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IN VITRO AND IN SILICO CHARACTERIZATION OF SURFACTIN PRODUCED BY PAENIBACILLUS AQUISTAGNI OP288247 ISOLATED FROM BALIOSPERMUM MONTANUM

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ABSTRACT:
Novel antimicrobial agents are urgently needed due to the rising incidence of antibiotic-resistant infections. Although medicinal plants like Baliospermum montanum are endangered and scarce, plant-associated bacteria offer a promising source of bioactive compounds. The antibacterial capability of B. montanum plant-associated bacteria was assessed in this investigation. A total of 17 bacterial isolates have been examined for antibacterial activity against 6 pathogenic bacteria: Proteus vulgaris, Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae. The isolate with most potent antibacterial activity was S4S2, which was identified as Paenibacillus aquistagni OP288247 by 16S ribosomal RNA gene sequencing. Four fractions were obtained by column chromatography; the second fraction exhibited strong antibacterial activity. Thin-layer chromatography (TLC) and bioautography have been utilised to localize active ingredient, which was identified as surfactin by liquid chromatography-mass spectrometry (LC–MS) analysis. The TLC-purified active fraction demonstrated dose–dependent inhibition of S. aureus biofilm formation, with maximum inhibition of 33.33% at 1.0 mg/mL. Molecular docking demonstrated that surfactin exhibited a favorable interaction with the S. aureus virulence protein CifB, with a binding affinity of -6.4kcal/mol. This study identifies surfactin as a promising candidate and demonstrates, for the first time, that plant-associated bacteria from B. montanum contribute to the antimicrobial potential of this medicinal plant.

KEYWORDS: Plant-associated bacteria; Bioactive metabolites; Antibiofilm activity; TLC-Bioautography; Molecular docking

INTRODUCTION

The need for novel and effective antimicrobials has improved due to emergence of multidrug-resistant (MDR) microorganisms1. Bioactive chemicals from medicinal plants are valuable, although seasonal change, environmental conditions, and compound isolation time and expense limit their use2,3. As a result, researchers are increasingly exploring alternative, sustainable, and environmentally friendly biological sources for discovering potent antimicrobial metabolites. Microorganisms represent valuable resources for drug discovery because they produce structurally diverse secondary metabolites, particularly those linked to plants 4,5. Baliospermum montanum (B. montanum) (Wild.) Muell. -Arg., a perennial shrub in Euphorbiaceae family, is commonly used in traditional Ayurvedic medicine. It grows up to 1.8m in height and is distributed across India, Myanmar, and Malaysia, from the Himalayan foothills to peninsular regions up to 1800 m in altitude 6. The roots, seeds, and leaves of the plant contain bioactive compounds with reported anti-inflammatory, antimicrobial, and digestive health benefits. B. montanum is listed in the Red Book of Endangered Species despite its therapeutic significance, underscoring the urgent need to investigate alternate sources of its bioactive compounds. The isolation of surfactin-producing Paenibacillus aquistagni (P. aquistagni) from B. montanum promotes plant-associated bacteria as bioactive antibacterial sources. The antibacterial activity of this plant has been studied extensively, however its plant-associated bacteria have not. To our knowledge, B. montanum plant-associated bacteria have not been widely studied for their antibacterial properties. These investigations demonstrate biochemical and ecological importance of plant-associated bacteria in producing new antibacterial chemicals. The objective of the present study has been for isolating and identifying plant-associated bacteria from B. montanum capable of producing biologically active antibacterial compounds.

2 MATERIALS AND METHODS

2.1 Collection and isolation of plant-associated bacteria

Fresh and healthy Baliospermum montanum (Wild.) Muell.-Arg. plant tissues, including leaves, stems, and roots, were collected on the 25th of June 2020 from Raniamba forest region of Dang District, Gujarat, India. The plant

materials were delivered to the lab in sterile zip-lock bags for processing. To remove debris, the samples have been rinsed under running water, air-dried, then cut into 1 cm pieces. Surface sterilization was performed progressively with 95% ethanol for 1min, 4% sodium hypochlorite for 3min, and 95% ethanol for 30s, followed by 3 sterile distilled water rinses 7,8. Ethanol and sodium hypochlorite were obtained from HiMedia Laboratories, Mumbai, India. The effectiveness of surface sterilization was assessed by gently pressing sterilized tissue segments onto nutrient agar plates and incubating them at 28±2 °C for 5 days. The plates have been examined for microbial growth as a sterility control9. The surface-sterilized tissues were aseptically macerated utilising a sterile mortar and pestle in 10mL of sterile 0.9% NaCl solution. The resulting tissue homogenate was serially diluted tenfold from 10-1 to 10-6. 20 µL of each dilution had been placed on HiMedia Laboratories nutrient agar plates (Mumbai, India) and incubated at 28±2 °C for 24 to 48 hrs. Observing and streaking bacterial colonies on nutrient agar revealed morphology and microscopic features 10. The purified isolates were sub-cultured twice on nutrient agar and stored at 4 °C until additional analysis11.

