

Combating antimicrobial resistance with lipopeptide biosurfactant produced by newly isolated *Bacillus stercosis* NT5 and *Bacillus subtilis* NT5

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1 **Combating antimicrobial resistance with lipopeptide biosurfactant**
2 **produced by newly isolated *Bacillus stercosis NT5* and *Bacillus***
3 ***subtilis NT5***

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Abstract

The rising prevalence of antimicrobial resistance (AMR) has become a major global health concern, therefore, demanding immediate attention. This current study brings forth an unprecedented approach to overcome the antimicrobial resistance of microbes by introducing novel lipopeptide biosurfactant LPA2 and LPA3 locally isolated from *Bacillus stercosis* NT5 and *Bacillus subtilis* NT5 respectively. Both LPA2 & LPA3 were tested for antimicrobial activity against AMR *Staphylococcus aureus* (MTCC3160) and *Pseudomonas aeruginosa* (MTCC424) through fluorescence-based viability assay and broth dilution assay. On evaluation, LPA2 showed the rfu and OD of 10232 and 0.861 respectively against *S. aureus* with MIC of 0.647mg/ml after 36 hours of incubation and LPA3 displayed rfu and OD of 5812 and 0.639 against *P. aeruginosa* respectively, with MIC of 0.323 mg/ml after 36 hours of incubation, which is significantly higher than reported one. Microbial cell examined before and after LP treatment through SEM revealed that LP acts on plasma membrane of AMR bacteria and lyse it which is different from the mode of action of antibiotics in use. Therefore, this difference in mode of action of LP against AMR microbes, supports the high AMA of LPA 2&3, which may be a breakthrough for pharmaceutical industry.

1 Introduction

The early 19th century marked the discovery of the first antibiotic Penicillin and from there the trail of antibiotic discoveries specifically an era between 1940-1960, uncovered new knowledge in therapeutic sector and saved human civilizations (Gaynes, 2017). These low molecular weight bioactive compounds brought about a revolution as it shifted the focus of health care providers from confining the spread of fatal infections to curing those infections (Antony *et al*, 2026). Their discovery even turned fatal diseases into a curable one giving hope to millions of people. However, excessive and repeated use of antibiotics led to revamping of the bacterial genes which in turn made them tolerant and resistant to the antibiotics (Muteeb *et al*, 2023). This led to prevalence of antibiotic-resistant bacteria, especially the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species), which have raised serious concerns about the efficacy of conventional antibiotics (Miller *et al*, 2024). Many first line and even second line medications are unsuccessful against these infections because they are notable for "escaping" the effects of widely used antibiotics (Varma *et al*, 2020). Consequently, mortality rates from infections that were previously treatable have gone up dramatically. Therefore, this testing time urgently calls for discovery of novel antimicrobial agents which can overcome the problem of antimicrobial resistance in bacteria and at the same time are safe for humans.

Unconventional antimicrobials agents such as biosurfactants are becoming more popular as viable substitutes against AMR pathogens. Given the amphiphilic character of biosurfactants and ability to reduce the surface tension, they have become a possible substitute in the fight against microbes that are resistant to antibiotics (Puyol McKenna *et al*, 2024). However, conventional antibiotics tend to target the DNA/ RNA synthesis or enzyme to kill the bacteria (Yang *et al*, 2014). Overtime the bacteria mutate and evolve to become resistant to these antibiotics but it

cannot change the amphipathic nature of its plasma membrane. Therefore, biosurfactants are being widely used and considered as a plausible substitute to the conventional antibiotics (**Ceresa *et al.*, 2021**). Compared to other biosurfactants, lipopeptide biosurfactants (LPBS) are known to demonstrate strong and broad-spectrum antimicrobial activity because of the presence of cyclic peptide residues which provides membrane specificity and interaction (**Wiman *et al.*, 2023**). **Fig. 1** illustrates the mode of action of LPBS.

In gram positive bacteria, LPBS interact with the negatively charged phospholipids bilayer comprising of phosphatidylglycerol and cardiolipin via electrostatic attraction and cause cell lysis (**Sreedharan *et al.*, 2023**). However, in gram-negative bacteria, LPBS competes with the divalent cations such as Ca^{2+} and Mg^{2+} ions to bind to negatively charged lipopolysaccharides thereby increasing membrane permeability. Without the divalent cations, lipopolysaccharide molecule starts repelling each other leading to destabilization of membrane and cell lysis (**Gaubha and Rahman, 2023**). The high selectivity of LPBS for bacteria arises due to the biochemical differences between bacterial and other membranes. Membranes such as mammalian plasma membranes majorly contain amphipathic phospholipids such as phosphatidylcholine and sphingomyelin along with cholesterol (**Armas *et al.*, 2018**) whereas bacterial membranes contain anionic phospholipids, generating a net negative surface charge that promotes preferential electrostatic binding of LPBS. Furthermore, the microbe producing LPBS does not get affected by it due to the presence of modified membrane molecules such as amino acylated phospholipid and highly saturated fatty acids that prevent autotoxicity. In addition to it, presence of exporters on the membrane rapidly translocates surfactin and related LPBS across the cytoplasmic membrane, preventing intracellular accumulation (**Reygaert, 2018**).

LPBS are reported to be majorly produced by genus *Bacillus*. Amongst this genus, *Bacillus subtilis* has been extensively researched for the production of surfactin, fengycin and iturin (**Jha *et***

95 *al.*, 2016). Giri *et al.* (2019) reported a MIC of 2mg/ml of LPBS against *Staphylococcus*
96 *aureus* ATCC29523 and *Bacillus cereus* MTCC7190. *Bacillus licheniformis* was observed to be
97 potent producer of lipopeptide lichenysin by Gomaa (2013). He investigated AMA of LPBS by disc
98 diffusion method against 2 strains of gram-positive and 2 strains of gram-negative bacteria and
99 found it to be most effective against *S. aureus* ATCC 25928 with MIC of 48µg/ml. Adhikary *et*
100 *al.*, 2025 critically reviewed iturin, a cyclic heptapeptide and reported it to have broad spectrum
101 antimicrobial and antibiofilm properties. It's MIC values ranged from 0.1 to 1.6 mg/ml and a biofilm
102 inhibition concentration (BIC) of 0.8–1.6 mg/ml in 18 h old cells.

103 This study reports a newly isolated species of *Bacillus* i.e. *Bacillus stercosis* NT5 and
104 *Bacillus subtilis* NT5 which have been observed to produce lipopeptide biosurfactants i.e. LPA2
105 and LPA3. To determine the antimicrobial activity of LPA2 and LPA3, this study conducted broth
106 dilution assay, fluorescence-based viability assay and viable plate count assay. Further, the
107 minimum inhibitory concentration (MIC) of the LPA2 and LPA3 was also calculated followed by
108 carefully analyzing their antimicrobial effect on pathogenic microbes using scanning electron
109 microscope.

110 2 Material and methods

111 2.1 Chemicals and reagent

112 Propidium iodide (Sigma Aldrich), Surfactin (Merck), Luria Bertani broth (Himedia), Nutrient
113 agar (Himedia), Petriplates (Himedia), 24 well plate (Thermo- Fischer) and 96 well plate (Bio-
114 Rad), Scanning Electron Microscope (ZEISS, Model: EVO MA 15).

115 2.2 Microbial strains

Escherichia coli (MTCC6748), *Paenibacillus thiaminolyticus* (MTCC3088), representative of ESKAPE pathogens: *Staphylococcus aureus* (MTCC3160) and *Pseudomonas aeruginosa* (MTCC424) were procured from the BET lab under Prof. Sangeeta Negi, MNNIT Allahabad.

2.3 Production of LPBS

Solid state fermentation for production of lipopeptide biosurfactant (LPBS) was carried out in 500ml flask using cassava flour and mustard cake as solid substrate in 1:1 ratio. The czapekdox media, a minimal media was added onto the solid substrate. The solid substrate is a good source of carbon while the minimal media is a source of nitrogen and essential minerals required for the growth of microbes. The autoclaved flasks were then inoculated with *Bacillus stercosis* NT5, and *Bacillus subtilis* NT5 respectively and incubated for 7days at 37°C.

2.4 Extraction of LPBS

After 7 days of incubation, the lipopeptide biosurfactant (LPBS) was extracted by addition of 20ml of autoclaved distilled water into each flask, followed by incubation at 150rpm, 37°C for 2 hours in shaker incubator. After 2 hours, the slurry was filtered using muslin cloth and centrifuged at 7000rpm for 20min. After centrifugation the pellet containing cell debris was discarded and the supernatant was used for further study.

2.5 Partial purification of LPBS

After extraction, the supernatant (containing LPBS) was further purified using ammonium sulphate precipitation. Using 80% ammonium sulphate precipitation, the supernatant was precipitated and kept overnight at 4°C. The extract was then centrifuged and the pellet containing the purified LPBS was dissolved in autoclaved distilled water. and then MIC was calculated.

