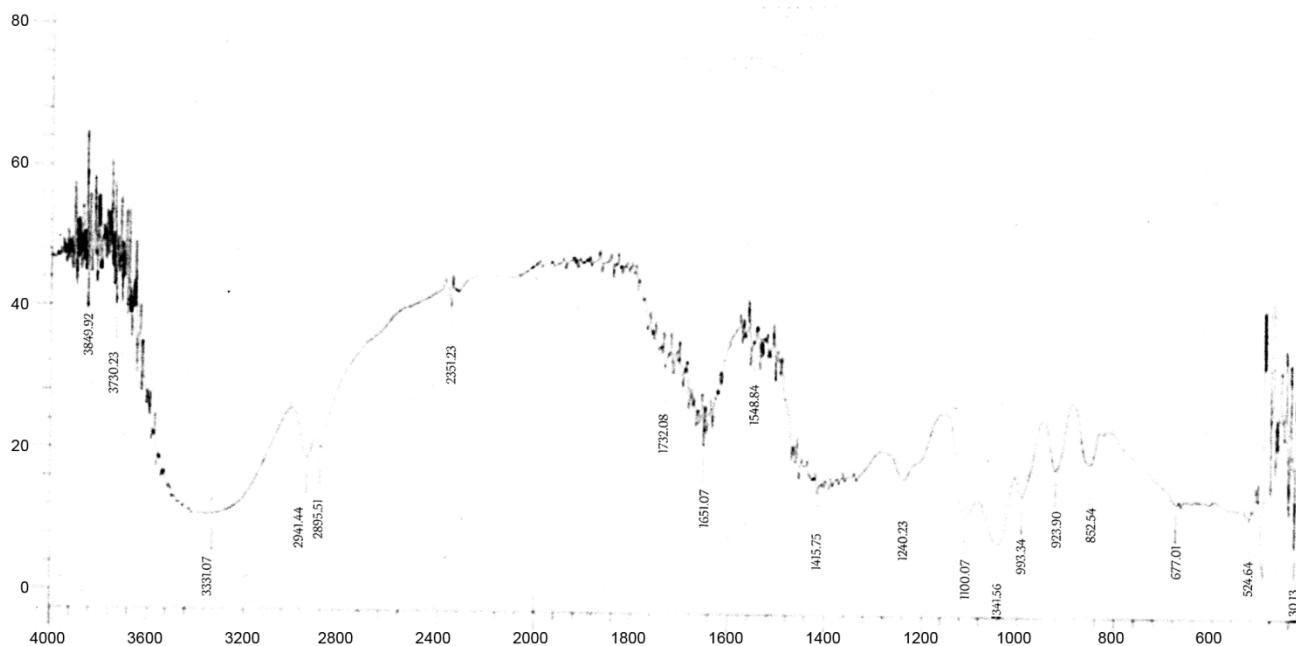


Fig. 5 — FTIR Spectrum of *Bacillus subtilis* (MF582633)

has serious deleterious impacts on non-targeted biotic and abiotic factors of environment.

#### Spectral characterization of biosurfactant by FTIR and $^1\text{H}$ NMR

The biosurfactant was characterized spectroscopically using FTIR and NMR techniques (Fig. 5 and 6). In FTIR (Fig. 5) the peak at  $1734\text{ cm}^{-1}$  is the characteristic of carbonyl group. The band appeared at 524 and  $677\text{ cm}^{-1}$  indicates the alkyl halides group in the surfactin. Peak range  $550\text{--}850\text{ cm}^{-1}$  shows the presence of C-Cl stretch which appears at  $852\text{ cm}^{-1}$ . The peak range  $910\text{--}950\text{ cm}^{-1}$  indicates O-H bend while  $650\text{--}1000\text{ cm}^{-1}$  peak range is characteristic of =C-H bond in aromatic ring. The peaks appeared at 1109 and  $1240\text{ cm}^{-1}$  indicates C-N stretch in the biosurfactant. The peak appeared at  $1020\text{--}1250\text{ cm}^{-1}$  range is the characteristics of aliphatic amines while peak  $1415\text{ cm}^{-1}$  indicates the aromatic stretch ring. Band at  $1651\text{ cm}^{-1}$  indicates the stretching mode of CO-N bond. Stretching peaks of  $-\text{CH}_3$  and  $-\text{CH}_2$  appeared in region  $3000\text{--}2000\text{ cm}^{-1}$ .  $1651$  indicates  $-\text{C}=\text{C}$  stretch of amines. The C=O of saturated fatty acids at peak  $1732$  while  $2885$  and  $2941\text{ cm}^{-1}$  peak indicates the presence of C-H stretch and assigned to alkanes<sup>41</sup>.