2.2 In vitro antibacterial activity of crude and solvent extracts

2.2.1 Preparation of crude and solvent extracts

A 5430 R centrifuge (Eppendorf, Hamburg, Germany) was used to centrifuge a five-day-old bacterial broth culture at 10,000 rpm for 15 minutes in order to produce a cell-free supernatant. The crude extract was defined as the cell-free supernatant. Whatman No. 1 filter paper had been utilized to further filter supernatant that contained the bioactive metabolite. The filter was gathered and combined with an equivalent volume of ethyl acetate, hexane, or chloroform in a 1:1 ratio. A separating funnel separated bioactive compound-containing organic solvent from aqueous phase. The solvent phase was evaporated on a Buchi-Model R-300 vacuum rotary evaporator. The dried extract is dissolved with dimethyl sulfoxide for antibacterial activity (DMSO; Merck, Darmstadt, Germany) 12,13.

2.2.2 Screening of antimicrobial activity by agar well diffusion assay

The antibacterial activity of crude and the solvent extracts has been evaluated utilising the agar well diffusion method 14. Six pathogenic bacterial strains have been utilised: Staphylococcus aureus MTCC 96 and Streptococcus pyogenes MTCC 442 are two Gram-positive bacteria, while Proteus vulgaris MTCC 8427, Escherichia coli MTCC 443, Klebsiella pneumoniae MTCC 109, and Pseudomonas aeruginosa MTCC 1688 are four Gram-negative bacteria. Fresh cultures of the test bacterial strains were prepared, and the inoculum was standardized to 0.5 McFarland turbidity. Agar plates have been inoculated with standardized bacterial suspensions, and 6 mm-diameter wells have been prepared in agar. Each well received 100µL of the corresponding crude extract. Positive control was streptomycin, negative control was DMSO. The plates were incubated at 37±2 °C for 24 hrs. The inhibitory zones' millimeter diameter has been measured after incubation. Results from the triple antibacterial assay have been expressed using the mean±standard deviation. Solvent extraction of the most active crude extract was performed, and antibacterial activity was assessed as before14. The isolate exhibiting the strongest antibacterial activity was selected for molecular identification and further investigation.

2.3 Molecular identification

The most antimicrobial bacterial isolate was found by 16S rRNA gene sequencing. Genomic DNA has been used to amplify the bacterial 16S rRNA gene using universal primers. Forward primer was 5′-AGAGTTTGATCCTGGCTCAG-3′F and reverse primer was 5′-AAGGAGGTGATCCAAGCCGCA-3′R. A 25µL reaction volume was used for PCR, including 2.5µL of template DNA (<1000 ng), 2.5µL of each primer (10µM), and 4µL of 2X PCR Master mix with standard buffer and nuclease-free water. Every PCR experiment includes a negative control that contains the PCR mixture devoid of DNA. For amplification, a MyCycler Thermal Cycler (Bio-Rad, USA) was used. PCR settings included initial denaturation at 94°C for 3mins, thirty-five cycles of denaturation at 94°C for 1 min, annealing at 48°C for 1 min, extension at 72°C for 2 mins, and a final extension at 72°C for ten minutes. The amplified PCR products have been purified using ExoSAP-IT (Thermo Fisher Scientific, USA).

2.3.1 Phylogenetic analysis

Consensus sequences were obtained by processing the raw 16S rRNA gene sequence data using BioEdit Sequence Alignment Editor version 7.2.615. The resultant sequences have been analyzed utilising Basic Local Alignment Search Tool (BLAST) against the National Centre for Biotechnology Information's (NCBI) rRNA sequence database (Bacteria and Archaea) (available at http://blast.ncbi.nlm.nih.gov) for comparing with closest related bacterial species. For phylogenetic analysis, only bacterial species with the highest similarity identity percentage (99%) have been chosen. MEGA 7 has been used to create phylogenetic trees16. After alignment with MUSCLE17. Evolutionary distances were calculated using the Tamura Nei model, as well as phylogenetic connections have been inferred utilising neighbor-joining method. Using 1000 bootstrap replications, statistical confidence of the nodes in phylogenetic trees was determined. Outgroups have been taken into account for supporting species differences. 16S rRNA gene nucleotide sequences have been assigned accession codes and uploaded to GenBank (https://www.ncbi.nlm.nih.gov/genbank/).

2.4 Column chromatography and antimicrobial activity of collected fractions

Fractionation of antibacterial compounds has been carried out utilising silica gel column chromatography. Silica gel (230 to 400 mesh, Merck, Germany) has been prepared as a slurry in methanol and used to pack a glass column (40 cm length, 1.5 cm internal diameter), resulting in a final column height of 30 cm. Dichloromethane: methanol (7:3 v/v) was used to elute a sample amount of no more than 5 mL of the ethyl acetate extract onto column at a

flow rate of 0.36mL/min.18. Fractions have been gathered and assessed for antibacterial efficacy in accordance with the prior methodology. The fractions were chosen for TLC and bioautography according to the activity.