2.6 Confirmatory tests for LPBS

The purified LPBS was subsequently used to evaluate emulsification index, oil displacement assay, drop collapse assay and surface tension. Further, the quantity of LPBS was assessed using the CPC-BTB assay. Gradually, the antimicrobial activity against various pathogens was examined using three methods i.e. Broth dilution method, Fluorescence- based viability assay and Viable plate count assay.

2.7 Antimicrobial activity of LPBS

The antimicrobial activity of the partially purified LPBS was tested against gram positive bacteria such as *Escherichia coli*, *Paenibacillus thiaminolyticus* and *Staphylococcus aureus* and gram-negative bacteria such as *Pseudomonas aeruginosa*. Three methods were used to evaluate the effect of LPBS treatment on pathogenic microbes. Broth dilution method was first used to detect the actual number of live cells. However, it gave an OD of live cells, dead cells and cell debris. Therefore, fluorescence-based viability assay was performed and the results of this assay were further checked using viable plate count method. The assays were performed in duplicates. This was followed by determining the minimum inhibitory concentration (MIC) of each of these isolates.

2.7.1 Broth dilution assay

The pathogenic culture of microbes such as *Escherichia coli*, *Paenibacillus thiaminolyticus*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* were inoculated in sterilized LB broth media and its OD₆₀₀ was maintained at 0.5 (McFarland standard). Gradually, following reaction mixture was prepared in a 24-well microtiter plate: 800µL inoculated LB broth and 200µL LPBS (4mg/ml) and incubated for 15 hours at 34° C. Optical density was measured at 600nm. The negative control was prepared by mixing 800µL of fresh LB broth and 200µL of inoculum. The positive control comprised of 800µL of LB broth and 200µL of commercial surfactin (Vanegas *et al.*, 2022).

2.7.2 Fluorescence-based viability assay

Fluorescence-based viability assay was carried out by pelleting 1.5ml of inoculated LB broth ($OD_{600} \leq 0.5$) upon centrifugation at 4000rpm for 15min. The supernatant was discarded and the cell pellet was treated with 200 μ L of LPBS (4mg/ml) and incubated for 15hours at 34° C. From the LPBS treated cells, 100 μ L of the aliquot was transferred to 96 well culture plate and 4ul of propidium iodide dye was added. The fluorescence intensity was thus measured at the excitation maxima of 535nm and the emission maxima of 617nm and results were obtained in form of relative fluorescence unit (rfu). The mode of action of PI dye is by entering the dead cells and intercalating itself with the DNA/RNA of the dead cells. As a result of this intercalation, it shows fluorescence. The higher the relative fluorescence unit (rfu) of the broth, the higher the number of dead cells in it. For negative control, pelleted cells were dissolved in 200 μ L of fresh LB broth and for positive control 200 μ L of commercial surfactin was used (Hossain, 2024).

2.7.3 Viable plate count assay

For viable plate count assay, known number of microbial cells were used to determine the antimicrobial activity of LPBS. For negative control, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* cells were first serially diluted till 10^{-5} dilutions and later each dilution was spread on LB agar plates and incubated for 15 hours at 34°C. The cells were then counted using colony counter. Using the following formula cfu/ml was calculated:

$$\frac{\text{cfu}}{\text{ml}} = \frac{\text{Number of colonies}}{\text{Volume plated(ml)}} * \text{Dilution Factor}$$

The antimicrobial activity of LPBS was evaluated by pelleting 1ml of the culture upon centrifugation at 4000rpm for 15min. The supernatant was discarded and the cell pellet was treated with 200 μ L of LPBS and incubated for 6hours and 12hours. After the treatment, the cells were

plated onto the fresh LB agar plates and incubated at 34°C for 5 hours. The colonies were then counted using colony counter. For negative control, the pelleted cells were dissolved in 200 µL of fresh LB broth and for positive control pelleted cells were treated with 200 µL of commercial surfactin (Fenton *et al.*, 2013).

2.8 Determination of minimum inhibitory concentration (MIC)

To calculate the MIC of LPA2 and LPA3 against *Escherichia coli* (MTCC6748), *Paenibacillus thiaminolyticus* (MTCC3088), *Staphylococcus aureus* (MTCC3160) and *Pseudomonas aeruginosa* (MTCC424), 100 µL of each was inoculated in 50 ml LB broth and incubated for 12 hours at 37°C. The culture broth was centrifuged at 4000 rpm for 15 min and the supernatant was discarded. The pelleted cells were dissolved in PBS buffer (Phosphate Buffer Saline; pH 7.2) and were used for further experiments. In the 96-well plate, 100 µL of each culture (in PBS buffer) was treated with different concentrations of LPBS (5.18 mg/ml, 2.5 mg/ml, 1.29 mg/ml, 0.647 mg/ml and 0.323 mg/ml) and incubated for 12, 24 and 36 hours at 34°C. The LPBS dilutions were prepared for both LPA2, and LPA3. Further, dead cells were stained using 4 µL of PI dye and incubated for 10 min. The fluorescence intensity in the form of rfu was thus measured at the excitation maxima of 535 nm and the emission maxima of 617 nm. The experiments were performed in duplicates. For positive control, 100 µL of cells (dissolved in PBS) were treated with 100 µL of commercial surfactin (1 mg/ml) and incubated for 12, 24 and 36 hours at 34°C. Gradually cells were stained with 4 µL of PI dye and rfu was calculated (Kowalska-Krochmal and Dudek-Wicher, 2021). Minimum concentration of LPBS showing consistent increase in number of dead pathogenic cells (increase in rfu) at 12, 24 and 36 hours of incubation were selected as MIC of LPBS against a particular microbe.

2.9 Thin Layer Chromatography (TLC)

The LPA2 and LPA3 produced from *Bacillus stercosis* NT5 and *Bacillus subtilis* NT5 respectively were analysed by thin layer chromatography (TLC). The stationary phase was TLC aluminum sheet and the mobile phase was Butanol: acetic acid: water (4:3:2 [v/v]). The glass capillary was used for spotting of the sample on TLC plate. The plate was sprayed with 0.2% ninhydrin reagent to detect the presence of free amino acid groups and later placed in closed iodine chamber to detect the presence of lipids in the sample. The plates were heated for 10min in hot air oven at 100°C until the corresponding bands appear and R_f value was calculated (Ghazi Faizal *et al.* 2022).

2.10 Fourier-Transform Infrared Spectroscopy (FTIR)

The FTIR spectra were obtained and recorded using Shimadzu IR Spirit FTIR spectrophotometer at the Department of Chemical Engineering, Motilal Nehru National Institute of Technology Allahabad, Prayagraj, India. The FTIR scans enabled the structural elucidation of LPA2 and LPA3 respectively. The scans were performed at the wavelength range of 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1} (Varadavenkatesan *et al.*, 2013).

2.11 Scanning electron microscopy

To prepare the samples for scanning electron microscope, 100 μL of aliquot from cell culture (dissolved in PBS) was centrifuged at 4000rpm for 15min. The supernatant was discarded and pellet was dissolved in 100 μL of LPA2 (5.8 mg/ml) and LPA3 (1.29mg/ml) and incubated for 12 hours at 34°C. After 12 hours, cells were pelleted upon centrifugation at 4000 rpm for 15 minutes. The cell pellet was washed twice with PBS. For primary fixation of the cells, the pellet was resuspended in 2.5% glutaraldehyde and incubated for 2 hours at 4°C. After 2 hours, the cells (suspended in glutaraldehyde) were centrifuged and the pellet was washed three times with PBS (10 minutes each) to remove excess fixative. The fixed cells were then dehydrated using a graded ethanol series: 30%, 50%, 70%, 90% for 10 minutes each, and 100% ethanol twice for 10 minutes

each. The pellet was then lyophilized overnight using lyophilizer (MAC freeze dryer, MSW-137).
The dried pellet was then used to perform scanning electron microscopy (Golding *et al.*, 2016).

3 Results

3.1 Characterization of LPBS using confirmatory assays

The bacterial isolates i.e. *Bacillus stercosis* NT5 and *Bacillus subtilis* NT5 produced LPA2 and LPA3 respectively. On examining LPA2 and LPA3 quantitatively using CPC-BTB assay, 10.41 µg/ml and 57.42 µg/ml of LPBS was reported. Moreover, the LPA2 was reported to have 60% emulsification index while LPA3 showed 58%. The oil displacement assay of LPA2 and LPA3 was 2.5cm and 2cm respectively. When placed on hydrophobic surface, the drops of LPA2 and LPA3 collapsed in 45sec and 58sec respectively, and when checked for surface tension using breakaway method, 29 mN/m and 28 mN/m was observed (Thapliyal and Negi, 2026).

3.2 AMR and lipopeptide biosurfactants

The antimicrobial potential of LPA2 and LPA3 was evaluated using three assays i.e. broth dilution method, fluorescence-based viability assay, and viable plate count method. In broth dilution method, the optical density of the LB broth inoculated with bacteria (OD₆₀₀ maintained at 0.5; McFarland method) detected live and dead cells along with the cell debris. This was evident because after LPA2 treatment, the reported OD₆₀₀ for pathogens *S. aureus*, *P. aeruginosa*, *E. coli* and *Paenibacillus* was 0.561, 0.69, 0.634 and 0.953 which was more than 0.5. Similar results were reported for LPA3. Therefore, fluorescence-based viability assay was performed which reported the dead cells only with the help of fluorescent dye i.e. propidium iodide dye (PI). Further, using viable plate count method, the number of live cells were counted using colony counter.