#### $^1\text{H}$ NMR spectrum of extracted biosurfactant

$^1\text{H}$  NMR (Fig. 6) revealed the region  $3.5\text{--}5.5\text{ ppm}$  which corresponds to alpha-carbon protons. The

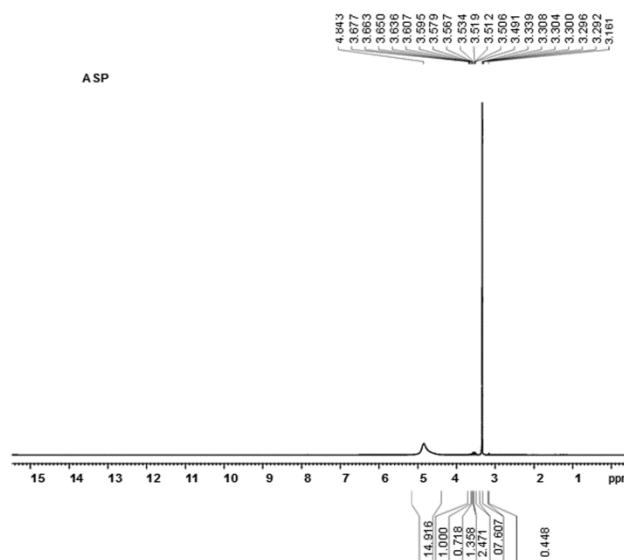


Fig. 6 — Chemical shift assignments of biosurfactant in  $^1\text{H}$  NMR spectra produced by *B. subtilis* (MF582633)

differences of the peaks in comparison with standard surfactin are at chemical shifts of  $3.2\text{--}3.5\text{ ppm}$  (i.e.,  $3.292$ ,  $3.296$ ,  $3.300$  etc, ppm). The small variations in the ppm may be due to bound surfactin with the trioctylmethyl ammonium cations of bacterial isolate<sup>42</sup>. The sharp singlet at  $3.579\text{ ppm}$  indicates presence of  $-\text{CH}_3$  singlet methyl proton<sup>43</sup>. The intense singlet at  $3.47\text{ ppm}$  assigned to  $\text{OCH}_3$  observed in different strains of *Bacillus* i.e. strain #311 and #573 is the characteristics of lipopeptide monoesters.

Instead of peak occurring at 3.47 ppm in the strain MF582366 it appeared at 3.49 ppm which might be due to presence of minor impurities instrumental specifications. The peaks at 3.59, 3.60, 3.63, 3.65, 3.66, 3.67 ppm matches with the peaks obtained from the different strains of *B. subtilis*. It has been reported elsewhere, *B. subtilis* strains produces a diversified homologues of surfactin differing in length, ramification of fatty acid chains, isoforms and peptidic sequences<sup>44,45</sup>.

### Conclusion

The present investigation aim biosurfactant production for its various biocidal activities like UTI inhibition, antioxidant, larvicidal and antitermite. It is evident that biosurfactant obtained at 40 µg/mL concentration play a significant role in inhibition of UTI pathogens. Biosurfactant at particular concentration gives 100% antitermite and larvicidal effects. Further studies however should be focused on natural microbial biosurfactants in a combinations with other herbs, enzymes, antibiotics or other powerful antioxidants which might play a key role in the destabilisation of cell membrane of UTI pathogens and act effectively as a antimicrobial and insecticidal agents in less time period.

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