2.5 Thin layer chromatography (TLC)-Direct Bioautography assay

It was utilized for determining antibacterial activity of bioactive compounds isolated on a chromatogram19,20. To disperse TLC sheet chemicals into the agar medium, the chromatogram has been positioned over NA seeded with test pathogens (0.5 McFarland standard) for 30 minutes. Next, NA plate was incubated for 24 hrs at 37°C. Iodonitrotetrazolium p-violet (INT, Sigma) was sprayed onto the plates following incubation. On the inhibitory zone of the medium, antibacterial bands were observed. The active band was dissolved in methanol after the TLC plate was removed. To remove silica, the mixture was filtered and centrifuged. The supernatant, which included an antibiotic chemical, has been utilised for characterization.

2.6 Identification of Active Compounds with Liquid Chromatography-Mass Spectrometry (LC–MS)

LC-MS analysis has been performed on the active chemical from TLC-bioautography experiment. An Agilent 1260 Infinity Series HPLC System (Agilent Technologies, Waldbronn, Germany) and a 6540 Ultra High-Definition Accurate Q-TOF-MS (Agilent Technologies, Singapore) with an ESI source were used to analyze the material. The mobile phase has been made up of 0.1% formic acid in water (A) and acetonitrile (B) under certain isocratic conditions. The injection volume was 1µL, the flow rate was 0.5mL/min, the column compartment was set to 35°C, and run time was 1 min. Positive-ion mode mass spectra were obtained in the m/z region of 100 to 1000 amu. 10L/min drying nitrogen gas (N2) flow rate, 350°C gas temperature, 30 psi nebulizer gas pressure, 3.5 kV capillary voltage, 100V fragment potentials, 65 V skimmer, 3500 V Vcap, and 750 V Octopole RFP were the mass spectrometric settings. Software for Qualitative Analysis and Data Acquisition: Agilent MassHunter B.05.01 Software B 06.0 (Agilent Technologies, Santa Clara, CA, USA) was utilized for mass acquisition and analysis following calibration and modification. The system was calibrated and modified using Agilent calibration standard standards A and B for MS prior to use. The peak has been analyzing and identifying to compare mass spectra with HMDB and FooDB databases18.

2.7 In vitro and in silico validation study of the identified antibacterial compound

2.7.1 Antibiofilm activity under in vitro conditions

The antibiofilm activity of the TLC-purified active fraction was evaluated against S. aureus utilising a 96-well microtiter plate assay. A 24-h bacterial culture of S. aureus has been adjusted to a bacterial inoculum of 0.5 McFarland. An aliquot of different concentration of bacterial suspension was added to each well, followed by overnight incubation 21. After incubation, free-floating cells were removed, and sterile phosphate-buffered saline (PBS; [HiMedia Laboratory, Mumbai, India]) was used to gently rinse the wells three times. After that, plates have been allowed to air dry for 30 minutes at room temperature. After staining the adherent biofilm for ten to fifteen minutes with 0.1% (W/V) crystal violet ([HiMedia Laboratories, Mumbai, India]), any excess stain has been removed by rinsing with sterile PBS.

Bound dye was subsequently solubilized in 200µL of 95% ethanol, and biofilm formation has been measured by determining the absorbance at 620nm utilising a Multiskan microplate reader (Thermo Fisher Scientific, Waltham, MA, USA) 22.

2.7.2 In silico analysis

Ligand preparation. The RCSB Protein Data Bank has provided crystal structure of S. aureus CifB (PDB ID: 4F24). Surfactin's molecular structure was obtained in SDF format from PubChem database.

Ligand conversion and optimization. The surfactin structure was converted to PDB format utilising Open Babel GUI (version 2.4.1) from ligand structures in SDF format. The 3D structure of surfactin was further optimized in Avogadro (version 1.2.0) and PyMOL (version 3.1.3.1) for obtaining an accurate spatial arrangement for molecular docking studies. Using the AutoDock Vina algorithm and PyRx software (version 0.8), docking simulations were performed.

Docking grid parameters. Grid sizes were X = 17.528 Å, Y = 9.500 Å, and Z = 5.222 Å, while grid center coordinates were X = 9.437, Y = -3.636, and Z = 4.243. The exhaustiveness value was set to 8.

Post-docking interaction analysis. UCSF Chimera (version 1.8) was used to analyze molecular interactions between S. aureus CifB (PDB ID: 4F24) and surfactin. The ligand binding characteristics, orientation, stability inside active site were investigated by analyzing the many types of interactions, comprising hydrophobic interactions, hydrogen bonding, and electrostatic interactions 23,24.

3 RESULTS AND DISCUSSION

3.1 Collection and isolation of plant associated bacteria

Healthy specimens of Baliospermum montanum were collected from the Raniamba forest region of Dang, Gujarat, India. Following surface sterilization and culture-based isolation, a total of 17 plant-associated bacterial isolates have been obtained from different tissues of B. montanum. No microbial growth has been observed on the control isolates used for assessing the effectiveness of surface sterilization, indicating that the recovered bacterial colonies were associated with the plant tissues.