3.2.1 Antimicrobial potential of LPA2

The antimicrobial activity of LPA2, tested against *Escherichia coli*, *Paenibacillus thiaminolyticus*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, was reported to be most effective against *S. aureus*. Broth dilution assay showed an OD₆₀₀ of 0.561 for *S. aureus* whereas the fluorescence-based viability assay showed a rfu of 10,232. The negative control in broth dilution assay and fluorescence-based viability assay showed an OD of 1.95 and rfu of 2125. A2 treated *E. coli* cells displayed an OD₆₀₀ of 0.634 and a relative fluorescence unit of 6736. The negative control for it showed an OD₆₀₀ of 1.5. and a rfu of 2000. On treating *Pseudomonas aeruginosa* cells with A2, an OD of 0.69 and rfu of 6076 was observed and its negative control displayed an OD of 1.906 and rfu of 2085 respectively. However, *Paenibacillus thiaminolyticus* reported contrasting results with the OD of 0.953 and a rfu of 3066 (**Fig.2 (a) and (b)**). These results addressed the ineffectiveness of the LPA2 against the *Paenibacillus thiaminolyticus* as the cells were growing in number despite treatment with LPA2 and with no visible cell death. **Fig. 3 (a)** shows colony count of the negative control for *S. aureus* with 23,970cfu/ml cell population and **Fig. 3 (b)** display 12 hours after treatment with LPA2 with a clear plate with no microbial growth.

3.2.2 Antimicrobial potential of LPA3

The LPA3 showed very high antimicrobial activity against the *P. aeruginosa* with optical density (using broth dilution method) of 0.439 and rfu (fluorescence-based viability assay) of 8812. The negative control's OD was observed to be 1.906 and rfu of 2025 was observed. For *E. coli*, an OD of 0.858 and rfu of 6772 was observed and *Staphylococcus aureus* gave OD of 0.857 and rfu of 6663 which showed that LPA3 was effective antimicrobial agent against them (**Fig.2 (c) and (d)**). However, LPA3 was found to be ineffective against *Paenibacillus* as the number of dead cells were very less when stained with propidium iodide (with 2770 rfu). Therefore, minimum inhibitory concentration (MIC) for *Paenibacillus* was not conducted. Viable plate count images in **Fig 3 (c)**

display colony count of negative control for *P. aeruginosa* with 24,720cfu/ml. However, **Fig, 3 (d)** displays 12 Hours after treatment with LPA3 with no visible bacterial growth.

3.3 Minimum Inhibitory Concentration

3.3.1 MIC of LPA2

The MIC of LPA2 against the pathogenic strains (performed using fluorescence- based viability assay) reported that LPA2 strongly and consistently inhibited the growth of *S. aureus* (even after 36 hours of incubation) when compared with *E. coli* and *P. aeruginosa*. The MIC of LPA2 against *S. aureus* was reported to be 0.647mg/ml with rfu of 2761, 3396 and 3749 at 12, 24 and 36 hours of incubation respectively (**Fig. 4a**). At LPA2 concentration 5.18mg/ml, the number of dead *S. aureus* cells was found to be very significant and LPA2 was reported to have very strong antimicrobial activity against them. The rfu of 8306, 9505 and 11155 at 12, 24 and 36 hours of incubation respectively was observed at LPA2 concentration of 5.18mg/ml. On the contrary *E. coli* was observed to be temporarily susceptible to LPA2 at 0.323mg/ml as it produced a rfu of 6561 but cells regained their growth (rfu=5501) after 36 hours of incubation (**Fig. 4c**) It was only after treatment of *E. coli* cells with 5.18mg/ml of LPA2, could we see stable decrease in *E. coli* cell number. However, the number of dead *E. coli* cells was not more than that of *S. aureus* (can be seen in rfu value), therefore we can say LPA2 showed strong antimicrobial activity against *S. aureus*. In context of *P. aeruginosa*, the MIC of A2 was observed to be 5.18 mg/ml with a rfu of 7944 on the 36th hour (**Fig. 4b**). The LPA2 was observed to have temporary antimicrobial activity against *P. aeruginosa* cells at concentration below 0.323 mg/ml. This is because the number of dead *P. aeruginosa* cells increased till 24 hours of incubation but after 24 hours, the number of dead cells decreased. Since, the number of *S. aureus* cells killed by LPA2 was very close to those killed by commercial surfactin (rfu of cells killed by LPA2 was 11155 and that by commercial surfactin was 13534), it can be concluded that LPA2 showed maximum antimicrobial activity

against *S. aureus* followed by *P. aeruginosa* and then *E. coli*. The heat map shown in **Fig.4** very evidently shows the antimicrobial activity of the LPA2 biosurfactant on the pathogenic cells with dark red color depicting the maximum number of dead cells and dark blue color showing the maximum number of living cells.

3.3.2 MIC of LPA3

Minimum inhibitory concentration of LPA3, in case of *S. aureus* cells was reported to be 5.8mg/ml as the number of cells were found to steadily decrease at LPA3 concentration of 5.18 mg/ml (**Fig.5a**). Below this concentration of LPA3, i.e. at 2.5mg/ml, the cells death was observed till 24 hours of incubation. However, cells regained their number after completing 36 hours of incubation. Therefore, LPA3 displayed temporary antimicrobial activity against *S. aureus* cells at 2.5mg/ml concentration. The MIC of LPA3 was reported to be 0.323mg/ml against *P. aeruginosa* cells after 36 hours of incubation (rfu of 5878). At LPA3 concentration above 0.323mg/ml, consistent decline in number of *P. aeruginosa* cells was observed. For instance, LPA3 concentration of 5.18 mg/ml, strong decline in number of cells with rfu 9869 at 12th hour, 9979 at 24th hour and 10838 at 36th hour was witnessed (**Fig. 5b**). In context of *E. coli*, the LPA3 was observed to have temporary antimicrobial activity at 2.5mg/ml as the number of dead cells increase after 24hours of incubation with rfu increasing from 5865 to 7278. However, the decline in rfu was observed after 36 hours of incubation with rfu of 7195. Similar pattern of antimicrobial activity was observed at 5.18mg/ml of LPA3 against *E. coli* cells. The heat map shown in **Fig.5** clearly depicts the antimicrobial activity of the LPA3 biosurfactant on the pathogenic cells.

3.4 Characterization of LPA2 and LPA3

The partially purified extracts of LPA2 and LPA3 were characterized using TLC and their R_f value was recorded. The presence of lipid and amino acid was confirmed with the revelation of yellow and ruhemann's purple-colored spots on the TLC plate. Their R_f values were 0.84 and 0.5

respectively (**Fig. 6 (a)**). Further, characterization of LPA2 and LPA3 was accomplished with FTIR spectral scans where the presence of C=O, N-H, C-H groups was observed. The LPA2 peak at 1728 cm^{-1} and LPA3 peak at 1719 cm^{-1} denoted C=O stretching vibrations. The LPA2 and LPA3 peaks at 3396 and 3381 cm^{-1} indicated the presence of N-H stretching of amide bond. A small peak at 2832 and 2838 cm^{-1} showed the presence of C-H stretching vibrations which corresponds to the alkyl groups (CH_2 , CH_3), mainly found in lipids and fatty acids. Hence, location of the peaks at their respective positions proved the presence of peptide CO-NH bond along with the presence of fatty acid chains and as a result we can say that the extracts LPA2 and LPA3 are lipopeptide biosurfactant. **Fig. 6 (b)** shows the FTIR peaks of LPA2 and LPA3 extracts.

3.5 Visualization of impact of LPA2 and LPA3 using SEM

To examine the morphological changes in the treated and untreated bacterial cells, scanning electron microscopy (ZEISS, Model: EVO MA 15) was used. SEM images of the LPA2 (5.18mg/ml) and LPA3 (5.18mg/ml) treated *S. aureus* and *P. aeruginosa* cells and their control images are shown in **Fig.7**. The negative control of *S. aureus* is shown in **Fig 7(a)** where intact coccus cells can be observed with no physical damage on the bacterial membrane. However, in **Fig 7(b)** LPA2 treated *S. aureus* can be seen with compromised plasma membrane which would have led to cell lysis. Whereas **Fig 7(c)** displays the negative control of *P. aeruginosa* control cells. In the image, *P. aeruginosa* can be seen to be surrounded by small circular shaped substance called extracellular polymeric substance (EPS). In **Fig 7(d)** cell lysis is visible in the cells which could be due to the treatment with LPA3.