The recovery of plant-associated bacteria from B. montanum indicated that this medicinal plant harbored culturable bacterial populations25,26. Previous studies have reported that healthy plant tissues can harbor diverse bacterial communities that may interact with their host and contribute to plant-associated functions. The diversity and recovery of plant-associated bacteria can be influenced by the plant species, tissue type, geographical location,

sampling season, surface sterilization procedure, culture medium, and incubation conditions25. In the recent research, use of nutrient agar and samples collected from the Dang region may have limited the recovery of culturable bacterial diversity. The isolation of these bacteria provided a basis for subsequent screening of their antibacterial potential.

3.2 Screening of crude and solvent extracts for antibacterial activity

The crude extracts obtained from the 17 bacterial isolates were initially screened against six pathogenic bacterial strains: P. vulgaris, S. pyogenes, S. aureus, P. aeruginosa, E. coli, and K. pneumoniae. Among isolates tested, S4S2 exhibited the strongest antibacterial activity against S. aureus, P. vulgaris, and E. coli and was therefore selected to further investigate.

The crude extract of S4S2 produced inhibition zones of 15.10 ± 0.10mm against S. aureus, 15.03 ± 0.05 mm against P. vulgaris, and 23.13 ± 0.15 mm against E. coli. Solvent extraction was subsequently performed using hexane, chloroform, and ethyl acetate. With inhibition zones of 34.50 ± 0.50mm against S. aureus, 39.50±0.50mm against P. vulgaris, and 32.16±1.00mm against E. coli, ethyl acetate extract had the most antibacterial activity among the solvent extracts. The chloroform extract produced inhibition zones of 25.00 ± 0.10, 25.00 ± 0.05, 23.12 ± 1.00 mm, respectively, whereas the hexane extract produced inhibition zones of 7.00 ± 0.50, 5.00 ± 0.10, and 6.00 ± 0.10mm, respectively (Table 1).

Table 1. Antibacterial activity of crude and solvent extracts of isolate S4S2 against pathogenic bacterial strains.

Bacterial strain	Crude extract Zone of inhibition (mm)	Ethyl acetate extract Zone of inhibition (mm)	Chloroform extract Zone of inhibition (mm)	Hexane extract Zone of inhibition (mm)	Streptomycin Zone of inhibition (mm)
S. aureus	15.10 ± 0.10	34.50 ± 0.50	25.00 ± 0.10	7.00 ± 0.50	16
P. vulgaris	15.03 ± 0.05	39.50 ± 0.50	25.00 ± 0.05	5.00 ± 0.10	14
E. coli	23.13 ± 0.15	32.16 ± 1.00	23.12 ± 1.00	6.00 ± 0.10	19

A Zone of inhibition is expressed as the diameter (mm). Values are presented as mean ± SD (n = 3).

Abbreviations: SD, standard deviation.

The comparatively greater activity of the ethyl acetate extract indicated that antibacterial metabolites produced by S4S2 were efficiently recovered in this solvent extract. Ethyl acetate can extract compounds with a range of polarities and may therefore recover several bioactive metabolites from microbial cultures27. The observed differences in activity among the solvent extracts further supported the selection of the ethyl acetate extract for subsequent fractionation and characterization.

3.3 Molecular identification

Based on its antibacterial activity, isolate S4S2 was selected for molecular identification. Analysis of 16S rRNA gene sequence identified isolate as Paenibacillus aquistagni, with 99% sequence similarity to the corresponding reference sequence. The isolate has been identified as P. aquistagni S4S2 and assigned accession number OP288247 in GenBank. Fig. 1 displays the evolutionary relationship between isolate S4S2 and closely related Paenibacillus species.

The identification of S4S2 as P. aquistagni was consistent with previous reports describing members of the genus Paenibacillus as producers of biologically active metabolites, including antimicrobial compounds28-30. The antibacterial activity observed in present study therefore supported further investigation of S4S2 as a potential source of bioactive secondary metabolites5.

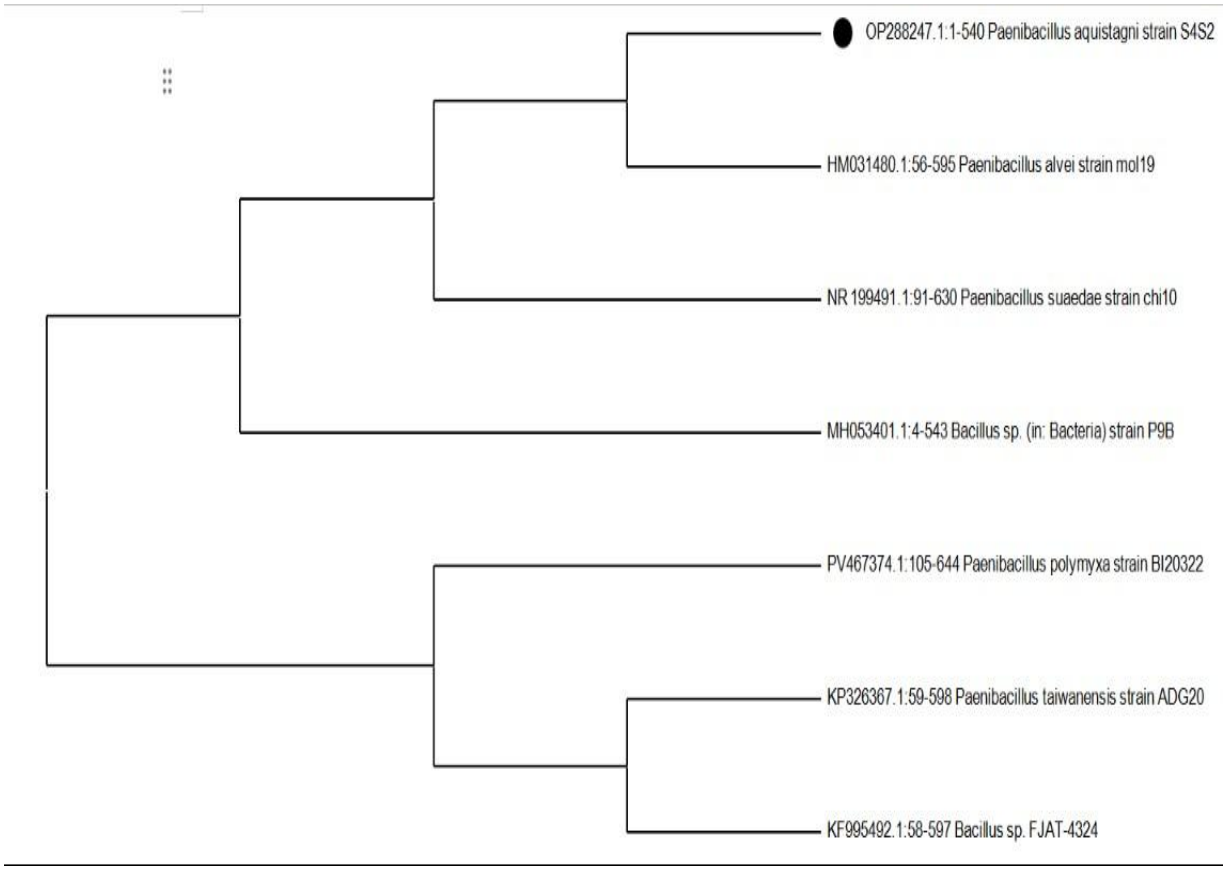

Fig 1. Phylogenetic relationships of Paenibacillus aquistagni OP288247 and related strains inferred from 16S rRNA gene sequences using the neighbor-joining method.

3.4 Column chromatography and antimicrobial activity of collected fractions

The ethyl acetate extract of P. aquistagni S4S2 has been subjected to silica gel column chromatography, and 4 fractions have been collected. Each fraction has been evaluated for antibacterial activity against S. aureus Fig. 2. Among the four fractions, fraction 2 exhibited a clear inhibition zone against S. aureus and was therefore selected for further TLC and bioautographic analysis.

The antibacterial activity detected in fraction 2 indicated that chromatographic fractionation facilitated the enrichment of antibacterial constituents from ethyl acetate extract18. The selection of the active fraction enabled subsequent localization and characterization of the antibacterial compound.

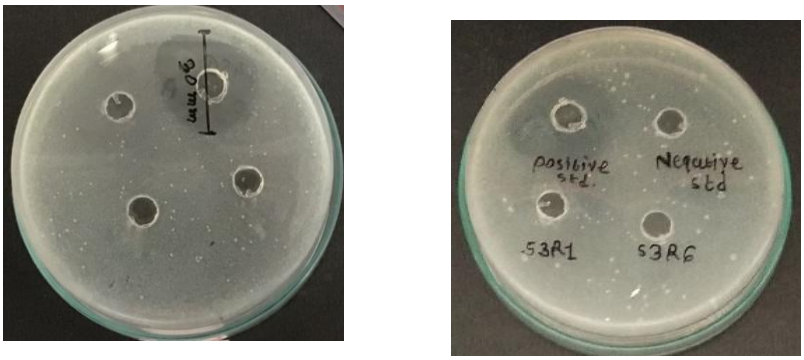

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Fig 2. Column chromatography and antibacterial activity of collected fractions.

3.5 TLC–Bioautographic identification of antibacterial constituents in the extract

TLC analysis of the bacterial ethyl acetate extract resulted in two distinct bands on the silica gel plate. The bands exhibited Rf values of 0.31 and 0.64 when visualized under UV light at 254 and 366nm, respectively. The dichloromethane: methanol solvent system at a ratio of (7:3, v/v) provided separation of constituents present in the extract (Fig. 3).