4 Discussion

This study brings forth an unprecedented approach to overcome the antimicrobial resistance of microbes by introducing novel lipopeptide biosurfactant LPA2 extracted for the first time from

Bacillus stercosis NT5 and LPA3, extracted from *Bacillus subtilis* NT5. Although the antimicrobial properties of LPBS isolated from *Bacillus* spp. have been reported previously but LPBS extracted from *Bacillus stercosis* NT5 and *Bacillus subtilis* NT5 have not been studied. Also, there are very few studies where fluorescence-based viability, viable plate count and broth dilution assay were performed simultaneously which showed a very visible number of dead cells. Therefore, to evaluate the AMA of LPA2 and LPA3, broth dilution method was first used to detect the actual number of live cells. However, it revealed an OD of not just live cells but also of dead cells and cell debris. Therefore, fluorescence-based viability assay was performed and the results of this assay were further checked using viable plate count method.

The results revealed that LPBS, show stronger antimicrobial activity majorly through bacterial membrane disruption, owing to their amphipathic structure (**Zakharova *et al.*, 2025**). The detailed mechanism behind this has been discussed in the introduction section. These lipopeptides molecules are synthesized by the bacterial cell as a defense mechanism to inhibit biofilm formation and also the growth of other neighboring bacteria. This is also done to have full access to nutrients and surrounding space for colonization (**Kourmentza *et al.*, 2021**). Although LPBS has potent antimicrobial activity, however, these molecules do not backfire the producer cells because of inbuilt self-resistance mechanisms such as modifications in lipid composition of plasma membrane which narrows the chances of LPBS binding on the membrane (**Moretta *et al.*, 2021**). LPBS producing bacteria also have active efflux pumps which exports the LPBS out of the cell and regulates the continuous secretion LPBS out of cell to prevent accumulation inside the cell (**Mulani *et al.*, 2019**). Broadly, these multiple mechanisms explain the wide-range of antimicrobial efficacy of bacterial LPBS and highlights their potential as favorable alternative to conventional antibiotics for combating antimicrobial-resistant pathogens.

When the antimicrobial activity of LPA2 was studied, it was reported to show maximum antimicrobial activity against *S. aureus* at 5.18mg/ml with a MIC of 0.647mg/ml. This observation was evident in the results of fluorescence-based viability assay as well. However, MIC was not performed using broth dilution method because after incubation period, OD₆₀₀ of the LB broth (containing LPA2 treated *S. aureus* cells) shifted from 0.5 (Mc Farland standard) to 0.561 whereas, fluorescence-based viability method for the same sample reported a rfu of 10232 and its results were further supported by the results of viable plate count assay (**Fig. 3b**). This anomaly in broth dilution assay could be due to high chances of the dead cells and cell debris getting detected along with live cells.

On evaluating the antimicrobial activity of LPA3, similar pattern was observed. In case of *E. coli* and *S. aureus* cells wherein an increase in OD₆₀₀ from 0.5 to OD₆₀₀ 0.858 and 0.857 (broth dilution assay) indicate an increase in number of cells (**Fig.2c**). However, their rfu values of 6772 and 6663 points towards the decline in cell number (**Fig. 2d**). On examining the AMA of these compounds using viable plate count method, fewer colonies were observed which testified the detection of dead cells and cell debris along with living cells in broth dilution method. In case of *Paenibacillus thiaminolyticus*, it was observed that both LPA2 and LPA3 were ineffective against these bacteria, as their optical density was found to be increasing and their rfu was reported to decrease. Therefore, *Paenibacillus thiaminolyticus* was not explicitly used for evaluating antimicrobial activity of LPBS.

After understanding about the antimicrobial potential of LPA2 & LPA3 their minimum inhibitory concentration (MIC) was determined. It was reported that LPA2 strongly inhibited the growth of *S. aureus* at about 5.18mg/ after 36 hours of incubation and its MIC was reported (against *S. aureus*) as 0.647mg/ml with rfu of 2761, 3396 and 3749 at 12, 24 and 36 hours of incubation respectively. The antimicrobial activity of LPA3 was observed to be maximum on *P.*

aeruginosa. Its MIC was found to be 0.323mg/ml after 36 hours of incubation (rfu of 5878) (Fig.5b). The SEM images of LPA2 (5.18mg/ml) and LPA3 (5.18mg/ml) treated *S. aureus* and *P. aeruginosa* cells revealed the significant differences between the LPBS treated and untreated cells (Fig.6). The intactness of the outer plasma membrane in the control cells and the disintegration of the membrane in both *S. aureus* and *P. aeruginosa* bacteria clearly reveal high antimicrobial activity of the LPA2 and LPA3.

It can be deduced from the results that LPA2 & LPA3 might be different at molecular level, because LPA2 showed high AMA against gram-positive *Staphylococcus aureus*, while LPA3 deadly for gram-negative *Pseudomonas aeruginosa*, as a gram-negative and positive bacteria are different at their cell membrane chemical composition. The MIC values of LPA2 (0.647mg/ml) & LPA3 (0.323mg/ml) were found to be significantly better than MIC of 2mg/ml of LPBS (against *Staphylococcus aureus* ATCC29523 and *Bacillus cereus* MTCC7190, (Giri *et al.* (2019)) and very close to the MIC (0.1mg/ml) of Plantaricin NC8 $\alpha\beta$ produced by *Lactobacillus plantarum* (Winman *et al.*, 2023). However, MIC of conventional antibiotic such as streptomycin was reported to be 0.8mg/ml against *S. aureus* which signifies that LPBS are better than these antibiotics (Field *et al.* 2016). Table 1 shows MIC values of existing lipopeptide biosurfactants.

Hence, we can conclude that LPA2 has stronger antimicrobial activity against gram positive *Staphylococcus aureus* (MTCC3160) with MIC of 0.647mg/ml after 36 hours of incubation whereas LPA3 showed very strong inhibitory against the gram-negative *P. aeruginosa* with MIC of 0.323 mg/ml after 36 hours of incubation. MIC of both the lipopeptide makes them potential future antibiotics to combat AMR.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contribution

All authors have contributed equally as follows: Lab experiments conducted by UT; Design and concept conceived by SN; Manuscript written and edited by UT and SN; Both authors read and approved the final manuscript. The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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References

- Adetunji AI, Olaniran AO. Production and potential biotechnological applications of microbial surfactants: An overview. Saudi J Biol Sci. 2021 Jan;28(1):669-679. doi: 10.1016/j.sjbs.2020.10.058. Epub 2020 Nov 4. PMID: 33424354; PMCID: PMC7783833.
- Adhikary, Rajsekhar & Maiti, Pulak & Ghosh, Narendra & Rajbanshi, Biplab & roy, professor mahendra & Mandal, Sukhendu & Mandal, Vivekananda. (2025). Lipopeptide iturin C3 from endophytic Bacillus sp. effectively inhibits biofilm formation and prevents the adhesion of topical and food-borne pathogens in vitro and on biomedical devices. Archives of Microbiology. 207. 10.1007/s00203-025-04253-y.

437 Ambust, Shweta & Purohit, Ashwrya & Das, Amar & Kumar, Rajesh & Ghosh, Debashish. (2023).
 438 Glycolipid Biosurfactant-Assisted Remediation Strategy to Minimize the Petroleum
 439 Contaminant Footprint in Environmental Compartments. *Water, Air, & Soil Pollution*. 234.
 440 10.1007/s11270-023-06771-1.

441 Antony BT, Osborne JW, Benjamin LK. Antibiotic: a boon to mankind and doom to the
 442 environment - an insight on biological and non-biological degradation processes. *Front*
 443 *Microbiol.* 2026 Apr 7;17:1801898. doi: 10.3389/fmicb.2026.1801898. PMID: 42022108;
 444 PMCID: PMC13095611.

445 Calvo LG, Corral-Orbe S, Villarino R-A, Sánchez S, de Miguel T. Employment of a Resazurin
 446 Viability-Based Assay for Minimum Inhibitory and Bactericidal Concentration
 447 Determination. *Pharmaceuticals*. 2026; 19(3):505. <https://doi.org/10.3390/ph19030505>

448 Ceresa C, Rinaldi M, Tessarolo F, Maniglio D, Fedeli E, Tambone E, Caciagli P, Banat IM, Diaz
 449 De Rienzo MA and Fracchia L (2021) Inhibitory Effects of Lipopeptides and Glycolipids on
 450 *C. albicans*–*Staphylococcus* spp. Dual-Species Biofilms. *Front. Microbiol.* 11:545654. doi:
 451 10.3389/fmicb.2020.545654.

452 Chen, Aaron & Byvank, Tom & Chang, Woo-Jin & Bharde, Atul & Vieira, Gregory & Miller,
 453 Brandon & Chalmers, Jeffrey & Sooryakumar, Ratnasingham. (2013). On-Chip Magnetic
 454 Separation and Encapsulation of Cells in Droplets. *Lab on a chip*. 13. 10.1039/c2lc41201b.

455 Fenton, Mark & Keary, Ruth & Mcauliffe, Olivia & Ross, R & O'Mahony, Jim & Coffey, Aidan.
 456 (2013). Bacteriophage-Derived Peptidase CHAPK Eliminates and Prevents Staphylococcal
 457 Biofilms. *International journal of microbiology*. 2013. 625341. 10.1155/2013/625341.