The antibacterial component was then localized using TLC-bioautography. The band with an Rf value of 0.64 produced a clear zone of inhibition against S. aureus, whereas the other band did not show detectable antibacterial activity under the conditions tested. The active band has been scraped from TLC plate, eluted with methanol, recovered for subsequent LC–MS analysis.

The combination of chromatographic separation and bioautography allowed the antibacterial activity to be directly associated with a specific chromatographic band. Similar TLC-bioautography approaches have been used for the rapid localization of antibacterial metabolites in microbial extracts. 18,31. The distinct inhibition zone associated with the band at Rf 0.64 confirmed the presence of a major antibacterial component suitable for subsequent chemical characterization.

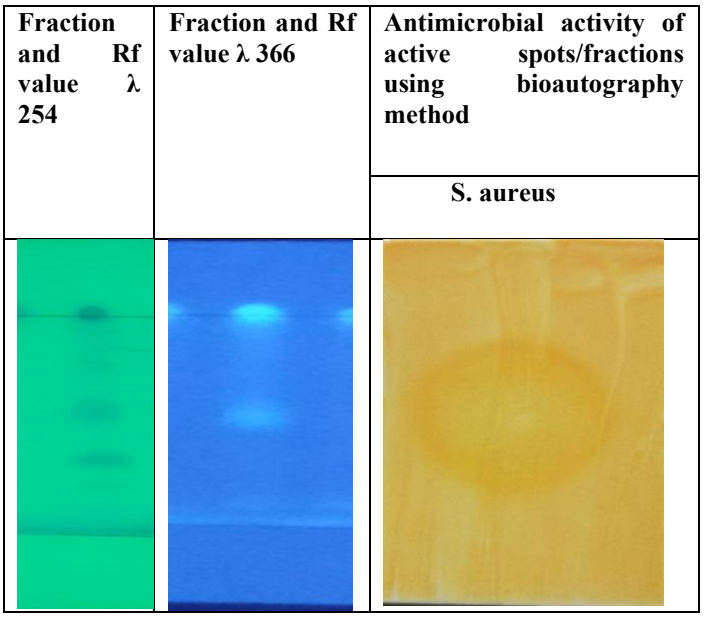
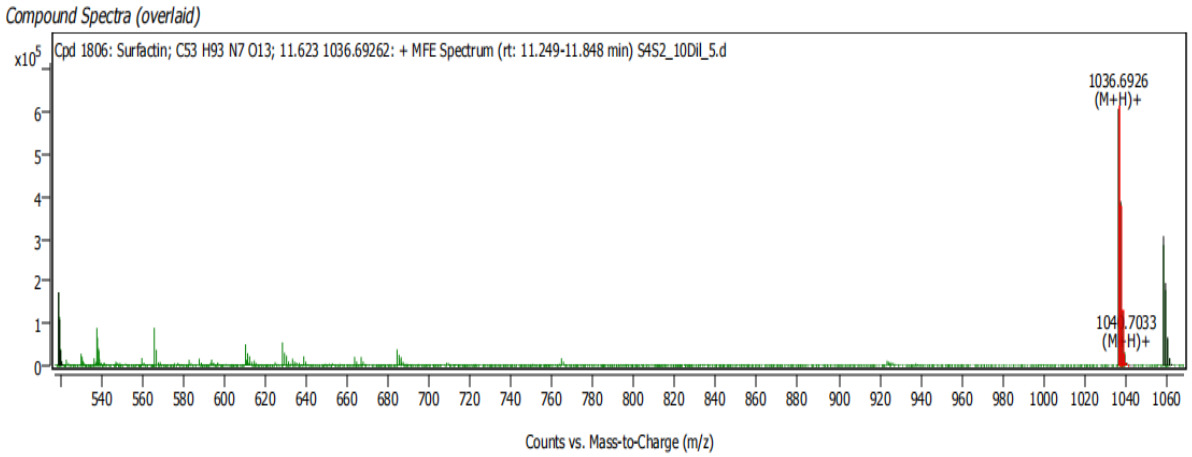


Fig 3. TLC-bioautographic identification of antibacterial constituents in the ethyl acetate extract

3.6 LC–MS Profiling for the identification of bioactive compounds

A chemical with a predicted molecular formula of C53H93N7O13 and a protonated precursor ion at m/z 1036.6926 ([M+H] +) has been identified by LC–MS analysis of active fraction. Analysis using tandem mass spectrometry revealed a distinctive fragment ion at m/z 285.0844. The metabolite was possibly identified as surfactin by comparing the observed mass spectral data with reference spectra in the HMDB and FOODB databases (Fig. 4).

Surfactin is a cyclic lipopeptide biosurfactant that has been widely investigated for its antibacterial properties32,33. Its amphiphilic structure enables interaction with bacterial membranes and has been associated with membrane destabilization and inhibition of bacterial growth33,34. The putative identification of surfactin in the active fraction was consistent with the antibacterial activity observed for the S4S2 extract and supported further evaluation of the compound for antibiofilm activity and molecular interactions.



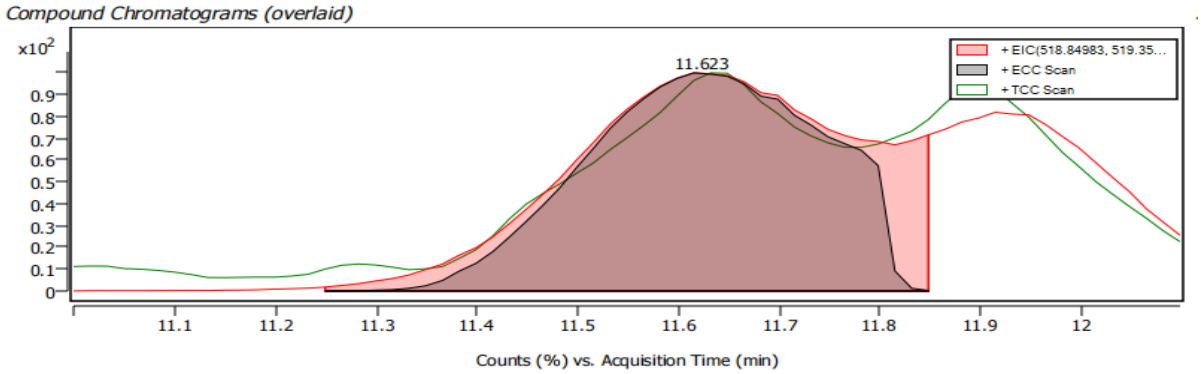


Fig 4. Bioactive compounds were identified using LC-MS

3.7 In vitro and in silico validation study of the identified antibacterial compound

3.7.1 Antibiofilm activity under in vitro conditions

The TLC-purified active fraction was evaluated for antibiofilm activity against S. aureus. Surfactin concentrations of 0.25, 0.5, 0.75, and 1.0mg/mL were tested. At 0.25, 0.5, 0.75, and 1.0mg/mL, the drug reduced biofilm formation by 3.03%, 24.07%, 27.24%, and 33.33% (Fig. 5).

Biofilm inhibition increased with increasing surfactin concentration, and the highest inhibition of 33.33% was observed at 1.0mg/mL. These findings indicated a concentration-dependent effect of the tested fraction on S. aureus biofilm formation.

The antibiofilm activity of surfactin likely attributed to its biosurfactant properties, which facilitate disruption of the extracellular polymeric substance (EPS) matrix and reduce bacterial adhesion. Previous studies have similarly demonstrated that surfactin inhibits biofilm formation and disrupts mature biofilms, supporting its potential application as an antibiofilm agent against pathogenic bacteria35,36.

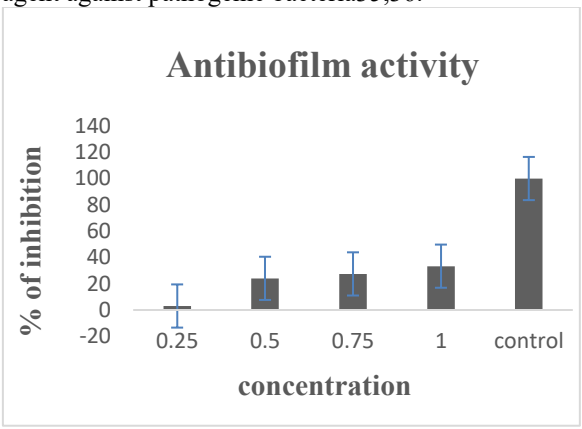


Fig 5. Antibiofilm activity under in vitro conditions.

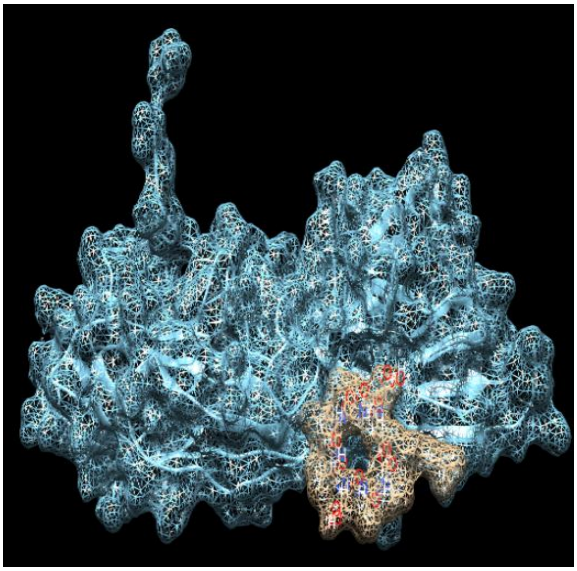
3.7.2 In silico analysis

Molecular docking has been performed for investigating the interaction of surfactin with the S.aureus CifB protein (PDB ID: 4F24). Surfactin has a predicted -6.4 kcal/mol binding affinity. Docking showed that surfactin occupied CifB's predicted binding area and interacted with multiple amino acid residues (Fig. 1).