458 Field D, O' Connor R, Cotter PD, Ross RP and Hill C (2016). In Vitro Activities of Nisin and Nisin
 459 Derivatives Alone and In Combination with Antibiotics against Staphylococcus Biofilms.
 460 Front. Microbiol. 7:508. doi: 10.3389/fmicb.2016.00508

461 Gaynes R. The Discovery of Penicillin—New Insights After More Than 75 Years of Clinical Use.
 462 Emerg Infect Dis. 2017 May;23(5):849–53. doi: 10.3201/eid2305.161556. PMCID:
 463 PMC5403050.

464 Ghazi Faisal Z, Sallal Mahdi M, Alobaidi KH. Optimization and Chemical Characterization of
 465 Biosurfactant Produced from a Novel *Pseudomonas guguanensis* Strain Iraqi ZG.K.M. Int J
 466 Microbiol. 2023 Feb 1;2023:1571991. doi: 10.1155/2023/1571991. PMID: 36776762;
 467 PMCID: PMC9908352.

468 Giri, S.S., Ryu, E.C., Sukumaran, V., Park, S.C. Antioxidant, antibacterial, and anti-adhesive
 469 activities of biosurfactants isolated from Bacillus strains, Microbial Pathogenesis, Volume
 470 132, 2019, Pages 66-72, ISSN 0882-4010, <https://doi.org/10.1016/j.micpath.2019.04.035>.

471 Golding, C., Lamboo, L., Beniac, D. *et al.* The scanning electron microscope in microbiology and
 472 diagnosis of infectious disease. *Sci Rep* 6, 26516 (2016). <https://doi.org/10.1038/srep26516>

473 Gomaa, Eman. (2013). Antimicrobial activity of a biosurfactant produced by Bacillus licheniformis
 474 strain M104 grown on whey. Brazilian Archives of Biology and Technology. 56. 259-268.
 475 10.1590/S1516-89132013000200011.

476 Hossain TJ. Methods for screening and evaluation of antimicrobial activity: A review of protocols,
 477 advantages, and limitations. Eur J Microbiol Immunol (Bp). 2024 Apr 22;14(2):97-115. doi:
 478 10.1556/1886.2024.00035. PMID: 38648108; PMCID: PMC11097785.

479 Hossain, T. J. (2024). Methods for screening and evaluation of antimicrobial activity: A review of
 480 protocols, advantages, and limitations. *European Journal of Microbiology and Immunology*,
 481 14(2), 97-115. <https://doi.org/10.1556/1886.2024.00035>

482 Jha SS, Joshi SJ, S J G. Lipopeptide production by *Bacillus subtilis* R1 and its possible applications.
 483 *Braz J Microbiol.* 2016 Oct-Dec;47(4):955-964. doi: 10.1016/j.bjm.2016.07.006. Epub 2016
 484 Jul 26. PMID: 27520530; PMCID: PMC5052371.

485 Kourmentza K, Gromada X, Michael N, Degraeve C, Vanier G, Ravallec R, Coutte F, Karatzas KA
 486 and Jauregi P (2021) Antimicrobial Activity of Lipopeptide Biosurfactants Against
 487 Foodborne Pathogen and Food Spoilage Microorganisms and Their Cytotoxicity. *Front.*
 488 *Microbiol.* 11:561060. doi: 10.3389/fmicb.2020.561060

489 Kowalska-Krochmal B, Dudek-Wicher R. The Minimum Inhibitory Concentration of Antibiotics:
 490 Methods, Interpretation, Clinical Relevance. *Pathogens.* 2021 Feb 4;10(2):165. doi:
 491 10.3390/pathogens10020165. PMID: 33557078; PMCID: PMC7913839.

492 Kuiper, Irene & Lagendijk, Ellen & Pickford, Russell & Derrick, Jeremy & Lamers, Gerda &
 493 Thomas-Oates, Jane & Lugtenberg, Ben & Bloemberg, Guido. (2004). Characterization of
 494 two *Pseudomonas Putida* lipopeptide biosurfactants, putisolvin I and II, which inhibit
 495 biofilm formation and break down existing biofilms. *Molecular microbiology.* 51. 97-113.
 496 10.1046/j.1365-2958.2003.03751.

497 Michels K, Heinke R, Schöne P, Kuipers OP, Arnold N, Wessjohann LA. A fluorescence-based
 498 bioassay for antibacterials and its application in screening natural product extracts. *J Antibiot*
 499 (Tokyo). 2015 Dec;68(12):734-40. doi: 10.1038/ja.2015.71. Epub 2015 Jul 8. PMID:
 500 26152282.

501 Miller, W.R., Arias, C.A. ESKAPE pathogens: antimicrobial resistance, epidemiology, clinical
502 impact and therapeutics. *Nat Rev Microbiol* **22**, 598–616 (2024).
503 <https://doi.org/10.1038/s41579-024-01054-w>.

504 Mnif I, Ghribi D. Review lipopeptides biosurfactants: Mean classes and new insights for industrial,
505 biomedical, and environmental applications. *Biopolymers*. 2015 May;104(3):129-47. doi:
506 10.1002/bip.22630. PMID: 25808118.

507 Moretta A, Scieuzo C, Petrone AM, Salvia R, Manniello MD, Franco A, Lucchetti D, Vassallo A,
508 Vogel H, Sgambato A and Falabella P (2021) Antimicrobial Peptides: A New Hope in
509 Biomedical and Pharmaceutical Fields. *Front. Cell. Infect. Microbiol.* 11:668632. doi:
510 10.3389/fcimb.2021.668632

511 Mulani MS, Kamble EE, Kumkar SN, Tawre MS and Pardesi KR (2019) Emerging Strategies to
512 Combat ESKAPE Pathogens in the Era of Antimicrobial Resistance: A Review. *Front.*
513 *Microbiol.* 10:539. doi: 10.3389/fmicb.2019.00539

514 Muteeb G, Rehman MT, Shahwan M, Aatif M. Origin of Antibiotics and Antibiotic Resistance, and
515 Their Impacts on Drug Development: A Narrative Review. *Pharmaceuticals (Basel)*. 2023
516 Nov 15;16(11):1615. doi: 10.3390/ph16111615. PMID: 38004480; PMCID:
517 PMC10675245.

518 Puyol McKenna P, Naughton PJ, Dooley JSG, Ternan NG, Lemoine P, Banat IM. Microbial
519 Biosurfactants: Antimicrobial Activity and Potential Biomedical and Therapeutic Exploits.
520 *Pharmaceuticals (Basel)*. 2024 Jan 22;17(1):138. doi: 10.3390/ph17010138. PMID:
521 38276011; PMCID: PMC10818721.

522 Rufián-Henares, J. A. & Morales, F. J. A new application of a commercial microtiter plate-based
 523 assay for assessing the antimicrobial activity of Maillard reaction products. Food Res. Int.
 524 39,33–39 (2006).

525 Tawfik, Kamilia & Jeffs, Peter & Bray, Brian & Dubay, George & Falkinham, Joseph & Mesbah,
 526 Mostafa & Youssef, Diao & Khalifa, Sherief & Schmidt, Eric. (2010). Burkholderia 1097
 527 and 1229, Potent Antifungal Peptides from Burkholderia ambifaria 2.2N. Organic letters.
 528 12. 664-6. 10.1021/ol9029269.

529 Teh CH, Nazni WA, Nurulhusna AH, Norazah A, Lee HL. Determination of antibacterial activity
 530 and minimum inhibitory concentration of larval extract of fly via resazurin-based
 531 turbidometric assay. BMC Microbiol. 2017 Feb 16;17(1):36. doi: 10.1186/s12866-017-
 532 0936-3. PMID: 28209130; PMCID: PMC5314469.

533 Thakur B, Kaur S, Dwibedi V, Albadrani GM, Al-Ghadi MQ and Abdel-Daim MM (2024)
 534 Unveiling the antimicrobial and antibiofilm potential of biosurfactant produced by newly
 535 isolated Lactiplantibacillus plantarum strain 1625. Front. Microbiol. 15:1459388. doi:
 536 10.3389/fmicb.2024.1459388

537 Vanegas D, Abril-Novillo A, Khachatryan A, Jerves-Andrade L, Peñaherrera E, Cuzco N, Wilches
 538 I, Calle J, León-Tamariz F. Validation of a method of broth microdilution for the
 539 determination of antibacterial activity of essential oils. BMC Res Notes. 2021 Dec
 540 2;14(1):439. doi: 10.1186/s13104-021-05838-8. Erratum in: BMC Res Notes. 2022 Feb
 541 14;15(1):53. doi: 10.1186/s13104-022-05915-6. PMID: 34857039; PMCID: PMC8638534.

542 Varadavenkatesan T, Murty VR. Production of a Lipopeptide Biosurfactant by a Novel *Bacillus* sp.
543 and Its Applicability to Enhanced Oil Recovery. *ISRN Microbiol.* 2013 Sep
544 24;2013:621519. doi: 10.1155/2013/621519. PMID: 24205445; PMCID: PMC3800565.