The protein–ligand interface was predominantly characterized by van der Waals and hydrophobic interactions, (Table 2). The binding pocket of CifB comprised key amino acid residues including Ser158, Asp265, Ile184, and Gly159, which established multiple van der Waals contacts with surfactin, whereas Gln235 and Ser236 participated in hydrogen-bond interactions. Phe328 and Trp522 were identified as aromatic residues that may contribute to stabilization of the docked complex through π–π stacking interactions (Table 2).

The predominance of hydrophobic interactions was consistent with the amphipathic nature of surfactin and suggested that hydrophobic interactions may contribute to its association with the CifB binding region. The predicted binding affinity indicated a favorable protein–ligand interaction under the docking conditions used. However, molecular docking provides predictive evidence supports the possibility that surfactin may interfere with the biological function of CifB.

(A)



(B)

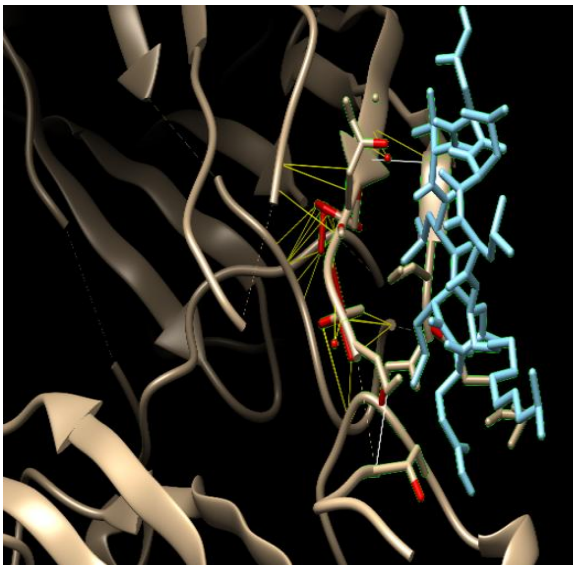


Fig. 1 Molecular docking analysis of surfactin with CifB. (A) Mesh representation illustrating the binding pocket and the accommodation of surfactin within the active site of CifB. (B) Surface representation of the CifB binding cavity showing docked surfactin and the spatial arrangement of the interacting amino acid residue

Table 2. Molecular interactions between surfactin and amino acid residues of CifB identified by molecular docking.

CifB amino acid residue	Interaction type	Interacting molecule	Interaction description
Ser158	Van der Waals	Surfactin	Multiple close contacts contribute to stable ligand binding.
Asp265	Van der Waals	Surfactin	Multiple van der Waals contacts contribute to ligand stabilization.
Ile184	Van der Waals	Surfactin	Involved in hydrophobic interaction interface.
Gly159	Van der Waals	Surfactin	The backbone contributes to stabilization of the binding interface.
Gln235, Ser236	Hydrogen bond	Polar group of surfactin	Important for ligand specificity.
Phe328, Trp522	Potential aromatic interaction	surfactin	May contribute to stabilization of the protein–ligand complex.

b Interactions were identified from the molecular docking analysis of surfactin with CifB (PDB ID: 4F24)
Abbreviation: CifB: clumping factor B; PDB: Protein Data Bank

CONCLUSION

The recent research showed that the plant-associated Paenibacillus aquistagni isolate S4S2, obtained from Baliospermum montanum, is a promising source of bioactive antimicrobial metabolites. The isolate exhibited broad-spectrum antibacterial activity against clinically important pathogenic bacteria and showed significant antibiofilm activity, highlighting its potential for controlling bacterial infections associated with biofilm formation. Bioassay-guided purification, supported by TLC analysis, followed by LC-MS characterization identified surfactin as the major bioactive lipopeptide responsible for the observed antibacterial activity. Furthermore, molecular docking analysis demonstrated a favorable interaction between surfactin and virulence-associated CifB protein, providing computational evidence for its ligand-protein interactions. These findings demonstrate the therapeutic potential of plant-associated Paenibacillus-derived surfactin as an antibacterial and antibiofilm agent.

Further studies involving purification to homogeneity, mechanistic investigations, toxicity evaluation, and in vivo validation are warranted to establish its suitability for pharmaceutical and biomedical applications.

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Conflict of Interest Disclosure

Nil.

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Author's Contribution

Krishna Chaudhari: Conceptualisation, methodology, investigation, sample collection, laboratory experiments, data curation, formal analysis, visualization, and writing-original draft.

Naga Rathna Supriya G.: Conceptualisation, supervision, methodology, validation, project administration, resources, writing-review and editing.

The final manuscript was reviewed and approved by all authors.

Data Availability Statement

With accession number OP288247, the 16S ribosomal RNA (16S rRNA) gene sequence of Paenibacillus aquistagni has been added to the NCBI GenBank database. The corresponding author will make datasets produced and/or analyzed during the recent work available upon reasonable request.

Ethical Approval Statement

N/A

Informed Consent Statement

N/A

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