545 Varma DM, Zahid MSH, Bachelder EM, Ainslie KM. Formulation of host-targeted therapeutics
546 against bacterial infections. *Transl Res.* 2020 Jun;220:98-113. doi:
547 10.1016/j.trsl.2020.03.009. Epub 2020 Mar 16. PMID: 32268128; PMCID: PMC10132281.

548 Wei-Chuan Chen, Kung-Hao Kan, Li-Fen Wang, Yu-Hong Wei, Characterization and production
549 of serrawettin, a lipopeptide biosurfactant, from *Serratia marcescens* SMΔR, *Journal of the*
550 *Taiwan Institute of Chemical Engineers*, Volume 160, 2024, 105232,
551 <https://doi.org/10.1016/j.jtice.2023.105232>.

552 Wiman E, Zattarin E, Aili D, Bengtsson T, Selegård R, Khalaf H. Development of novel broad-
553 spectrum antimicrobial lipopeptides derived from plantaricin NC8 β. *Sci Rep.* 2023 Mar
554 13;13(1):4104. doi: 10.1038/s41598-023-31185-8. PMID: 36914718; PMCID:
555 PMC10011573.

556 Yang S-C, Lin C-H, Sung CT and Fang J-Y (2014) Antibacterial activities of bacteriocins:
557 application in foods and pharmaceuticals. *Front. Microbiol.* 5:241. doi:
558 10.3389/fmicb.2014.00241.

559 Zakharova AA, Efimova SS, Ostroumova OS. State of the Art of Cyclic Lipopeptide-Membrane
560 Interactions: Pore Formation and Bilayer Permeability. *Pharmaceutics.* 2025 Aug
561 31;17(9):1142. doi: 10.3390/pharmaceutics17091142. PMID: 41012479; PMCID:
562 PMC12473245.

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Fig.1 (I) Mode of action of LPBS (a) Insertion of Cyclic Lipopeptide (CLP) surfactant onto bacterial membrane in Calcium dependent manner (b) Release of Ca^{2+} ions (c) Oligomerization of CLP leading to formation of pores (d) Loss of membrane potential eventually leading to cell death **(II)** Mode of action of conventional antibiotics

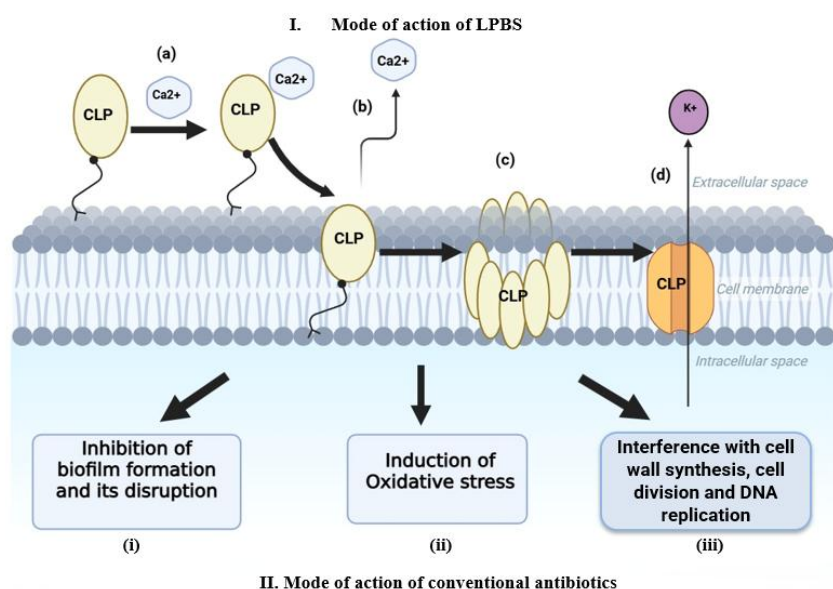
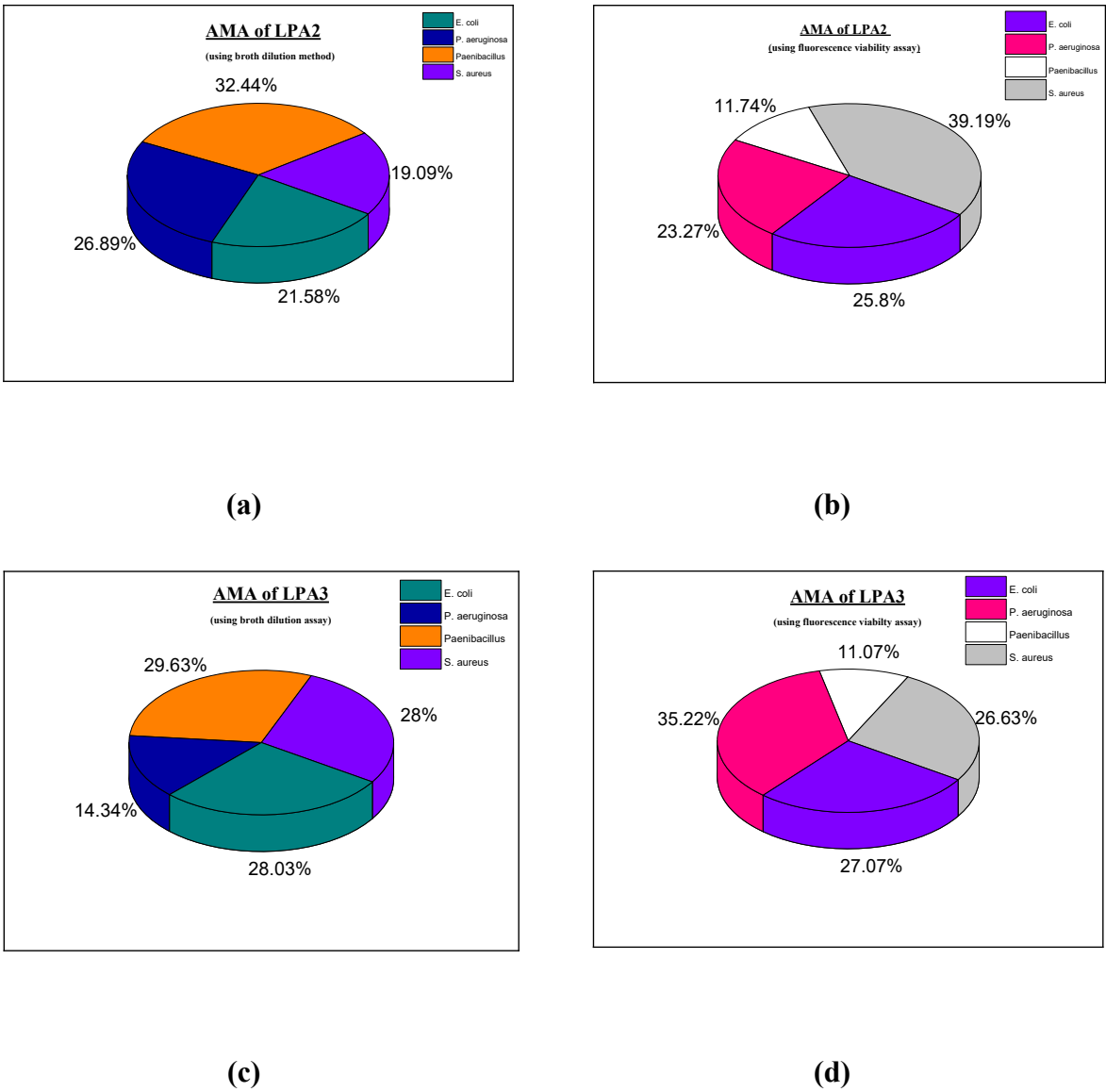


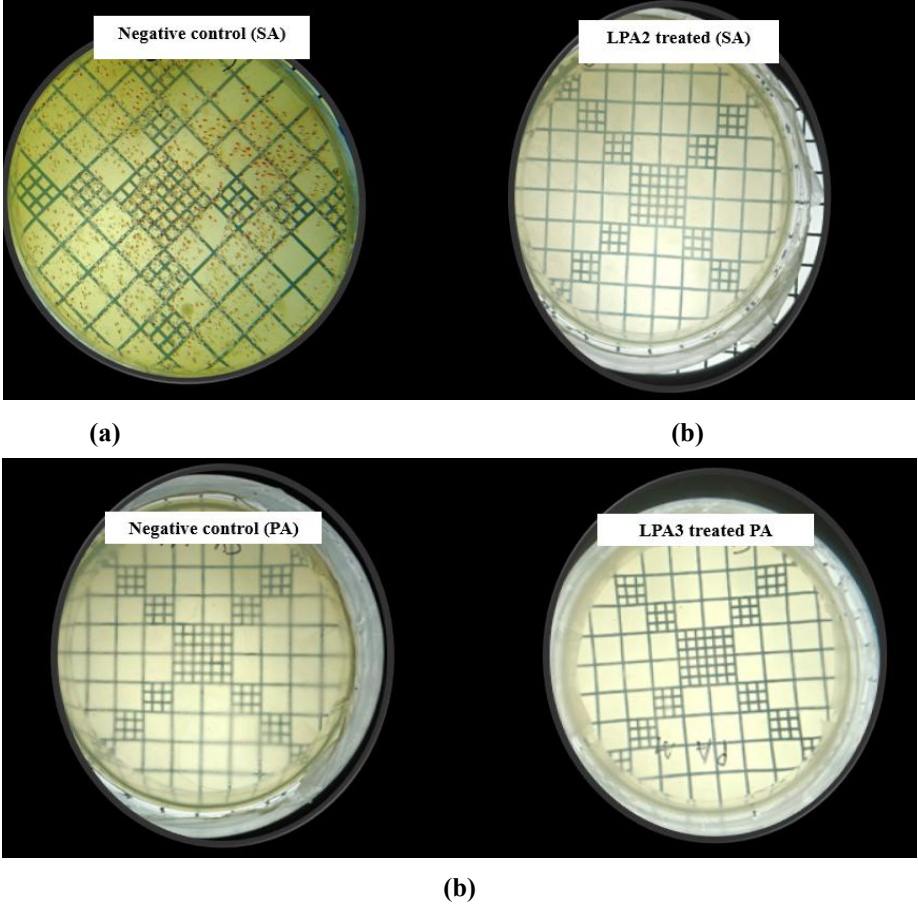
Fig.2: Antimicrobial activity of LPA2 on pathogens via (a) broth dilution assay (b) fluorescence-based viability assay (c) Antimicrobial activity of LPA3 on pathogens via broth dilution assay (d) fluorescence-based viability assay



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Fig. 3: Viable plate count assay (a) Negative control for *S. aureus* (b) After 12 hours of LPA2 treatment
(c) Negative control for *P. aeruginosa* (d) Cells after 12 Hours treatment of LPA3



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Fig. 4: MIC of LPA2 against (a) *S. aureus* (b) *P. aeruginosa* and (c) *E. coli* strains of bacteria (d) Positive control

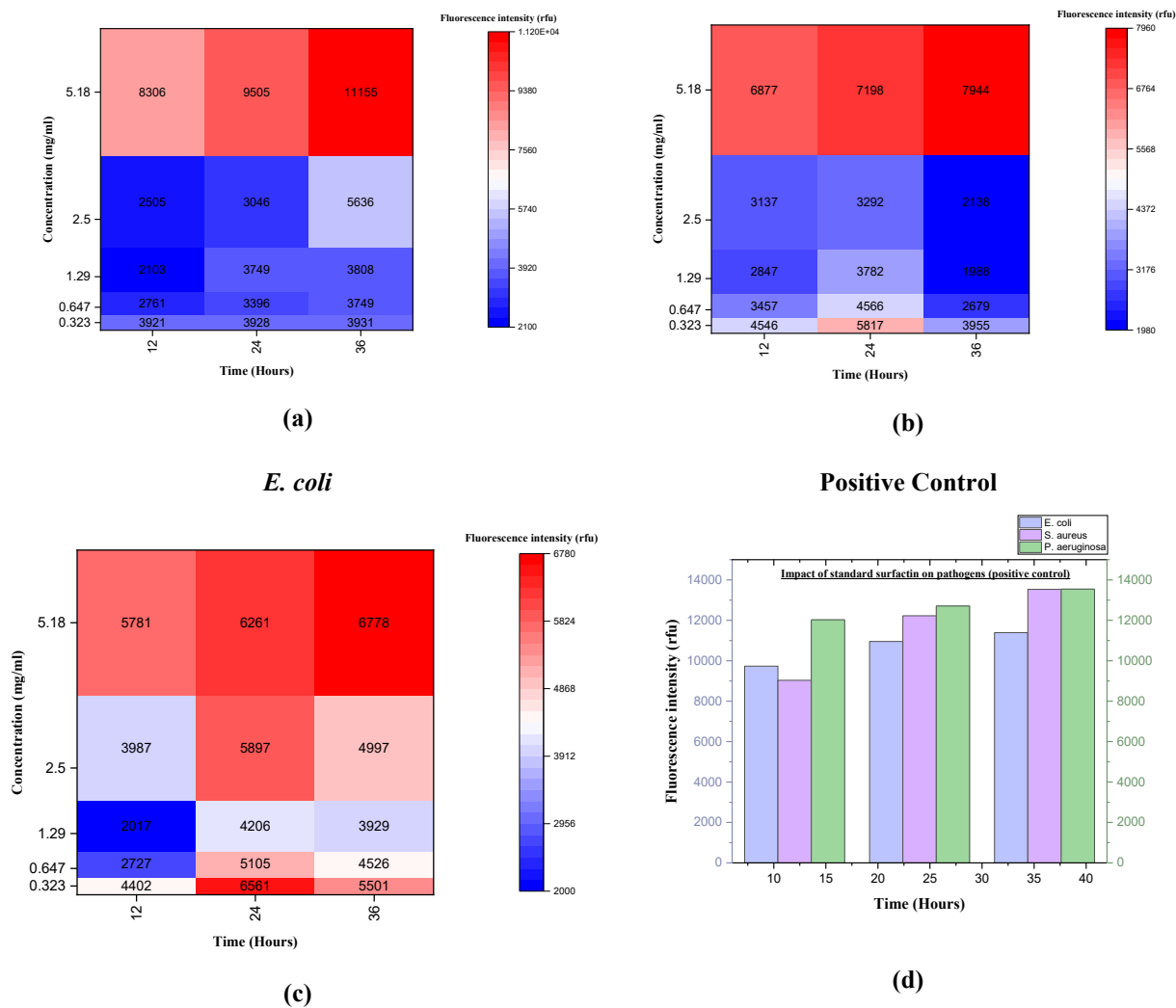
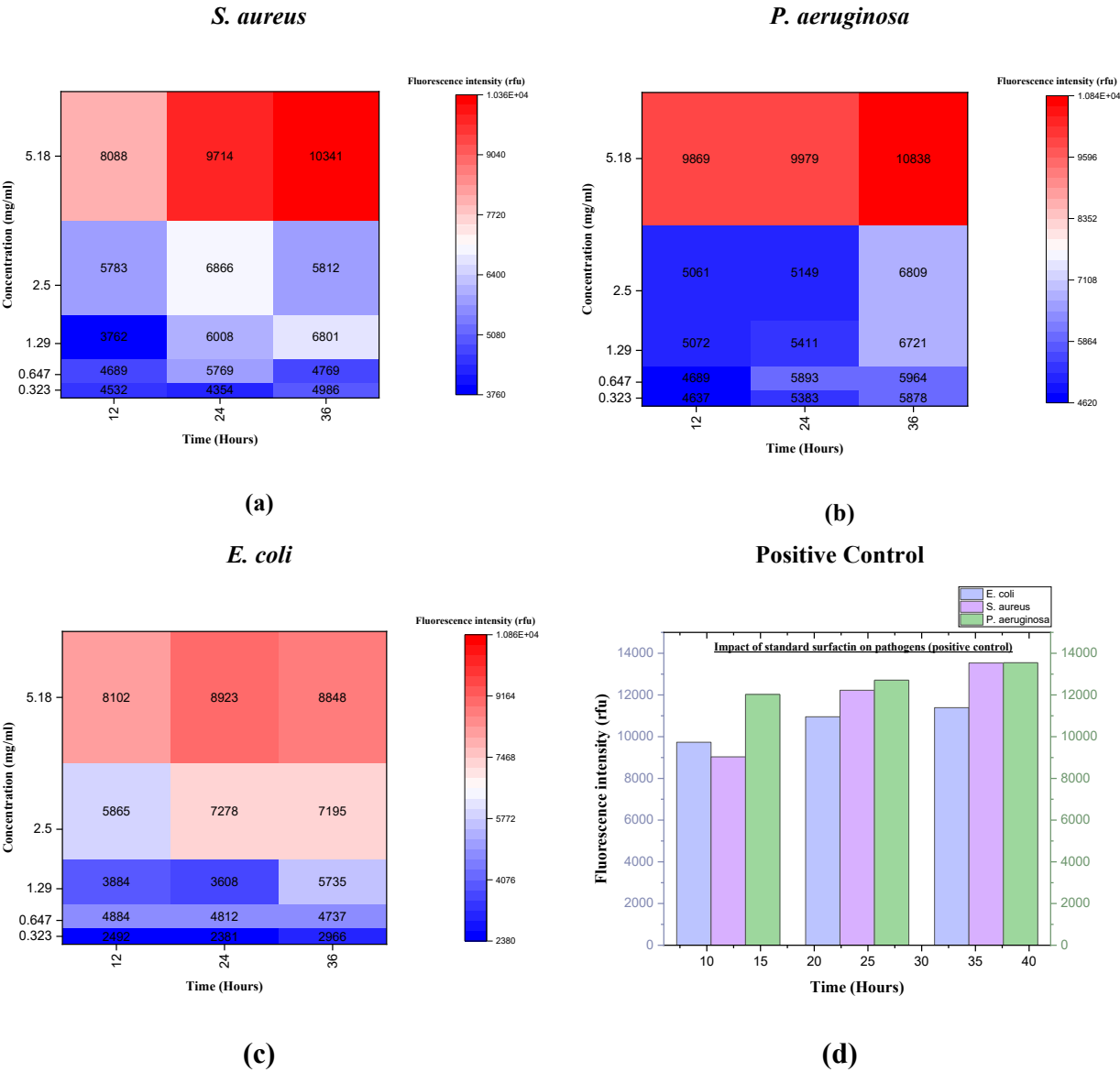


Fig. 5: MIC of LPA3 against (a) *S. aureus*, (b) *P. aeruginosa*, (c) *E. coli* strains of bacteria (d) Positive control



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Fig.6 (a): TLC analysis of LPA2 and LPA3

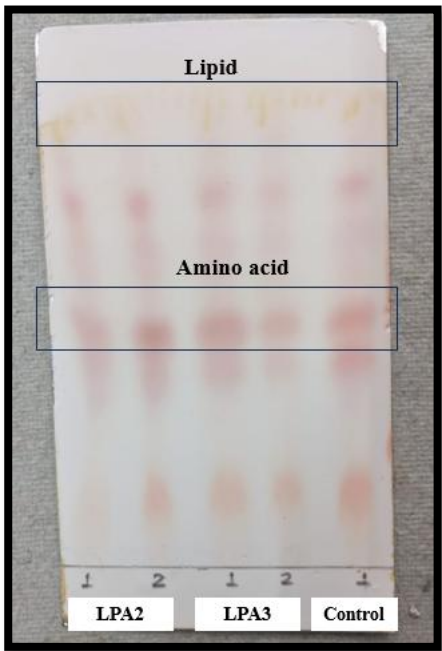
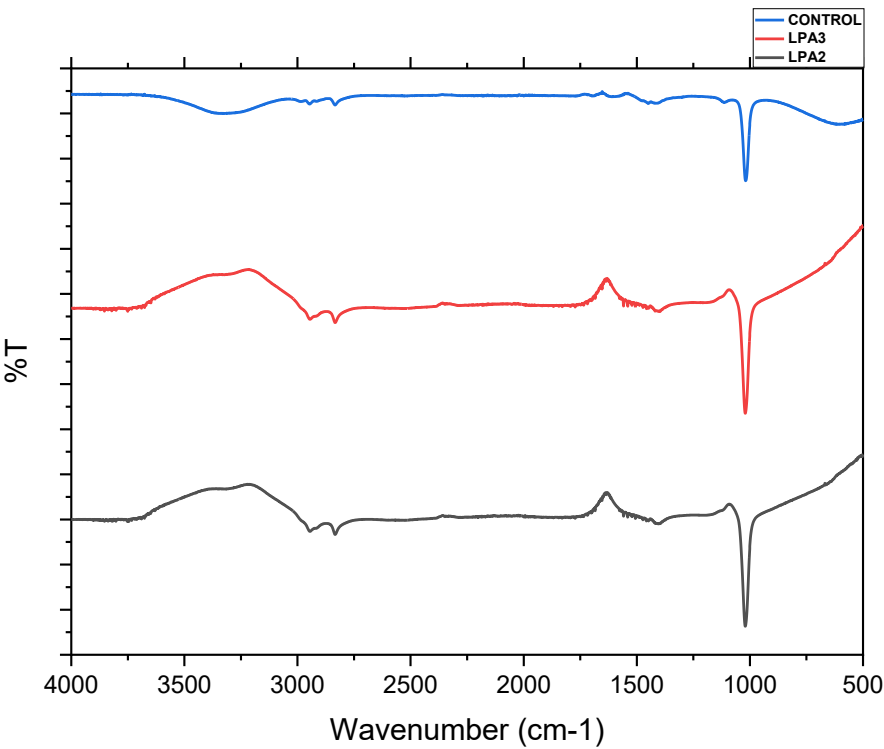


Fig.6(b): FTIR peaks of LPA2 and LPA3



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Fig.7: SEM images of control for *S. aureus* (a) and LPA2 treated *S. aureus* cells (b) and control image of *P. aeruginosa* (c) and LPA3 treated *P. aeruginosa* cells (d)

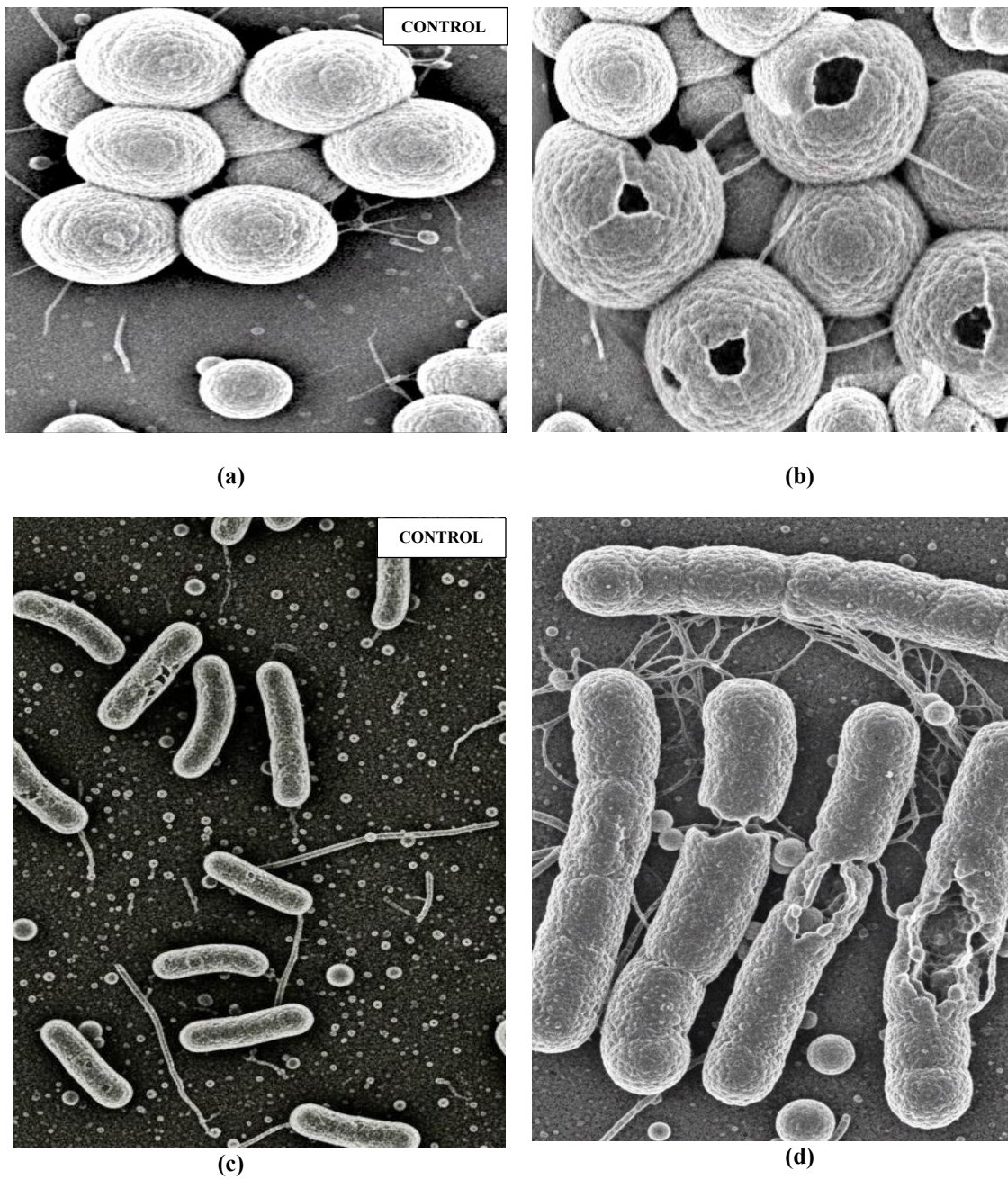


Table.1: MIC of different Lipopeptide biosurfactant

Bacterial strains	Lipopeptide produced	Reported MIC	Anti-microbial activity	References
<i>Lactiplantibacillus plantarum</i> 1625	LPBS	0.1 mg/ml	<i>S. aureus</i> , <i>E. coli</i> and <i>P. putida</i>	Thakur <i>et al.</i> , 2024
<i>Lactobacillus plantarum</i>	Plantaricin NC8 $\alpha\beta$	0.1 mg/ml	<i>S. aureus</i> and <i>E. coli</i>	Winman <i>et al.</i> , 2023
<i>Pseudomonas putida</i> PCL1445	Putisolvin I and II	0.064 mg/ml	Biofilm-forming <i>C. albicans</i>	Kuiper <i>et al.</i> , 2004
<i>Burkholderia thailandensis</i> E264	Burkholdines 1097 and 1229	2–8 μ g/ml	<i>C. albicans</i> , <i>Cryptococcus neoformans</i>	Tawfik <i>et al.</i> , 2010